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NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet 2021; 11; 398(10304): 957-80.

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World Health Organization, Geneva. 2019. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: seventh report of a WHO study group. WHO Technical Report Series, No. 1015.

NCD Risk Factor Collaboration (NCD-RisC). Rising rural body-mass index is the main driver of the global obesity epidemic in adults. Nature 2019; 569: 260–64.

World Health Organization. Novel Coronavirus (2019-nCoV) Situation Report 85, April 14, 2020. [cited April 2020] Accessed from: https://www.who.int/docs/defaultsource/coronaviruse/situationreports/20200414-sitrep-85-covid-19.

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# Factors associated with mortality among retinoblastoma patients in Malaysia: A retrospective cohort study

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## ABSTRACT

**Introduction: Retinoblastoma is the most common primary intraocular cancer worldwide. Research into factors associated with mortality in retinoblastoma from the Southeast Asian region is currently limited. The present study aims to identify the associated factors that predict mortality among retinoblastoma patients in Malaysia.**

**Materials and Methods: A retrospective cohort study was conducted on retinoblastoma patients diagnosed between January 2004 and April 2023 at hospitals with paediatric ophthalmology services in Malaysia. Data were collected from the Retinoblastoma Registry of the National Eye Database and patients' medical records. The adjusted hazard ratio (AHR) was used in the multivariable Cox regression model to identify the factors associated with mortality among retinoblastoma patients.**

**Results: A total of 402 retinoblastoma patients were included in the study. Of these, 22 (5.5%) patients died, while 353 (87.8%) were alive and under follow-up at the end of the study, and 27 (6.7%) were lost to follow-up. The univariable Cox regression model identified laterality, lag time, and recurrence as the associated factors. The multivariable Cox regression model confirmed that bilateral (AHR: 3.64; 95% CI: 1.46, 9.02; p=0.004) and longer lag time (AHR: 4.03; 95% CI: 1.57, 10.35; p=0.004) were independent predictors of mortality.**

**Conclusion: This study found that bilateral and longer lag time were independent prognostic factors associated with higher mortality among retinoblastoma patients in Malaysia. These results highlight the utmost importance of early diagnosis and access to appropriate treatment to improve survival outcomes for retinoblastoma patients.**

**KEYWORDS:**
*Retinoblastoma, factors associated, mortality, Cox model, Malaysia*

## INTRODUCTION

Retinoblastoma is a tumour that develops in the retina and is the most common intraocular malignancy in children, contributing significantly to mortality worldwide. This type of cancer accounts for around 3% of all childhood cancers and usually affects children under the age of five when diagnosed.[1] The incidence rate of retinoblastoma is approximately 1 case per 15,000 to 20,000 live births, resulting in around 9000 new cases annually.[2] Early detection and treatment are essential for retinoblastoma to prevent severe consequences such as blindness, loss of the affected eye, or even death.[3] Prompt treatment is essential for improving the overall prognosis of patients diagnosed with retinoblastoma.[4]

Previous studies have examined the prognostic factors associated with mortality across various regions. In high-income countries, factors such as age at diagnosis, gender, laterality of the disease, disease extension, and treatment abandonment were identified.[5-8] Meanwhile, studies from middle-income countries highlighted the presenting signs, laterality of the disease, and disease extension as predictors of mortality.[3,9-11] On the other hand, a study in low-income countries found that disease extension and acceptance of chemotherapy were significant predictors of mortality.[12]

Retinoblastoma poses a significant burden in paediatric patients in low- and middle-income countries, leading to a less favorable prognosis and increased chances of mortality.[13] In Malaysia, delays in diagnosis and refusal of treatment can lead to lower survival rates.[14] Given these disparities, a region-specific understanding of retinoblastoma prognosis is essential for optimizing patient management and improving survival outcomes. To the best of our knowledge, there is limited research investigating the factors associated with retinoblastoma mortality in Southeast Asian countries. Although a study in Malaysia has examined prognostic factors in patients with unilateral retinoblastoma, the combination evaluation of prognostic factors in both unilateral and bilateral cases remains underexplored.[15] Thus, this study aimed to identify key prognostic factors that predict

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mortality in both unilateral and bilateral retinoblastoma patients in Malaysia.

## MATERIALS AND METHODS

### Study design and setting

A retrospective cohort study involved a record review of retinoblastoma patients diagnosed in hospitals with paediatric ophthalmology services in Malaysia between January 2004 and April 2023. These include Hospital Kuala Lumpur, Hospital Tunku Azizah in Kuala Lumpur, Hospital Wanita dan Kanak-Kanak Sabah, Hospital Queen Elizabeth in Sabah, Hospital Umum Kuching in Sarawak, Hospital Sultanah Nur Zahirah in Terengganu, and Hospital Pakar Universiti Sains Malaysia in Kelantan.

### Inclusion and exclusion criteria

All patients with retinoblastoma were clinically diagnosed using indirect ophthalmoscopy, and those with tumour extension were further evaluated by imaging through computed tomography scan (CT scan) or magnetic resonance imaging (MRI). In some cases, the diagnosis was confirmed through histopathological examination. The study included patients who were clinically diagnosed with retinoblastoma. Those diagnosed with retinoblastoma who had an indefinite date of diagnosis or indefinite date of last clinical visit were excluded from the analysis.

### Sample size and sampling method

A post-hoc sample size calculation for survival analysis was performed using Power and Sample Size Software to evaluate whether the study had sufficient power to detect the effect of disease extension between extraocular and intraocular retinoblastoma on mortality.[16] The analysis was based on the prognostic factor of disease extension observed in the current study. Using the final sample size of 402 retinoblastoma patients and a median survival time of 12 months, the power to detect a hazard ratio comparing extraocular to intraocular disease of 8.43 was recalculated. A two-tailed significance level of 0.05 was applied. The ratio of intraocular to extraocular retinoblastoma patients, 1.41, was incorporated based on the study findings. The accrual period was 232 months, with an additional 12 months of follow-up. The post-hoc analysis yielded an observed power of 0.98, indicating that the study had sufficient statistical power to detect the effect between extraocular and intraocular disease and mortality in retinoblastoma patients. These findings suggest that the study was well-powered to support its conclusions regarding the prognostic impact of disease extension. Given the rarity of retinoblastoma and the limited number of available cases for study, probability sampling methods were not feasible to implement in order to maximize statistical power.

### Data collection

This study obtained relevant data from the Retinoblastoma Registry of the National Eye Database (NED) and patients' medical records using a standardized data collection tool. The data collected encompassed patients' demographic characteristics, clinical features, treatments received, and outcomes. For cases of bilateral retinoblastoma, the analysis included only the eye with the more advanced disease, as determined by the clinical staging criteria of the International Retinoblastoma Staging System (IRSS). These stages denote orbital extension or metastatic spread, indicating more severe disease progression. Additionally, the date of the last clinical visit and any missing information were retrieved from the patients' medical records.

### Statistical analysis

This study conducted data analyses using the STATA/SE software version 15. The association between two categorical variables was compared using either the Pearson Chi-square test or Fisher's exact test. Survival analyses were based on time-to-event data, with survival time measured from the date of retinoblastoma diagnosis until the date of death or the last clinical visit date. Censored observations referred to patients who were alive at the end of the study period or were lost to follow-up. The event was defined as a patient who died from retinoblastoma. A preliminary analysis using the simple Cox regression model was conducted to identify potential prognostic factors with a p-value less than 0.25. Subsequently, an advanced multivariable Cox regression model was conducted to identify the independent prognostic factors associated with mortality among retinoblastoma patients. This analysis was guided by the principles of parsimony, biological plausibility, and statistical significance. The full stepwise selection method was employed for variable selection to ensures that the final model includes only the most significant predictors, and all possible multicollinearity and interaction effects were thoroughly examined.

Hazard function plot, log minus log plot, Schoenfeld partial residuals plot, scaled and non-scaled Schoenfeld, and C-statistics were performed to check the proportional hazards assumptions. Regression diagnostics were also conducted, using Cox-Snell residuals to evaluate model fitness, Martingale residuals to assess adequate functional form, and deviance residuals and influence analysis to determine the presence of outliers and its influence on the model fitness. The final best model was chosen based on the consideration of statistically significant and clinically important variables. Crude hazard ratios (CHR) and adjusted hazard ratios (AHR) were estimated with 95% confidence intervals (CI), Wald statistics, and corresponding p-values. The level of significance was set at 0.05 in a two-tailed test.

### Ethical approval

The study was approved by the Medical Research Ethics Committee, Ministry of Health Malaysia (NMRR ID-22-00956-GJE (IIR)), UniSZA Human Research Ethics Committee (UniSZA/UHREC/2023/495(1)), and the USM Human Research Ethics Committee (USM/JEPeM/KK/24030236). Informed consent was not required as the secondary data were used in this study. However, permission to access patients' medical records was obtained from the participating hospitals. Confidential codes were used in the data collection sheet to represent each patient to protect their privacy and confidentiality.

**Table I: Comparison of demographic and clinical characteristics of retinoblastoma patients (n = 402)**

| Characteristics | Total (%) | Died (%) | Alive (%) | p-value |
|---|---|---|---|---|
| Age at diagnosis | | | | |
| Less than 12 months | 96 (23.9) | 3 (13.6) | 93 (24.5) | 0.641 |
| 12-23 months | 111 (27.6) | 8 (36.4) | 103 (27.1) | |
| 24-36 months | 105 (26.1) | 6 (27.3) | 99 (26.0) | |
| More than 36 months | 90 (22.4) | 5 (22.7) | 85 (22.4) | |
| Gender | | | | |
| Female | 174 (43.3) | 10 (45.5) | 164 (43.2) | 0.833 |
| Male | 228 (56.7) | 12 (54.5) | 216 (56.8) | |
| Ethnicity | | | | |
| Non-malay | 147 (36.6) | 9 (40.9) | 138 (36.3) | 0.664 |
| Malay | 255 (63.4) | 13 (59.1) | 242 (63.7) | |
| Family history | | | | |
| No | 387 (96.3) | 21 (95.5) | 366 (96.3) | 0.577 |
| Yes | 15 (3.7) | 1 (4.5) | 14 (3.7) | |
| Leading sign | | | | |
| Proptosis | 20 (5.0) | 3 (13.6) | 17 (4.5) | 0.101 |
| Leukocoria | 334 (83.1) | 18 (81.8) | 316 (83.2) | |
| Strabismus | 48 (11.9) | 1 (4.6) | 47 (12.3) | |
| Laterality | | | | |
| Bilateral | 143 (35.6) | 15 (68.2) | 128 (33.7) | 0.001 |
| Unilateral | 259 (64.4) | 7 (31.8) | 252 (66.3) | |
| Disease staging | | | | |
| Stage A-E | 345 (85.8) | 0 (0.0) | 345 (90.8) | 0.001 |
| Stage 0-II | 30 (7.5) | 1 (4.5) | 29 (7.6) | |
| Stage III | 8 (2.0) | 2 (9.1) | 6 (1.6) | |
| Stage IV | 19 (4.7) | 19 (86.4) | 0 (0.0) | |
| Disease extension | | | | |
| Intraocular | 344 (85.6) | 0 (0.0) | 344 (90.5) | 0.001 |
| Extraocular | 58 (14.4) | 22 (100.0) | 36 (9.5) | |
| Treatment received | | | | |
| Radiotherapy | 18 (2.4) | 1 (2.3) | 17 (2.3) | 0.230 |
| Focal therapy | 136 (17.8) | 4 (8.9) | 132 (18.4) | |
| Chemotherapy | 304 (39.8) | 20 (90.9) | 284 (39.6) | 0.853 |
| Enucleation | 305 (40.0) | 20 (44.4) | 285 (39.7) | |
| Recurrence | | | | |
| No | 382 (95.0) | 19 (86.4) | 363 (95.5) | 0.055 |
| Yes | 20 (5.0) | 3 (13.6) | 17 (4.5) | |
| Lag time | | | | |
| Less than 6 months | 285 (70.9) | 7 (31.8) | 278 (73.2) | 0.001 |
| More than 6 months | 117 (29.1) | 15 (68.2) | 102 (26.8) | |
| Years of diagnosis | | | | |
| 2004-2008 | 84 (20.9) | 4 (18.2) | 80 (21.1) | 0.766 |
| 2009-2013 | 90 (22.4) | 6 (27.3) | 84 (22.1) | |
| 2014-2018 | 104 (25.9) | 7 (31.8) | 97 (25.5) | |
| 2019-2023 | 124 (30.8) | 5 (22.7) | 119 (31.3) | |

## RESULTS

A total of 402 retinoblastoma patients were included in the final analysis. Of these, 353 (87.8%) were alive and under follow-up at the end of the study and 27 (6.7%) were lost to follow-up, while 22 (5.5%) patients died. Table I shows the comparison of demographic and clinical characteristics of retinoblastoma patients. Most of the patients (27.6%) were between 12-23 months old when diagnosed with retinoblastoma. Approximately 63.4% of patients were Malays, and 56.7% were males. Only one patient with family history died from retinoblastoma. The majority of patients with stage IV died from retinoblastoma (86.4%). The analysis showed statistically significant differences in disease staging (p=0.001), disease extension (p=0.001), laterality (p=0.001), lag time (p=0.001) between the two groups.

Table II displays the factors associated with mortality among retinoblastoma patients using the univariable Cox regression model. The factors were considered important if the p-value < 0.250. The results revealed that three predictors were deemed significant as prognostic factors. These include disease staging (p<0.250), laterality (p=0.004), lag time (p=0.001), and recurrence (p=0.138).

The proportional hazards assumptions were met. Table III exhibits the factors associated with mortality among retinoblastoma patients using the multivariable Cox regression model. The analysis identified two predictors as independent prognostic factors of mortality. These include laterality and lag time. Patients with bilateral cases had a significantly 3.64 times higher risk of dying than patients with unilateral cases (AHR: 3.64; 95% CI: 1.46, 9.02; p=0.004). Furthermore, patients who had lag time more than 6 months had a 4 times higher risk of dying than those who had lag time less than 6 months (AHR: 4.03; 95% CI: 1.57, 10.35; p=0.004).

**Table II: Factors associated with mortality among retinoblastoma patients using the univariable Cox regression model (n = 402)**

| Characteristics | Regression coefficient (b) | Crude HR (95% CI) | Wald statistic | p-value |
|---|---|---|---|---|
| Age at diagnosis | | | | |
| Less than 12 months | 0.95 | 2.58 (0.68-9.74) | 1.40 | 0.162 |
| 12-23 months | 0.68 | 1.98 (0.50-7.92) | 0.97 | 0.334 |
| 24-36 months | 0.66 | 1.94 (0.46-8.14) | 0.91 | 0.363 |
| More than 36 months | 0 | 1 | | |
| Gender | | | | |
| Female | 0.06 | 1.06 (0.46-2.45) | 0.13 | 0.892 |
| Male | 0 | 1 | | |
| Ethnicity | | | | |
| Non-malay | 0 | 1 | | |
| Malay | -0.30 | 0.74 (0.32-1.73) | -0.69 | 0.488 |
| Family history | | | | |
| No | 0 | 1 | | |
| Yes | 0.26 | 1.30 (0.18-9.67) | 0.26 | 0.797 |
| Proptosis | | | | |
| No | 0 | 1 | | |
| Yes | 0.70 | 2.02 (0.47-8.63) | 0.95 | 0.344 |
| Strabismus | | | | |
| No | 0 | 1 | | |
| Yes | -0.97 | 0.38 (0.05-2.83) | -0.94 | 0.345 |
| Leukocoria | | | | |
| No | 0 | 1 | | |
| Yes | 0.18 | 1.19 (0.35-4.03) | 0.28 | 0.777 |
| Laterality | | | | |
| Unilateral | 0 | 1 | | |
| Bilateral | 1.32 | 3.76 (1.53-9.23) | 2.89 | 0.004 |
| Disease staging | | | | |
| Stage 0-I | 0 | 1 | | |
| Stage II | 1.77 | 5.85 (0.65-52.35) | 1.58 | 0.114 |
| Stage III | 2.02 | 20.51 (2.39-175.78) | 2.76 | 0.016 |
| Stage IV | 3.76 | 38.22 (10.40-250.40) | 3.47 | 0.0012 |
| Recurrence | | | | |
| No | 0 | 1 | | |
| Yes | 0.92 | 2.51 (0.74-8.51) | 1.48 | 0.138 |
| Lag time | | | | |
| Less than 6 months | 0 | 1 | | |
| More than 6 months | 1.77 | 5.86 (2.39-14.38) | 3.86 | 0.001 |
| Years of diagnosis | | | | |
| 2004-2008 | 0 | 1 | | |
| 2009-2013 | 0.26 | 1.30 (0.37-4.62) | 0.41 | 0.682 |
| 2014-2018 | 0.39 | 1.48 (0.43-5.06) | 0.62 | 0.535 |
| 2019-2023 | 0.58 | 1.78 (0.46-6.92) | 0.84 | 0.402 |

CI: Confidence interval

**Table III: Factors associated with mortality among retinoblastoma patients using the multivariable Cox regression model (n = 402)**

| Characteristics | Regression coefficient (b) | Crude HR (95% CI) | p-value | Regression coefficient (b) | Adjusted HR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| Laterality | | | | | | |
| Extension | 1.32 | 3.76 (1.53-9.23) | 0.004 | 1.27 | 3.64 (1.46-9.02) | 0.004 |
| Lag time | 1.77 | 5.86 (2.39-14.38) | 0.001 | 1.39 | 4.03 (1.57-10.35) | 0.004 |

HR: Hazards ratio
CI: Confidence interval
Backward stepwise Cox proportional hazards regression model applied, multicollinearity and interaction term were unlikely, the preliminary final model was properly specified, hazard function plot, Nelson-Aalen cumulative hazard function, log-minus-log plot, partial residuals, scaled and non-scaled Schoenfeld residuals plot, and C-statistics were applied to check for model assumptions, regression diagnostics were conducted by Cox-snell residuals, Martingale residuals, Deviance residuals, and influence analysis, no influential outliers were detected in the model.

## DISCUSSION

This is the comprehensive study on determining the key associated factors that predict mortality among retinoblastoma patients in Malaysia. Retinoblastoma patients in high-income countries have experienced a remarkable improvement in prognosis over the past five decades, with survival rates now approaching 100%. These advancements are attributed to multiple factors, such as the establishment of specialized treatment institutions and the implementation of targeted screening initiatives.[17] A global study by Tomar et al. found that retinoblastoma patients in upper-middle-income and lower-middle-income countries demonstrated a 10.3-fold and 9.3-fold greater risk of mortality respectively, compared to those from high-income countries.[14] This study further reported that low-income countries were significantly associated with a higher risk of poor treatment outcomes for retinoblastoma patients.

In the current study, bilateral and longer lag time were found to have an impact on the mortality of retinoblastoma patients. Our findings indicated that patients with bilateral had an 3.64-fold higher risk of mortality from retinoblastoma compared to those with unilateral cases. Previous studies on regional disparities have consistently reported that laterality is associated with increased mortality for retinoblastoma patients globally.[6,7] The AHR for mortality was found to be 3.78 in high-income countries,[9] and 1.70 in middle-income country.[10] Our findings are comparable to those seen in high-income countries. This occurs in patients who presented late and their prognosis are poor. This is likely due to a delayed presentation in seeking appropriate modern treatment, as some parents may opt for traditional treatment of care rather than pursuing the recommended medical interventions. Thus, patient education initiatives and financial support programs should be introduced to increase awareness and accessibility of retinoblastoma care. These strategies can help empower patients and their families, ensuring timely diagnosis and enabling access to critical treatment options, particularly for those from lower-income backgrounds.

Another important prognostic factor identified in this study was longer lag time. The risk effect of longer lag time with an AHR of 4 is noteworthy. TA longer lag time between symptom onset and diagnosis was associated with mortality among patients with retinoblastoma. This delay likely reflects late recognition of symptoms, limited awareness, and challenges in accessing specialized care, leading to advanced disease at presentation. Similar findings have been reported in other developing countries, where prolonged lag time contributes to higher mortality. Notably, our study findings indicate that the tumour has remained intraocular suggesting that early treatment intervention was effective. The possible reasons for parents who had history of default treatment may be reluctance towards surgical interventions, preference for alternative traditional therapies, financial constraints, and concerns about the potential consequences of the treatment outcome.

In this series, the majority of retinoblastoma patients died at an advanced stage IV. The higher proportion of mortality observed in stage IV underscores the critical need for early detection and treatment to improve survival outcomes. A recent study reported a higher frequency of patients diagnosed with the more advanced stage among the older age groups.[22] In contrast, a study conducted by Zhang et al. in China showed that the advanced disease was found highly significant prognostic factor in the mortality of retinoblastoma patients ($p<0.001$).[10] This discrepancy could be attributed to the differences in healthcare access, and socioeconomic factors between the two countries.

In the present study, age at diagnosis was not identified as an independent prognostic factor for mortality. This contrasts with a study in the United States that found higher mortality risk among patients diagnosed between 1 to 9 years of age, compared to those below 1 year.[8] This observation is further supported by Fernandes et al. who reported that retinoblastoma patients aged 1 to 3 years faced greater mortality risk than those younger than 1 year.[23] The differences observed between our study and others may be attributed to the relatively older age of diagnosis and the progression of the disease in the Malaysian population compared to high-income countries. This is attributed to the advancements in decoding the genetic basis of the retinoblastoma disease, which have enabled improved understanding and treatment approaches in high-income countries.[14]

The selection of appropriate treatment modalities for retinoblastoma is partially influenced by the tumour's laterality.[24,25] A previous study conducted in China reported that patients with bilateral retinoblastoma exhibited a statistically significantly higher mortality risk compared to those with unilateral disease.[11] This finding was further supported by another study in the United States, which similarly indicated that bilateral cases were more likely to develop secondary tumours, leading to a higher risk of mortality.[23] This highlights that the majority of retinoblastoma patients in the current study setting had unilateral disease, which may have contributed to their relatively better prognosis compared to those with bilateral involvement.

One of the key strengths of this study is the large sample size of retinoblastoma patients and the involvement of the majority of paediatric ophthalmology services across Malaysia. The researchers were able to analyze a sample of 402 retinoblastoma patients registered in hospitals with specialized paediatric ophthalmology departments throughout the country, which allows for a more robust and comprehensive statistical analysis. This broader representation of the retinoblastoma population in Malaysia enhances the generalizability and significance of the study findings.

The limitation of this retrospective study was the potential introduction of selection bias due to incomplete or missing information in the patient registry. The retrospective nature of the data collection process may have resulted in the unavailability of some relevant details, which could have influenced the analysis and interpretation of the findings. The observations that were excluded from the analysis may have had a worse prognosis, which could have resulted in an underestimation of potential confounding factors and

influenced the prediction of prognostic factors associated with mortality by the Cox regression model. These limitations should be taken into consideration when interpreting the findings of this study.

## CONCLUSION

This large-scale study on retinoblastoma in Malaysia identified bilateral and longer lag time as independent prognostic factors associated with higher mortality. These findings underscore the importance of timely diagnosis and access to appropriate treatment for improving survival outcomes of retinoblastoma patients in Malaysia. Thus, this study's findings may contribute to the body of knowledge on survival outcomes and is of paramount importance as it lays the groundwork for future studies. Healthcare systems are encouraged to develop comprehensive nationwide or regional screening programs to detect retinoblastoma in its earliest stages, particularly among high-risk populations. Additionally, policymakers should prioritize increasing funding and resources for paediatric oncology services, ensuring all families, regardless of socioeconomic background, can access life-saving treatments and genetic counselling for children at risk of this disease.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORIGINAL ARTICLE

# Depression prevalence and its associated factors among sub-urban residents in Kuala Nerus

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## ABSTRACT

**Introduction: Depression is a prevalent mental health disorder and a growing public health concern. Understanding its burden, this study aimed to determine the prevalence of depression and identify its associated factors among suburban adult residents of Kuala Nerus, Malaysia.**

**Materials and Methods: A community-based cross-sectional study was conducted among 689 adults aged 18 and above who had resided in a suburban area for at least one month and were proficient in Bahasa Malaysia. Data were collected through a house-to-house survey using a pre-tested, validated questionnaire administered via Google Form. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9), and multiple logistic regression analysis was performed to identify associated factors.**

**Results: The prevalence of depression among respondents was 26.4% (95% CI: 23.1, 29.7). Multiple logistic regression analysis identified several significant factors associated with depression. Older age (adjusted OR: 0.972, 95% CI: 0.948, 0.996; p=0.020), being married (adjusted OR: 0.377, 95% CI: 0.188, 0.754; p=0.006), engaging in physical activity (adjusted OR: 0.669, 95% CI: 0.459, 0.976; p=0.037), and higher total knowledge scores (adjusted OR: 0.883, 95% CI: 0.825, 0.945; p < 0.001) were less likely to have depression. Conversely, being female (adjusted OR: 1.614, 95% CI: 1.076, 2.421; p=0.021) and having a history of depression (adjusted OR: 2.977, 95% CI: 1.122, 7.899; p=0.028) were more likely to have depression.**

**Conclusion: The study highlights the substantial burden of depression in suburban communities and underscores the need for targeted mental health interventions. Efforts to improve mental health literacy and promote protective factors, particularly among vulnerable groups such as younger individuals and females, are essential. Further research is warranted to develop effective prevention and treatment strategies.**

**KEYWORDS:**
*Prevalence, depression, cross-sectional, PHQ-9*

## INTRODUCTION

Depression is a widespread mental health disorder affecting approximately 280 million people globally.[1] It manifests as persistent sadness, a loss of interest in daily activities, and various physical and emotional symptoms. Without proper treatment, depression can lead to significant impairment, including social isolation, disability, and an increased risk of mortality.[1] The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) provides clear criteria for diagnosing depression, emphasizing the need for early detection and effective intervention.[2]

In Malaysia, depression poses a critical public health challenge, further complicated by societal stigma and underreporting.[3] Despite existing mental health initiatives, the prevalence of depression is on the rise, particularly among socioeconomically disadvantaged groups. The COVID-19 pandemic has exacerbated this issue, leading to a notable increase in depression rates nationwide.[4] Although Terengganu is not among the states with the highest prevalence, the risk of depression remains considerable in suburban areas where public awareness and access to mental health services may be limited.[3]

Stigma and a lack of understanding about mental health issues often prevent individuals from seeking appropriate care, contributing to the rising suicide rates in Malaysia.[5] For years, the stigmatization of mental illness has made depression a taboo subject among Malaysians.[6]

This study aims to assess the prevalence of depression and its associated factors among adults in Kuala Nerus, Terengganu. By analysing sociodemographic characteristics, clinical profiles, community knowledge, attitudes, and behaviours toward depression, this research seeks to identify key risk factors and inform targeted interventions. The findings will contribute to a deeper understanding of depression in this region, address existing knowledge gaps, and support the development of more effective public health strategies. Ultimately, the study aims to reduce the burden of depression and enhance the mental health and well-being of the community.

## MATERIALS AND METHODS

The community-based cross-sectional survey was conducted among adults in a suburban area, Kampung Felda Belara, in Kuala Nerus with ethics approval from the Universiti Sultan Zainal Abidin Human Research Ethics Committee (UHREC) under Reference no: UniSZA.800-1/1/2 Jld. 2(20). The survey

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targeted participants aged 18 years and older, who had resided in the study area for at least one month and were proficient in reading Bahasa Malaysia. Individuals experiencing communication difficulties were excluded from the study.

Data collection was conducted using a structured questionnaire via Google Forms. The questionnaire comprised five sections, assessing socio-demographic characteristics, clinical profiles, knowledge, attitudes, and depression status. Part I contained 9 questions on socio-demographic data, and Part II included 4 questions on clinical profiles. Parts III and IV, assessing knowledge and attitudes respectively, were adapted primarily from previous studies by Jha and Mandal, 2021[7] and Mulango et al., 2018.[8] Part V, evaluating depression status, consisted of 9 items from the validated Patient Health Questionnaire (PHQ-9) in the Malay language, with a sensitivity of 87% and specificity of 82%.[9]

Initially, the questionnaire's Part III on knowledge encompassed 15 items, while Part IV addressing attitude contained 23 items. A panel of public health specialists validated the questionnaire to ensure its relevance to the studied community. Originally developed in English, the questionnaire was translated into Malay and then back-translated into English by bilingual experts following standard guidelines. Face validity was assessed with 10 individuals to evaluate the clarity and comprehension of the questionnaire. A validation study was subsequently conducted among 147 respondents in Kampung Bukit Tunggal. Based on the results, only 11 items were retained for the knowledge section, and 24 items were retained for the attitude domain. Items with factor loadings of 0.30 or higher were deemed acceptable. Consequently, the finalized questionnaire comprised a total of 57 items in total.

In the knowledge section, responses were scored with two marks for correct answers, one mark for "not sure," and zero marks for incorrect answers, yielding total scores ranging from 0 to 22. In the attitude section, a five-point Likert scale was used, ranging from "strongly disagree" to "strongly agree," with scores assigned from 1 to 5. For negatively worded statements, reverse scoring was applied. The total attitude scores ranged from 24 to 120.

Part V used a Likert scale format to assess depression status, with responses scored from 0 to 3 based on an increasing order of frequency: 0 (not at all), 2-7 days (several days), 8-14 days (more than half the days), and more than 14 days (nearly every day). Negative statements were reverse scored. Then, the total scores ranged from 0 to 27, and classified into normal mental status (0-4 marks) and having depression (5-27).[9]

Data collection was conducted through a house-to-house survey using a pre-tested, validated questionnaire in Google Form. To enhance inclusivity and representativeness, data were collected from all eligible and available adults in Felda Belara using two different approaches, without employing any probability sampling method. Participants with internet access and digital literacy were provided with the questionnaire link via WhatsApp for self-administration. Meanwhile, for elderly respondents and those without internet access, trained data collectors facilitated the survey using their tablets, ensuring that responses were accurately recorded. To minimize the bias, all data collectors—third-year medical students from Universiti Sultan Zainal Abidin (UniSZA)—underwent comprehensive training before the survey. The training focused on standardized data collection procedures, neutral guidance techniques, and ethical considerations to ensure consistency across all data collection efforts.

Prior to the survey, all participants were informed about the study's objectives, the confidentiality of their responses, and the voluntary nature of their participation. The required sample size was determined using the two-proportions formula, incorporating key parameters from a prior validation study. Sample size calculations were performed using Power and Sample Size (PS) software10, with a statistical power of 80% and a margin of error of 5%. Based on these considerations, a minimum of 672 participants was required for the study.

Data cleaning, exploration and analysis were conducted using SPSS version 25. Descriptive statistics were used to present categorical data with frequencies (percentages) and numerical data with means (standard deviations). The prevalence of depressed individuals was reported with percentage and its 95% confidence intervals (CI). Initially, a simple logistic regression was utilized to identify potentially significant variables. Subsequently, multiple logistic regression, employing the forward likelihood ratio method, was employed to determine the associated factors with depression among the respondents. Checks for multicollinearity and interactions among independent variables were performed. The fit of the final model was evaluated using various indexes, including the Hosmer-Lemeshow goodness-of-fit test, overall correctly classified percentage, and the area under the receiver operating characteristic (ROC) curve. The final results were presented through crude and adjusted odds ratios along with their respective 95% CI, and corresponding P value. The significance level (α) was set at less than 0.250 for univariable analysis and less than 0.05 for multivariable analysis.

## RESULTS

A total of 689 participants who met the inclusion criteria were included in this study. Table I presents the socio-demographic characteristics of the study population. Regarding the clinical profile of the participants, the majority did not have any comorbidities (67.3%) and they did not need to take any pill everyday (79.2%). Moreover, almost all of the participants did not have a history of depression (96.8%), and the majority of them did not have a family history of depression (93.2%).

*Exploratory factor analysis and internal consistency*

Table II presents the construct validity and reliability analyses of the questionnaire assessing knowledge and attitudes toward depression among adult respondents. The

**Table I: Socio demographic characteristics of the respondents in Kampung Felda Belara (n =689)**

| Variables | Frequency (%) | Mean (SD) |
|---|---|---|
| Age (in years) | | 34.60 (15.94) |
| Gender | | |
| Male | 253 (36.7) | |
| Female | 436 (63.3) | |
| Race | | |
| Non-Malay | 64 (9.3) | |
| Malay | 625 (90.7) | |
| Marital status | | |
| Single | 378 (54.9) | |
| Married | 311 (45.1) | |
| Education level | | |
| Primary | 46 (6.7) | |
| Secondary | 217 (31.5) | |
| Tertiary | 426 (61.8) | |
| Occupational status | | |
| Unemployed | 390 (56.6) | |
| Employed | 299 (43.4) | |
| Monthly income | | |
| B40 (< RM4070) | 413 (59.9) | |
| M40 (RM4070-RM8109) | 212 (30.8) | |
| T20 (> RM8109) | 64 (9.3) | |
| Living status | | |
| Alone | 31 (4.5) | |
| With family | 658 (95.5) | |
| Physical activity | | |
| No | 273 (39.6) | |
| Yes | 416 (60.4) | |

reliability analysis indicated that Cronbach's alpha values for the knowledge and attitude sections were 0.685 and 0.750, respectively, demonstrating good internal consistency. The overall Cronbach's alpha for these two domains was 0.759, confirming that the questionnaire is both valid and reliable.

*Prevalence of depression among adults in Kampung Felda Belara*
Based on the PHQ 9 questionnaire, out of 689 respondents, 182 people (26.4% with 95% CI: 23.1, 29.7) was found to have depression. Table III displays the descriptive statistics of answers to PHQ 9 questionnaire among the study's participants.

*To assess the knowledge about depression among adult respondents*
The majority of respondents (90.6%) recognized depression as a component of mental health. Nevertheless, almost all of the respondents (95.4%) remained uncertain about the prevalence of undetected depression among many individuals. Around two-thirds (63.6%) were aware of the likelihood of middle-aged and elderly individuals developing depression. Moreover, the majority acknowledged key aspects of depression, including its potential to lead to breakdowns (87.1%) and its impact on concentration (61.1%). Additionally, a substantial number (90.4%) understood the association between depression and suicide or suicide attempts.

Most of the respondents (76.3%) were aware that depression is not confined to a specific age group. They were also aware of the triggering factors such as psychological stress or major life events (89.8%) and emphasized the importance of seeking treatment upon suspecting depression (95.6%). Majority of the respondents claimed that depression can be cured (81.7%) and individuals with depression are dangerous to themselves and others (86.9%). The total knowledge scores among participants had a mean value of 18.55 (SD = 2.70).

*To assess the attitude towards depression among adult respondents*
Regarding attitude towards depression, more than half of the respondents (70.8%) disagreed that mental health facilities in a society will downgrade the prestige, and majority of them (92.0%) feel that society needs to adopt a more tolerant stance towards depressed people. Additionally, they (82.3%) believed in the effectiveness of integrating depressed individuals into society, while half (51.7%) were open to marrying someone who had recovered from depression.

The majority disagreed with the perception of depressed individuals as societal burdens (83.2%) or as incapable of shouldering responsibilities (65.9%). Instead, the majority of them supported the idea that nobody should ignore depressed individuals from society (88.5%) and disagreed that depressed individuals do not deserve sympathy (92.0%).

A large percentage perceived equal job opportunity for depressed individuals (75.5%) and agreed that almost everyone can get depression (74.6%), advocating for their fair treatment akin to those with other illnesses (69.6%). However, opinions varied among the respondents regarding the ease of identifying depressed individuals: 36.2% agreed, 33.7% disagreed, and 30.2% remained neutral.

Notably, 39.8% disagreed that lack of discipline causes depression, whereas 45.0% agreed that lack of determination

**Table II: Construct validity and reliability analyses of questionnaire on knowledge, and attitude towards depression among respondents in Kampung Bukit Tunggal (n =147)**

| Items | Factor loading | | Reliability |
|---|---|---|---|
| **Knowledge** | **Factor 1 (Knowledge)** | **Factor 2 (Attitude)** | **Cronbach's alpha** |
| Q1: Depression is a component of mental health. | 0.692 | | **0.685** |
| Q2: Many people have depression but do not realise it. | 0.498 | | |
| Q3: *Middle-aged and elderly individuals are unlikely to develop depression. | 0.506 | | |
| Q4: Individuals with depression can break down at any time. | 0.668 | | |
| Q5: *Individuals with depression have a good concentration. | 0.417 | | |
| Q6: Depression can lead to suicide and suicide attempts. | 0.563 | | |
| Q7: *Depression affects people of a particular age group. | 0.597 | | |
| Q8: Depression may occur when an individual is under psychological stress | 0.339 | | |
| Q9: Treatments should be sought if one suspects the presence of depressions. | 0.533 | | |
| Q10: *Depression cannot be cured. | 0.52 | | |
| Q11: Individuals with depression are dangerous to themselves and others. | 0.589 | | |
| **Attitude** | | | **0.750** |
| Q12: *In my opinion, mental health facilities in a society downgrade the prestige. | | 0.348 | |
| Q13: I feel that society needs to adopt a far more tolerant attitude towards depressed people. | | 0.633 | |
| Q14: I think treating depressed people as a part of society is effective | | 0.601 | |
| Q15: *I do not want to marry depressed people even after they are fully recovered. | | 0.547 | |
| Q16: *I believe depressed people are a burden to the society. | | 0.623 | |
| Q17: *I feel that depressed people should not assign any responsibilities. | | 0.579 | |
| Q18: I believe that nobody should ignore depressed individuals from society. | | 0.753 | |
| Q19: *I believe depressed individuals do not deserve sympathy from society. | | 0.577 | |
| Q20: I think that depressed individuals have an equal right to get a job. | | 0.694 | |
| Q21: In my opinion, almost everyone can get depression. | | 0.641 | |
| Q22: I believe that depressed individuals should be treated just like people with other diseases. | | 0.589 | |
| Q23: I believe depressed individuals are easy to be recognised because they are different. | | 0.529 | |
| Q24: I believe lack of discipline can cause depression. | | 0.743 | |
| Q25: I believe lack of determination can cause depression. | | 0.568 | |
| Q26: *In my perspective, spending on treating depressed people is a waste of money. | | 0.743 | |
| Q27: *I think that mental hospitals are out-dated means of treating depression. | | 0.704 | |
| Q28: I believed that mental health facilities should be community-based | | 0.504 | |
| Q29: I think that individuals with symptoms of depression should be hospitalised immediately | | 0.586 | |
| Q30: *I feel that depressed people living in the neighbourhood are frightening. | | 0.587 | |
| Q31: *I do not want to live next door to someone who has been depressed. | | 0.585 | |
| Q32: In my opinion, society should not be afraid of depressed people receiving mental health service in our neighbourhood | | 0.628 | |
| Q33: I think depressed people are less dangerous than most people suppose. | | 0.469 | |
| Q34: I believe depressed people can be trusted as babysitters after recovery. | | 0.509 | |
| Q35: Healthy lifestyle can help maintain mental health. | | 0.395 | |

*Negative statement

could be a contributing factor. A vast majority (88.7%) rejected the idea that investing in the treatment of depressed individuals is a waste of resources, and 69.7% did not consider mental hospitals as outdated means of treating depression. Moreover, most of them (68.0%) advocated for community-based mental health facilities while 90.9% believed that individuals exhibiting depressive symptoms should receive immediate hospitalization. Interestingly, 44.2% disagreed that having depressed individuals in the neighbourhood is frightening, and 61.8% expressed willingness to live next to someone who has experienced depression. A substantial portion of respondents (77.2%) agreed that society should not be afraid of depressed individuals accessing mental health services in their communities and 64.3% believed that depressed individuals are less dangerous than commonly perceived. Additionally, 43.4% believed that individuals recovered from depression could be trusted as babysitters. Lastly, most respondents (90.2%) concurred that maintaining a healthy lifestyle contributes to preserving mental health.

**Table III: Descriptive statistics of PHQ-9 responses assessing depression among adults in Kampung Felda Belara (n=689)**

| No | Items | Never (0) | Sometimes (2-7) | Often (8-14) | Always (>14) |
|---|---|---|---|---|---|
| | | n (%) | n (%) | n (%) | n (%) |
| 1. | Lack of interest or fun in doing things | 469 (68.1) | 193 (28.0) | 7 (1.0) | 20 (2.9) |
| 2. | Feeling sad, moody or hopeless | 512 (74.3) | 146 (21.2) | 15 (2.2) | 16 (2.3) |
| 3. | Problems going to bed or during sleep or oversleeping | 405 (58.8) | 227 (32.9) | 26 (3.8) | 31 (4.5) |
| 4. | Feeling tired or less energized | 283 (41.1) | 358 (52.0) | 21 (3.0) | 27 (3.9) |
| 5. | Lack of appetite or overeating | 486 (70.5) | 167 (24.2) | 24 (3.5) | 12 (1.7) |
| 6. | Having negative feelings about yourself- or feeling like you are a failure or have disappointed yourself or your family | 481 (69.8) | 167 (24.2) | 11 (1.6) | 20 (2.9) |
| 7. | Difficulty in focusing on something like reading newspapers or watching television | 520 (75.5) | 138 (20.0) | 11 (1.6) | 20 (2.9) |
| 8. | Moving or speaking too slowly until others noticed. Or vice versa - being too restless or anxious to cause you to move more than usual | 594 (86.2) | 76 (11.0) | 12 (1.7) | 7 (1.0) |
| 9. | Thinking that it's better if you're dead or want to hurt yourself in some way | 632 (91.7) | 37 (5.4) | 4 (0.6) | 16 (2.3) |

**Table IV: Associated factors of depression status among sub-urban residents in Kampung Felda Belara (n =689)**

| Variables | Simple Logistic Regression | | Multiple Logistic Regression[a] | |
|---|---|---|---|---|
| | Crude OR (95% CI) | p value | Adjusted OR (95% CI) | p-value |
| Age (years) | 0.945 (0.930, 0.960) | <0.001 | 0.972 (0.948, 0.996) | 0.020 |
| Gender | | | | |
| Male | 1.00 | | 1.00 | |
| Female | 1.586 (1.100, 2.286) | 0.014 | 1.614 (1.076, 2.421) | 0.021 |
| Ethnicity | | | | |
| Non-Malay | 1.00 | | | |
| Malay | 0.909 (0.512, 1.613) | 0.745 | | |
| Marital status | | | | |
| Single | 1.00 | | 1.00 | |
| Married | 0.208 (0.139, 0.312) | <0.001 | 0.377 (0.188, 0.754) | 0.006 |
| Highest completed education | | | | |
| Primary | 1.00 | | | |
| Secondary | 3.856 (1.144, 12.993) | 0.029 | | |
| Tertiary | 6.506 (1.983, 21.348) | 0.002 | | |
| Occupation | | | | |
| Unemployed | 1.00 | | | |
| Employed | 0.407 (0.282, 0.587) | <0.001 | | |
| Monthly income | | | | |
| B40 | 1.00 | | | |
| M40 | 0.965 (0.665, 1.400) | 0.849 | | |
| T20 | 0.544 (0.275, 1.079) | 0.082 | | |
| Living status | | | | |
| Alone | 1.00 | | | |
| With family | 1.914 (0.724, 5.060) | 0.191 | | |
| Physical activity | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 0.577 (0.410, 0.813) | 0.002 | 0.669 (0.459, 0.976) | 0.037 |
| Having Comorbidities | | | | |
| No | 1.00 | | | |
| Yes | 1.054 (0.736, 1.511) | 0.773 | | |
| Medications | | | | |
| 0 | 1.00 | | | |
| 1-2 | 0.620 (0.372, 1.034) | 0.067 | | |
| ≥3 | 0.761 (0.338, 1.712) | 0.509 | | |
| History of Depression | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 2.895 (1.233, 6.797) | 0.015 | 2.977 (1.122, 7.899) | 0.028 |
| History of family depression | | | | |
| No | 0.00 | | | |
| Yes | 1.480 (0.789, 2.775) | 0.222 | | |
| Total knowledge scores | 0.919 (0.865, 0.975) | 0.005 | 0.883 (0.825, 0.945) | <0.001 |
| Total attitude scores | 0.984 (0.968, 0.999) | 0.045 | | |

[a]Forward likelihood ratio was applied; No collinearity and interaction existed between variables. The model reasonably fits well. The model fitness was checked by Hosmer-Lemenshaw test (p=0.547), overall, correctly classified percentage (75.4%) and the area under ROC curve (75.2%; 95% CI: 71.1, 79.3; p<0.001).

*Factors associated with depression among sub-urban residents in Kampung Felda Belara*

Through univariate analysis employing simple logistic regression, several potential associated factors of the depression status of respondents were identified: age, being female, being married, educational milestones such as secondary school attainment and tertiary education, being employed, those having monthly income of T20, cohabitation with family, engagement in physical activity, intake of 1-2 medications, those with previous history of depression, family history of depression, total knowledge scores and total attitude scores.

After controlling the confounding factors employing multiple logistic regression, several factors were identified to link with the respondents' depression status. The results of the final model implied that the factors of being female and those having a previous history of depression were associated with a higher likelihood of experiencing depression among the respondents. On the other hand, older individuals, married participants, those engaged in physical activities and those with higher total knowledge scores were less likely to have depression (Table IV).

## DISCUSSION

The current study highlights a concerning prevalence of depression among adults in this study based on the PHQ-9 questionnaire. Moreover, the study identified age, gender, marital status, physical activity, history of depression, and total knowledge scores as the significant associated factors of the respondents' depression status.

*Prevalence of depression*

The results of the current study revealed that the prevalence of depression among sub-urban adults in Kuala Nerus was found to be 26.4% (95% CI: 23.1–29.7). Comparatively, previous studies in Malaysia have reported varying prevalence rates depending on study populations, instruments, and cut-off points. For instance, Ong, 2022[11] found a higher prevalence (38.5%) among the general Malaysian community using DASS-21, while Yeoh et al. (2017)[12] reported 36.0% among adults in Selangor, Penang, and Terengganu using Beck's Depression Inventory. However, a much lower prevalence of depression was observed in the adult population in rural and urban areas (3.7%),[13] rural communities (15.8%)[14] and adolescents (19%),[15] possibly due to variations in study populations and measurement tools. Meanwhile, Abdul Manaf et al. (2016)[16] found a prevalence of 27.8% among elderly Malays, aligning closely with the current study findings.

Despite these variations, the persistently high prevalence of depression across multiple studies underscores the need for urgent mental health interventions in Malaysia. The findings from Kuala Nerus further reinforce the importance of targeted mental health strategies in sub-urban populations, where depression appears to be a significant public health concern.

*Predictors of depression*

This study identified several significant predictors of depression. This current study revealed that female gender and a previous history of depression were positively associated with experiencing depression. Conversely, older age, being married, engaging in regular physical activity, and higher total knowledge scores were found to be protective factors against depression.

The role of knowledge in influencing depression was evident in this study. Participants with lower knowledge scores about depression exhibited a higher prevalence of the condition, aligning with prior research that highlights poor mental health literacy as a key contributor to stigma and discrimination against individuals living with depression.[17] This underscores the importance of educational interventions in mitigating depression risks within communities.

Interestingly, despite the critical role of knowledge, no significant association was found between attitudes and depression prevalence among adults in Kuala Nerus (p=0.045). However, the overall attitude distribution revealed a high prevalence of positive attitudes toward depression, with 97.0% of participants exhibiting favourable views compared to only 3.0% with negative attitudes. This is a striking contrast to findings from Southern Ethiopia, where only 39.9% of participants held positive attitudes towards individuals with depression.[18] The higher level of positive attitudes in Kuala Nerus suggests reduced stigma and greater acceptance of mental health issues within the community.

Age also emerged as a significant predictor, with younger participants displaying a higher prevalence of depression compared to their older counterparts (p<0.001). Participants with depression had a mean age of 25.59 years, while those without depression had a mean age of 35.17 years. These findings are consistent with data from both national[4,19] and international[20] sources, which report higher rates of depression among younger adults. The social isolation often faced by younger adults may contribute to this trend, highlighting the need for social support networks as a protective factor against depression.[21]

Gender differences in depression were also notable, with women more likely to experience depression than men. This aligns with global patterns where women consistently show higher depression rates than men. For instance, a study in Al Ain found lifetime depression rates of 9.5% in females compared to 2.5% in males.[22] The observed gender disparity may be attributed to various factors, including the stress associated with household responsibilities, especially among homemakers in Kuala Nerus. National studies, such as NMHS 2019[3] and NMHS 2023 reports,[4] also revealed similar findings, further highlighting the vulnerability of women to depression.

Marital status was another significant determinant, with single individuals more likely to experience depression than married ones (p=0.001). The protective effect of marriage may stem from the social support and companionship it provides, reducing feelings of loneliness and psychological distress.[23] This is consistent with previous research showing

that marital stability contributes to lower depression rates, while serious marital problems can increase the risk.[24]

Physical activity was inversely associated with depression, with those engaging in regular exercise showing lower prevalence rates ($p<0.001$). This supports existing literature on the mental health benefits of physical activity, which include neuro-modulation and the release of mood-enhancing hormones.[25] Regular exercise also helps manage stress and fosters social connections, both of which are vital in preventing depression.[26]

Comorbidities, even though it was not a significant predictor, were also linked to depression, with individuals suffering from other health conditions exhibiting higher depression rates ($p=0.772$). This relationship highlights the bidirectional nature of mental and physical health, where one often exacerbates the other.[27] Effective management of both mental and physical health conditions is crucial for improving overall quality of life.

Finally, while a previous history of depression was a significant predictor ($p=0.015$), family history was not ($p=0.222$). This finding contrasts with some studies that suggest a strong genetic component to depression, indicating that environmental factors may play a more critical role in this population.28 These results emphasize the multifactorial nature of depression, where both genetic predispositions and external triggers contribute to the onset of the disorder.

## LIMITATIONS

This study acknowledged some limitations. While we examined attitudes toward depression, no significant association with depression prevalence was found. This suggests that additional factors may play a more prominent role, and further research is needed to explore the complex interplay between knowledge, attitudes, stigma, and depression.

Additionally, in this study, we could not collect data on environmental or occupational stressors, which may contribute to depression, particularly in suburban settings. Including these factors in future studies could provide a more comprehensive understanding of the determinants of depression in this population.

Lastly, our findings indicated a higher prevalence of depression among females, consistent with global trends. However, this study did not specifically investigate gender-related stressors such as domestic responsibilities, cultural expectations, or employment disparities in Malaysia. Future research should explore these aspects to better understand their potential impact on mental health outcomes.

## CONCLUSION

This community-based exploratory cross-sectional survey involved 689 adults in Kuala Nerus. The study identified the female and having a history of depression were predictors of depression, whereas older age, marital status, doing physical activity, and higher knowledge scores were less likely to have depression. These insights inform the development of targeted strategies for depression management. Public health interventions should focus on enhancing awareness of depression risk factors and improving knowledge within the community. Continued research is crucial to refine prevention and treatment approaches, particularly for younger individuals and females.

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ORIGINAL ARTICLE

# Apoptosis and anti-migratory effects of Methyl gallate combined with Cisplatin in human breast cancer (MCF-7) cells

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## ABSTRACT

**Introduction: Breast cancer is among the leading causes of cancer-related death in women worldwide. Cisplatin is a widely used chemotherapeutic drug because of its strong efficacy in killing various cancer cells. However, it presents several toxicity effects and development of drug resistance. Looking at these shortcomings, there is growing interest in combining conventional chemotherapeutic drugs with natural compounds. Therefore, a natural phenolic compound, methyl gallate, known to have strong anticancer and antioxidant properties, is a potential candidate for enhancing the therapeutic efficacy and reducing the side effects of current chemotherapy drugs.**

**Materials and Methods: This study aims to determine the antiproliferative effect of methyl gallate and its combination with cisplatin on MCF-7 cells using MTT assay. The combination effect was evaluated using MTT assay and then analysed using CompuSyn software. Besides, this study was done to evaluate the morphology of apoptotic cells using AO/PI stain and to determine the ability of the combination treatment to inhibit the migration of MCF-7 cells using wound healing assay.**

**Results: The cell viability was inhibited dose-dependently with $IC_{50}$ values of methyl gallate and cisplatin were 20.21 µg/mL and 13.38 µg/mL respectively. Methyl gallate at fixed concentration combined with the lowest concentration of cisplatin inhibited greater MCF-7 cell growth compared to cisplatin alone with a combination index value of less than 1 indicating the synergistic effect. The MCF-7 cells treated with single and combination drugs exhibited several apoptotic characteristics. Migration of MCF-7 cells was also significantly inhibited by single and combination treatments. Conclusion: In conclusion, this study suggested that methyl gallate in combination with cisplatin has a good potential to be developed as a chemotherapeutic agent for breast cancer.**

**KEYWORDS:**
*Apoptosis, migration, cisplatin, methyl gallate, combination treatment, synergistic effect*

## INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women in 2022 where it accounts for an estimated 2.29 million new cases globally. Furthermore, breast cancer has become the leading cause of cancer death with approximately 665,684 deaths.[1] Meanwhile, with 34.1% of all cancers among women, breast cancer ranks as the first most common cancer in Malaysia. There are estimated 21,634 cases of female breast cancer were diagnosed from 2012 to 2016.[2]

There are different treatment options for breast cancer depending on the type of breast cancer and the severity such as its stage and cancer cells size. The available breast cancer treatments include chemotherapy, radiotherapy, hormonal therapy, and immunotherapy. Breast cancer is strongly associated with females, late menopause, older age, early menarche, nulliparity, positive family history and lack of breastfeeding.[3] Chemotherapy is the most common cancer therapy that uses drugs to destroy the cancer cells. It functions by inhibiting and slowing the growth of cancer. Unfortunately, chemotherapy has a lot of unfavorable side effects despite its effectiveness against cancer cells. It destroys the nearby healthy cells in addition to the cancerous ones. Because multiple drugs kill cancer cells in different ways, chemotherapy is now often administered as a combination of agents rather than a single agent.[4]

Cisplatin is one of the well-known platinum-based chemotherapeutic drugs used to treat numerous human malignancies including those of the bladder, head and neck, lung, ovarian, and testicular cancers. The ability of this compound to crosslink with purine bases on DNA has been connected to its mode of action. This causes DNA damage, disrupts DNA repair processes, and ultimately triggers apoptosis in cancer cells.[5] Nevertheless, cisplatin treatment has inherent limitations that hinder its efficacy. These limitations include the development of dose-limiting toxicities due to drug accumulation in non-targeted areas of the body. Several non-cancerous sites vulnerable to cisplatin toxicity include the kidneys, auditory system and brain.[6]

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Methyl gallate (MG) is a gallate ester derived from formal condensation of gallic acid. It is a polyphenolic compound that is commonly present in natural plants such as Meliaceae species, *Galla rhois*, and seed coats of *Givotia rottleriformis* Griff. MG has been extensively studied due to its numerous biological activities such as antiplatelet activity, ability to protect DNA damage from oxidative stress, ability to reduce lung injury caused by phosgene, ability to attenuate diabetic and antiapoptotic activity.[7] Moreover, MG has exhibited significant anticancer activity on various cancer cells such as liver, skin, cervix, lung, brain, and lymph nodes.[8] MG has been shown to induce apoptosis through a variety of mechanisms, including the production of reactive oxygen species (ROS), regulation of apoptotic and anti-apoptotic proteins, suppression and promotion of oncogenes, inhibition of matrix metalloproteinases (MMPs), and cell cycle arrest.[9]

Combination chemotherapy is one of the strategies to increase the efficacy of anticancer drugs by combining and administering multiple drugs. The combination of chemotherapy may enhance the efficiency of the drug since it impacts the cancer cells through different stages of the cell cycle.[10] The synergistic effect of the combination may increase the tumor responses, thereby overcoming the side effects by lowering the drug dosage. The combination therapy of cisplatin with other anticancer agents has been proven to be more effective as it will produce fewer toxic effects and prevent cancer cells from developing resistance.[11] The use of natural products, such as plants, herbs, and other substances derived from nature, for their potential anti-cancer properties has been a subject of interest and research. Numerous clinical trials are being conducted to evaluate the effectiveness and safety of using natural remedies to cure or prevent cancer.[12] Hence, combining MG which is known to have high antioxidant capacity, with cisplatin is a proposed alternative to enhance the cytotoxicity effect on MCF-7 cells.

To the best of our knowledge, there is no study has reported the combination of MG and cisplatin on MCF-7 cells. Hence, in this current study, MG in combination with cisplatin was investigated for its antiproliferative effects on MCF-7 cells. The apoptosis features and anti-migratory effect of MCF-7-treated cells by single and combination compounds were also evaluated through AO/PI staining and wound healing assay to postulate its potential role as an anticancer agent for breast cancer.

## MATERIALS AND METHODS

*Chemicals*

Cisplatin, MG and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), streptomycin and penicillin and trypsin were purchased from Gibco Thermo scientific. 3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) was purchased from Nacalai Tesque, Inc. (Japan).

*Cell culture*

MCF-7 cells were obtained from the stock culture at the School of Health Sciences, Universiti Sultan Zainal Abidin. The cell lines were grown in a humidified atmosphere at 370C with 5% (v/v) $CO_2$. The complete medium containing DMEM, 10% FBS, and 1% penicillin-streptomycin was used to culture cells.

*Determination of cell viability and $IC_{50}$*

The MTT assay was conducted to determine the $IC_{50}$ values of cisplatin and MG. MCF-7 cells ($4 \times 10^3$ cells/ml) were seeded in 96-well plates and incubated for 24 hours in a $CO_2$ incubator at 37°C. Upon reaching 80-90% confluency, the used medium was discarded. Subsequently, the cells were treated with different concentrations of cisplatin and MG, with 1% DMSO that served as the negative control. The treated cells were incubated for 72 hours in a $CO_2$ incubator.

Following the treatment period, 10 µl of MTT solution (5 mg/ml in PBS) were added to each well. The plate was covered with aluminum foil and placed in a humidified incubator with 5% $CO_2$ at 37°C for 4 hours. After this incubation period, the contents in the well were discarded, and 100 µl absolute DMSO was added to dissolve the blue formazan crystals produced by viable cells. Then, the absorbance (OD) of each well was measured using a microplate reader at a wavelength of 570 nm. The percentage of cell viability (%) was calculated using the formula:

$$Percentage\ of\ cell\ viablity\ (\%) = \frac{Absorbance\ in\ a\ well}{Absorbance\ of\ negative\ control} \times 100$$

The $IC_{50}$ values of cisplatin and MG were determined from the dose-response curve, generated by plotting the percentage of cell viability (%) against log10 concentration (µg/ml) using GraphPad Prism software. These $IC_{50}$ values were used in the combination treatment of MCF-7 cells.

*Combination treatment*

A non-constant combination of compounds MG and cisplatin method was applied to determine the combination effects of these two compounds. MCF-7 cells were treated with MG at a constant dose of $IC_{50}$ (20.21µg/mL) combined with cisplatin at 5 serial concentrations starting range from its $IC_{50}$ value, (13.38 to 6.69, 3.35, 1.67 and 0.84 µg/mL) using two-fold dilution. The MTT assay was used to calculate the percentage of cell viability (%). CompuSyn software was then used to calculate the combination effect. The pharmacological interaction of drug combinations was evaluated using a mathematical analysis and reported as a combination index (CI), The CI values can be expressed as additive (CI=1) synergism (CI<1) and antagonism (CI>1). The best combination was chosen to be used for treatment in the subsequent assays.

*AO/PI staining*

The morphological changes in apoptotic nuclei of MCF-7 treated cells were evaluated using Acridine Orange/ Propidium Iodide (AO/PI) staining. $2 \times 10^5$ MCF-7 cells per well were seeded into 6-well plates. The cells were incubated in a humidified incubator with 5% $CO_2$ at 37°C for 24 hours. Before treatment, the confluency of the seeded cells was checked to ensure it was 80-90% in each well. The cells were then treated with MG and cisplatin at their respective $IC_{50}$ concentrations and a combination treatment. The cells were incubated again in a humidified incubator with 5% $CO_2$ at

**Table I: Combination index for combination of cisplatin and MG on MCF-7 cells**

| Dose Cis | Dose MG | Effect | CI |
|---|---|---|---|
| 13.38 | 20.21 | 0.62 | 2.42340 |
| 6.69 | 20.21 | 0.55 | 1.95718 |
| 3.35 | 20.21 | 0.45 | 1.85391 |
| 1.67 | 20.21 | 0.42 | 1.29350 |
| 0.84 | 20.21 | 0.4 | 0.93842 |

37°C for 72 hours. The cells were detached using trypsin and were transferred to the 15 mL tube used before and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and 20 µL of AO solution was added to the tube and mixed with the cell pellet, followed by 20 µL of PI solution. The mixed solutions were immediately cooled by placing them in an icebox. A 10 µL of the cell solution was placed onto a clean slide and covered with a coverslip. The cells were quickly viewed under a fluorescence microscope to observe and identify apoptotic, condensed, and fragmented nuclei, distinguished by green and orange fluorescence. The entire procedure was performed without light exposure.

*Wound healing assay*

The determination of migratory inhibition of MCF-7 cells was performed using wound healing assay. MCF-7 cells were seeded at density 2.5 $\times 10^5$ per well into a 6-well plate and the plate was maintained in a humidified incubator with 5% $CO_2$ at 37°C for 24 hours or until they reached 100% confluency. After 24 hours, a sterile SPLScar™ Scratcher and its lid were used to create a straight scratch, or wound, across the center of each well. The wound area was observed and captured starting from 0 hours using an inverted microscope equipped with a digital camera. The cells were then treated with single and combination treatment. The cells were incubated again in a humidified incubator with 5% $CO_2$ at 37°C. The wound area was observed and captured at 0, 12, and 24 hours. The captured images were analyzed using, Image J to measure the wound width. The percentage of wound closure were calculated using the formula:

$$\text{Percentage of wound closure} = \frac{(\text{space at time 0} - \text{space at (t) hours}) \times 100}{\text{Space at time 0}}$$

*Statistical analysis*

Statistical analysis was performed using GraphPad Prism (version 10) statistical software. All data were presented as the mean ± standard deviation (SD) from triplicate experiments. The combination effects of the compounds were determined using CompuSyn software. The statistical significance of the data between the three groups was determined using one-way ANOVA with Turkey post hoc test where $p<0.05$ was considered statistically significant.

## RESULTS

*Antiproliferative effect of cisplatin and MG*

The antiproliferative effects of cisplatin and MG on MCF-7 cells were assessed using MTT assay after 72 hours of incubation treatment with different concentrations of cisplatin and MG ranging from 100 µg/mL to 6.25 µg/mL. Figure 1 illustrates the antiproliferative effects of both compounds on MCF-7 cells after the treatment. The results showed that cisplatin and MG inhibited MCF-7 cell growth in a dose-dependent manner where the higher the concentration led to the greater cell inhibition. The $IC_{50}$ values of cisplatin and MG on MCF-7 cells obtained from the dose-response curve of percentage of cell viability (%) against log10 concentration were 13.38 µg/mL and 20.21 µg/mL, respectively.

*Combination effect of cisplatin and MG*

The analysis of the combination effects using CompuSyn software was reported as combination indexes (CI) and expressed as synergistic, antagonistic or additive effects. Table I shows the CI values of different combinations of cisplatin and MG. Based on this result, the CI values of the combination of MG with cisplatin at concentrations 13.38 µg/mL, 6.69 µg/mL, 3.35 µg/mL and 1.67 µg/mL were more than 1, indicating antagonistic effects. However, the combination with cisplatin at 0.84 µg/mL showed the lowest CI which was less than 1. Thus, this combination reaction is considered to have a synergistic effect. Hence, it was chosen as the best combination and was used for the treatment in further assay.

*Morphological observation of apoptotic cells (AO/PI staining)*

The morphological changes and apoptotic features of cells treated with single and combination drugs was evaluated using Acridine Orange/ Propidium Iodide (AO/PI) staining. This staining method allows visualization of viable and non-viable cells using green and orange/ red fluorescent dyes. Viable cells appear with a green and uniform nucleus, while apoptotic cells exhibit an irregular bright green nucleus or irregular orange fluorescence. After 72 hours, the observation revealed that MCF-7 cells treated with the $IC_{50}$ concentrations of cisplatin, MG and a combination of cisplatin and MG (CIS-MG) showed significant apoptotic characteristics compared to the untreated control cells. As shown in Figure 2, typical features of apoptotic cells, such as irregular or fragmented nuclei, nuclear condensation, irregular cell shapes and membrane blebbing, were visible in the treated cell groups (B-D), marked by bright green and orange dyes. Several dead cells also present in these treated groups marked by red dyes. In contrast, the untreated control cells (A) maintained its oval shape with an intact nucleus uniformly stained with green fluorescence.

*Anti-migratory effect of CIS-MG on MCF-7 cells*

MCF-7 cell migratory inhibition after treatment was evaluated using wound healing or scratch assay. The results of the wound healing assay on MCF-7 cells (Figure 3) demonstrated marked differences in cell migration between

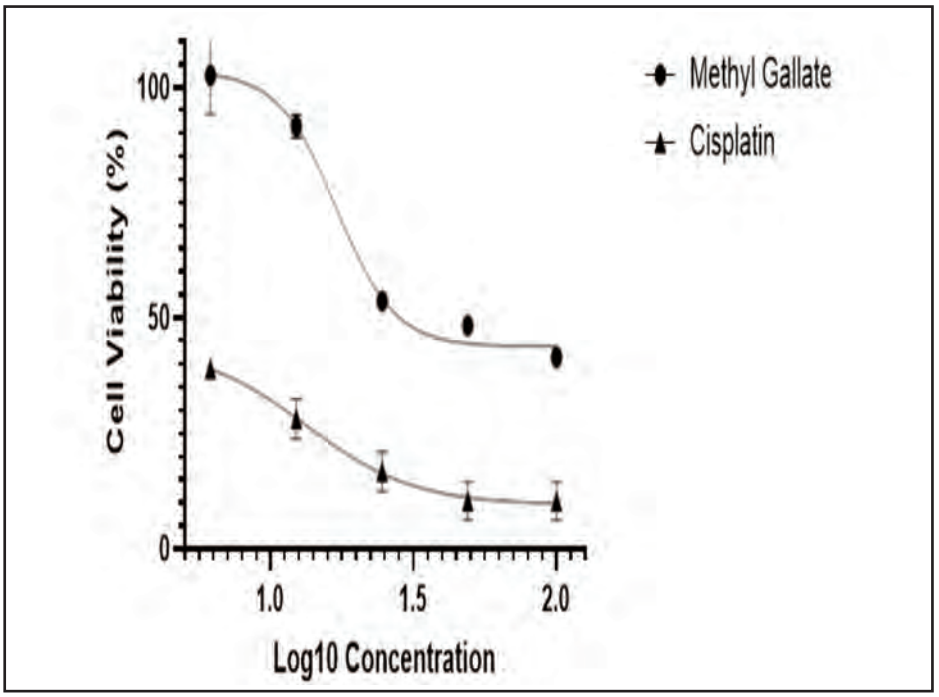


**Fig. 1:** Antiproliferative activity of cisplatin and MG on MCF-7 cells

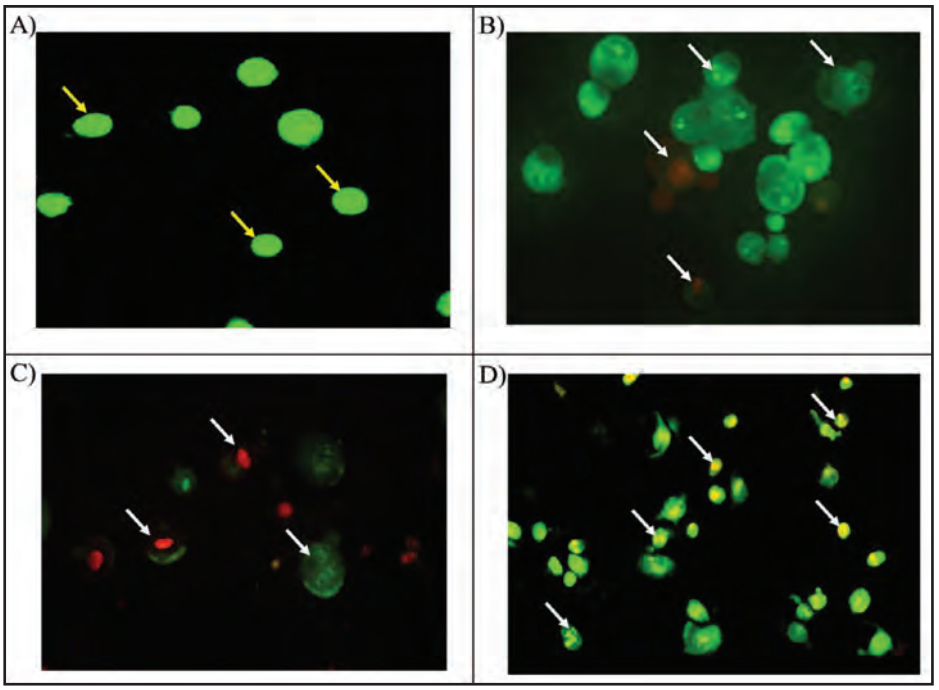


**Fig. 2:** The morphology of apoptotic cells with irregular or fragmented nucleus, nuclear condensation, irregular cell shape, membrane blebbing and apoptotic bodies characteristics (white arrow) presented in B) cisplatin, C) MG and D) CIS-MG groups, stained by AO/PI staining after 72 hours incubation. The oval shape and uniform nucleus maintained (yellow arrow) in A) untreated control cells. The images of cells were captured under 200x and 400x magnification using a fluorescence microscope

untreated and treated groups over time. As presented in Figure 4, the percentages of wound closure in cisplatin (70.1 ± 4.97), MG (58.58 ± 2.20) and CIS-MG (59.56 ± 0.58) were significantly lower than in untreated cells (79.87±5.35) at 12 hours of incubation treatment, indicating that all the treatments demonstrated migration-inhibitory effects on MCF-7 cells. At 24 hours of incubation, untreated cells and cells treated with cisplatin and MG alone were completely closed. Interestingly, only cells treated with CIS-MG were not completely closed with 73.74 ± 3.48% of wound closure. This finding indicated that CIS-MG has a higher ability in inhibiting and slowing MCF-7 cell migration.

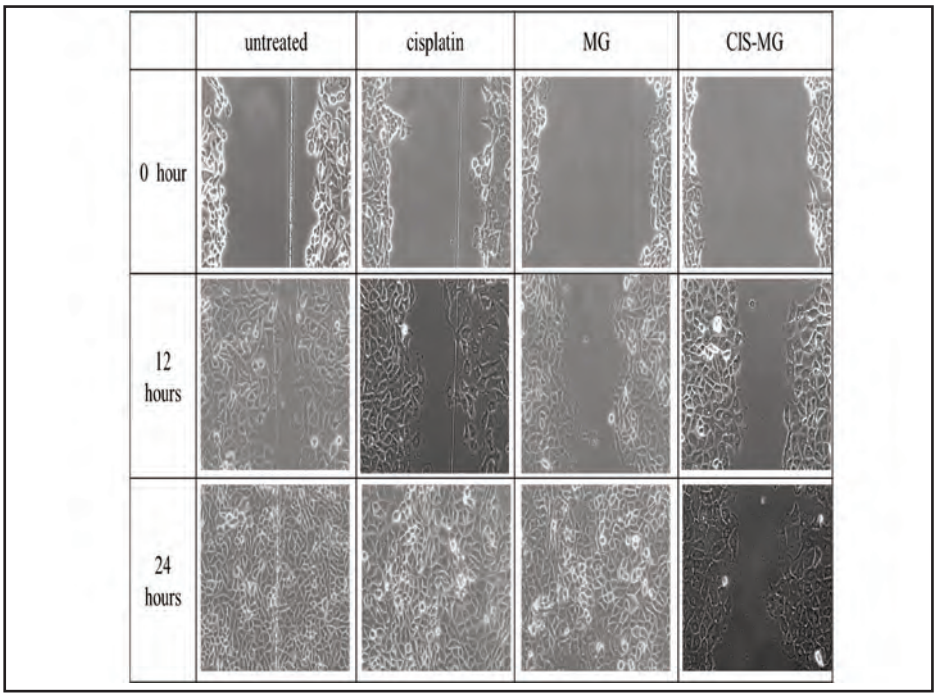


**Fig. 3:** The effect of single and combination treatments of MG with CIS on MCF-7 cell migration. The wound area of the cells was photographed at 0, 12 and 24 hours (40x original magnification). The wound area at 24 h for untreated, cisplatin and MG showed complete closure compared to cells treated with CIS-MG

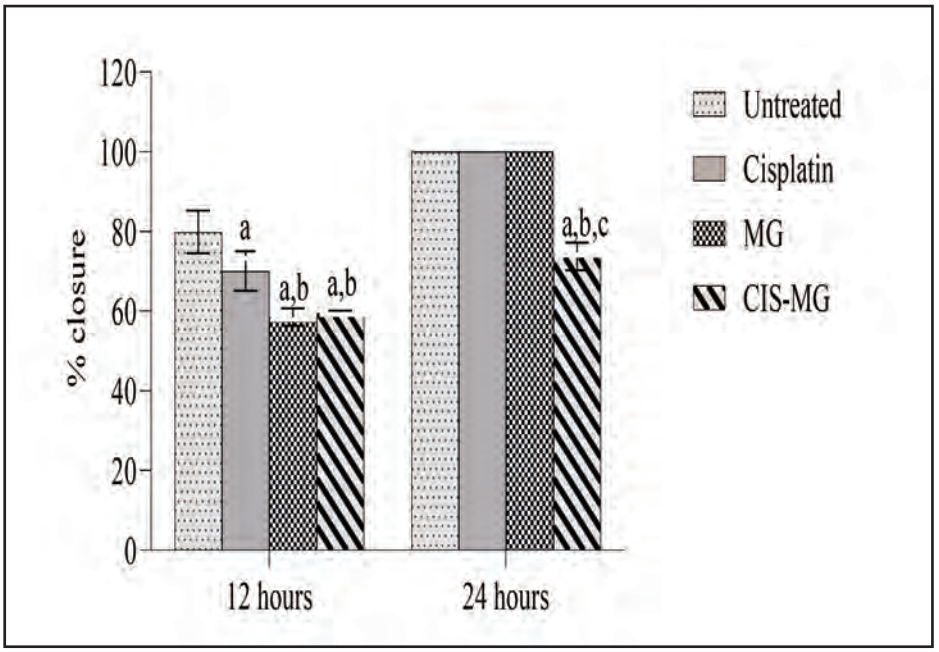


**Fig. 4:** Relative wound closure after 24 hours of incubation treatment. Results are presented as mean ± SD from three separate experiments. a: p<0.05 as compared to the untreated group, b: p<0.05 compared to cisplatin, c: p<0.05 compared to MG alone

## DISCUSSION

*Antiproliferative effect of Methyl Gallate on MCF-7 cells*

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related deaths among women. The rising number of cases highlights the urgent need for more effective treatments. Many conventional chemotherapeutic drugs face challenges such as drug resistance, cancer relapse, and significant side effects. Consequently, researchers are increasingly exploring natural compounds for the treatment and prevention of breast cancer. These natural compounds, derived from living organisms, can promote apoptosis and inhibit metastasis, thereby preventing cancer growth. Therefore, they have the potential to suppress cancer progression, improve patient survival rates, and reduce the number of deaths associated with breast cancer.[13]

MG, a natural phenolic compound, commonly found in plants such as maple leaves and *Camellia sinensis*[14] demonstrates various biological activities. These include antioxidant, anti-inflammatory, antimicrobial, and antitumor properties.[15] Based on the MTT assay results of cell viability, it was observed that both MG and cisplatin inhibited the proliferation of MCF-7 cells. Cell viability decreased in a dose-dependent manner compared to the untreated group, demonstrating a positive antiproliferative effect on MCF-7 cells. Both MG and cisplatin exhibited low $IC_{50}$ values indicating their efficiency in inhibiting proliferation of MCF-7 cells. Despite its effectiveness on cancer cells, cisplatin presents several toxicity issues as it causes harm to normal cells. However, MG was shown to have cytoselective effects on cancer cells as it was reported to exhibit low cytotoxic effects on HaCat normal skin and Vero cells[16] and MDCK cells.[17]

The antiproliferative activity of MG on other cells has been reported previously. Treatment with MG on HeLa cells after 72 hours demonstrated a good cytotoxicity effect with $IC_{50}$ values of 11.00 ± 0.58 µg/mL.[16] Similarly, a study by Mamat et al. showed the same antiproliferative effect of MG and cisplatin with $IC_{50}$ values of 16.55 µg/mL.[18] MG, a major compound in *Syzygium coriaceum* leaf extract inhibited the proliferation of human hepatocellular carcinoma cells (HepG2) with an $IC_{50}$ value of 24.2 ± 2.8 µg/mL.[19] On the other hand, another study found that MG significantly reduced the proliferation of Hep3B, Mahlavu, and HepJ5 Hepatocellular carcinoma (HCC) cells in a dose-dependent manner. The 50% inhibitory concentrations were >40 µg/ml for Hep3B, ~40 µg/ml for Mahlavu, and ~20 µg/ml for HepJ5 at 48 hours, indicating significant inhibition of cells.[17] Moreover, a study on melanoma mouse models and B16F10 cells also demonstrated the antitumor effect of MG.[20]

*Combination effect of Cisplatin and Methyl gallate*

Conventional cancer therapy offers clear benefits, yet despite the development of new anticancer agents, chemotherapy often has limitations including chemotherapy-induced toxicity, adverse reactions, inadequate target specificity, and, most critically, drug resistance during cancer progression. Combination therapy has emerged as a promising strategy for addressing these issues. By simultaneously delivering two or more therapeutic agents, such as chemotherapeutic drugs and phytochemical compounds, different signaling pathways in cancer cells can be targeted, leading to synergistic effects, improved targeting specificity, enhanced therapeutic outcomes, and overcoming multidrug resistance (MDR).[21] Due to the minimal side effects of natural compounds and their availability, there is a growing interest in pursuing combination therapy as a new strategy.[22]

In this study, the combination effects of cisplatin and MG on MCF-7 cells varied according to the different concentrations of cisplatin, the combination indexes were identified as synergistic and antagonistic reactions. The lowest concentration of cisplatin at 0.84 µg/mL, combined with MG in this study presents a CI value of less than 1 indicating a synergistic effect. This low CI value provided evidence that MG can enhance the cytotoxicity effects of cisplatin, even at the lowest concentration. Hence, this synergistic interaction shows that the combination treatment is more effective in killing cancer cells or inhibiting their cell growth compared to drug alone. This obtained result may suggested that when the drugs work better together, only lower doses of each drug are required to achieve the desired therapeutic effect and the risk of toxicity associated with higher doses of chemotherapy can be reduced. Hence, the combination of MG with lowest concentration of cisplatin has been used for further assays.

In a study conducted on cervical cancer cells (HeLa), increased inhibition of the cells was observed when combining MG with cisplatin, indicating that this combination can enhance cisplatin's efficacy. CI values of less than 1 suggest that MG works synergistically with cisplatin to inhibit HeLa cell proliferation. These combinations may cause DNA damage and activate the intra-S-phase checkpoint, which inhibits DNA synthesis and results in apoptosis or cellular senescence.[18] Moreover, MG has demonstrated synergistic interaction and stronger anti-cancer effects on EL4 murine lymphoma in combination with cisplatin compared to MG or cisplatin alone. The study suggests that MG inhibited Treg cell migration, thus enhancing the antitumor immune response and also caused no renal toxicity even in combination with cisplatin.[23]

*Morphological observation of apoptotic cells (AO/PI staining)*

Apoptosis, or programmed cell death, is an active physiological process resulting in cellular self-destruction. In cancer therapy, inducing apoptosis in cancer cells is a crucial strategy to eliminate malignant cells and prevent tumor progression effectively. Apoptosis can be initiated through two main pathways: the intrinsic and extrinsic pathways. The intrinsic pathway involves the release of cytochrome c from mitochondria, leading to the formation of the apoptosome and activation of caspases that execute cell death. The extrinsic pathway involves the formation of the death-inducing signaling complex (DISC) and the activation of initiator caspases, which also lead to apoptosis.[24] Apoptotic cell death is typically marked by chromatin condensation, DNA fragmentation, cell shrinkage, membrane blebbing, and detachment from the extracellular matrix.[25]

AO/PI staining was performed to evaluate cell nuclei and demonstration of apoptosis after treatment with the specific compounds. This dual staining technique is essential for distinguishing between live, apoptotic, and necrotic cells based on their membrane integrity and chromatin condensation. AO, a nucleic acid-binding dye, stains all nucleated cells green, allowing for the visualization of both live and apoptotic cells. PI, on the other hand, penetrates only cells with compromised membranes, which are characteristic of late apoptotic or necrotic cells, staining them red.[26]

In this study, there were various apoptotic characteristics in MCF-7 cells after being treated with single and combination treatments compared to untreated cells. These treated cells displayed several morphological changes such as nuclear irregularities and fragmentation, loss of cellular membrane integrity, nuclear condensation, damaged cell membranes and membrane blebbing indicated by green and orange staining. While the untreated cells remain healthy, viable

cells with a green, intact nuclear structure. These results suggested that the combination of cisplatin and MG may demonstrate its cytotoxicity effect on MCF-7 cells through the induction of apoptosis.

The apoptosis induction of MG has been reported previously on various cancer cell lines. Treatment with MG has induced apoptosis in human epidermoid carcinoma (A431) skin cancer cells by upregulating caspase-3 and upregulating bcl-2 expressions.[27] Another study by Abdullah et al. revealed that MG significantly inhibited the growth of HeLa cells by inducing apoptosis through upregulation of p53 and Bax, while Bcl-2 was significantly downregulated.[16] Moreover, MG also triggered apoptosis in B16F10 cells by elevating the expression of cleaved caspase-3.[20]

*Anti-migratory effect of combination treatment on MCF-7 cells*

One of the main causes of morbidity and mortality in solid cancer is metastases. The migration of cancer cells is one of the most important contributors to cancer metastasis. Therefore, inhibiting migration is an important approach for preventing or slowing cancer metastases.[28] Therefore, anti-cancer medications should be able to prevent cancer cells from penetrating the extracellular matrix (ECM) and developing into secondary tumors. Since these processes differ, it is crucial to differentiate between antiproliferative approaches and those that target motility, migration, invasion, and metastasis when concentrating on drug development efforts.[29]

The wound healing assay was conducted in this study to evaluate the migration inhibitory effect of single and combination treatments on MCF-7 cells. This study showed that the migration of MCF-7 cells was inhibited by single and combination treatments. Untreated MCF-7 cells, as expected, showed rapid cell migration, with high percentages of wound closure at 12 hours and complete closure within 24 hours. This result is consistent with previous reports, indicating that MCF-7 cells possess a very high migratory capacity, which forms the basis of their invasive and metastatic potential.[30]

In contrast, wound closure at 12 hours in cells treated with cisplatin, MG, and CIS-MG were much lower indicating their inhibitory effects on MCF-7 cell migration. Interestingly, CIS-MG demonstrated the most prolonged wound area for 12 and 24 hours. The combination treatment showed a greater inhibition effect on MCF-7 cell migration than the single treatment, indicating its synergistic potential. This finding aligns with previous studies that demonstrated the migration and invasion inhibitory on other cancer cell lines. MG has been reported to inhibit the migration and invasion of migration of hepatocellular carcinoma cells (BEL-7402) invitro and in vivo through the AMPK/NF-κB signaling pathway.[31]

## CONCLUSION

In conclusion, MG demonstrated growth inhibition on breast cancer (MCF-7) cells with an $IC_{50}$ value of 20.21 µg/mL. Moreover, a greater inhibition was observed when MG was combined with cisplatin. MG combined with the lowest concentration of cisplatin synergistically inhibited the growth of MCF-7 cells. Cells treated with combination treatment exhibited several apoptotic features thus suggesting its apoptosis-inducing effect. Furthermore, the combination also demonstrated anti-migratory effect on MCF-7 cells. This discovery might suggest that MG has potential as a natural source candidate to be combined with cisplatin in developing a novel therapeutic medication for breast cancer treatment. The findings may provide the basis for further studies in elucidating the underlying mechanism of synergistic reaction of the combinations on MCF-7 cells.

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ORIGINAL ARTICLE

# Practices related to dietary salt intake and associated factors among adults in Kuala Terengganu

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**ABSTRACT**

**Introduction: Sodium plays a crucial role in regulating blood volume and osmotic balance; however, excessive intake can lead to fluid retention and increased blood pressure. Assessing knowledge, attitudes, and practices (KAP) related to salt consumption is a fundamental step in developing effective public health strategies to reduce excessive salt intake.**

**Materials and Methods: This cross-sectional study was conducted among 1,001 adults in Kuala Terengganu using a snowball sampling method to assess salt-related KAP and the factors associated with good dietary salt intake practices. Eligible participants were aged 18 years and above, proficient in Bahasa Malaysia, and had access to a smartphone with internet connectivity. Data were collected through a structured, pre-tested questionnaire administered via Google Forms and analysed using multiple logistic regression to identify factors associated with good dietary salt intake practices.**

**Results: Among the total respondents, 28.8% (95% CI: 26.0% - 31.6%) demonstrated good dietary salt intake practices. The study revealed a disconnect between knowledge and practice, indicating that awareness alone did not translate into healthier dietary behaviours. Multiple logistic regression analysis identified employment status (adjusted OR: 1.541, 95% CI: 1.166 - 2.038; p<0.001) and higher attitude scores (adjusted OR: 1.138, 95% CI: 1.065 - 1.215; p<0.001) as significant positive predictors of good dietary salt intake practices.**

**Conclusion: The findings provide valuable insights into salt consumption behaviours and associated factors among the self-selected adults in Kuala Terengganu. The results underscore the importance of targeted public health interventions, particularly those promoting positive attitudes and addressing employed populations, to encourage healthier dietary salt intake practices.**

**KEYWORDS:**
*Dietary salt intake, knowledge, attitude, practice, Kuala Terengganu*

## INTRODUCTION

Noncommunicable diseases (NCDs) are the leading cause of death globally,[1] necessitating cost-effective strategy to mitigate their impact. High sodium intake is a significant contributor to NCDs, particularly hypertension and its cardiovascular complications.[2] Sodium is essential for regulating blood volume and osmotic balance; however, excessive consumption can lead to fluid retention and increased blood pressure. According to the National Health and Morbidity Survey (NHMS) 2019, approximately 6.4 million Malaysians were living with hypertension.[3] However, compared to other countries, awareness and control of hypertension among Malaysians remain relatively low.[4]

Salt consumption behaviours are influenced by various sociodemographic factors, such as cultural practices, age, educational level, and income.[5] Individual knowledge, attitudes, and behaviours also play a role in salt intake.[6] Additionally, the increasing availability and affordability of highly processed, energy-dense foods rich in saturated fats, trans fats, sugars, and salt have contributed to changing dietary patterns.[7]

Recent studies have established a strong link between high sodium intake and adverse health outcomes, including cardiovascular diseases, gastric cancer, osteoporosis, cataracts, kidney stones, and diabetes.[8] Research indicates that reducing salt intake by less than 2g per day can lower systolic and diastolic blood pressure by approximately 3.47 mmHg and 1.81 mmHg, respectively.[9] The World Health Organization (WHO) recommends a daily salt intake of less than 5 grams (approximately 2g sodium) per person to prevent cardiovascular diseases.[10]

In Malaysia, the Malaysian Community Salt Survey (MyCoSS)[11] reported an average daily salt intake of 7.9 grams (3,167 mg sodium), surpassing WHO guidelines. While a majority of Malaysians are aware of the negative health impacts of excessive salt consumption and acknowledge the importance of reducing intake, their dietary practices are often not reflective of this awareness. For instance, 72.9% of MyCoSS participants reported adding salt during cooking, and 47.7% reported using additional salt, sauces, or condiments at the table.[11]

Recognizing the urgency of addressing high salt intake, the Ministry of Health Malaysia has implemented the Salt Reduction Strategy 2021-2025, aiming to reduce salt consumption by 30% by 2025 and alleviate the burden of NCDs. This initiative emphasizes creating environments that support healthy eating practices.[12] Understanding

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sociodemographic factors, along with community knowledge and attitudes toward dietary salt intake practice, is critical for cultivating such lifestyle changes. Insights from this research can guide stakeholders and drive actions to enhance public health outcomes.

This study aims to assess practices related to dietary salt intake and determine its associated factors among adults in Kuala Terengganu, Malaysia. By assessing their knowledge, and attitudes, the influencing factors of salt intake practice, this research seeks to fill existing gaps in understanding and contribute to the ongoing efforts to reduce NCD prevalence in Malaysia.

## MATERIALS AND METHODS

A cross-sectional study was conducted from 27th November 2022 to 12th January 2023 to assess prevalence of dietary salt intake and its associated factors among adults in Kuala Terengganu. The study included individuals aged 18 years and above who could read and understand Bahasa Malaysia and had access to a smartphone with internet connectivity. The required sample size was calculated using the two-proportions formula, with a power of 80% and a significance level (α) set at $p<0.05$. This calculation considered several independent variables identified from previous literature, and after accounting for a 10% non-response rate, the final sample size was determined to be 1,001 participants. The study protocol and ethical aspects were approved by Universiti Sultan Zainal Abidin Human Research Ethics Committee (UHREC) (Study protocol code: UniSZA/UHREC/2022/444).

A snowball sampling method was applied to recruit eligible respondents in Kuala Terengganu. The recruitment process began with an initial distribution of the survey link through professional and social networks, including healthcare professionals, community groups, and public forums. Participants were then encouraged to share the survey link with their eligible contacts. The researchers did not have direct contact with all respondents, as the survey was disseminated primarily through peer sharing via digital platforms such as WhatsApp, Facebook, and email. A structured, pre-tested, and validated questionnaire developed in Google Forms was used as the data collection tool from all the eligible respondents for this cross-sectional study. The questionnaire items were gathered from prior studies,[11,13] and refined following discussions with experts and approval from UHREC. As for face validity, the first draft of the questionnaire was reviewed by two medical lecturers and three staff from the UniSZA Medical Faculty. Feedback was used to improve the questions, and a validation study was subsequently conducted in Kg. Teluk Pasu, Kuala Terengganu, involving 100 participants with characteristics similar to the actual study population. Amendments to the questionnaire were made based on their responses and reliability analysis.

The final questionnaire consisted of 42 items divided into four sections (I, II, III, IV). The first section comprised nine items on socio-demographic variables. The second section assessed knowledge about dietary salt intake, including the recommended daily amount, the relationship between salt and sodium, health risks of high salt consumption, and sources of information. Of the 23 items in this section, 17 assessed knowledge, while the remaining six explored sources of information. Correct answers were awarded two points, "Not sure" responses received one point, and incorrect answers received zero points, with a total possible score of 0 to 34. The third section evaluated attitudes towards dietary salt intake through four items using a five-point Likert scale ranging from "Strongly Agree" to "Strongly Disagree." Scores ranged from 4 to 20. The final section measured salt intake practices through six items on a Likert scale from "Never" (zero points) to "Always" (ten points), with total scores ranging from 0 to 60. Then, practices were categorized as "good practice" (0–30) or "poor practice" (31–60).

Participants were informed about the study objectives, the measures taken by the investigators to ensure strict confidentiality, and the voluntary nature of their participation. Consent was obtained prior to participation, and those who failed to complete all questions or did not respond within the data collection period were excluded.

*Statistical analysis*

Data were analysed using SPSS version 25. Descriptive statistics were used to summarize categorical variables as frequencies and percentages, and numerical variables as means with standard deviations or medians with interquartile ranges, depending on their distribution. Simple logistic regression was conducted to identify potential factors associated with dietary salt intake. Variables with a p-value $<0.250$ in univariable analysis and clinically significant variables were further analysed using multiple logistic regression. Forward and backward selection methods were employed, with variables entering the model at $p<0.05$ and removed at $p>0.10$. Ultimately, the forward selection method provided a parsimonious model which was used for further data analysis. Then, the model was subsequently evaluated using the Hosmer-Lemeshow goodness-of-fit test, overall classification accuracy, and the area under the receiver operating characteristic (ROC) curve. Results were reported as crude and adjusted odds ratios with 95% confidence intervals (95% CI), and p-values. Statistical significance for multivariable analysis was set at $\alpha<0.05$.

## RESULTS

The survey included 1,001 participants who met the inclusion criteria, with ages ranging from 18 to 70 years. Table I presents the socio-demographic profile of the respondents. The majority of respondents were Malay (74.3%), single (63.0%), and female (61.0%). Regarding education, 1.6% had attained primary school, 11.4% secondary school, and 87.0% higher education. Most participants (59.7%) were unemployed, comprising students, housewives, retirees, and others, while 40.3% were employed. In terms of each respondent's total household income level, based on the 2019 Household Income and Basic Amenities (HIS/BA) Survey Report classification,14 57.4% of respondents fell into the B40 income category, 30.5% into M40, and 12.1% into T20. Additionally, 652 respondents (65.1%) reported a positive family history of hypertension, while 34.9% had no such history.

**Table I: Socio-demographic characteristics of the adults in Kuala Terengganu (n =1001)**

| Variables | Frequency (%) | Mean (SD) |
|---|---|---|
| Age (in years) | | 29.66 (11.59) |
| Gender | | |
| Female | 611 (61.0) | |
| Male | 390 (39.0) | |
| Ethnicity | | |
| Malay | 744 (74.3) | |
| Non-Malay | 257 (25.7) | |
| Marital status | | |
| Single | 631 (63.0) | |
| Married | 370 (37.0) | |
| Highest completed education | | |
| Primary | 16 (1.6) | |
| Secondary | 114 (11.4) | |
| Tertiary | 871 (87.0) | |
| Occupation | | |
| Unemployed | 598 (59.7) | |
| Employed | 403 (40.3) | |
| Total household income levels of the respondents | | |
| B40 (< RM4850) | 575 (57.4) | |
| M40 (RM4850-RM10959) | 305 (30.5) | |
| T20 (> RM10959) | 121 (12.1) | |
| Family history of hypertension | | |
| No | 349 (34.9) | |
| Yes | 652 (65.1) | |

**Table II: Construct validity and reliability analyses of questionnaire on knowledge, and attitude and practices related to dietary salt intake among respondents in Kampung Teluk Pasu (n =100)**

| Items | Factor loading | | |
|---|---|---|---|
| | Factor 1 (Knowledge) | Factor 2 (Attitude) | Factor 3 (Practice) |
| **Knowledge** | | | |
| Q1: The recommended amount of salt is 5g/day (about 1 teaspoon). | 0.464 | | |
| Q2: The relationship between salt and sodium is salt contains sodium. | 0.331 | | |
| Q3: Food that contains highest salt consumed by Malaysian adults are: | | | |
| a: Fried rice | 0.684 | | |
| b: Omelette | 0.571 | | |
| c: Curry noodle | 0.606 | | |
| d: Light soy sauce | 0.667 | | |
| e: Fried vegetables | 0.799 | | |
| f: Fried instant noodle | 0.861 | | |
| g: Vegetable with soy sauce | 0.399 | | |
| h: White/ wholemeal bread | 0.528 | | |
| i: Fried chicken with spices | 0.476 | | |
| Q4: Diet that contains high amount of salt could cause health problem/s such as: | | | |
| a: Stroke | 0.303 | | |
| b: Kidney stones | 0.515 | | |
| c: Stomach cancer | 0.354 | | |
| d: Heart disease | 0.532 | | |
| e: Osteoporosis | 0.420 | | |
| f: High blood pressure | 0.421 | | |
| **Attitude** | | | |
| Q5: In my opinion, identifying food that has high amount of salt is very important. | | 0.592 | |
| Q6: In my opinion, reducing the amount of salt in diet is very important. | | 0.852 | |
| Q7: In my opinion, my health will improve if I reduce the intake of salt in my diet. | | 0.508 | |
| Q8: In my opinion, checking for salt content on food labels is very important. | | 0.583 | |
| **Practice** | | | |
| Q9: I do not add salt when cooking at home. | | | 0.629 |
| Q10: I ask for less salt in the food that I ordered when eating out. | | | 0.559 |
| Q11: I do the followings as routine to control my salt intake: | | | |
| a: Not adding salt when cooking. | | | 0.383 |
| b: Buy alternative food with low salt. | | | 0.549 |
| c: Read the salt content on the food labels. | | | 0.675 |
| d: Avoid/minimize the consumption of processed food. | | | 0.318 |

**Table III: Factors influencing dietary salt intake practice among adults in Kuala Terengganu (n = 1001)**

| Variables | Simple Logistic Regression | | Multiple Logistic Regression[a] | |
|---|---|---|---|---|
| | Crude OR (95% CI) | p-value | Adjusted OR (95% CI) | p-value |
| Age (in years) | 1.148 (0.869, 1.518) | 0.331 | | |
| Sex | | | | |
| Male | 1.00 | | | |
| Female | 1.007 (0.995, 1.018) | 0.258 | | |
| Ethnicity | | | | |
| Non-Malay | 1.00 | | | |
| Malay | 1.224 (0.900, 1.664) | 0.198 | | |
| Marital status | | | | |
| Single | 1.00 | | | |
| Married | 1.296 (0.979, 1.716) | 0.070 | | |
| Highest completed education | | | | |
| Primary | 1.00 | | | |
| Secondary | 1.024 (0.306, 3.424) | 0.970 | | |
| Tertiary | 1.242 (0.397, 3.887) | 0.710 | | |
| Occupation | | | | |
| Unemployed | 1.00 | | 1.00 | |
| Employed | 1.495 (1.133, 1.971) | 0.004 | 1.541 (1.166, 2.038) | <0.001 |
| Household Monthly income | | | | |
| B40 (< RM4850) | 1.00 | | | |
| M40 (RM4850-RM10959) | 1.445 (1.069, 1.953) | 0.017 | | |
| T20 (> RM10959) | 1.153 (0.746, 1.781) | 0.521 | | |
| Having family history of hypertension | | | | |
| No | 1.00 | | | |
| Yes | 0.869 (0.654, 1.156) | 0.335 | | |
| Total knowledge scores | 1.032 (1.007, 1.058) | 0.013 | | |
| Total attitude scores | 1.132 (1.060, 1.208) | <0.001 | 1.138 (1.065, 1.215) | <0.001 |

[a]Forward likelihood ratio approach was used, confirming no collinearity or interactions among variables. The model demonstrated a reasonable fit, as assessed by the Hosmer-Lemeshow test (p=0.050). The model's performance was further supported by an overall correctly classified percentage of 71.2% and an area under the ROC curve of 59.2% (95% CI: 55.4, 63.0; p<0.001).

*Exploratory factor analysis and internal consistency*

Table II presents the analysis results of construct validity and reliability of the questionnaire evaluating knowledge, attitude and practice related to dietary salt intake among respondents. The Cronbach's alpha values for the knowledge, attitude, and practice sections were 0.673, 0.731, and 0.513, respectively, with an overall reliability score of 0.50 for all 27 items.

*Knowledge and attitude towards dietary salt intake among adults in Kuala Terengganu*

Of the total respondents, 486 (48.6%) correctly identified the recommended daily salt intake as 5g. Regarding the relationship between salt and sodium, 402 respondents (40.2%) understood that salt contains sodium, yet more than one-third (36.5%) selected the wrong answer. A majority of respondents recognized foods with high salt content commonly consumed by Malaysian adults, including fried rice (69.4%), omelette (76.6%), light soy sauce (67.5%), fried vegetables (47.9%), fried instant noodles (72.1%), vegetables with soy sauce (86.3%), and white or wholemeal bread (75.1%). However, only 43.4% identified curry noodles, and just 15.7% acknowledged fried chicken with spices as high-salt foods. When asked about health problems associated with high salt consumption, most respondents recognized conditions such as stroke (75.8%), kidney stones (53.5%), heart disease (70.2%), and high blood pressure (94.3%). In contrast, fewer respondents identified stomach cancer (29.0%) and osteoporosis (24.5%) as potential health risks related to excessive salt intake.

*Sources of information regarding the dietary salt intake*

Most of the respondents (93.0%) acquired information regarding dietary salt intake from the Internet followed by social media such as Facebook, twitter and Instagram (87.8%), television (67.3%), healthcare personnel (67.2%), newspaper (46.0%) and magazine (40.2%).

*Attitude towards dietary salt intake among adults in Kuala Terengganu*

Regarding the attitudes toward salt intake, the majority of participants agreed on the importance of identifying foods high in salt (91.7%) and reducing salt intake in their diet (92.9%). Additionally, most respondents believed that reducing salt consumption would improve their health (90.6%). Regarding the practice of checking salt content on food labels, 89.5% of participants agreed that it is very important.

*Total knowledge and attitude scores towards dietary salt intake among adults in Kuala Terengganu*

According to the scoring system outlined in the methodology, respondents' knowledge scores ranged from 0 to 34, with a mean score of 22.61 (SD=5.68). Attitude scores ranged from 8 to 20, with a mean score of 18.15 (SD=2.32).

*Dietary salt intake practice among residents in Kuala Terengganu*

Among the participants, 102 (10.2%) reported never adding salt when cooking at home, while 54 (5.4%) consistently avoided this practice. The majority, 239 (23.9%), exhibited occasional adherence to this behaviour, falling into the mid-

range of the scale. Regarding the practice of requesting less salt in food when dining out, 408 (40.8%) participants reported never doing so, whereas only 38 (3.8%) consistently followed this habit. This suggests that a significant portion of participants rarely prioritized salt reduction when eating out.

In terms of controlling salt intake, 205 (20.5%) participants moderately refrained from adding salt when cooking, and 41 (4.1%) consistently adhered to this practice. Similarly, 207 (20.7%) participants reported moderate adherence to buying low-salt alternatives, while only 40 (4.0%) consistently purchased such products. Reading food labels for salt content was another behaviour assessed; 169 (16.9%) participants never engaged in this practice, whereas 57 (5.7%) consistently checked food labels for salt content. The avoidance or minimization of processed food consumption was consistently practiced by 75 (7.5%) participants, with 232 (23.2%) moderately adhering to this behaviour. These findings indicate varying levels of adherence to practices aimed at reducing dietary salt intake, with only a minority of participants consistently following recommended habits. Based on the study results, out of 1001 respondents, 28.8 % of them (95% CI: 26.0%, 31.6%) had good dietary salt intake practice.

*Associated factors of having good dietary salt intake practice among residents in Kuala Terengganu*

Based on the univariable analysis results, several factors were identified as potential predictors of having good dietary salt intake practices when no confounding variables were identified. These included ethnicity (Malays, p=0.198), marital status (being married, p=0.070), employment status (employed, p=0.004), socioeconomic status (M40 family, p=0.017), total knowledge scores (p=0.013), and total attitude scores (p<0.001).

After performing multiple logistic regression analysis, only employment status (being employed, p<0.001) and total attitude scores (p<0.001) remained significantly associated with having good dietary salt intake practices. The results of the final model implied that employed respondents and those with higher total attitude scores were more likely to exhibit good dietary salt intake practices compared to their counterparts. (Table III).

## DISCUSSION

Excessive salt consumption is a significant public health concern, contributing to hypertension and an increased risk of cardiovascular diseases.[7] Recognizing the urgency of addressing high salt intake, it is crucial to implement interventions that promote environments supportive of healthy eating practices. Understanding individuals' dietary salt-related practices, along with their knowledge and attitude towards salt intake, is essential in formulating effective strategies to reduce overall salt consumption.[15] Therefore, this study was conducted to assess salt-related knowledge, attitude, and practices among the adult population in Kuala Terengganu. Our findings contribute to the growing body of evidence on salt intake practices, offering insights into potential areas for intervention within this population.

While nearly half of the respondents correctly identified the recommended daily salt intake of 5g (48.6%) and understood the relationship between salt and sodium (40.2%), their knowledge did not always extend to recognizing hidden sources of salt in everyday foods. Although many participants were aware of high-salt food sources such as vegetables with soy sauce, fried instant noodles, and light soy sauce, they were less aware of the salt content in local dishes like curry noodles and fried chicken with spices. This suggests that while general knowledge about salt is present, people may underestimate their actual sodium intake from commonly eaten foods.

Moreover, although nearly all participants acknowledged the link between excessive salt intake and its negative health consequences, particularly hypertension and cardiovascular diseases, fewer recognized other potential risks such as stomach cancer and osteoporosis. Similar patterns have been observed in previous studies conducted in Malaysia among public university staff in Selangor,16 fast food consumers in Klang Valley[13] and Chinese adults in Johor.[17] This indicates that while hypertension is widely recognized, awareness of other salt-related health risks remains limited, emphasizing the need for broader public health campaigns. This necessity has also been highlighted in the National Strategic Plan for Non-Communicable Disease 2016–2025 by the Ministry of Health Malaysia.[18]

Despite this level of awareness, no significant association was found between knowledge and actual dietary salt intake practices. This finding aligns with previous studies,[19,20] which have shown that knowledge alone may not be sufficient to drive behavioural change. The gap between awareness and practice highlights the influence of external factors such as cultural dietary habits, taste preferences, and food availability.[21] Even individuals who understand the risks of excessive salt intake may continue consuming high-salt foods due to social influences or the convenience of salty, processed foods. These findings underscore the need for targeted interventions that go beyond education alone, incorporating policy measures, food environment modifications, and community-based initiatives to effectively reduce salt intake among the population.

Although no association was found between knowledge and practice, the study findings revealed that attitude was significantly associated with dietary salt practices. This is consistent with a study conducted among Turkish adults, which also found that higher attitude scores were predictive of better salt reduction behaviours.[22] This suggests that individuals with a more positive attitude toward salt reduction are more likely to adopt healthier dietary habits.

In this study, the majority of respondents exhibited a positive attitude toward reducing salt intake. A high percentage of participants agreed on the importance of identifying foods high in salt (91.7%), reducing salt intake in their diet (92.9%), and recognizing the potential health benefits of doing so (90.6%). Additionally, 89.9% expressed a positive opinion about checking salt content on food labels. These findings align with previous studies conducted in Malaysia[13] and North India.[23] The positive perceptions may encourage

individuals to make healthier dietary choices, reinforcing the role of attitude in shaping better salt-related behaviour.16 Therefore, fostering positive attitudes through targeted interventions and behavioural change strategies may be a key approach to improving salt reduction practices.

Employment status was another significant factor associated with good dietary salt intake practices, as identified through multiple logistic regression analysis. This finding is consistent with previous research indicating that socio-economic factors, such as employment and income, play a role in shaping dietary behaviours. Employed individuals may have better access to resources, information, or healthier food choices, which could contribute to have good practice compared to the unemployed individuals.[24,25]

Despite high levels of awareness and positive attitudes toward dietary salt reduction, the findings indicate a gap between knowledge, attitude, and actual practice behaviour. The study revealed that only 10.2% of participants reported never adding salt when cooking at home, and merely 3.8% requested less salt when dining out. Furthermore, only a small proportion consistently adhered to recommended salt reduction behaviours, including refraining from adding salt while cooking (4.1%), purchasing low-salt alternatives (4.0%), reading food labels for salt content (5.7%), and minimizing processed food consumption (7.5%). These findings suggest that while participants recognize the importance of reducing salt intake, they often fail to implement these practices in daily life.

This discrepancy between knowledge, attitude, and practice is consistent with findings from previous studies in Malaysia and other countries, where salt reduction behaviours remain challenging despite positive attitudes toward dietary changes.[13,23] One possible explanation is the deep-rooted dietary habits and taste preferences within Malaysian food culture, where salty and savoury flavours are commonly preferred. Additionally, the availability and affordability of high-sodium processed foods may encourage habitual salt intake rather than active dietary modifications. Furthermore, the low adherence to reading food labels for salt content (5.7%) suggests that many consumers may either lack awareness of food labelling information or find it difficult to interpret. This aligns with findings from previous studies indicating that food labelling alone is often insufficient to drive behavioural change unless accompanied by public education and policy interventions.[26]

## LIMITATIONS

This study has several limitations. Firstly, the reliance on self-reported data may introduce reporting biases, as participants could underreport or overreport their dietary practices.[27] Secondly, the use of snowball sampling limits the generalizability of the findings, as this method does not approximate probability sampling and may not fully represent the broader adult population. Thirdly, the study was restricted to adults in Kuala Terengganu who had access to a smartphone with internet connectivity. As a result, the findings should be interpreted cautiously, as they reflect a self-selected sample and may not be generalizable to all adults in Kuala Terengganu.

## CONCLUSION

This study highlights the dietary salt intake knowledge, attitudes, and practices among self-selected adults in Kuala Terengganu, revealing key gaps in salt reduction behaviours. While a majority of respondents demonstrated awareness of the recommended salt intake and its health implications, actual adherence to salt reduction practices remained low, with only 28.8% exhibiting good dietary salt intake behaviours. Multiple logistic regression analysis identified employment status and attitude scores as significant predictors of good dietary salt intake practices, suggesting that those who are employed and have positive attitudes toward salt reduction are more likely to adopt healthier habits.

Given these findings, multi-level interventions are necessary to improve salt intake practices of the studied population in Kuala Terengganu. Public health strategies should not only focus on raising awareness but also on creating an environment that facilitates healthier choices. This could involve policy-driven approaches such as mandatory salt reduction in processed foods and clearer front-of-package labelling. Moreover, educational campaigns emphasizing the importance of reading food labels, reducing salt usage when cooking and dining out, and choosing low-sodium alternatives should be intensified, particularly among unemployed individuals and those with lower attitude scores.

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# Prevalence of Haemoglobin Constant Spring in the Malaysian population: Insights from a single-center study

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## ABSTRACT

**Introduction: Thalassemia is a significant public health concern in Malaysia, with an estimated 6.8% of the population being carriers. These individuals may experience varying severities of anaemia. Alpha thalassemia, in particular, results from deletions or mutations within the α-globin gene complex, leading to reduced or absent α-globin chain production. Haemoglobin Constant Spring (Hb CS) is one of the most common non-deletional alpha-thalassemia variants, characterized by a specific mutation in the HBA2 gene.**

**Materials and Methods: A cross-sectional study utilized data from 159 samples collected during the National Thalassemia Screening Program for Form 4 students in Terengganu from 2019 till 2022. Haemoglobin analysis was conducted with the capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) methods. DNA analysis was performed using multiplex PCR and ARMS to detect both deletional and non-deletional α-thalassemia.**

**Results: Three different types of Hb CS were observed. Among the 159 samples, 137 (86.2%) were heterozygous, 20 (12.6%) were compound heterozygous, and 2 (1.3%) were homozygous for Hb CS.**

**Conclusion: These findings offer valuable insights for improving genetic counselling practices and public health strategies, especially in regions with a high prevalence of thalassemia.**

**KEYWORDS:**
*Thalassemia, haemoglobin constant spring, high-performance liquid chromatography, capillary electrophoresis*

## INTRODUCTION

Thalassemia is the most common inherited haemoglobin disorder worldwide, with an exceptionally high prevalence in Southeast Asia, including Malaysia.[1,2] It is one of Malaysia's most prevalent inherited blood disorders, where approximately 4.5% of the population are carriers of either beta thalassemia or alpha thalassemia.[3]

Alpha thalassemia results from impaired production of alpha-globin chains, an essential component of haemoglobin. While the deletional forms of alpha thalassemia are well-documented, non-deletional variants such as Haemoglobin Constant Spring (Hb CS) are less recognized but equally significant due to their clinical implications.[4]

Hb CS is caused by a point mutation in the alpha-globin gene (α2), resulting in a termination codon being replaced by a glutamine codon. This leads to an elongated alpha-globin chain that is unstable and inefficiently produced, exacerbating globin chain imbalance.[5,6] Individuals with Hb CS may present with a spectrum of clinical phenotypes, from asymptomatic carriers to severe forms of alpha thalassemia, especially when co-inherited with other alpha-thalassemia mutations.[4]

Several methods for screening thalassemia include capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). In CE, a peak will appear in Zone 2 for Hb CS, while in HPLC, Hb CS sometimes produces a small peak in the C window at a retention time of 4.90 to 5.30 minutes.[7,8] Screening programs for thalassemia have been set up for all form four students between the ages of 15 and 16 by the Malaysian Government.[9] This screening program aims to determine the thalassemia carrier rate among students in a secondary school and educate these healthy carriers on the risks and options for preventing the birth of children with severe thalassemia syndromes.[10]

The prevalence of Hb CS varies significantly across different populations, reflecting genetic, geographic, and cultural influences. In Malaysia, some communities' multi-ethnic composition and high consanguinity rates contribute to a diverse hemoglobinopathy landscape. However, comprehensive data on Hb CS prevalence remains limited, particularly at a population level. This study aimed to determine the prevalence of Hb CS, a subtype of alpha thalassemia, among form four students in Terengganu, Malaysia. By employing advanced diagnostic techniques such as capillary electrophoresis and molecular analysis, we seek to provide insights into the epidemiology of Hb CS in this region. The findings will enhance the understanding of this condition and inform strategies for genetic counselling, population screening, and public health initiatives.

## MATERIALS AND METHODS

This cross-sectional study involved Form Four students aged 15 to 16 who were screened for thalassemia as part of the National Thalassemia Screening Program in Terengganu.

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**Table I: Distribution of types of Hb CS based on genotypes findings (n = 159)**

| Types and co-inheritance | Number of samples (n) | % |
|---|---|---|
| Heterozygous Hb CS | 137 | 86.2 |
| Homozygous Hb CS | 2 | 1.3 |
| Compound heterozygous Hb CS | 20 | 12.6 |

**Table II: Distribution of compound heterozygous Hb CS according to genotypes (n = 20)**

| Types and co-inheritance | Number of samples (n) | % |
|---|---|---|
| Beta thalassemia trait | 1 | 5.0 |
| Hb CS Quang Sze | 1 | 5.0 |
| Het IVS-1 | 1 | 5.0 |
| Het with α-3.7 | 11 | 55.0 |
| Het with α-4.2 | 2 | 10.0 |
| Hb CS Malay | 1 | 5.0 |
| Hb CS with α SEA | 1 | 5.0 |
| Hb CS Adana | 1 | 5.0 |
| Hb CS D Punjab | 1 | 5.0 |
| Total | 20 | 100.0 |

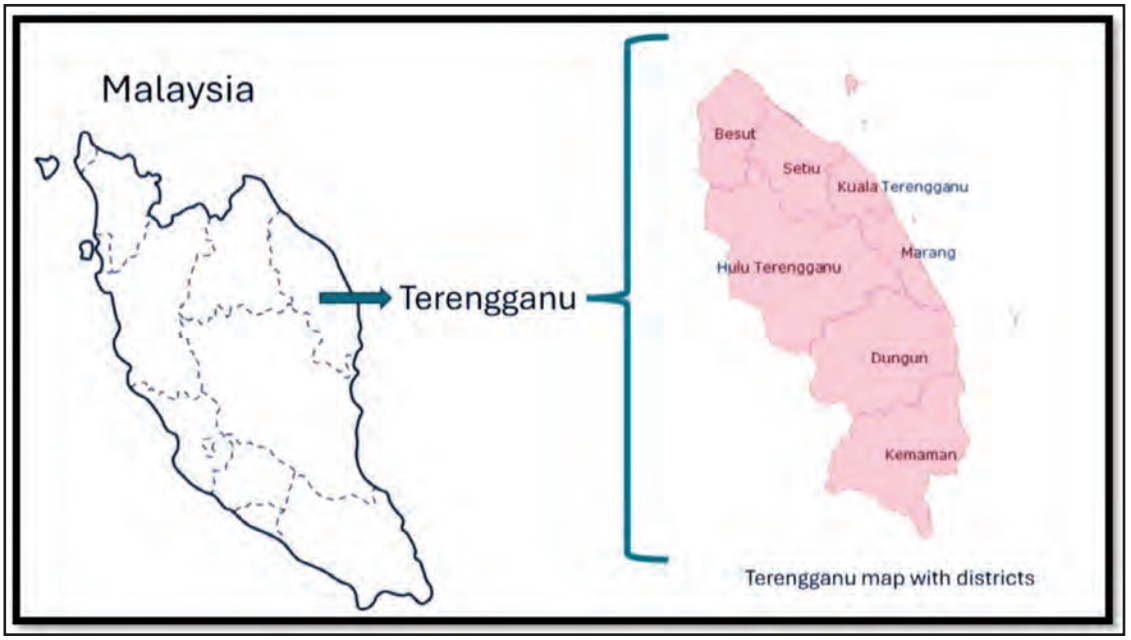


**Fig. 1:** Map highlighting Terengganu and its districts in Malaysia

Data for the study were collected from the laboratory information system covering the period from January 2019 to December 2022. Access to this data was granted only after obtaining the necessary ethical clearances. Ethical approval for data collection and analysis was secured from the Medical Research and Ethics Committee (NMRR ID-23-00137-ACP) and the Human Research Ethics Committee of Universiti Sultan Zainal Abidin (UHREC) (UniSZA/UHREC/2023/569) prior to the commencement of the study. Additionally, the study adhered to the ethical principles outlined in the Declaration of Helsinki. A total of 159 samples were selected based on a peak at Zone 2 on CE and a small peak at the C window on HPLC. DNA or molecular analysis was conducted using multiplex polymerase chain reaction (PCR) and amplification-refractory mutation system (ARMS) techniques to identify both deletional and non-deletional α-thalassemia mutations. The molecular analysis specifically targeted common α-thalassemia mutations prevalent in Malaysia, including deletional mutations such as $-\alpha^{3.7}$, $-\alpha^{4.2}$, and —SEA, as well as non-deletional mutations such as Haemoglobin Constant Spring (Hb CS; HBA2: c.427T>C) and Hb Adana (HBA2: c.179G>A).

## RESULTS

Ethnicity analysis in this study found that 158 (99.4%) of the students with Hb CS were Malays, and 1 (0.6%) was Chinese. No cases were observed among Indian or other ethnic groups. Molecular studies of the 159 samples with a positive peak in Zone 2 on CE confirmed the presence of haemoglobin Constant Spring (Hb CS). Among these, 137 samples (86.2%) were heterozygous for Hb CS, 20 samples (12.6%) were

compound heterozygous, and two samples (1.3%) were homozygous. Of the 159 samples, 54 (34.0%) were from male students, and 105 (66.0%) were from female students (Table I).

The distribution of compound heterozygous Hb CS cases was further analysed and classified based on their genotypes and co-inheritance with alpha or beta-thalassemia. Among these cases, the most common co-inheritance involved Hb CS with the α-3.7 deletion (55.0%) and Hb CS with the α-4.2 deletion (10.0%). One case of Hb CS co-inherited with specific beta-thalassemia variants, including Hb Malay, Hb D Punjab, Hb Adana, and β-thalassemia (Table II).

## DISCUSSION

Terengganu, located on the east coast of Peninsular Malaysia, is part of the Southeast Asian thalassemia belt, where hemoglobinopathies are relatively prevalent. It is divided into seven administrative districts with the population predominantly Malay, with smaller Chinese and Indian communities (Figure 1). This ethnic distribution mirrors the demographic composition of Terengganu, where Malays represent the overwhelming majority, followed by minority Chinese and Indian populations.

Terengganu actively participates in the Malaysian National Thalassemia Screening Program, which focuses on identifying early carriers among high-risk groups, such as school students and pregnant women, to prevent disease transmission. Despite these efforts, hemoglobinopathies remain a significant public health challenge due to associated morbidity and the demand for regular transfusions and iron chelation therapy in affected individuals.

Thalassemia traits are highly prevalent in the state, with significant occurrences of both alpha and beta thalassemia carriers. Hb CS, a non-deletional alpha-thalassemia mutation, is one of the common variants in the region. Additionally, other hemoglobinopathies such as Hb E and Hb H disease are frequently reported, often in compound forms, due to co-inheritance of multiple mutations.

This study investigated the prevalence of Hb CS among form four students in Terengganu, Malaysia. The results confirmed Hb CS through molecular studies in all 159 samples collected. Of these, the majority were heterozygous Hb CS, accounting for 86.2%. Additionally, 12.6% were identified as compound heterozygous Hb CS, while 2 samples (1.3%) were homozygous Hb CS. This prevalence aligns with previous studies in Malaysia but highlights the regional genetic burden within the east coast states. The high frequency of Hb CS necessitates targeted public health strategies, including enhanced genetic counselling and community education on hemoglobinopathies.[11]

In cases of compound heterozygous Hb CS, the majority were found to have co-inheritance with the -$\alpha^{3.7}$ deletion. This observation aligns with findings reported by Ramli et al., who also identified the co-inheritance of the -$\alpha^{3.7}$ deletion as the most prevalent form of thalassemia in Malaysia.[12] Among Malaysians, the -$\alpha^{3.7}$ deletion is the most common type of alpha thalassemia, leading to a higher incidence of co-inheritance with Hb CS. Given that Hb CS can lead to clinically significant conditions when co-inherited with other alpha-thalassemia mutations, early detection is essential.[13,14] Public health initiatives, such as the National Thalassemia Screening Program, are instrumental in identifying carriers at an early age, allowing for timely interventions, particularly in high-risk populations. The incorporation of molecular diagnostics into routine screening could further improve the detection of non-deletional alpha-thalassemia variants, ultimately reducing the burden of severe thalassemia syndromes.[15]

This study has several limitations. First, using both capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) for initial screening may introduce variability in case selection. Second, the sampling was limited to Form Four students from a single Malaysian state, which restricts the generalizability of the findings to the entire country. Additionally, the ethnic distribution was predominantly Malay, reflecting local demographics, but this limits the ability to extrapolate the results to multiethnic populations. Finally, the small number of homozygous Hb CS cases restricts phenotypic analysis for this specific genotype.

## CONCLUSION

This study highlights the significant prevalence of heterozygous Hb CS among Form Four students in Terengganu, Malaysia. The co-inheritance of Hb CS with α-thalassemia deletions, particularly the –$\alpha^{3.7}$ deletion, emphasizes the importance of molecular diagnostics in population-based screening. These findings offer valuable insights for enhancing genetic counselling practices and public health strategies in similar demographic areas.

## DECLARATION

Authors declare that there is no conflict of interest.

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# D-dimer value for predicting pulmonary embolism in COVID-19 patients: A retrospective study

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## ABSTRACT

**Introduction: Pulmonary embolism (PE) is a significant complication in patients with COVID-19, often associated with elevated D-dimer levels. However, there remains uncertainty around the D-dimer threshold for predicting PE in COVID-19 patients as it is influenced by multiple disease factors. This study aimed to establish the D-dimer cut-off value for predicting pulmonary embolism (PE) in patients with COVID-19, evaluate the sensitivity and specificity of this cut-off value, and describe the occurrence of PE and its significant factors.**

**Materials and Methods: A retrospective analysis was conducted on 320 patients with COVID-19 who underwent computed tomography pulmonary angiography (CTPA) due to clinical suspicion of PE between 2020 and 2021 at a single centre in Malaysia. Clinical and biological factors associated with PE were analyzed, including age, sex, race, and D-dimer levels.**

**Results: Among the study population, 23.4% of males and 15.4% of females tested positive for PE, with no significant differences noted across racial groups. Age was significantly associated with PE development (p = 0.013). D-dimer levels in PE-positive patients were five times higher than in PE-negative patients (p = 0.001). An optimal D-dimer cut-off of 2799 ng/ml level was identified with an area under the curve (AUC) of 0.744 (95% CI: 0.676–0.813), and sensitivity of 81%, and specificity of 51%.**

**Conclusion: This study highlights the role of D-dimer as a predictive biomarker for PE in COVID-19 patients. The identified cut-off value offers a practical threshold for clinical decision-making, balancing sensitivity and specificity. Further studies are needed to validate these findings in broader population.**

**KEYWORDS:**
*Computed Tomography Pulmonary Angiogram (CTPA), Pulmonary Embolism (PE), D-Dimer, COVID-19*

## INTRODUCTION

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), posed a significant public health challenge. This was particularly due to its link with thromboembolic and hypercoagulable events, such as pulmonary embolism (PE). COVID-19 infection triggers a complicated state of increased clotting and inflammation, which results in elevated levels of D-dimer. These D-dimer levels serve as biomarkers to assess the risk of developing PE. Recent studies have highlighted the critical role of D-dimer levels in predicting PE in COVID-19 patients, a D-dimer cut-off of 2903 ng/mL demonstrated an 81% sensitivity for predicting PE in COVID-19 patients undergoing computed tomography pulmonary angiography (CTPA).[1] Another study also recommended that higher D-dimer cut-off value, such as 2000–5000 ng/mL, can highly predict PE while minimizing unnecessary CTPA.[2] Furthermore, in the emergency department unit, thresholds as low as 1815 ng/mL represent 93% sensitivity of PE.[3]

The associated coagulopathy nature of COVID-19 and the various presentations of PE in different patient populations underscore the need to understand the D-dimer cut-off value. This value and characterization of PE such as the location will be helpful for clinicians to determine the likelihood of PE and the next steps in management. Peripheral PE locations are more common in COVID-19 patients, which may highlight a localized thrombotic process differences from traditional embolic pathways.[4]

This study aimed to determine the optimal D-dimer cut-off value for predicting PE in hospitalized COVID-19 patients and to evaluate its diagnostic performance in terms of sensitivity and specificity. Additionally, the study sought to explore demographic and clinical factors associated with the occurrence of PE. Given the burden of COVID-19 and its evolving clinical presentations, understanding the predictive value of D-dimer levels remains challenging for improving diagnostic strategies and minimizing resource overuse.

## MATERIALS AND METHODS

*Study Design and Participants*

A retrospective analysis was conducted on 320 patients at Hospital Sultanah Nur Zahirah in Kuala Terengganu, Malaysia, during January 2020 and December 2021. The inclusion criteria included those who confirmed COVID-19 diagnosis with clinical suspicion of PE and underwent CTPA. The exclusion criteria are incomplete CTPA or missing D-dimer data. The clinical suspicion is based on clinical

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**Table I: Location of pulmonary embolism (PE) in the CTPA**

| Location of PE | | N (%) |
|---|---|---|
| Laterality | Unilateral | 46 (73) |
| | Bilateral | 17 (26.9) |
| Affected artery | Pulmonary artery trunk | 1(1.6) |
| | Main pulmonary arteries | 10 (15.9) |
| | Lobar pulmonary arteries | 16 (25.4) |
| | Segmental pulmonary arteries | 25 (39.7) |
| | Subsegmental pulmonary arteries | 3 (4.8) |
| Location | Right upper lobe | 16 (26.7) |
| | Right lower lobe | 20 (33.3) |
| | Middle lobe | 4 (6.7) |
| | Left upper lobe | 7 (11.7) |
| | Left lower lobe | 13 (21.7) |

**Table II: PE comparison among COVID-19 patients**

| Variable | PE-positive (63) | PE-negative (257) | p value |
|---|---|---|---|
| Mean age (SD) years | 59.89 (13.61) | 54.91 (15.93) | 0.013 [a] |
| Gender | | | |
| Male | 40 (23.4%) | 131 (76.6%) | 0.074 [b] |
| Female | 23 (15.4%) | 126 (84.6%) | |
| Race | | | |
| Malay | 61 (19.2%) | 256 (80.8%) | 0.100 [c] |
| Chinese | 2 (66.7%) | 1 (33.3%) | |
| D-dimer (Median (IQR)) | 17000 (28801) | 3073 (5249) | <0.001 [d] |

[a] Independent t-test, [b] Pearson chi-square test, [c] Fisher's exact test, [d] Mann Whitney test

symptoms of dyspnoea, chest pain, hypoxia and elevated D-dimer.

The approval for the research was obtained from the Research Ethics Committee of the Ministry of Health Malaysia (NMRR ID-23-01789-5R6). The requirement for informed consent was waived, given the retrospective nature of the analysis. All collected data were fully anonymized to maintain confidentiality and prevent any potential identification of the patients.

To ensure a standardized and secure method of data collection, allowing for comprehensive analysis while upholding ethical research standards, patient imaging and associated clinical information were systematically retrieved from the hospital picture archiving and communication system.

*CTPA image acquisition*

A 640-multislice CT scan (Aquilion One, Canon Medical Systems LTD) was used for the CTPA assessment. For the image aquisition, the entire thorax was scanned in a craniocaudal direction from the top of the lung to the bottom of the diaphragm. To reduce breathing artefact, all patients were instructed to temporarily hold their breath during the scanning. The pulmonary trunk was used as the region of interest for an automatic bolus-tracking approach, with a threshold of 100HU and a fixed latency of 5 s before picture capture. A single breath-hold was used to acquire CTPA pictures.

*Image interpretation*

The location of the pulmonary thrombus was identified for each patient. Information which was recorded includes the laterality (unilateral or bilateral), the affected pulmonary artery (trunk, main, lobar, segmental, or subsegmental) and the affected lobe (right upper lobe, right lower lobe, middle lobe, left upper lobe or left lower lobe).

*Statistical analysis*

The interquartile range (IQR) was used to depict the median of continuous variables. Numbers and percentages were used to describe categorical variables. Fisher's exact test, Pearson chi-square test, Mann-Whitney test, and Independent t-test were all used to evaluate patient characteristics between the PE-positive and PE-negative groups.

The optimal D-dimer cutoff point for PE was determined based on Receiver operating characteristic (ROC) analysis and area under the curve (AUC). The p-value was considered statistically significant of less than 0.05. Statistical analyses were done using SPSS 21.0 software.

## RESULTS

*PE Imaging findings*

A total of 320 patients were included in this study. There were 63 patients (19.7%) who were diagnosed with PE while the remaining were negative for PE. Unilateral PE was 3-fold higher than bilateral involvement. The right lower lobe was the commonest affected site. The distribution of the affected arteries were shown in Table I

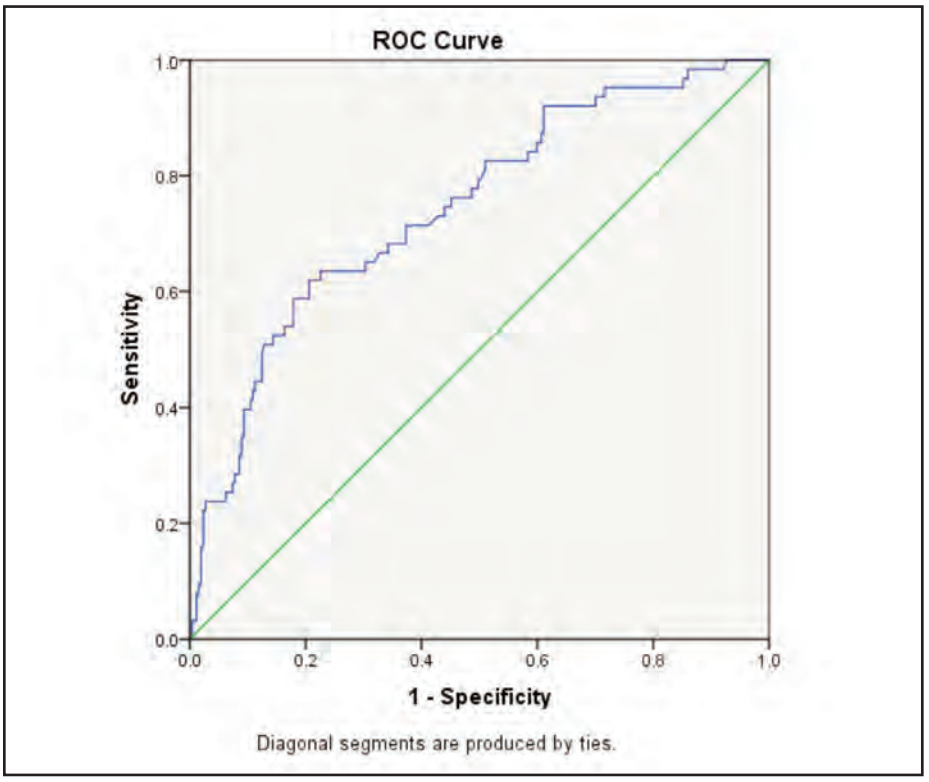


**Fig. 1:** ROC curve for specificity and sensitivity based on D-dimer value

*Comparison of Pulmonary Embolism (PE) among COVID-19 Patients*

The mean age was higher among patients diagnosed with PE compared to PE-negative patients and it was statistically significant (p=0.013). There was a higher percentage of males than females diagnosed with PE. However, this difference was not statistically significant (p=0.074). The demographic data was shown in Table II.

The D-dimer values were almost five times higher in PE-positive patients, with a median of 17,000 ng/mL (IQR: 28,801), than in PE-negative patients (3,073 ng/mL; IQR: 5,249) (p<0.001) as shown in the Table II.

*ROC Analysis for D-dimer Cutoff*

The ROC curve analysis for D-dimer values indicated an optimal cut-off value of 2,799 ng/mL, achieving an Area Under the Curve (AUC) of 0.744 (95% CI: 0.676–0.813). At this cut-off value for predicting PE, the D-dimer demonstrated a sensitivity of 81% and a specificity of 51% (Figure 1).

## DISCUSSION

D-dimer level is crucial in predicting PE in COVID-19 patients. D-dimer with a cut-off value of 2799 ng/mL, can be used to predict the presence of PE in patients with COVID-19. This finding was consistent with other studies, include a multi-centre study with the D-dimer cut-off value of 2494 ng/mL, while other studies reported a cut-off value of between 2600 - 2903 ng/mL.[1,5-6] Those findings highlighted the value of D-dimer as biomarker for diagnosing PE among COVID-19 patients. However, there was a study with a lower D-dimer cut-off value of 1815 ng/mL, with sensitivity of 93% for diagnosis of PE.[3]

A clinical scoring system with the integration of biomarkers with the Wells or Geneva criteria was used in diagnosing PE among patients with COVID-19 in few studies. Those studies have demonstrated that the clinical scoring system enhanced diagnostic precision without requiring a CTPA, mostly in older populations.[7] An elevated D-dimer level is also present in other diseases such as malignancy, sepsis, and autoimmune disorders.

While the D-dimer cut-off value may be useful in predicting the presence of PE, it can be used to avoid unnecessary CTPA. An adjusted D-dimer cut-off value of 2000 ng/mL was found to have a high negative predictive value for critically ill patients to be diagnosed with PE in a study, hence avoiding any unnecessary imaging.[8]

This study also suggested the need to treat a high-risk patient for PE earlier with an anticoagulation. A study has showed that in high risk patients with PE, the PE risk increased to four-fold in the absence of anticoagulation.[9] Other studies have also found that patients on therapeutic-dose anticoagulation prior to hospitalization or who have received prophylactic-dose anticoagulation during their hospital stay had reduced incidences of pulmonary embolism.[10]

Another important finding of this study was the incidence of PE in segmental arteries, with right-sided and unilateral involvement being the most prevalent. These findings were consistent with previous worldwide studies pointing to

morphological and hemodynamic predispositions in PE linked to COVID-19.[11-13] Further studies may be required to study localized thrombosis processes, which diverge from conventional embolic pathways. There was emerging evidence of microangiopathy involving small vessels, mainly in peripheral and unilateral PE among patients with COVID-19. We was not able to study such changes due to the limited information of the macrovascular findings with a standard CTPA.

Microvascular diseases such as microangiopathy, which are often associated with microembolism, cannot be accurately evaluated by using a conventional computed tomography (CT) imaging. Future studies incorporating dual-energy CT and perfusion imaging could give further insight into these changes in small vessels and their clinical impact.[11-13]

The findings in this study reinforce the utility of D-dimer as a critical biomarker across various thrombotic illnesses, including COVID-19. The integration of clinical probability models with biomarkers serves as a framework for more efficient diagnostic strategies.

## LIMITATION

Limitations in this retrospective study include a single-centre study design and absence of information related to the co-morbidities. Further studies are required to identify age-adjusted and population-specific D-dimer cut-off values in high risk patients especially among patients with COVID-19.

## CONCLUSION

This study suggested that the D-dimer value can be used as a predictive biomarker of diagnosis of PE in COVID-19 patients. This biomarker can be used particularly in the determining the need for pulmonary imaging and the initiation of anticoagulation. Future multi-centre studies should be carried out with broader datasets to validate these conclusions and investigate the impact of comorbid conditions on the D-dimer cut-off values.

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# Factors influencing mammogram uptake among women attending primary care clinics in Terengganu, Malaysia

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## ABSTRACT

**Introduction: Breast cancer ranks as the most common cancer among women in Malaysia with its incidence rate steadily rising over the years. Mammograms are crucial for early detection, yet their usage remains low in Malaysia, especially in Terengganu where the services are scarce. This study seeks to explore the factors influencing mammogram uptake among women visiting public primary care clinics in Terengganu.**

**Materials and Methods: A cross-sectional study was conducted at six public primary care clinics in Terengganu, selected using cluster random sampling from both urban and rural areas. A total of 739 women participated, of whom 231 met the eligibility criteria for mammogram screening according to Ministry of Health guidelines. A validated questionnaire was utilized to gather information on sociodemographic factors, knowledge and attitudes towards breast cancer and screening behaviours. Both univariable and multivariable logistic regression analyses were conducted to examine the relationships between independent variables and mammogram uptake.**

**Results: Only 16.5% of the eligible participants reported having undergone a mammogram at some point. The multivariable analysis indicated that age and education level significantly predicted mammogram uptake. Women with higher education levels exhibited 2.6 times greater odds (95% CI: 1.22, 5.43) of having undergone mammography, and each additional year of age increased the probability of screening by 7% (aOR: 1.07, 95% CI: 1.01, 1.13).**

**Conclusion: The uptake of mammograms among women in Terengganu is alarmingly low and mirrors national trends. Women who possess higher education and are older were more likely to participate in mammography, proving the critical role of health literacy in screening behaviour. Addressing access and awareness are vital challenges in areas where mammogram services are limited. Implementing targeted educational initiatives and enhancing healthcare access, particularly in rural settings is crucial for improving breast cancer screening rates.**

**KEYWORDS:**
*Breast cancer, cancer screening, cancer, mammogram, screening*

## INTRODUCTION

Breast cancer remains the most prevalent cancer in Malaysia, accounting for 17.6% of all cancer cases and is the most common cancer among women, with an incidence rate of 38.9 per 100,000 population.[1] This incidence rate shows a steady increase from 31.1 per 100,000 population between 2007 to 2011[2] to 34.1 per 100,000 between 2012 to 2016,[3] which could be partly attributed to improved detection and reporting mechanisms.[1] The incidence begins to rise at the age of 30, plateaus at around 50 years, peaks between the ages of 60-64 and decreases thereafter.[1]

Early detection of breast cancer in Malaysia faces significant challenges, as many patients present at advanced stages. The percentage of late-stage (stage III and IV) breast cancer diagnoses increased from 43.2% between 2007 and 2011[2] to 47.9% between 2012 to 2016.[3] Moreover, one-third of patients delay seeking medical attention for over three months after recognizing symptoms.[4] This delay highlights the need for enhanced awareness and access to early screening services, such as mammograms, as delayed diagnosis significantly affects survival outcomes. Mammography, especially among women aged 50–74, has been shown to reduce breast cancer mortality (relative risk [RR] 0.77) with smaller reductions among younger women (RR 0.88).[5] Early diagnosis through mammogram screening allows for more effective therapy, less invasive interventions, and reduced treatment costs, thereby improving overall cancer outcomes.

Malaysia's breast cancer screening guidelines recommend a risk-stratified approach, where biennial mammograms are advised for women aged 50-74.[6] For women at high risk of breast cancer, screening mammogram may be initiated between 30-39 years of age, followed annually between 40-59, and biennially after age 60. Carriers of BRCA1, BRCA2, and PALB2 are advised to undergo annual magnetic resonance imaging from ages 30-49, annual mammograms from 40-69 and biennial mammograms after age 70.[6]

In Malaysia, mammogram services are available at public hospitals, private hospitals and certain private clinics. Patients may walk in or receive a referral for these services, with government hospitals offering mammograms at minimal charges, while private centres charge between RM100 and RM400, depending on the type of mammogram. The Ministry of Health, in collaboration with other government bodies and private organizations, has launched several initiatives aimed at increasing public awareness and promoting early screening. The annual Pink October campaign, in conjunction with Breast Cancer Awareness Month, is a major event that emphasizes the importance of

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screening, early detection and treatment of breast cancer. Public and private organizations, including hospitals and non-profit groups, participate by organizing educational programs on breast self-examinations and offering free or subsidized mammograms.

Despite these ongoing campaigns and subsidized screening programs, mammogram uptake remains low in Malaysia.[7] The National Health and Morbidity Survey 2023 reported a national mammogram uptake rate of only 12.8% within the past three years.[8] Disparities exist between urban and rural areas, with uptake rates slightly higher in Selangor (27%)[9] and Putrajaya (30%)[10] while significantly lower in Kedah (2.8%).[11] Barriers to mammogram screening include lack of knowledge, poor attitudes, financial constraints and perceived low risk.[12]

Terengganu, located on the east coast of Malaysia, has a population of approximately 1.2 million, most of whom live in urban areas.[13] The female population aged 30-74 is around 200,000.[14] Geographically, Terengganu is divided into eight districts, spanning about 200 kilometres along the coastline, with Kuala Terengganu as the capital.[13] There are 52 health clinics throughout the state,[15] but at the moment, only two public facilities, one in Kuala Terengganu and one in Kemaman offer mammogram services. Additionally, there are two private hospitals, both in Kuala Terengganu, providing mammograms. This limited accessibility predominantly restricts mammogram services to Kuala Terengganu. According to the National Health and Morbidity Survey, 2019, Terengganu ranks among the lowest in mammogram uptake, following Kelantan, Pahang and Perak.16 The present study aims to determine factors that influence mammogram uptake among women attending public primary care clinics in Terengganu.

## MATERIALS AND METHODS

A cross-sectional study was conducted among women attending six primary care clinics in Terengganu. The clinics were selected via cluster random sampling from 52 health clinics in all eight districts in the state. To ensure representation from both rural and urban settings, the districts were first categorized into urban (Kuala Terengganu, Kuala Nerus, and Marang) and rural (Setiu, Besut, Hulu Terengganu, Dungun, and Kemaman) groups. Three health clinics were selected from each group using simple random sampling. The selected urban clinics were Bukit Tunggal Health Clinic, Hiliran Health Clinic, and Seberang Takir Health Clinic, while the rural clinics were Bukit Besi Health Clinic, Kuala Berang Health Clinic, and Telemong Health Clinic.

The questionnaire used in the study comprised of sections on sociodemographic characteristics, breast cancer knowledge and attitudes toward breast cancer screening and breast cancer screening practices. The breast cancer knowledge section, developed by the researcher, consisted of 24 items related to symptoms, risk factors, and screening methods. Attitudes toward screening were measured using 56 items adapted from the Champion Health Belief Model (CHBM)[17], assessing perceived susceptibility, severity, benefits, barriers, self-efficacy, and cues to action. Responses were rated on a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The practice section assessed engagement in breast self-examinations, clinical breast examinations, and mammogram screening, aligned with guidelines from the Ministry of Health.[6]

A pilot study involving 30 university librarians was conducted to assess the reliability of the questionnaire, yielding Cronbach's alpha values of 0.7 for the knowledge section and 0.9 for the attitude section.

The study recruited 739 consecutive women aged 18 years and above attending the selected clinics. Of these, 231 women met the Ministry of Health's criteria for mammogram screening: (1) age 30 or older with a first-degree relative diagnosed with breast cancer or (2) age 50 years and above.

### Data Analysis

The data were analyzed using IBM SPSS Statistics (Version 21.0). Descriptive statistics were used to summarize sociodemographic characteristics, with means and standard deviations were reported for continuous variables while frequencies and percentages were used for categorical variables. Total scores were calculated for the knowledge and attitude domains. Univariable logistic regression was used to examine the associations between independent variables and mammogram uptake. Variables with a p-value <0.25 or those deemed clinically relevant were included in the multivariable logistic regression model. The final adjusted model was constructed, and statistical significance was set at $p<0.05$. Interaction effects, multicollinearity, and model fit (using the Hosmer-Lemeshow goodness-of-fit test) were assessed for the final model. Crude and adjusted odds ratios (OR and aOR) were used to report associations between the independent variables and mammogram uptake.

## RESULTS

A total of 231 eligible participants were included in the final analysis.

### Socio-demographic characteristics of respondents

All 231 respondents were Malay, with the majority being married (95.7%) and having attained either primary or secondary education (72.3%). Approximately 85.3% reported a history of breastfeeding, while only 3.9% had a first-degree relative diagnosed with breast cancer. The sociodemographic details of the participants are presented in Table I.

### Mammogram Uptake

Only 38 out of 231 participants (16.5%) reported having undergone mammogram screening at least once in their lifetime. This measure did not account for the frequency of screening or adherence to recommended intervals (i.e., annually or biennially).

### Factors associated with mammogram uptake

Univariable logistic regression was conducted to examine the association between each independent variable and mammogram uptake. As shown in Table II, age, marital status, and educational level were associated with a p-value

**Table I: Demographic data of the respondents (n=231)**

| Variable | Mean (SD) | No. (%) |
|---|---|---|
| Age | 34.36 (9.59) | |
| No. of children | 2.31 (2.42) | |
| Knowledge score | 81.53 (9.13) | |
| Attitude score | 178.24 (21.27) | |
| Race | | |
| Malay | | 231 (100.0) |
| Education level | | |
| Primary/secondary school | | 167 (72.3) |
| Certificate & higher | | 64(27.7) |
| Marital status | | |
| Single | | 10 (4.3) |
| Married/ever married | | 221 (95.7) |
| Occupation | | |
| Employed | | 87 (37.7) |
| Housewife | | 144 (62.3) |
| Breastfeeding Practice | | |
| Yes | | 197 (85.3) |
| No | | 34 (14.7) |
| 1st degree with breast cancer | | |
| Yes | | 9 (3.9) |
| No | | 222 (96.1) |

**Table II: Association between respondents' demographics and mammogram practice (n=231)**

| Variable | Mammogram | | Chi-square (df) | Wald statistics (df) | p-value |
|---|---|---|---|---|---|
| | Done n (%)/mean | Not done n (%)/mean | | | |
| Malay | 38 (16.5%) | 193 (83.5%) | - | - | |
| Marital status | | | 2.06 | 0.01 | *0.151 |
| Single | 0 (0.0%) | 10 (100.0%) | | | |
| Married/ever married | 38 (17.2%) | 183 (82.8%) | | | |
| Occupation | | | 0.72 | 0.71 | 0.397 |
| Housewife | 26 (18.1%) | 118 (81.9%) | | | |
| Working | 12 (13.8%) | 75 (86.2%) | | | |
| Educational status | | | 4.71 | 4.57 | *0.030 |
| Primary/ secondary | 22 (13.2%) | 145 (86.8%) | | | |
| Certificate & above | 16 (25.0%) | 48 (75.0%) | | | |
| Breastfeeding practice | | | 0.42 | 0.04 | 0.838 |
| Yes | 32 (16.2%) | 165 (83.8%) | | | |
| No | 6 (17.6%) | 28 (82.4%) | | | |
| 1st degree with breast cancer | | | 0.22 | 0.82 | 0.634 |
| Yes | 2 (22.2%) | 7 (77.8%) | | | |
| No | 36 (16.2%) | 186 (83.8%) | | | |
| Age | 49.24 | 47.42 | - | 2.95 | *0.086 |
| No. of children | 4.58 | 4.47 | - | 0.06 | 0.813 |
| Knowledge score | 81.84 | 81.47 | - | 0.05 | 0.816 |
| Attitude score | 178.68 | 178.16 | - | 0.20 | 0.888 |

*p-value <0.25

**Table III: Association between particiants' demographics and mammogram practice using multivariable logistic regression**

| Variable | Exp (B) (odds ratio) | 95% CI | p-value |
|---|---|---|---|
| Age | 1.07 | 1.01, 1.13 | 0.035 |
| Education level | 2.6 | 1.22, 5.43 | 0.013 |

[a]Forward LR Multiple Logistic Regression model was applied
Hosmer-Lemeshow test (p=0.720), classification table (overall correctly classified percentage=83.5%) and area under the ROC curve were applied to check the model fitness.

less than 0.25 and were subsequently included in the multivariable analysis. Knowledge and attitude scores were not significantly associated with mammogram uptake and were excluded from further analysis.

Multivariable logistic regression revealed that both age and educational level were significantly associated with mammogram uptake. Older age was associated with a higher likelihood of screening, with each additional year of age increasing the odds by 7% (adjusted Odds Ratio [aOR]: 1.07; 95% Confidence Interval [CI]: 1.01, 1.13). Participants with higher education levels were also more likely to undergo mammogram screening (aOR: 2.60; 95% CI: 1.22, 5.43). These findings are summarized in Table III.

## DISCUSSION

### Mammogram Screening Uptake

This study found that only 16.5% of eligible women in Terengganu had undergone mammogram screening at least once. This rate is notably lower than the prevalence reported in the National Health and Morbidity Survey (NHMS) 2023, which indicated that 25.9% of women aged 40 and above in Terengganu had ever participated in mammogram screening.[8] However, it is important to note that the study population here differed slightly, focusing on women aged 50 years and above or women aged 30 years and above with a first-degree family history of breast cancer. Despite these differences, both findings suggest that mammogram screening rates remain low and have not improved substantially in recent years. This represents a significant public health concern, given the established role of mammography in the early detection and treatment of breast cancer.

Mammogram uptake across Malaysia varies considerably, ranging from as low as 3.6% among the general population to 80.3% among healthcare professionals in tertiary hospitals.[18] The lowest uptake was reported among Malay women aged 40 and above in rural Kedah, with a mere 2.8% participation rate.[11] These findings highlight a significant disparity in mammogram uptake, which is influenced by various demographic and socioeconomic factors.

### Factors Influencing Mammogram Uptake

In the current study, age and education level were identified as significant factors associated with mammogram uptake. The association between increasing age and higher mammogram participation may be linked to greater awareness of breast cancer risk as women age. Several studies have found that age is an important factor influencing mammogram uptake.[18-22] Increased awareness of breast cancer risk with advancing age could drive women to seek screening more actively. Additionally, higher education levels were found to positively correlate with better breast cancer knowledge and a higher likelihood of undergoing mammography, consistent with other studies.[20,23-26]

Education plays a pivotal role in enhancing health literacy, which is directly linked to better health-seeking behaviours. Women with higher education are more likely to understand the importance of early detection and adhere to screening recommendations. This finding shows the need for educational campaigns to improve knowledge about breast cancer and the benefits of mammography, particularly among women with lower educational backgrounds.

While this study did not find a significant association between knowledge or attitude scores and mammogram uptake, previous research has identified perceived susceptibility, such as having a family history of breast cancer, as a strong motivator for mammogram screening.[27-29] The perception of breast cancer risk, coupled with healthcare provider recommendations, significantly influences screening behaviours. Women who perceive themselves to be at higher risk are more likely to undergo mammograms, but this alone may not be sufficient to prompt action. The role of healthcare providers in offering clear recommendations during routine check-ups is crucial in encouraging mammogram participation. Consistent advice from healthcare professionals has been shown to positively impact screening uptake by reducing uncertainty and reinforcing the importance of early detection.[12,19,21]

### Barriers to Mammogram Screening

A number of studies have identified key barriers to mammogram screening. Fear of a cancer diagnosis remains one of the most significant barriers.[10,12,25,30] This fear is often compounded by stigma and the perception of cancer as a death sentence, which can deter women from seeking early screening. Cultural beliefs, particularly in some rural areas, may also contribute to the perception that a cancer diagnosis leads to poor prognosis, resulting in avoidance of mammograms. A study by Mohan (2021) found that up to 74.8% of women reported fear of cancer as the primary reason for not undergoing mammogram screening.[25] However, fear of cancer diagnosis and cultural beliefs were not looked into in our study.

Geographical access to healthcare services, especially in rural areas, remains another significant barrier. A key challenge in Terengganu is, access to mammography services is limited due to the concentration of services in only two public hospitals and two private facilities, three of which are in the state capital, Kuala Terengganu. This geographical disparity forces women to travel long distances, adding time and financial burdens that further discourage screening participation. Furthermore, long waiting times for mammogram appointments, particularly in public hospitals, add another layer of inconvenience, especially for women in rural areas. The limited availability of mammography services in rural and semi-urban areas has been consistently linked to lower screening rates.[31-35] This issue mirrors findings from other regions, where proximity to healthcare facilities is strongly associated with mammogram participation.[11,19]

### Role of Health Education and Awareness Campaigns

Several studies have demonstrated that breast cancer knowledge and awareness among women remain low and this lack of knowledge negatively influences screening behaviour.[10,12,27,30,36] Several factors affect knowledge and awareness, which include race, marital status, educational level, income and whether women live in rural or urban areas.[24,26,37]

Current awareness campaigns, such as the annual Pink October initiative, aim to address misconceptions and promote breast cancer screening. However, these campaigns may not adequately reach women in rural and semi-urban areas. More localized and culturally appropriate interventions are necessary to address the specific barriers faced by these populations. A national mass media campaign promoting breast cancer symptoms, screening benefits and addressing perceived barriers has been shown to significantly improve awareness and participation in screening programs.[20,38] The effectiveness of such campaigns can be enhanced through partnerships with non-governmental organizations and healthcare providers. For instance, collaborating with organizations such as the National Cancer Society Malaysia could improve women to participate in screening programs through subsidized mammograms and incentives.[32]

A review of national mammogram screening data from 2016 to 2020 concluded that the rate of mammogram uptake is closely linked to the intensity of health promotion campaigns.[34] Sustainable awareness programs and community involvement are critical for the success of breast cancer screening initiatives. Annual national campaigns, particularly during Pink October, have been associated with a significant increase in screening rates.[39]

Primary care providers play a critical role in promoting breast cancer screening. Referral for mammograms should be made mandatory in indicated women during routine consultation and as one of the key indicators to be achieved in primary care settings. In addition, addressing patients' concerns about the screening process, healthcare providers can significantly influence women's decisions to undergo mammography.

Public awareness campaigns need to be more targeted and localized to effectively reach populations in rural areas. These campaigns should aim to correct myths and address cultural misconceptions about breast cancer and its screening. Expanding subsidized mammogram programs and financial incentives, particularly for women from lower socioeconomic backgrounds, can further increase screening rates by reducing financial barriers. Lastly, further research should be conducted to explore how psychosocial factors such as fear and stigma impact mammogram uptake, with the aim of developing more effective interventions to address these barriers.

## LIMITATIONS

One of the limitations of this study is that all participants were Malay women attending public primary care clinics. Approximately 95% of the population in Terengganu is Malay.[40] Thus, the ethnic homogeneity and the healthcare setting may limit the generalisability of the findings to Malaysia's more diverse multi-ethnic population and to women who seek care in private or alternative healthcare settings. Furthermore, the sampling strategy might have systematically excluded women who do not engage with the healthcare system, who may be at higher risk of not undergoing mammogram screening.

This study was based on a cross-sectional design, which provides a single-point evaluation of mammogram uptake. Without longitudinal follow-up, it does not allow for assessment of whether participants adhered to recommended screening intervals or maintained consistent screening behaviour over the years.

This study also did not assess the major barriers for mammogram screening which was fear of diagnosis and cultural beliefs. However, geographical barrier may indirectly implicate mammogram uptake due to poor accessibility. Efforts should focus on increasing accessibility, particularly for women living in rural and semi-urban areas. Expanding mammogram services through mobile units or establishing more screening centres in rural clinics can help reduce the geographical barriers that many women face. Since 2024, a newly established Hospital Sultan Zainal Abidin in Kuala Nerus is offering its mobile mammogram service. The new service should benefit women especially in the outlying areas.

## CONCLUSION

This study highlights the critically low mammogram uptake among women in Terengganu, with only 16.5% of eligible participants had ever undergone mammogram screening. This rate reflects broader national trends where breast cancer screening remains suboptimal, despite efforts to promote early detection. Factors such as age and education were found to be significantly associated with mammogram uptake, with older women and those with higher education levels being more likely to participate in screening. These findings suggest that awareness of breast cancer risks and the importance of early detection increases with age and educational attainment.

Women living in semi-urban and rural parts of Terengganu face challenges related to long distances to screening facilities, additional financial and time burdens and a lack of localized awareness campaigns. This disparity in access and knowledge reinforces the need for more targeted interventions to improve screening participation.

Efforts to increase mammogram uptake must focus on improving access to screening facilities, particularly for rural populations and addressing the psychosocial barriers that deter women from seeking screening. Healthcare providers can play a pivotal role by actively recommending mammograms and providing clear information on the benefits of early detection. Public health campaigns should be tailored to the specific needs and cultural contexts of underserved populations, with an emphasis on dispelling fears and correcting misconceptions about breast cancer. A comprehensive approach involving enhanced healthcare access, education, and community engagement is essential to increasing mammogram uptake and improving breast cancer outcomes in Terengganu and similar regions.

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ORIGINAL ARTICLE

# Psychometric evaluation of the Malay Health Belief Model for COVID-19 booster dose vaccination intention scale among Malaysians

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## ABSTRACT

**Introduction: The COVID-19 pandemic has highlighted the importance of vaccination, with booster doses now integral to public health strategies. However, there is a lack of validated tools to assess vaccine intention for booster doses, despite the Health Belief Model (HBM) being widely used for primary doses. This study aims to address this gap by validating a Malay version of the HBM-based COVID-19 booster dose vaccine intention scale to measure factors influencing booster vaccination decisions in Malaysia.**

**Materials and Methods: The study was conducted in three phases. Phase 1 involved translation, content, and face validity. The translation process employed a forward-backward method, utilising two bilingual translators and an independent back-translator. Content validity was assessed by five experts from psychology, public health, and medicine, and face validity was evaluated through a pre-test with 40 laypersons. Phase 2 used Exploratory Factor Analysis (EFA) with 160 participants to identify the tool's underlying factor structure. Phase 3 employed Confirmatory Factor Analysis (CFA) with 556 participants to assess construct validity and reliability. Internal consistency was measured using Cronbach's alpha and Composite Reliability (CR). Participants aged 18 and above were recruited via online platforms, with eligibility determined by receipt of primary doses of the COVID-19 vaccine.**

**Results: The translation process resulted in a 16-item Malay COVID-19 HBM-Booster Dose Intention Scale (HBM-BDIS). Phase 1 confirmed content and face validity, with the Content Validity Index (CVI) for all items exceeding 3.0, and a satisfactory Face Validity Index (FVI). EFA in Phase 2 revealed a clear factor structure with factor loadings meeting the 0.33 threshold, and internal consistency was confirmed with Cronbach's alpha of 0.82. CFA in Phase 3 confirmed the scale's construct validity, with improved fit indices after revision (CFI = 0.926, TLI = 0.909, RMSEA = 0.084). The final model showed strong internal consistency, with CR values above 0.80 for all factors.**

**Conclusion: The 16-item Malay COVID-19 HBM-BDIS is a valid and reliable measure for assessing COVID-19 booster dose vaccination intention in Malaysia, providing a solid foundation for future research and public health interventions.**

**KEYWORDS:**
*COVID-19 vaccination, Booster dose, Health Belief Model, Vaccine Intention, Public Health Intervention*

## INTRODUCTION

The COVID-19 pandemic has underscored the importance of vaccination as a vital public health measure, yet vaccine hesitancy remains an obstacle, including in Malaysia, where sociocultural and behavioural factors shape vaccine attitudes.[1] While primary doses have generally been well-accepted[2], the uptake of booster doses presents unique challenge. These doses are crucial for maintaining immunity as the effectiveness of the initial vaccine wanes, yet they face resistance driven by hesitancy and misinformation.[3] A meta-analysis of eight studies involving 12,995 participants revealed that while 79% intended to receive a booster dose, only 31% actually did, indicating a substantial gap in uptake.[4] This highlights the need for increased efforts to promote booster dose uptake and underscores the importance of exploring the psychological factors that influence decisions to receive COVID-19 booster doses.

A significant gap in the literature exists regarding the development of psychometrically sound tools to assess vaccine intention in the specific context of booster doses. While the Health Belief Model (HBM) has been widely applied to primary vaccine doses[5], there is a paucity of validated tools designed to capture the unique psychological and behavioural factors influencing decisions about booster doses. Several HBM-based questionnaires have been developed to assess COVID-19 vaccination intention, but most focus solely on the primary dose.

Ren et al. developed the COVID-19 Vaccine Beliefs Scale, which demonstrated strong reliability and validity in assessing vaccine beliefs within the Chinese population.[6] However, its applicability to Malaysia is uncertain due to

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cultural differences. Similarly, Huynh et al. developed an HBM-based 15-item scale to assess vaccine intention among Vietnamese adults with chronic conditions, focusing on perceived threat, benefits, barriers, and cues to action.[7] Although the scale showed good reliability and effectively identified vaccine intention in high-risk groups, its relevance to the broader Malaysian population, especially regarding booster doses, is limited. In Malaysia, Wong et al. used a 15-item HBM-derived questionnaire to identify predictors of COVID-19 vaccine intention, finding perceived susceptibility, severity, benefits, and cues to action to be significant determinants.[8] However, their study primarily focused on the primary dose, and no validity information for the tool was provided.

These studies underscore the need for context-specific, psychometrically sound tools to assess vaccine intentions for COVID-19 booster doses in Malaysia. Developing such a tool would deepen our understanding of vaccine behaviours and inform targeted public health interventions to address hesitancy and improve vaccine uptake.

Therefore, the present study seeks to provide a comprehensive development and validation of a contextually appropriate tool for Malaysians, in the specific context of COVID-19 booster dose vaccination intention. The results will provide empirical support for the scale's utilisation in public health interventions aimed at increasing vaccine uptake.

## MATERIALS AND METHODS

The study was conducted in three distinct phases. Phase 1 focused on the translation, content validity, and face validity of the HBM questionnaire. Phase 2 involved Exploratory Factor Analysis (EFA) to identify the underlying factor structure of the questionnaire, while Phase 3 employed Confirmatory Factor Analysis (CFA) to assess the construct validity of the instrument.

### Phase 1: Translation, Content Validity, and Face Validity

In Phase 1, a 15-item HBM questionnaire8 was translated into Bahasa Malaysia using the forward-backward translation method to ensure both linguistic and conceptual equivalence. Two bilingual translators, one with expertise in psychological medicine and the other a professional translator, performed the forward translation. An independent translator conducted the back translation, and discrepancies were resolved by a panel of researchers and translators to ensure consistency.

To assess content validity, a panel of five experts in psychology, public health, and medicine evaluated the clarity, simplicity, ambiguity, and relevance of each item. The feedback provided was incorporated into the final version of the questionnaire, and the Content Validity Index (CVI) was used to quantify the results.[9] Face validity was evaluated through a pre-test involving 40 laypersons who assessed the clarity and relevance of the items using the Face Validity Index (FVI).

### Phase 2: Exploratory Factor Analysis and Internal Reliability

Phase 2 involved a cross-sectional study design, with data collected from 160 participants using online platforms, including WhatsApp, Facebook, Twitter, Telegram, Instagram, and email. Inclusion criteria required participants to be Malaysian citizens aged 18 years and above who had received the first and second doses of the COVID-19 vaccine. Individuals who were illiterate or had been advised by medical professionals not to receive the COVID-19 booster dose were excluded.

Participants completed the questionnaire independently on Google Forms after providing informed consent. Data were analysed using RStudio statistical software. Descriptive statistics summarized the sociodemographic characteristics. The EFA was conducted to explore the underlying factor structure of the HBM questionnaire8. The Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy was employed to evaluate the adequacy of the sample for factor analysis, with a threshold value of >0.50 considered acceptable.[10] Additionally, Bartlett's Test of Sphericity was performed to test the hypothesis that the correlation matrix is an identity matrix, with a p-value <0.05 indicating that the data are suitable for factor analysis.[11] Factor retention was determined using Cattell's Scree Test and Horn's Parallel Analysis, and factor loadings ≥0.32 were considered significant.[12] Cronbach's alpha was used to evaluate the internal consistency of the constructs, with a value ≥0.60 indicating acceptable reliability.[13]

### Phase 3: Confirmatory Factor Analysis and Composite Reliability

In Phase 3, a cross-sectional survey was conducted with 556 participants recruited via online platforms. Stratified quota sampling was employed to ensure representation from all 14 states in Malaysia, based on population proportions reported by the Department of Statistics Malaysia.[14] Field enumerators from each state recruited participants.

Descriptive statistics were used to summarise the demographic data, with age reported as the mean and standard deviation, and other sociodemographic variables presented as percentages. CFA was performed to assess the factor structure, with parameters for model specification set to include factor loadings >0.50 and inter-factor correlations <0.85, in accordance with established guidelines for structural equation modelling.[15-16] Model fit was evaluated using the Comparative Fit Index (CFI >0.95), Tucker-Lewis Index (TLI >0.95), and Root Mean Square Error of Approximation (RMSEA <0.07), based on the recommendations of Hu and Bentler.[17] The internal consistency of the constructs was assessed through Composite Reliability (CR), with a CR value ≥0.60 considered indicative of adequate reliability for each construct.[18]

## RESULTS

The 15-item HBM questionnaire[8] underwent forward and backward translation to ensure linguistic and conceptual equivalence. Forward translation was done by an expert in psychological medicine and a professional translator, while

**Table I: Demographic information of the participants for Phase 1: Exploratory Factor Analysis (n = 160)**

| Criteria | % |
|---|---|
| Gender | |
| Female | 73.1 |
| Male | 26.9 |
| Race | |
| Malay | 93.1 |
| Chinese | 04.4 |
| Indian | 00.6 |
| Other Races | 01.9 |
| Employment Status | |
| Students | 20.0 |
| Unemployed | 06.2 |
| Employed | 71.9 |
| Retirees | 01.9 |
| Level of Education | |
| High School Diploma | 0 3.1 |
| College diploma | 34.4 |
| Bachelor's Degree | 45.6 |
| Master / Doctorate | 16.9 |
| Marital Status | |
| Single | 48.1 |
| Married | 51.3 |
| Divorced/Widowed | 00.6 |

**Table II: Factor loadings and internal consistency of the 19-item Malay COVID-19 HBM-Booster Dose Intention Scale from Exploratory Factor Analysis**

| No. | Item | Factor Loading | Cronbach's Alpha (a) |
|---|---|---|---|
| | **Perceived Benefits** | | |
| 1. | Booster vaccination is a good idea because it makes me feel less worried about catching COVID-19. (Item 7) | 0.94 | 0.93 |
| 2. | The COVID-19 booster vaccine can protect my family from COVID-19. (Item 20) | 0.85 | |
| 3. | Booster vaccination decreases my chance of experiencing complications from COVID-19. (Item 9) | 0.83 | |
| 4. | Booster vaccination decreases my chance of getting COVID-19. (Item 8) | 0.81 | |
| 5. | I believe that the booster dose can control the spread of the COVID-19 pandemic. (Item 18) | 0.78 | |
| | **Perceived Barriers** | | |
| 6. | I am concern about the safety of the COVID-19booster dose vaccination. (Item 12) | 0.90 | 0.90 |
| 7. | I am concern about the efficacy of the COVID-19 booster dose vaccination. (Item 11) | 0.87 | |
| 8. | Worry the possible side effects of COVID-19 booster dose. (Item 10) | 0.75 | |
| 9. | I am concern of the affordability (high cost) of getting the COVID-19 booster dose. (Item 13) | 0.73 | |
| | **Perceived Threat** | | |
| 10. | I am worried about the likelihood of getting COVID-19. (Item 2) | 0.87 | 0.83 |
| 11. | I am afraid of getting COVID-19. (Item 6) | 0.79 | |
| 12. | My chance of getting COVID-19 in the next few months is great. (Item 1) | 0.55 | |
| 13. | Getting COVID-19 is currently a possibility for me. (Item 3)0.55 | | |
| 14. | I will be very sick if I get COVID-19. (Item 5) | 0.46 | |
| 15. | Complications from COVID-19 are serious. (Item 4) | 0.46 | |
| | **Cues to Action** | | |
| 16. | I believe that the booster dose is a planned conspiracy. (Item 17) | 0.76 | 0.77 |
| 17. | I will only take the COVID-19 booster dose if the vaccine is taken by many in the public. (Item 16) | 0.52 | |
| 18. | I am concerned if the new COVID-19 booster dose vaccine is halal. (Item 14) | 0.47 | |
| 19. | I believe that the booster dose is a futile effort in protecting me from COVID-19. (Item 19) | 0.42 | |

**Table III: Demographic information of the participants for Confirmatory Factor Analysis (n = 556)**

| Criteria | % |
|---|---|
| Gender | |
| Female | 61.6 |
| Male | 38.4 |
| Race | |
| Malay | 69.5 |
| Chinese | 08.1 |
| Indian | 12.5 |
| Other Races | 09.9 |
| Level of Education | |
| Primary School | 0.3 |
| Secondary School | 09.8 |
| College Diploma | 18.5 |
| Bachelor's Degree | 48.6 |
| Master's / Doctorate | 02.9 |
| Employment Status | |
| Students | 56.1 |
| Unemployed | 06.1 |
| Employed | 35.5 |
| Retirees | 02.2 |
| Marital Status | |
| Single | 69.3 |
| Married | 27.3 |
| Divorced | 00.7 |
| Widowed | 00.4 |
| Front liner Status | |
| Not Applicable | 92.5 |
| Healthcare Workers | 07.0 |
| Police / Military | 00.5 |

**Table IV: Factor loadings and composite reliability of the 16-item Malay COVID-19 HBM-Booster Dose Intention Scale from Confirmatory Factor Analysis**

| No. | Item | Factor Loading | Composite Reliability (CR) |
|---|---|---|---|
| | **Perceived Benefits** | | |
| 1. | Booster vaccination is a good idea because it makes me feel less worried about catching COVID-19. (Item 7) | 0.92 | 0.88 |
| 2. | Booster vaccination decreases my chance of getting COVID-19. (Item 8) | 0.89 | |
| 3. | Booster vaccination decreases my chance of experiencing complications from COVID-19. (Item 9) | 0.89 | |
| 4. | The COVID-19 booster vaccine can protect my family from COVID-19. (Item 20) | 0.81 | |
| 5. | I believe that the booster dose can control the spread of the COVID-19 pandemic. (Item 18) | 0.76 | |
| | **Perceived Barriers** | | |
| 6. | I am concerned about the safety of the COVID-19 booster dose vaccine. (Item 12) | 0.88 | 0.89 |
| 7. | I am concerned about the efficacy of the COVID-19 booster dose vaccine. (Item 11) | 0.86 | |
| 8. | Worry about the possible side effects of COVID-19 booster dose vaccine. (Item 10) | 0.66 | |
| 9. | I am concerned about the affordability (high cost) of getting the COVID-19 booster dose vaccine. (Item 13) | 0.60 | |
| | **Perceived Threat** | | |
| 10. | I am afraid of getting COVID-19. (Item 6) | 0.81 | 0.84 |
| 11. | I am worried about the likelihood of getting COVID-19. (Item 2) | 0.79 | |
| 12. | Complications from COVID-19 are serious. (Item 4) | 0.76 | |
| 13. | I will be very sick if I get COVID-19. (Item 5) | 0.60 | |
| | **Cues to Action** | | |
| 14. | I believe that the booster dose is a planned conspiracy. (Item 17) | 0.64 | 0.82 |
| 15. | I am concerned if the new COVID-19 booster dose vaccine is halal. (Item 14) | 0.66 | |
| 16. | I believe that the booster dose is a futile effort in protecting me from COVID-19. (Item 19) | 0.60 | |

backward translation was performed independently by another bilingual translator. The translated version, which was subsequently labelled as the 15-item Malay HBM-Booster, was harmonised by the translators and study authors.

### Phase 1: Content and Face Validity

Content validity was assessed by a panel of five experts in psychology, medicine, and public health, using the CVI. All 15 original items received CVI scores above 3.0, indicating strong agreement on their relevance. The panel recommended adding five new items to broaden the scope of the questionnaire. These included Item 17, "I believe that

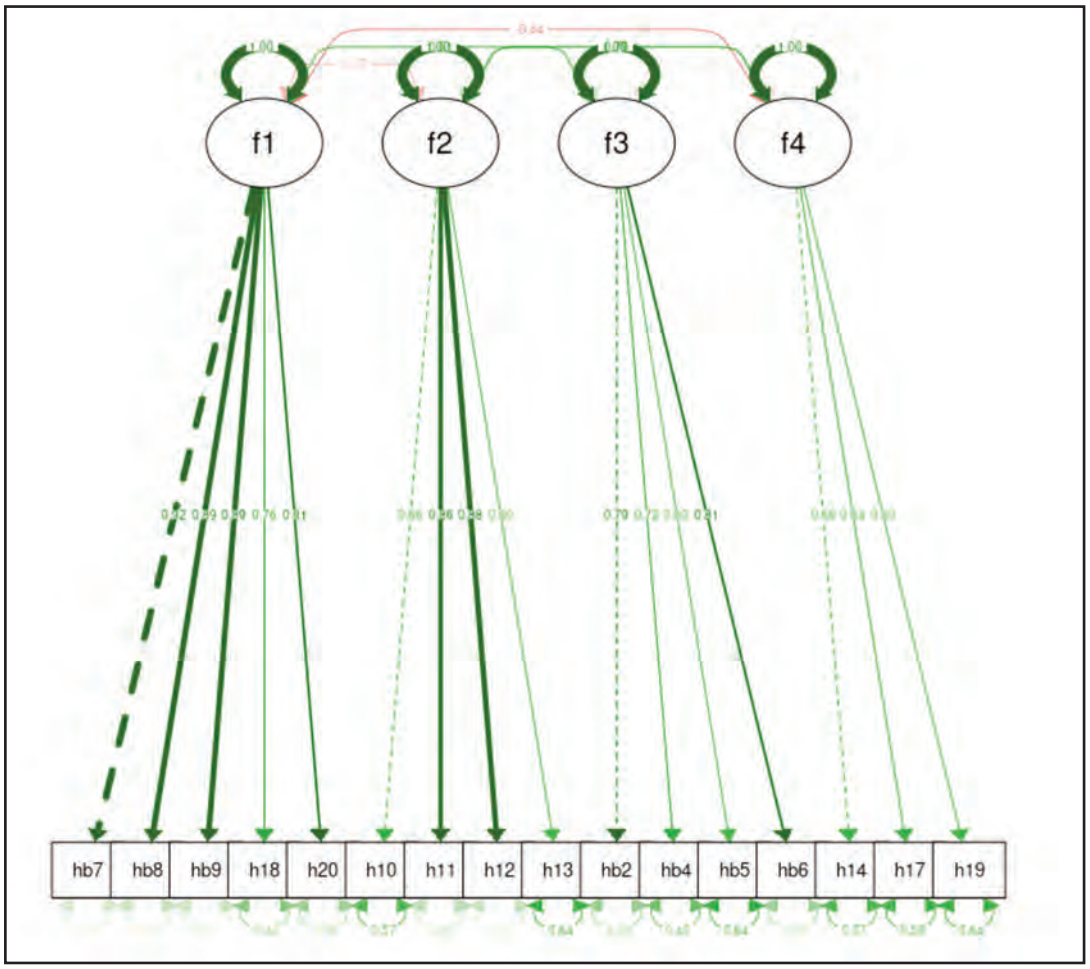


**Fig. 1:** Confirmatory Factor Analysis of 16-item Malay COVID-19 HBM-Booster Dose Intention Scale (n = 556)

booster doses are a planned conspiracy," Item 18, "I believe that booster doses are capable of controlling the spread of the COVID-19 pandemic," Item 19, "I believe that booster doses are a futile effort in protecting me from COVID-19," and Item 20, "The COVID-19 booster vaccine can protect my family from COVID-19." Additionally, Item 8 was split into two separate questions for clarity. The revised 20-item questionnaire was subsequently labelled as the 20-item Malay HBM-Booster Dose.

Face validity was evaluated with 40 participants (mean age=30.52 years, SD=5.22). The sample was 55% female and 45% male, all of whom were Malay. The FVI for each item showed scores above 3.0, confirming that the questionnaire was comprehensible and suitable for the target population.

**Phase 2: Exploratory Factor Analysis**

The demographic characteristics of the participants in Phase 2 included 160 participants (mean age = 31.3 years, SD = 8.9 years), with additional details provided in Table I. EFA was conducted on the 20-item Malay HBM-Booster Dose using oblique factor rotation. Preliminary analyses, including inter-item correlation checks, revealed several correlations above 0.30, confirming the suitability of the data for factor analysis. Item 15, with a correlation below 0.30, was excluded from further analysis, leaving 19 items for the subsequent EFA.

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO=0.85) and Bartlett's Test of Sphericity ($p < .001$) confirmed the suitability of the dataset for factor analysis. The retention of factors was determined using multiple criteria, including Cattell's Scree Plot, Horn's Parallel Analysis, factor loadings exceeding 0.33, and the interpretability of the extracted factors. The EFA yielded a well-defined factor structure for the 19-item Malay HBM-Booster, with factor loadings summarised in Table II. Internal consistency was evaluated using Cronbach's alpha, with an overall value of 0.82 indicating good reliability. The individual factors had alpha values of 0.93, 0.90, 0.83, and 0.77, further supporting the reliability of the tool.

**Phase 3: Confirmatory Factor Analysis (CFA)**

Phase 3 involved a CFA of the pre-finalised 19-item Malay HBM tool to assess construct validity. This phase involved 556 participants (mean age = 29.4 years, SD = 12.0 years), with demographic details provided in Table III. The initial CFA model showed poor fit, with several items (Item 1, Item 3, and Item 16) exhibiting low factor loadings below the 0.60 threshold. The model's fit indices were $\chi^2$=871.472, CFI=0.873, TLI=0.852, RMSEA=0.095, and BIC=28,961.231, all suggesting inadequate model fit.

Consequently, Items 1, 3, and 16 were removed, resulting in a revised 16-item model. This revised model demonstrated significant improvements in fit indices: $\chi^2$=475.165, CFI=0.926, TLI=0.909, RMSEA=0.084, and BIC=23,889.076, indicating a good fit to the data. In the structural model, latent factors were represented by large circles, and measured variables by rectangles, with statistically significant parameter estimates ($p<0.05$). Three notable correlations were observed between the latent factors: r=0.74 between Factor 1 (f1) and Factor 3 (f3), r=-0.49 between Factor 1 (f1) and Factor 4 (f4), and r=0.572 between Factor 4 (f4) and Factor 2 (f2). The results of the CFA are illustrated in Figure 1. The internal consistency of the revised 16-item model was evaluated using CR. The overall CR was 0.86, and the individual factors showed CR values of 0.88, 0.89, 0.84, and 0.82, demonstrating strong reliability across the tool. The factor loadings and construct reliability for the 16-item model are summarised in Table IV.

## DISCUSSION

The validation of the Malay version of the Health Belief Model COVID-19 booster dose vaccine intention scale (HBM-BDIS) was carried out in three phases to ensure its linguistic, content, and construct validity. In Phase 1, the translation and pre-testing procedures confirmed that the Malay HBM-BDIS was suitable for the Malaysian context, with content and face validity scores surpassing the recommended thresholds. Furthermore, EFA and CFA demonstrated a clear structure, highlighting the tool's reliability in measuring vaccination intention according to the HBM constructs. These results support the construct validity and internal consistency of the final 16-item Malay HBM-Booster Dose model, confirming its readiness for future research and practical use. Ren et al. developed and validated a reliable and valid HBM-based scale to assess COVID-19 vaccine beliefs in China, highlighting the need for cultural adaptation in vaccine belief measures.[6] However, their study was limited to the Chinese population, and its applicability to Malaysia, with its distinct sociocultural context, remains unclear. The present study addresses this gap by providing a validated tool for assessing vaccine intentions in Malaysia, where sociocultural factors significantly influence vaccine attitudes.[1]

Huynh et al. developed an HBM-based scale to assess vaccine intention among Vietnamese adults with chronic conditions, providing insights into vaccine hesitancy in high-risk groups.[7] However, their tool's applicability to the broader Malaysian population is limited due to its lack of cross-cultural focus. The present study addresses this by ensuring the tool's relevance across diverse demographic groups in Malaysia, including those with and without pre-existing health conditions.

Wong et al. applied the HBM to assess COVID-19 vaccine intention in Malaysia, identifying key predictors such as perceived susceptibility, severity, and benefits.[8] However, the study did not develop and validate a comprehensive scale specifically targeting booster dose vaccine intention, which limits its applicability in future research. The current study addresses this gap by offering a psychometrically validated, context-specific scale for booster doses, which can be utilised in subsequent public health interventions and studies targeting Malaysian adults.

Despite the robustness of the present study, some limitations must be acknowledged. One limitation is the focus on the Malaysian adult population, which may restrict the generalizability of the findings to other demographic groups, such as children and elderly individuals. Future studies should consider expanding the scope to include these populations for a more comprehensive understanding of vaccine intentions across different demographic groups. Another limitation is the cross-sectional design of this study, which prevents the examination of changes in vaccine intention over time. Longitudinal studies could provide further insights into how attitudes toward booster doses evolve and inform ongoing vaccination strategies.

Moreover, while the tool has been validated in the Malaysian context, its applicability in other Southeast Asian countries with similar sociocultural contexts remains to be explored. Future research should test the scale's applicability and psychometric properties in these settings to ensure broader utility.

## CONCLUSION

In conclusion, the 16-item Malay COVID-19 HBM-Booster Dose Intention Scale (HBM-BDIS) is a valid and reliable measure for assessing vaccination intention among Malaysian adults, particularly regarding booster doses. This study addresses key gaps in the literature by providing a culturally appropriate, psychometrically sound instrument, laying a strong foundation for future research and public health interventions.

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# A randomised controlled study on the synergistic effects of neuroplasticity-based interventions for trunk control in post-stroke rehabilitation

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## ABSTRACT

**Introduction: Trunk impairment after a stroke is a common complication in hemiplegic patients. Neuroplasticity-driven interventions, such as trunk rehabilitation exercises (TRE) and transcranial direct current stimulation (tDCS), have shown potential to enhance trunk control, balance, and mobility. This study evaluates the individual and combined effects of TRE and tDCS on trunk function and mobility in post-stroke rehabilitation.**

**Materials and Methods: A randomised controlled study was conducted among post-ischaemic stroke survivors from a single medical institution. Participants (aged 24–85 years) with first-ever stroke of at least six months' duration, without a history of drug abuse or antidepressant use, with well-controlled comorbidities, and able to stand with minimal support were included. Exclusion criteria included recurrent stroke, fractures, amputations, or other neurological conditions affecting balance. Participants were randomly assigned to four groups: (1) control, (2) TRE, (3) tDCS, and (4) combined intervention (CI). Effectiveness was evaluated using the Trunk Impairment Scale, Postural Assessment Stroke Scale, and Rivermead Mobility Index, measured at baseline and after 8 weeks.**

**Results: Sixty-nine participants were enrolled and randomly assigned to four groups: control (n=18), TRE (n=17), tDCS (n=17), and CI (n=17). Although no statistically significant differences were observed across groups, the combined intervention group demonstrated higher improvement in Trunk Impairment Scale, Postural Assessment Stroke Scale, and Rivermead Mobility Index scores compared to single interventions or control.**

**Conclusion: The combination of TRE and tDCS may be a promising approach to enhance trunk control and mobility in post-stroke patients. Further research with larger sample sizes is recommended to confirm these findings.**

**KEYWORDS:**
*Neuroplasticity, trunk rehabilitation exercises, transcranial direct current stimulation, stroke rehabilitation, balance training*

## INTRODUCTION

Stroke, a condition characterised by the rapid onset of focal or global cerebral dysfunction due to vascular causes, remains a leading cause of long-term disability worldwide.[1,2,3] Approximately 70% of stroke survivors face significant dependence, while 30% require assistance with daily activities. Among the most debilitating complications is trunk impairment, which affects multiple systems involved in postural control, including strength, proprioception, range of motion, vision, vestibular function, and endurance.[3,4]

Recovery of trunk function is crucial for restoring balance and functional independence in stroke survivors.[5] Neural pathways from the premotor cortex and supplementary motor area modulate spinal circuitry, enabling anticipatory postural responses.6 However, damage to these areas or hyperexcitability of the premotor regions disrupts normal postural sequencing, leading to impaired coordination.[7,8] Such dysfunction is exacerbated by hyperexcitability of premotor regions, which inhibits normal spinal sequencing and delays postural recovery.[7]

Recent advances in rehabilitation emphasise neuroplasticity-driven interventions, such as task-specific exercises and non-invasive brain stimulation, including transcranial direct current stimulation (tDCS).[9-11] Task-specific exercises, including trunk rehabilitation exercises (TRE), fosters motor learning and neuroplasticity through goal-oriented, repetitive tasks, while tDCS modulates cortical excitability to enhance recovery.[9,12,13] A recent pilot study highlighted the potential of combined task-specific exercises and tDCS interventions in elevating serum brain-derived neurotrophic factor levels, a biomarker for neuroplasticity. The study demonstrated a significant increase in serum brain-derived neurotrophic factor levels in the combined intervention group, underscoring the synergistic effects of these therapies in promoting neuronal recovery.[14]

Despite promising findings in motor rehabilitation, research exploring these interventions' impact on trunk performance remains scarce. Combining tDCS with TRE may amplify therapy-induced recovery through synergistic effects. This study aims to evaluate the individual and combined effects of TRE and tDCS on trunk function, balance, and mobility, in

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post-stroke patients, addressing this critical gap in rehabilitation strategies.

## MATERIALS AND METHODS

### Study Design and Participants

The randomised controlled study was conducted at Imamu Wali General Hospital, Kano, Nigeria. Recruitment of participants was made from this single institution among post-ischaemic stroke survivors after ethical approval was obtained from the Health Research Ethics Committee of the Ministry of Health, Kano State of Nigeria (NHREC Approval No.: NHREC/17/03/2018). The health research ethics committee approval was issued on the 12th of April 2024. The trial protocol was also registered with the Pan African Clinical Trial Registry (Reg. ID: PACTR202408592508053) prior to commencement. Participants were eligible if they were aged 25–85 years, had experienced a first-ever stroke at least six months prior, and were able to stand with minimal or without support. Additional criteria included the absence of drug abuse, antidepressant use, or significant visual/auditory deficits, and well-controlled hypertension or diabetes. Exclusion criteria included recurrent stroke, fractures, amputations, or other neurological conditions affecting balance.

Recurrent stroke patients were excluded to ensure homogeneity of the study population and to reduce confounding factors. Individuals with multiple strokes often present with more complex neurological impairments, varying lesion locations, and different recovery trajectories, which may affect the outcome measures. Including only first-ever stroke survivors allowed for a more accurate assessment of the effects of the interventions on trunk control and mobility.

### Sample Size Calculation

The sample size was determined using G*Power software version 3.1, based on an alpha level (α) of 0.05, statistical power of 0.80, and an effect size of 0.426, which yielded a minimum required sample of 68 participants. To account for a potential dropout rate of 10%, an additional seven participants were added (10% of 68 = 6.8, rounded to 7), resulting in a total estimated sample size of 75 participants.

### Randomisation and Blinding

After providing written informed consent, participants were randomly assigned into four groups using Microsoft Excel's random number function: the control intervention group (control), the trunk rehabilitation exercises group (TRE), the transcranial direct current stimulation group (tDCS), and the combined TRE + tDCS intervention group (CI). Both the treating physiotherapists and laboratory scientists conducting assessments were blinded to the group allocations.

### Procedure and Interventions

All participants received a standard 20-minute control intervention consisting of 5 minutes of infrared radiation therapy, followed by 15 minutes of proprioceptive neuromuscular facilitation exercises. These exercises were designed to target the neck, trunk, and scapular regions, following a cephalo-caudal sequence.

In the tDCS group, participants underwent the control intervention and received tDCS. The stimulation was delivered using the International 10-20% electroencephalography montage, applying a constant current for 20 minutes with anodal stimulation targeting the primary motor cortex. In the TRE group, participants performed selective trunk movements in supine and sitting positions for 30 minutes, as outlined by Karthikbabu et al.,[15] in addition to receiving the control intervention. These exercises were designed to improve trunk coordination and balance through goal-directed practice. The CI group received a sequential combination of therapies. This included 30 minutes of TRE, followed by 20 minutes of tDCS, and 20 minutes of the control intervention. A 5-minute rest period was observed between the TRE and tDCS sessions to prevent fatigue and ensure participant comfort.

The sequence of the combined intervention (CI group) was based on prior studies supporting the priming effect of task-specific movement on cortical excitability.[5,14] Initiating with TRE was intended to activate motor pathways and enhance cortical responsiveness to subsequent tDCS. The session concluded with proprioceptive-based control therapy to reinforce functional integration and consolidate motor learning.

All interventions were administered three times weekly over eight weeks. Baseline assessments of trunk control and functional mobility were conducted three days prior to the first intervention using the Postural Assessment Stroke Scale, Rivermead Mobility Index, and Trunk Impairment Scale. Procedures were explained to participants and caregivers, and clinical deficits were evaluated. Post-intervention assessments were conducted using the same standardised tools to monitor changes and assess improvements. All outcome assessments were conducted by two trained physiotherapists who were blinded to group allocation. To ensure consistency, each participant was assessed by the same physiotherapist for both pre- and post-intervention evaluations.

### Statistical Analysis

Data were analysed using IBM SPSS version 23.0. Descriptive statistics, including frequencies, means, standard deviations (SD), and percentages, were used to summarize patient characteristics. The Shapiro-Wilk test was applied to assess the normality of continuous variables. Factorial ANOVA was conducted to evaluate the effects of the interventions on trunk control measures. If ANOVA indicated statistically significant differences among group means, Tukey's post hoc test was performed to identify specific group differences. The level of statistical significance for all analyses was set at $p < 0.05$.

## RESULTS

A total of 72 post-ischaemic stroke patients were initially assessed for eligibility, but only 69 were recruited; three were excluded due to recurrent stroke. The mean age of the participants was 57.2 ± 11.83 years, with no significant differences across groups. Participants were randomly assigned to four groups, as described earlier (Figure 1). Table I summarises the demographic distribution across groups.

**Table I: Socio-demographic characteristics of study participants (n = 69)**

| Characteristics | Intervention Groups | | | | p-value |
|---|---|---|---|---|---|
| | Control (n = 18) n (%) | tDCS (n = 17) n (%) | TRE (n = 17) n (%) | CI (n = 17) n (%) | |
| Age (years) | | | | | 0.785 |
| 25-44 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| 45-64 | 15 (83.3) | 15 (88.2) | 15 (88.2) | 16 (94.1) | |
| 65-85 | 3 (16.7) | 2 (11.8) | 2 (11.8) | 1 (5.9) | |
| Mean ± SD | 57.94 ± 5.84 | 58.94 ± 5.99 | 58.12 ± 5.81 | 56.94 ± 5.29 | |
| Gender | | | | | 0.689 |
| Male | 10 (55.6) | 10 (58.8) | 11 (64.7) | 11 (64.7) | |
| Female | 8 (44.4) | 7 (41.2) | 6 (35.3) | 6 (35.3) | |
| Marital status | | | | | 0.934 |
| Single | 5 (27.8) | 4 (23.5) | 5 (29.4) | 5 (29.4) | |
| Married | 11 (66.1) | 11 (64.7) | 9 (52.9) | 9 (52.9) | |
| Divorced | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Widow | 2 (11.1) | 2 (11.8) | 3 (17.6) | 3 (17.6) | |
| Residence | | | | | 0.377 |
| Rural | 13 (72.2) | 9 (58.8) | 10 (58.8) | 10 (58.8) | |
| Urban | | | | | |
| 5 (27.8) | 8 (41.2) | 7 (41.2) | 7 (41.2) | | |
| SBP (mmHg) | | | | | 0.974 |
| < 140 | 14 (77.8) | 12 (70.6) | 12 (70.6) | 12 (70.6) | |
| ≥ 140 | 4 (22.2) | 5 (29.4) | 5 (29.4) | 5 (29.4) | |
| Mean ± SD | 135.0 ± 11.8 | 134.35 ± 11.0 | 135.72 ± 5.9 | 134.35 ± 9.1 | |
| DBP (mmHg) | | | | | 0.704 |
| < 90 | 15 (83.3) | 14 (82.4) | 13 (76.5) | 12 (70.6) | |
| ≥ 90 | 3 (16.7) | 3 (17.6) | 4 (23.5) | 5 (29.4) | |
| Mean ± SD | 80.47 ± 8.6 | 83.2 ± 6.1 | 81.0 ± 10.43 | 81.1 ± 8.2 | |

CI: combined intervention, DBP: diastolic blood pressure, SBP: systolic blood pressure, tDCS: transcranial direct current stimulation, TRE: trunk rehabilitation exercises.

**Table II: Pre- and post-intervention assessments and the mean differences of outcome measures in the study groups**

| Outcomes measures | Control (Mean ± SD) | tDCS (Mean ± SD) | TRE (Mean ± SD) | CI (Mean ± SD) |
|---|---|---|---|---|
| Pre-intervention (baseline) assessment | | | | |
| PASS | 18.94 ± 1.71 | 18.94 ± 1.82 | 19.06 ± 1.85 | 19.17 ± 1.47 |
| RMI | 8.71 ± 1.31 | 8.88 ± 1.05 | 9.05 ± 0.89 | 9.12 ± 0.93 |
| TIS | 14.41 ± 2.00 | 15.59 ± 1.69 | 15.06 ± 1.54 | 15.65 ± 1.62 |
| Post-intervention assessment | | | | |
| PASS | 24.76 ± 0.74 | 20.77 ± 2.28 | 26.06 ± 2.11 | 28.24 ± 1.72 |
| RMI | 11.06 ± 1.03 | 9.94 ± 1.14 | 12.06 ± 0.89 | 12.88 ± 0.61 |
| TIS | 18.41 ± 1.28 | 17.12 ± 2.03 | 18.24 ± 1.25 | 20.17 ± 1.55 |
| Pre- and post-assessment mean differences | | | | |
| PASS | 5.82 ± 1.54 | 1.83 ± 1.62 | 7.00 ± 1.43 | 9.07 ± 1.13 |
| RMI | 2.35 ± 0.87 | 1.06 ± 0.92 | 3.01 ± 0.93 | 3.76 ± 1.23 |
| TIS | 4.00 ± 2.20 | 1.53 ± 0.69 | 3.18 ± 1.41 | 4.52 ± 1.03 |

CI: combined intervention, PASS: Postural Assessment Stroke Scale, RMI: Rivermead Mobility Index, SD: standard deviation, tDCS: transcranial direct current stimulation, TIS: Trunk Impairment Scale, TRE: trunk rehabilitation exercises.

interventions, which consistently outperformed other groups in all outcome measures (Table II).

## DISCUSSION

Trunk performance plays a crucial role in postural control, balance, and overall stabilization, allowing stroke patients to perform activities independently. It also serves as a predictive factor for independence and good rehabilitation prognosis.[3,4,6,16] In this study, we observed modest improvements in trunk control across all outcome measures, including the Postural Assessment Stroke Scale, Rivermead Mobility Index, and Trunk Impairment Scale. While no statistically significant differences were detected among the intervention groups, patients receiving combined interventions (CI group) tended to demonstrate greater improvements compared to those receiving individual interventions or control treatments.

The three outcome measures were selected to comprehensively evaluate the multidimensional aspects of trunk function, balance, and functional mobility in stroke patients. Each tool offered distinct advantages in capturing specific components of trunk performance, making them collectively suitable for achieving the study's objectives.

**Table III: The effects of studied interventions on trunk control and performance of participants**

| Interventions | Mean ± SE | df | F-value | p-value |
|---|---|---|---|---|
| **Postural Assessment Stroke Scale (PASS)** | | | | |
| Control | 5.656 ± 0.731 | 3 | 2.109 | 0.111 |
| tDCS | 6.825 ± 0.789 | | | |
| TRE | 7.631 ± 0.767 | | | |
| CI | 8.661 ± 0.789 | | | |
| **Rivermead Mobility Index (RMI)** | | | | |
| Control | 2.250 ± 0.375 | 3 | 2.217 | 0.098 |
| tDCS | 2.530 ± 0.405 | | | |
| TRE | 3.012 ± 0.394 | | | |
| CI | 3.774 ± 0.405 | | | |
| **Trunk Impairment Scale (TIS)** | | | | |
| Control | 3.854 ± 0.484 | 3 | 2.195 | 0.101 |
| tDCS | 3.077 ± 0.523 | | | |
| TRE | 3.509 ± 0.509 | | | |
| CI | 4.893 ± 0.523 | | | |

CI: combined intervention, SE: standard error, tDCS: transcranial direct current stimulation, TRE: trunk rehabilitation exercise

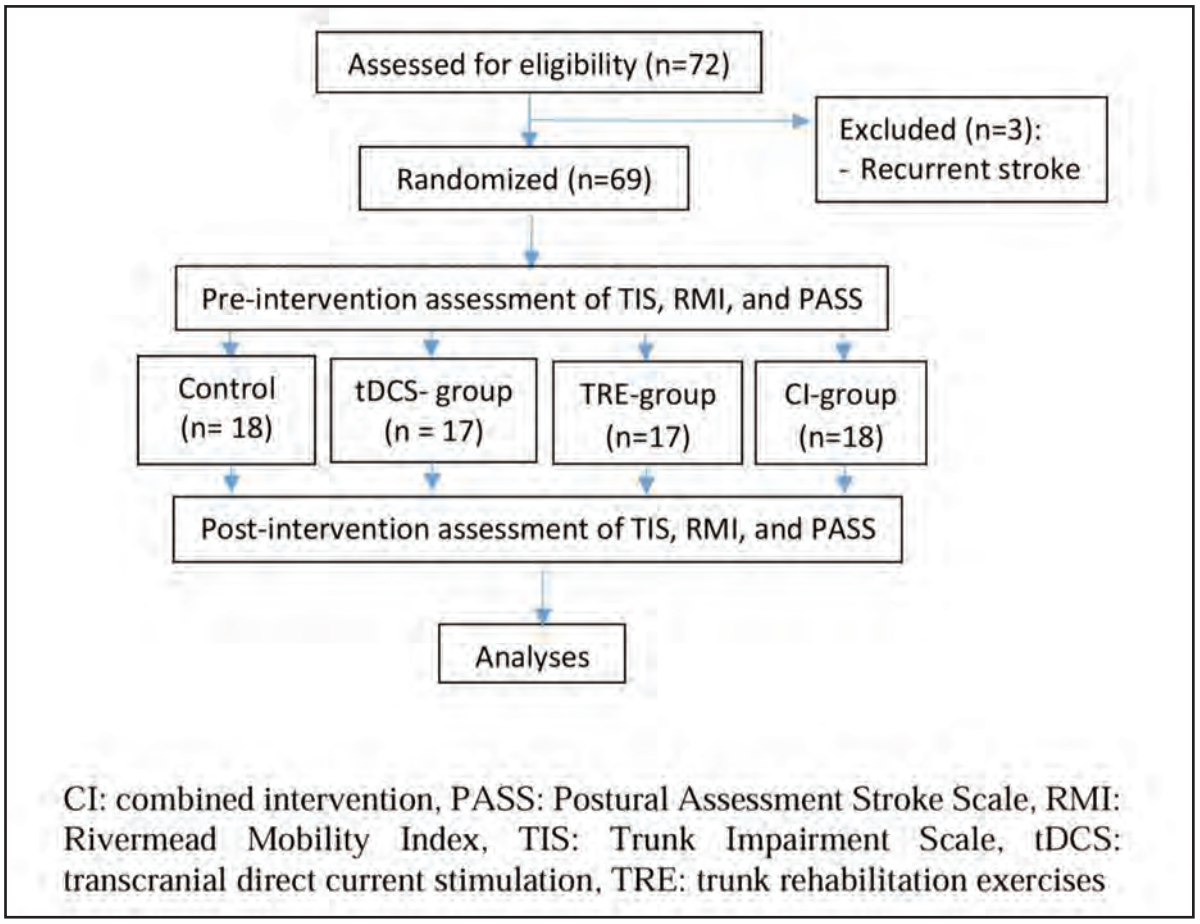


**Fig. 1:** Flow chart of the study

There was a modest improvement in trunk control and performance levels across all groups following eight weeks of intervention, as shown in Table II. Participants who received the combined interventions demonstrated the greatest improvements. Despite these observed trends, factorial ANOVA revealed no statistically significant differences in the effects of the interventions on trunk control and performance outcomes, including Postural Assessment Stroke Scale (F=2.109, p=0.111), Rivermead Mobility Index (F=2.217, p=0.098), and Trunk Impairment Scale (F=2.195, p=0.101) (Table III). As the ANOVA results did not indicate significant differences between groups, Tukey post hoc tests were not performed.

However, the pre-test to post-test differences in trunk control and performance measures were highest in the CI group, suggesting a potential synergistic effect. The mean differences in post-test scores indicated a trend favouring the combined

The Postural Assessment Stroke Scale was used to assess both static and dynamic balance, which are crucial for maintaining postural control in stroke patients. The scale comprises 12 items that evaluate the ability to maintain or change lying, sitting, or standing postures, providing a comprehensive assessment of functional equilibrium. PASS has demonstrated excellent reliability, with high internal consistency (Cronbach's alpha: 0.95) and inter- and intra-rater reliability (0.88 and 0.72, respectively). Its strong correlation with the Functional Independence Measure (FIM) underscores its validity as a balance assessment tool.[17,18]

The Rivermead Mobility Index was selected to evaluate functional mobility, encompassing gait, balance, and transfers. This 15-item scale provides a simple yet effective measure of mobility, with higher scores indicating better functional performance. RMI demonstrates strong psychometric properties, including excellent test-retest reliability (ICC: 0.98), inter-rater reliability (ICC: 0.93), and internal consistency (Cronbach's alpha: 0.93–0.96). Its predictive and content validity for stroke patients further establish its suitability for this study.[19,20]

The Trunk Impairment Scale was chosen to assess motor coordination and selective trunk movements, which are critical for core functionality in stroke rehabilitation. Its robust psychometric properties, including high reliability (ICC: 0.85–0.99) and internal consistency (Cronbach's alpha: 0.89), make it a reliable measure for both clinical and research applications. The scale evaluates static and dynamic sitting balance and trunk coordination, offering a detailed understanding of trunk motor impairment. Its strong correlations with the Barthel Index (Spearman rank: 0.86) and Trunk Control Test (Spearman rank: 0.83) further establish its validity.[11]

Together, these measures provided a comprehensive framework for evaluating the impact of the interventions on trunk control and functional recovery. By capturing complementary dimensions of trunk performance, the tools facilitated a multidimensional understanding of rehabilitation outcomes, aligning with the study's objectives to explore the effects of neuroplasticity-driven interventions.

The combined intervention appeared to leverage complementary mechanisms, potentially explaining the observed trends of improvement. TRE likely promoted neuroplasticity through repetitive, goal-directed movements that enhanced motor learning and neuromuscular re-education.[13] Stroke patients often experience postural disturbances, but recovery is achievable to some extent through intensive rehabilitation practices.10 Exercises like TRE, rooted in movement science and motor learning principles, emphasise goal-directed practice and repetition, making them effective for improving motor function in the arm, hand, and lower limb after a stroke.[13,21] These exercises are believed to induce behavioural changes that lead to structural neuronal adaptations, promoting neuroplasticity through processes such as neurogenesis, angiogenesis, and axonal sprouting.[22] Furthermore, increased physical activity enhances brain function, stimulates biochemical activity, and facilitates motor recovery after stroke.[23]

On the other hand, tDCS facilitates neuroplasticity by modulating cortical excitability, particularly in motor regions.[9,10,12,14] Animal studies further support this mechanism, showing that neurons in the subcortical ponto-medullary reticular formation are essential for anticipatory postural adjustments, transmitting signals from higher cortical levels before movement initiation.[6] However, in chronic stroke patients, abnormal hyperexcitability in the premotor areas may excessively inhibit the ponto-medullary reticular formation and spinal cord, disrupting the normal sequencing needed for coordinated posture and movement.[7,24] Previous research has shown that tDCS is effective in enhancing neuroplasticity even in the chronic phase of stroke rehabilitation.[25-28] Studies involving healthy controls revealed that tDCS could induce sustained cortical excitability in the primary motor cortex for up to 90 days following stimulation.[12,29] Moreover, several studies have demonstrated the benefits of tDCS applied over the primary motor cortex region in improving arm, leg, and hand performance in stroke patients, highlighting its potential for motor recovery.[30,31]

The synergistic effects of these interventions could be attributed to their capacity to reinforce bilateral trunk muscle activation and facilitate motor recovery through neural pathways that innervate both paretic and non-paretic muscles.[32] Researchers have postulated that changes in the musculoskeletal system may indirectly influence the formation and function of the brain-derived neurotrophic factor, a key marker of neuroplasticity.[14,21] The observed improvements in our study may also align with this hypothesis, as enhanced trunk control and stabilization could support neural recovery. However, the anatomical bilateral innervation of trunk muscles, which rarely contract unilaterally, may also explain the improvements in both the study and control groups. Axial muscle contraction on the paretic side may have been facilitated through irradiation during interventions, further contributing to functional gains.[32]

TRE, unlike tDCS, offers a practical and accessible approach that can be continued at home under caregiver supervision. Once properly instructed by a rehabilitation professional, caregivers can safely assist patients in performing TRE, which involves structured, goal-directed trunk movements in supported positions. This makes TRE a cost-effective and sustainable method for improving trunk control, postural stability, and overall functional independence in stroke survivors, particularly in settings with limited access to continuous physiotherapy. In contrast, tDCS requires trained personnel and clinical supervision due to its technical and safety considerations.

While the present study yielded encouraging findings, it is not without limitations. The small sample size might have reduced the statistical power, potentially obscuring meaningful differences between groups. Furthermore, the study exclusively focused on participants in the chronic phase of stroke recovery, which limits the generalisability of the results to those in the acute or subacute phases. The relatively short intervention duration and absence of long-term follow-up further constrained assessment of sustained

improvements and the potential cumulative benefits of the interventions. Additionally, the intervention sequence in the CI group was structured to maximise neuroplastic effects based on theoretical and prior research foundations. However, the influence of different sequencing strategies remains unclear and warrants further investigation in future research.

Future studies should aim to address these limitations by recruiting larger and more diverse cohorts that include acute and subacute stroke populations. Extending the duration of interventions and incorporating long-term follow-up assessments will provide greater insights into the durability of the observed improvements. Furthermore, exploring biomarkers such as brain-derived neurotrophic factor levels with trunk control interventions may offer a deeper understanding of the mechanisms driving neuroplasticity and recovery. Optimising intervention protocols and identifying patient-specific factors that enhance responsiveness to combined interventions should also be prioritized in future research.

## CONCLUSION

This study suggests that combining TRE and tDCS may offer a promising neuroplasticity-driven approach to improving trunk control and performance in post-stroke patients. Although no statistically significant differences were found, the trends favouring the CI group indicate it could leverage neuroplasticity to improve outcomes. Larger-scale studies are needed to validate these preliminary findings and refine the intervention protocols for broader clinical application.

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ORIGINAL ARTICLE

# Antiproliferative activity of *Neolamarckia cadamba* (kelempayan) leaves methanol extract on human glioblastoma (U-87 MG) cells

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## ABSTRACT

**Introduction: Glioblastoma is a malignant brain tumor with a poor prognosis and high fatality rates. Current chemotherapy, primarily with temozolomide (TMZ), is limited by resistance and toxic side effects, underscoring the need for alternative treatments. This study investigates the antiproliferative properties of *Neolamarckia cadamba* (NC) leaves methanol extract on glioblastoma (U-87 MG) cells as a potential alternative treatment.**

**Materials and Methods: NC leaves were collected, dried, and extracted using methanol via maceration. Phytochemical screening identified active compounds, including phenolics, flavonoids and tannins. U-87 MG cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum and 1% Penicillin-Streptomycin. The cells were treated with serial dilutions of NC extract and TMZ as a positive control for 24, 48 and 72 hours. Cytoselective of NC extract is conducted on non-cancerous (Vero) cells. Cytotoxicity was assessed using the MTT assay, with IC50 values determined from dose-response curves. Apoptotic induction was evaluated using Acridine Orange/Propidium Iodide (AO/PI) staining under a fluorescent microscope.**

**Results: Phytochemical screening of NC leaves extract revealed active compounds such as phenolics, flavonoids, tannins and saponins. The MTT assay demonstrated dose- and time-dependent cytotoxicity of NC extract, with IC50 values of 198.7 µg/mL (24 hours), 321.4 µg/mL (48 hours), and 126.3 µg/mL (72 hours). NC extract also shows cytoselective on Vero cells with an IC50 value of more than 100 µg/mL for all timeframes. AO/PI staining confirmed apoptosis induction of the NC extract on U-87 MG cells.**

**Conclusion: NC extract exhibited low potential as an alternative therapeutic agent for glioblastoma by inducing early apoptosis on U-87 MG cells.**

**KEYWORDS:**
*Neolamarckia cadamba, kelempayan, antiproliferative, anticancer, glioblastoma, medicinal plants*

## INTRODUCTION

Gliomas are the most common primary malignant brain tumours, with glioblastoma being the most prevalent and aggressive subtype. The global incidence rate of glioblastoma is approximately 3.23 per 100,000 population.[1] Gliomas account for about 24.5% of all primary brain tumours and 80.9% of all malignant brain tumours in adults.[1] The incidence of gliomas varies significantly by age, sex, and geographical region. For instance, the incidence rate is higher in males compared to females.[2,3] Additionally, the higher incidence observed in American and Northern European populations compared to Asian populations suggests a possible hereditary predisposition.[1,4] In Malaysia, gliomas are less common compared to other cancers. According to the Malaysia National Cancer Registry Report 2017-2021, gliomas are not listed among the top ten most common cancers in the country.[5] However, the incidence of brain and central nervous system (CNS) cancers, which include gliomas, has been documented.5 The report indicates that the age-standardized rate (ASR) for brain and CNS cancers in Malaysia is relatively low compared to other types of cancer. In 2022, there were 870 new cases of brain and CNS cancers reported, with an ASR of 0.23 per 100,000 population.[5] The mortality rate for these cancers was 2.5%, with 799 deaths reported.[5,6]

Glioma presents a significant therapeutic challenge due to its high invasiveness, poor prognosis, and resistance to conventional treatments.[7] Current therapies, including surgery, radiation, and chemotherapy with agents like temozolomide (TMZ), are often limited by systemic toxicity and the emergence of drug resistance, emphasizing the urgent need for novel therapeutic approaches.[8] TMZ is an oral alkylating agent that has become a cornerstone in the treatment of gliomas, particularly glioblastoma (GB). TMZ is most commonly used in combination with radiotherapy, after surgery and collectively known as the Stupp protocol. This combination has been shown to improve median survival rates for patients with GBM from approximately 12.1 months with radiotherapy alone to 14.6 months with the addition of TMZ. While the landmark Stupp protocol demonstrated an increase in 2-year survival of glioblastoma patients from 10.4% to 26.5% with combined radiotherapy

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and temozolomide, it also underscored the considerable systemic toxicity and economic burden associated with this treatment, highlighting the urgent need for safer, more cost-effective therapeutic alternatives.[6]

*Neolamarckia cadamba* (NC), commonly known as Kelempayan or Bur flower, is a fast-growing tropical deciduous tree native to South and Southeast Asia and widely distributed across India, Nepal, Bangladesh, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia, and the Philippines,[9] with introductions to other tropical regions for ornamental, timber, and medicinal purposes.[10,11] The species thrives in moist, evergreen, and mixed deciduous forests, particularly in fertile, well-drained alluvial soils. Traditionally, various parts of NC, including leaves, bark, and fruits,[12] have been employed in ethnomedicine, and modern studies have reported that its phytochemicals, particularly phenolics, flavonoids, and alkaloids[12] exhibit diverse bioactivities, including antioxidant,[13] antidiabetic,[14] anti-inflammatory,[15] antitumor,[16] and anticancer effects.[17,18] However, despite these promising findings, systematic investigations into the mechanisms underlying its anticancer activity and its potential for development as a therapeutic agent remain limited, highlighting the need for further research.

Therefore, the current study was designed to evaluate the anticancer effect of NC leaves methanol extract on cell cytotoxicity and apoptosis induction in human glioblastoma cell line.

## MATERIALS AND METHODS

*Plant material authentication*

The leaves of NC were collected and verified by local residents of Telemong, Terengganu, Malaysia. The plant materials were further authenticated at the International Islamic University Malaysia (IIUM) Herbarium Centre in Pahang, Malaysia, and the voucher specimen (PIIUM 0266-1) was deposited in the IIUM Herbarium Centre (Voucher No.: PIIUM 0266-1)

*Plant material extraction*

The leaves were properly washed and dried in a drying cabinet. The dried leaves were then finely powdered and 50 g of the powdered leaves were extracted using the maceration technique with 450 mL of methanol solvent. The leaves were soaked for six days and filtered every two days. The crude plant extract was evaporated to dryness in a rotary vacuum evaporator. The crude plant extract was dried and then collected into vials, with the amount being recorded.[19] The percentage of yield was calculated using the formula given below:

$$\text{Percentage yield (\%)} = \frac{\text{weight of crude extract (g)}}{\text{weight of plant material (g)}} \times 100$$

*Phytochemical analysis of NC extract*

NC leaves methanol extract was subjected to various qualitative phytochemical tests to screen for phytochemical constituents[20]

i. Detection of phenolic compound
250 mg (0.25g) of extract was dissolved in 5 ml distilled water. Then, 5 drops of 5% ferric chloride (FeCl3) were added. The appearance of intense green, purple or blue-black coloration indicates the presence of phenolic compound.

ii. Detection of flavonoid compound
100 mg (0.1 g) of extract was dissolved in 2 ml of distilled water. Then, the solution was mixed with 4 drops of 10% sodium hydroxide (NaOH) solution. Appearance of yellow coloration indicates presence of flavonoid compound.

iii. Detection of tannin compound
500 mg (0.5 g) of extract was dissolved in 10 ml of distilled water. In 2 ml of the solution, 4 drops of 1% ferric chloride (FeCl3) were added. Appearance of blue-black coloration indicates the presence of tannin compound.

iv. Detection of coumarin compound
100 mg (0.1 g) of extract was dissolved in 1 ml of distilled water. Then, 1 ml of 10% sodium hydroxide (NaOH) was added followed by 1 ml of chloroform. Appearance of yellowish brown shows the presence of coumarin.

v. Detection of alkaloid compound
250 mg (0.25 g) of extract was dissolved with 5 ml of 1% hydrochloric acid (HCl). Then, 3 drops of Dragendorff's reagent were added. Appearance of orange red precipitate indicate the presence of alkaloid compound.

vi. Detection of glycosides compound
2 ml of sulphuric acid was added into 250 mg (0.25 g) of extract. A reddish-brown colour formation indicates the presence of glycosides compound.

vii. Detection of saponin compound
300 mg (0.3 g) of extract was dissolved in 3 ml of distilled water and shaken vigorously for 2 minutes. Persistent frothing indicates the presence of saponin compound.

viii. Detection of steroid compound
250 mg (0.25 g) of extract was dissolved in 2.5 ml distilled water. Then, 1 ml of chloroform was added. Then, 1 ml of sulphuric acid was carefully added to form a layer. In the lower of chloroform layer, the appearance of red colour indicates the presence of steroid compound.

ix. Detection of terpenoid compound
250 mg (0.25 g) of extract was dissolved in 2.5 ml of distilled water. Then, 1 ml of chloroform and 1 ml of concentrated sulphuric acid (H2SO4) were added carefully to form a layer. After that, solution was mixed. A reddish-brown coloration indicates terpenoids compound.

x. Detection of quinone compound
100 mg of extract was dissolved in 1 ml of distilled water. Then, 1 ml of dilute sodium hydroxide (NaOH) was added. The appearance of blue-green or red coloration indicates the presence of quinones.

*Cell culture*

U-87 MG (human glioblastoma cells) and Vero (African Green Monkey normal kidney cells) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Both cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (ATCC, Manassas, VA) supplemented with 10% foetal bovine serum (FBS) and penicillin–streptomycin 1% (v/v) (Gibco, Grand Island, NY). The cells were incubated at 37°C and 5% CO2 in a humidified atmosphere.

*Antiproliferative assay*

U-87 MG and Vero cells were seeded into a 96-well plate at a density $5.0 \times 10^4$ cells per well and incubated for 24 hours, respectively. After 24 h, complete media with gradient concentrations (0, 3.9,7.8, 15.6, 31.3, 62.5, 125 and 250 µg/mL) of NC extracts and TMZ (used as positive control drug) were added into respective wells. Whereby, Vero cells (negative control cells) were treated with six different concentrations of NC extract ranging from 150µg/ml to 12.5µg/ml.

The plates were incubated at 37°C for 24, 48 and 72 hours. Then, MTT solution (5 mg/mL) was added into each of the wells. The plates were incubated in humidified incubator with at 37°C for 4 hours. Absolute DMSO was added into the wells and absorbance (OD) was measured at wavelength 570 nm. The half maximal inhibitory concentration (IC50) of each solvent was determined using graph percentage of viable cells against concentration (µg/mL). The percentage of viable cells was calculated using formula as follows:

$$\text{Percentage of viable cells (\%)} = \frac{\text{(OD value of treated cells)}}{\text{(OD value of untreated cells)}} \times 100$$

*Cell morphology analysis*

Morphological detection of apoptosis was examined using acridine orange and propidium iodide (AO/PI) staining. U-87 MG cells at a concentration of $3.0 \times 10^4$ cells/mL were treated with NC extract for 24, 48 and 72 hours at their IC50 concentrations in triplicates. The treated cells were trypsinised and harvested with 1 mL cold phosphate buffered saline (PBS), followed by centrifuged at 1500 rpm for 10 minutes at 4°C. This process was repeated twice. The cell suspension was mixed with 20 µL of AO/PI solution (1:1) and the mixture (10 mL) was transferred onto a slide and covered with a cover slip. Viable, apoptotic and necrotic cells were quantified in a population of 200 cells using a fluorescence microscope equipped with a B-2A filter (Nikon TE2000-U, Japan). Untreated U-87 MG cells served as a negative control and TMZ-treated cells were used as a positive control.

*Data analysis*

The statistical analysis was performed using the GraphPad PRISM software version 10 (GraphPad Software, Inc., San Diego, CA, USA). The dose-dependent curve analysis for the determination of $IC_{50}$ was analysed and plotted using GraphPad Prism. The differences between the means were assessed with the two-way analysis of variance (ANOVA) and Tukey's post hoc test. The difference with p values less than 0.05 was considered statistically significant ($p<0.05$).

## RESULTS

*Phytochemical analysis of NC extract*

The phytochemical analysis of NC leaves methanol extract revealed a diverse profile of bioactive compounds, which likely contribute to its observed anticancer effects. This qualitative screening identified the presence of key phytoconstituents, including phenolics, flavonoids, tannins, coumarins, alkaloids, glycosides, saponins, and steroids. However, terpenoids and quinones were not detected in the extract as represented in Table I.

*Antiproliferative activity of NC extract*

The MTT assay results demonstrated the antiproliferative effects of NC extract and TMZ on U-87 MG cells in a time- and dose-dependent manner, as shown in Figure 1. Table II shows the IC50 values of NC extract and TMZ on U-87 MG cells obtained from the dose-response curve of percentage of cell viability against log10 concentration. Based on the results, IC50 values of NC extract on U-87 MG cells were 198.7, 321.4, and 126.3 µg/mL at 24, 48, and 72 hours, respectively. TMZ achieved much lower IC50 value than NC extract; 18.70, 83.90 and 67.12 µg/mL at 24,48 and 72 hours, respectively.

Two-way ANOVA revealed that both NC extract and TMZ exerted concentration-dependent effects on U-87 MG cell viability, although with different patterns. For NC extract, concentration was the dominant factor ($F(7,48)=193.72$, $p<0.0001$), explaining 79.5% of the total variance, with a significant contribution from treatment duration ($F(2,48)=27.97$, $p<0.0001$; 3.3% variance) and a strong interaction effect ($F(14,48)=17.53$, $p<0.0001$; 14.4% variance). In contrast, TMZ activity was almost exclusively driven by concentration ($F(7,36)=247.53$, $p<0.0001$), which accounted for 87.9% of the variance, with no significant effect of treatment duration ($F(2,36)=0.57$, $p=0.571$), although the interaction between concentration and time remained significant ($F(14,36)=12.55$, $p<0.0001$; 8.9% variance). Collectively, these results indicate that while both agents are strongly concentration-dependent, NC extract's antiproliferative activity is more influenced by treatment duration compared to TMZ, suggesting differences in the kinetics or mechanisms of action between the crude extract and the chemotherapeutic drug.

Figure 2 shows the IC50 values of NC extract against non-cancerous (Vero) cells were 321.6, 479.9, and 169.6 µg/mL at 24-, 48-, and 72-hour treatment, respectively. Two-way ANOVA showed a significant main effect of concentration ($p<0.0001$) and a significant interaction between concentration and treatment duration ($p<0.0001$), whereas treatment duration alone was not significant ($p=0.0870$). Concentration accounted for 74.42% of the total variation, while the interaction contributed 18.71%, indicating that the effect of concentration on the response was dependent on treatment duration.

*Apoptosis analysis from AO/PI staining*

The Acridine Orange/Propidium Iodide (AO/PI) staining results provided valuable insights into the induction of apoptosis by NC extract in U-87 MG cells. Following 72 hours of treatment with the IC50 concentration of NC extract, a substantial increase in apoptotic cells was observed compared to untreated controls. As shown in Figure 3, NC-

**Table I: Phytoconstituent from Neolamarckia cadamba leaves methanol extract**

| Compound | Indicator | Result |
|---|---|---|
| Phenolic | Appearance of intense green, purple or blue -black color upon addition of 5% FeCl3. | a |
| Flavonoid | Solution turns yellow upon mixing with 10% NaOH solution. | a |
| Tannin | Appearance of blue-black coloration upon addition of 1% FeCl3. | a |
| Coumarin | Appearance of a yellowish-brown solution upon addition of chloroform. | a |
| Alkaloid | Appearance of orange-red precipitate upon addition of Dragendorff's reagent. | a |
| Glycoside | A reddish-brown color formation upon addition of H2SO4. | a |
| Saponin | Persistent frothing upon vigorous shake. | a |
| Steroid | Appearance of red color in the lower of chloroform layer upon addition of sulphuric acid. | a |
| Terpenoid | Formation of reddish-brown coloration upon mixing. | b |
| Quinone | Appearance of blue-green or red coloration upon addition of dilute NaOH. | b |

a: Presence of compound
b: Absence of compound

**Table II: IC50 value of Neolamarckia cadamba (NC) leaves methanol extract and Temozolomide (TMZ) on U-87 MG cells**

| Treatment | IC50 value (µg/mL) | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| NC extract | 198.7 | 321.4 | 126.3 |
| Temozolomide (TMZ) | 19.46 | 83.90 | 67.12 |

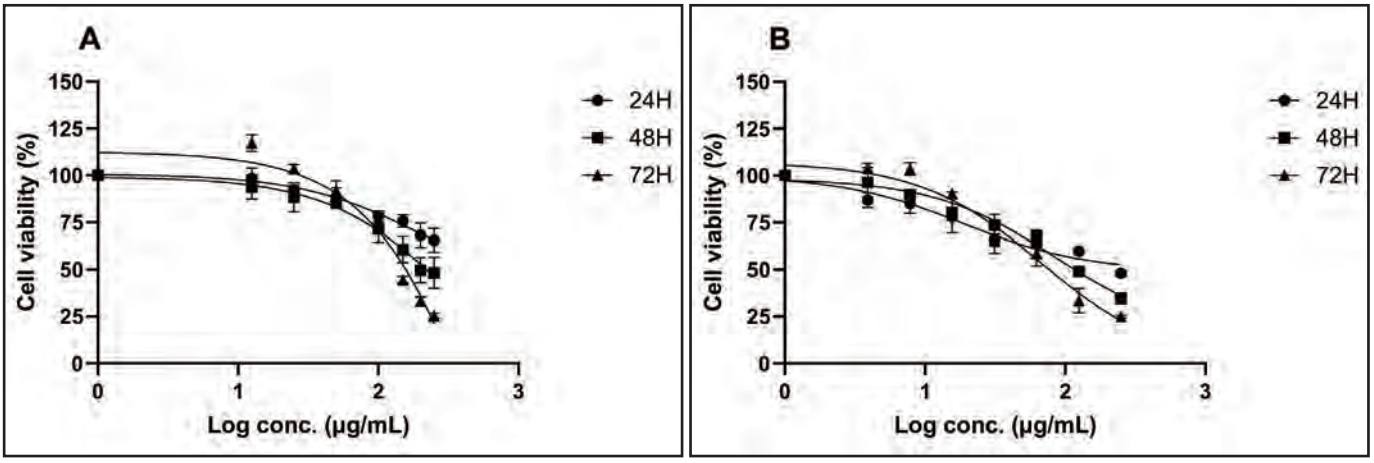


**Fig. 1:** Antiproliferative activity of different concentrations of (A) NC extract and (B) TMZ on U-87 MG cells at 24-, 48- and 72- hours treatment

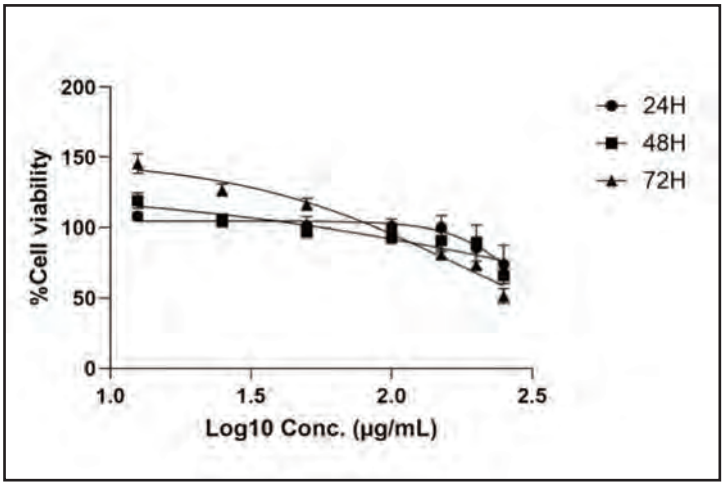


**Fig. 2:** Antiproliferative activity of different concentrations of NC extract on non-cancerous (Vero) cells at 24-, 48- and 72-hours treatment

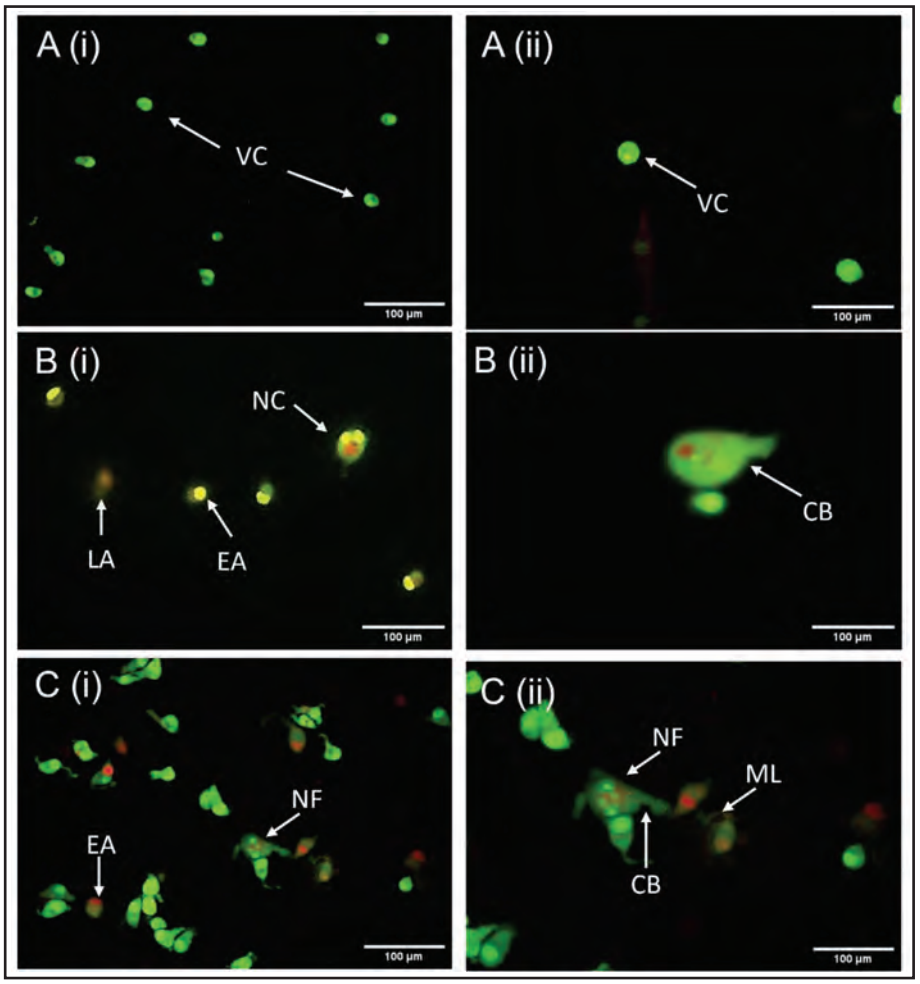


**Fig. 3:** Morphological changes of (A) untreated, (B) NC-treated (126.3 µg/mL) and (C) TMZ-treated (67.12 µg/mL) of U-87 MG cells stained with AO/PI staining after 72 hours. The cells were observed and captured under (i) 200x and (ii) 400x magnification, respectively, by a fluorescence microscope. EA; early apoptosis, LA; late apoptosis cell, NC; nuclear condensation; CB; cell blebbing; NF; nuclear fragmentation, ML; membrane loose, VC; viable cell

treated cells showed classic apoptotic morphological features, including chromatin condensation, membrane blebbing, and the formation of apoptotic bodies. These features were distinctly absent in untreated cells, which displayed uniformly green fluorescence indicative of healthy nuclei.

The apoptotic effect of NC extract was comparable to that of TMZ, the standard chemotherapeutic agent. NC-treated cells also exhibited a less pronounced necrotic response, suggesting that the extract primarily induces programmed cell death rather than uncontrolled necrosis. This finding underscores the potential of NC extract as a targeted, less toxic alternative therapy for glioblastoma.

## DISCUSSION

The interpretation of $IC_{50}$ values is essential to evaluate the cytotoxic potential of plant extracts and to benchmark their activity against established pharmacological standards. According to American National Cancer Institute (NCI) - based criteria, crude extracts with $IC_{50}$ values below 20–30 µg/mL are considered cytotoxic, while $IC_{50}$ values between ~21–200 µg/mL are viewed as moderately active and values greater than 200 µg/mL are deemed weakly cytotoxic.[21] This framework is consistently applied in recent studies.[22-24] Based on these criteria, the $IC_{50}$ values obtained for NC extract against U-87 MG cells fall within the moderate activity range, suggesting limited potency compared to standard chemotherapeutics, TMZ. However, the higher $IC_{50}$ values of NC extract in normal Vero cells relative to U-87 MG cancer cells indicate a modest safety margin, consistent with the principle that plant extracts demonstrating reduced cytotoxicity toward normal cells are considered safer and potentially more selective.[25,26]

The antiproliferative activity of NC extract against glioblastoma cells may be attributed to its phytoconstituents, particularly flavonoids. Quercetin, identified in NC extract, has been shown to suppress phospholipase D (PLD), a key regulator of proliferation and tumorigenesis in U-87 MG cells.[27] In addition, flavonoids inhibit matrix metalloproteinase-2 (MMP-2), thereby reducing glioma cell invasion and metastatic potential. Differences in $IC_{50}$ values across studies highlight the influence of both the plant part

used and the bioactive compounds present. Dolai et al. (2016) reported that methanolic NC extract reduced tumor volume and induced apoptosis in Dalton's lymphoma ascites cells, supporting its broad antitumor activity. Razali et al. (2021) demonstrated that ethanol extract of NC leaves inhibited proliferation of MCF-7 breast cancer cells ($IC_{50}$ ≈ 200 µg/mL at 72 H), with apoptosis mediated through mitochondrial pathways, identifying D-pinitol and myo-inositol as key contributors. Similarly, Fatima et al. (2016) found that NC bark extract exhibited time- and dose-dependent cytotoxicity in HeLa cervical cancer cells, with $IC_{50}$ values decreasing from 319 µg/mL (24 h) to 254 µg/mL (72 H), which was attributed to high phenolic content and strong antioxidant activity.

Extraction method and solvent type also influence cytotoxic potency. Singh et al. (2013) reported variable IC50 values of NC leaf extracts prepared with different solvents, with chloroform extract showing the most potent activity ($IC_{50}$ < 100 µg/mL) across multiple cancer cell lines (A-549, IGR-OV-1, PC-3, SF-295), and identified indole-based compounds as likely contributors. Taken together, these findings suggest that the antiproliferative potential of NC varies depending on (i) the plant part used, which determines the profile of bioactive compounds, (ii) the type of cancer cell line tested, as different molecular pathways may be engaged, and (iii) the extraction method, which influences the recovery of active metabolites (Le et al., 2018). Such variability underscores the importance of linking $IC_{50}$ values to phytochemical composition to better understand the mechanisms underlying NC's anticancer activity.

Although the $IC_{50}$ values of NC extract were higher compared to TMZ, its mechanism of action, mediated through apoptosis induction, aligns closely with the goals of conventional chemotherapeutics but with potentially fewer systemic toxicities. Importantly, NC extract induced morphological changes characteristic of apoptosis, including chromatin condensation and membrane blebbing, as observed through AO/PI staining. These findings align with a study by Razali et al. (2021), which reported the cell death mechanism by NC leaves extract via apoptosis induction and cell cycle arrest in breast cancer (MCF-7) cells. The extract also exerted anticancer effects in a dose- and time-dependent manner by triggering the mitochondrial cell death pathway.

The advantages of NC extract are rooted in its phytochemical composition. The presence of phenolics, flavonoids, tannins, and saponins, among others, is consistent with its antiproliferative and pro-apoptotic effects. These compounds have been extensively studied for their ability to modulate critical cancer pathways, including cell cycle arrest, reactive oxygen species (ROS) regulation, and the activation of apoptotic cascades.[13,20] For instance, flavonoids are known to inhibit tumor growth by modulating oxidative stress and inducing apoptosis through caspase activation, while phenolics contribute to DNA damage in cancer cells. These properties underscore the multifaceted mechanisms through which NC extract exerts its anticancer effects.[18]

The absence of terpenoids and quinones does not diminish the NC extract's therapeutic potential, as the detected compounds already offer a broad spectrum of anticancer mechanisms. The combination of all bioactive constituents of NC extract likely works synergistically, providing the extract with its potent antiproliferative and pro-apoptotic effects observed in glioblastoma cells.[31,32] In addition, studies suggest that the likelihood of developing resistance is lower when cancer is targeted using a combination of bioactive compounds compared to single active molecules.[33,17] This phytochemical diversity underscores the multifaceted therapeutic potential of NC extract, not only as an anticancer agent but also as a candidate for addressing other diseases linked to oxidative stress and inflammation.[18] Future studies should focus on isolating and quantifying these active compounds to better understand their individual contributions and explore their specific mechanisms of action. Moreover, advanced analytical techniques such as high-performance liquid chromatography and mass spectrometry could be utilized to confirm and characterize these phytochemical constituents in greater detail.

When compared to TMZ, which functions as a DNA-alkylating agent, NC extract offers a natural, less toxic alternative. TMZ indiscriminately targets both healthy and cancerous cells, leading to significant side effects such as gastrointestinal distress and myelosuppression.[8] In contrast, plant-derived compounds like those in NC extract often display selective toxicity toward cancer cells, sparing normal cells and reducing the risk of systemic toxicity. This selectivity is particularly advantageous in preserving the quality of life for cancer patients undergoing treatment.[18,19]

Furthermore, the economic and practical benefits of plant-based therapies make Neolamarckia cadamba (NC) extract an attractive candidate, especially in resource-limited settings. Unlike synthetic drugs, the cultivation, extraction, and preparation of plant-based therapies are generally more cost-effective, improving accessibility to a broader population.[34] Additionally, the traditional use of NC for treating various ailments supports both its cultural acceptance and perceived safety in communities familiar with its medicinal applications.[35,36]

While this study establishes a foundation for the antiproliferative potential of NC leaves extract, further research is necessary to optimize its application. Isolation and characterization of its active compounds will help identify the specific bioactive molecules responsible for its effects. Additionally, combining NC leaves extract with TMZ or other chemotherapeutics could offer synergistic benefits, enhance efficacy while mitigating the adverse effects of conventional treatments.

## CONCLUSION

The present study concluded the potential of Neolamarckia cadamba (NC) leaves methanol extract as a natural, accessible, and effective alternative for glioblastoma therapy. Its demonstrated ability to induce apoptosis, combined with its traditional medicinal use and bioactive composition, highlights its potential to complement or even partially replace conventional chemotherapeutics like TMZ. Therefore, a safety and toxicology profile of NC needs to be done. Future

investigations, including in vivo studies and clinical trials, will be critical to fully realize its potential in cancer treatment.

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ORIGINAL ARTICLE

# The effects of transcranial direct current stimulation on serum superoxide dismutase levels in ambulatory ischaemic stroke patients: A randomised pilot study

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## ABSTRACT

**Introduction: Stroke is one of the leading causes of disability worldwide, imposing a significant public health and economic burden. Oxidative stress, which results from an imbalance between free radical production and antioxidant defences, exacerbates stroke outcomes via its role in promoting neuroinflammation and tissue damage. Superoxide dismutase (SOD), a critical antioxidant enzyme, plays a key role in mitigating oxidative stress. This study evaluates the effect of transcranial direct current stimulation (tDCS) on serum SOD levels in ambulatory ischaemic stroke patients with mild cognitive impairment.**

**Materials and Methods: In this randomized pilot study, 30 ischaemic stroke survivors (aged 20–65 years) were divided into two groups: control intervention physiotherapy (CIP, n=15) and tDCS combined with CIP (tDCS+CIP, n=15). Each group underwent 30-minute sessions, three times weekly, over four weeks. Serum SOD levels were measured pre- and post-intervention using SOD Activity Assay Kit. Paired t-test was used to evaluate within-group changes, and independent t-test assessed between-group differences and the influence of stroke risk factors such as body mass index and blood pressure.**

**Results: The tDCS+CIP group exhibited a significant increase in serum SOD levels post-intervention (289.02±64.94 U/L from 215.58±53.65 U/L, p=0.001), compared to the CIP group (216.67±45.02 U/L from 198.04±40.74 U/L, p=0.001). No significant association was observed between serum SOD levels and the studied risk factors.**

**Conclusion: tDCS combined with conventional therapy significantly improves serum SOD levels, suggesting its potential as an adjunctive treatment for reducing oxidative stress in stroke rehabilitation. Further studies with larger sample sizes and extended follow-up are recommended to validate these findings and explore long-term benefits.**

**KEYWORDS:**
*Brain stimulation, cerebrovascular accident, neuromodulation, oxidative stress, superoxide dismutase*

## INTRODUCTION

Stroke is a leading cause of disability worldwide, profoundly affecting the quality of life of patients and their caregivers.[1-3] This debilitating condition often results in motor, sensory, and cognitive impairments.[4] Oxidative stress, marked by an imbalance between the production of reactive oxygen species and the body's antioxidant defences, is a critical factor in stroke pathology.[5,6] Superoxide dismutase (SOD), an essential antioxidant enzyme, plays a pivotal role in neutralising superoxide radicals, thereby mitigating oxidative stress and reducing cellular damage.[7] Stroke patients often experience oxidative stress due to an imbalance between free radical production and antioxidant defences, especially in ischemic regions such as the penumbra, where excessive free radicals exacerbate tissue damage and hinder recovery.[5,6,8] Reduced serum antioxidant levels, including SOD, have been linked to poorer outcomes, such as larger infarct sizes and heightened neuroinflammation.[9,10] Moreover, SOD deficiency correlates with elevated systemic inflammatory biomarkers such as erythrocyte sedimentation rate (ESR), interleukin-6 (IL-6), and C-reactive protein (CRP), which exacerbate post-stroke complications.[11] Its deficiency has also been inversely correlated with recovery potential, as its depletion may lead to increased inflammation and the progression of neurological deficits.[12] Genetic mutations or defects affecting SOD expression have also been associated with unfavourable outcomes.[11] Enhancing SOD levels could, therefore, mitigate stroke-related complications and improve recovery.[12]

Non-invasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), and deep brain stimulation, have emerged as powerful tools to modulate brain function.[13,14] The use of electrical currents in neuromodulation has a long history, dating back nearly a century,[15] but inconsistent results led to a temporary decline in interest. Recent advancements and promising findings have revived enthusiasm for tDCS,[13,16,17,18,19] particularly anodal stimulation, which enhances neuroplasticity, modulates oxidative stress, and induces long-lasting alterations in cortical excitability.[20,21] It is a simple, cost-effective, and safe brain stimulation technique with proven benefits for conditions involving impaired neuroplasticity, such as stroke.[13,22,23] By

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applying low-amplitude electrical currents through epicranially positioned electrodes, tDCS modulates cortical excitability. Anodal stimulation depolarizes neurons to enhance excitability and promote neuroplasticity, while cathodal stimulation hyperpolarizes neurons, exerting inhibitory effects.[13,14,24,25]

Anodal tDCS has been shown to reduce proinflammatory cytokines, including interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNFα),[26,27] and promote neuronal growth by increasing the expression of neuronal growth-promoting proteins such as GAP-43 and microtubule-associated protein 2 (MAP-2) in peri-infarct regions.[27] These mechanisms collectively position tDCS as a promising adjunctive therapy for stroke rehabilitation. Evidence also indicates that anodal tDCS can improve cognitive and motor functions in disorders like depression, Parkinson's disease, and stroke.[13,22,23] However, its effects on oxidative stress biomarkers such as SOD in stroke patients remain underexplored.

Given the potential of tDCS to improve stroke outcomes, this pilot study was designed to investigate its impact on serum SOD levels in ambulatory stroke patients. Pilot studies are essential to assess feasibility, optimize study design, and generate preliminary data for subsequent trials. Accordingly, this pilot study aims to evaluate the effect of a four-week tDCS intervention on serum SOD levels in ambulatory stroke patients with mild cognitive impairment. We hypothesize that tDCS combined with conventional therapy will significantly enhance serum SOD levels compared to conventional therapy alone, providing a foundation for future research.

## MATERIALS AND METHODS

*Study Design and Participant Selection*

This randomised, assessor-blinded, pilot-controlled study was conducted at Imamu Wali General Hospital, Kano, Nigeria, following ethical approval from the Kano State Ministry of Health (Ref. No.: SHREC/2023/3901, Approval No.: NHREC/17/03/2018) and was performed in accordance with the Declaration of Helsinki. Thirty ambulatory ischaemic stroke patients were recruited and randomly assigned into two groups: the control intervention physiotherapy group (CIP, n = 15) and the combined intervention group receiving tDCS with control intervention physiotherapy (tDCS+CIP, n = 15) (Figure 1). The study was conducted over a four-week period, with all participants giving written informed consent prior to the enrolment.

Eligible participants were male and female ischaemic stroke survivors, aged 20 - 65 years, with a Mini-Mental State Examination (MMSE) score of 22 or higher. Inclusion criteria required patients to be free of drug abuse, psychiatric comorbidities, visual or auditory deficits, and able to walk unaided or with mild assistive devices. Patients were required to achieve controlled systemic hypertension or diabetes with medication, and without the use of antidepressants. The onset of stroke was at least six months prior, and participants had to be responsive to verbal commands.

Participants were excluded if they had engaged if they had engaged in regular exercise or physiotherapy three months prior to enrolment, had moderate to severe musculoskeletal disorders in the upper or lower extremities, balance impairments, or concurrent participation in any other clinical research.

*Randomisation, Allocation Concealment, and Blinding*

The randomisation sequence was computer-generated using Microsoft Excel's random number function (=RAND( )) by an independent statistician not involved in the trial. The generated random number was sorted from smallest to largest, which was used to allocate the two studied groups repeatedly. To minimise selection bias, allocation concealment was ensured by using sequentially numbered, opaque, sealed envelopes (SNOSE) prepared by an independent researcher not involved in participant recruitment, intervention, or assessment. The envelopes were opened by the independent researcher only after the enrolled participant had completed all baseline assessments.

Due to the nature of the non-invasive brain stimulation, participants could not be blinded to their group assignment. This lack of blinding may introduce performance and detection bias, which is acknowledged as a limitation. However, to minimise assessment bias, the research personnel responsible for collecting blood samples, conducting laboratory analyses (serum SOD measurement), and performing all outcome assessments were blinded to group allocation throughout the study.

*Intervention Protocol*

Both groups participated in 30-minute sessions of control intervention physiotherapy three times a week for four weeks. These included 10 minutes of infrared radiation (IRR) to the affected limb and 20 minutes of static cycling using a Reebok (Z9BIKE) set at zero resistance. Stretching exercises were performed pre- and post-cycling.

Additionally, participants in the combined intervention group underwent anodal stimulation of the primary motor cortex (PMC) using the International 10-20% EEG electrode placement system.28 The anodal electrode was positioned over the PMC at a point 1 cm anterior and 4 cm lateral to the cortical vertex, which corresponds to the C3/C4 site in the EEG system.[28] To complete the circuit, the cathodal electrode was placed over the contralateral supraorbital region. A constant 2 mA current was delivered for 20 minutes using a tDCS stimulator (Medisystems tDCS DC-Stimulator Plus). Electrodes were secured with elastic straps and moistened with saline to ensure proper contact.

*Monitoring of Adverse Events*

tDCS is generally safe. However, participants were actively monitored for any adverse effects (e.g., skin redness, itching, burning sensation, headache, dizziness) during and after each tDCS session. Any reported adverse events were recorded.

*Data Collection*

Socio-demographic characteristics of the participants were recorded using a structured data collection sheet, capturing variables such as age, gender, marital status, education level,

**Table I: Socio-demographic and clinical characteristics of participants in the combined intervention and control groups**

| Characteristics | Combined Intervention | | Control | | p-value |
|---|---|---|---|---|---|
| | n (%) | Mean + SD | n (%) | Mean + SD | |
| Age (years) | | 56.70 ± 6.19 | | 56.87 ± 4.97 | 0.396 |
| 20-34 | 0 (0) | | 0 (0) | | |
| 35-49 | 3 (20) | | 2 (13.3) | | |
| 50-65 | 12 (80) | | 13 (86.7) | | |
| Gender | | | | | |
| Male | 11 (73.3) | | 10 (53.3) | | |
| Female | 4 (26.7) | | 5 (46.7) | | |
| Marital status | Single | 0 (0) | | 0 (0) | |
| Married | 9 (60.0) | | 12 (80) | | |
| Divorced | 5 (33.3) | | 2 (13.3) | | |
| Widow | 1 (6.7) | | 1 (6.7) | | |
| Residence | Rural | 8 (53.3) | | 9 (60) | |
| Urban | 7 (46.7) | | 6 (40) | | |
| Occupation | | | | | 0.090 |
| Small traders | 10 (66.7) | | 8 (53.3) | | |
| Civil servant | 3 (20.0) | | 4 (26.7) | | |
| Skilled labourers | 2 (13.3) | | 0 (0) | | |
| Unemployed | 0 (0) | | 3 (20.0) | | |
| Retired | 0 (0) | | 0 (0) | | |
| Education | | | | | 0.703 |
| Post-secondary | 4 (26.7) | | 4 (26.7) | | |
| Secondary | 7 (46.7) | | 5 (33.3) | | |
| Primary | 2 (13.3) | | 3 (20.0) | | |
| Non-formal | 2 (13.3) | | 3 (20.0) | | |
| None | 0 (0) | | 0 (0) | | |
| Laterality | | | | | 0.819 |
| Right hemiparesis | 13 (86.7) | | 12 (80.0) | | |
| Left hemiparesis | 2 (13.3) | | 3 (20.0) | | |
| SBP (mmHg) | | | | | 0.675 |
| < 140 | 7 (46.7) | | 10 (66.7) | | |
| > 140 | 8 (52.3) | | 5 (23.3) | | |
| DBP (mmHg) | | | | | 0.546 |
| < 90 | 14 (93.3) | | 12 (80) | | |
| > 90 | 1 (6.7) | | 3 (20) | | |
| BMI | | | | | 0.066 |
| Underweight | 0 (0) | | 0 (0) | | |
| Normal | 8 (53.3) | | 8 (53.3) | | |
| Pre-obesity | 7 (46.7) | | 7 (46.7) | | |
| Obesity | 0 (0) | | 0 (0) | | |
| Baseline SOD level (U/L) | - | 215.58 ± 53.60 | - | 198.04 ± 40.74 | 0.292 |
| MMSE | - | 23.27 ± 1.27 | - | 24.30 ± 1.09 | 0.432 |

Data analysed by paired t-test (within-group) and independent t-test (between-group). A *p-value of <0.05 was considered statistically significant.
BMI: body mass index, DBP: diastolic blood pressure, MMSE: Mini-Mental State Examination, SBP: systolic blood pressure, SD: standard deviation, SOD: superoxide dismutase.

**Table II: Within-group and between-group comparison of serum SOD levels pre- and post-intervention**

| Group | n | Serum SOD (U/L) Mean + SD | | Difference in serum SOD (U/L) | p-value | |
|---|---|---|---|---|---|---|
| | | Pre-intervention | Post-intervention | Mean + SD | Within-group | Between-group |
| Combined intervention | 15 | 215.58 ± 53.65 | 289.02 ± 64.94 | 73.44 ± 37.86 | 0.001* | 0.001* |
| Control | 15 | 198.04 ± 40.74 | 216.67 ± 45.02 | 18.63 ± 15.21 | 0.001* | |

Data analysed by paired t-test (within-group) and independent t-test (between-group). A *p-value of <0.05 was considered statistically significant.
SD: standard deviation, SOD: superoxide dismutase.

*Statistical Analysis*

Data were analysed using IBM SPSS 23.0 software. Descriptive statistics summarized demographic variables as means and standard deviations (SD). The Shapiro-Wilk test was employed to assess the normality of continuous variables. Paired t-tests compared pre- and post-intervention serum SOD levels within groups, while independent t-tests assessed between-group differences and associations with risk factors. A p-value of <0.05 was considered statistically significant.

## RESULTS

A total of 30 participants were included in the study, with 15 participants in each of the two groups. The mean age of participants was approximately 57 years. The age distribution varied, with the majority (80%) of participants in the combined intervention group aged 50–65 years,

**Table III: The effect of studied risk factors on serum SOD levels of participants**

| Variables | n (%) | Serum SOD (U/L) | Mean + SD | t-value | p-value |
|---|---|---|---|---|---|
| Age (years) | 35-49 | 5 (16.7) | 56.99 + 34.82 | 0.665 | 0.511 |
| | 50-65 | 25 (83.3) | 43.85 + 40.69 | | |
| BMI (kg/m$^2$) | 18.2-24.9 | 13 (43.3) | 53.23 + 47.10 | 0.885 | 0.398 |
| | 25.0-34.9 | 17 (56.7) | 40.54 + 33.18 | | |
| SBP (mmHg) | < 140 | 17 (56.7) | 41.72 + 32.33 | -0.671 | 0.508 |
| | > 140 | 13 (33.3) | 51.69 + 49.17 | | |
| DBP (mmHg) | < 90 | 26 (86.7) | 42.99 + 34.82 | -1.069 | 0.294 |
| | > 90 | 4 (13.3) | 65.87 + 68.69 | | |
| Laterality | Right hemiparesis | 24 (80) | 43.85 + 40.55 | 0.382 | 0.705 |
| | Left hemiparesis | 6 (20) | 40.45 + 40.58 | | |

Data analysed by an independent t-test. A *p-value of <0.05 was considered statistically significant.
BMI: body mass index, DBP: diastolic blood pressure, SBP: systolic blood pressure, SD: standard deviation, SOD: superoxide dismutase

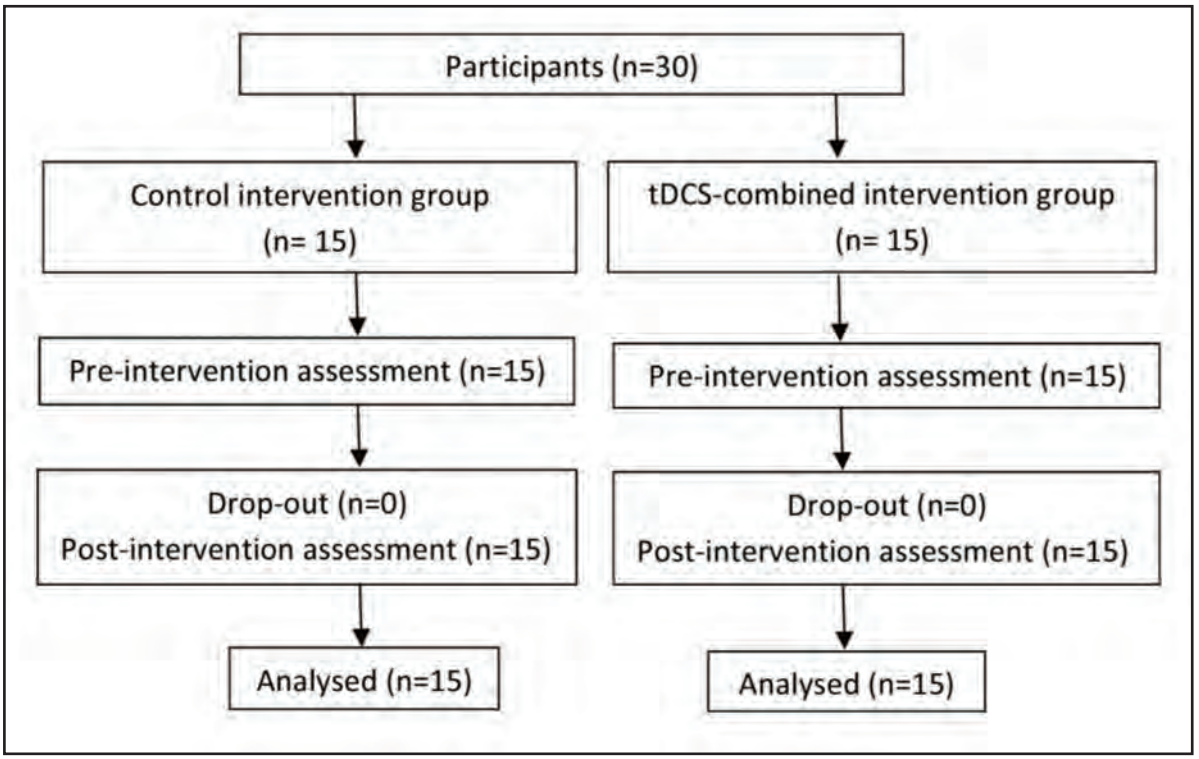


**Fig. 1:** Study flow chart

and residence. Blood pressure measurements were taken twice for each participant using validated and calibrated mercury and digital sphygmomanometers. The average values of systolic (SBP) and diastolic (DBP) blood pressure readings were recorded. Body mass index (BMI) was calculated based on each participant's weight and height. Weight was measured using a digital scale, with participants standing barefoot in the centre of the scale. Height was recorded against a calibrated wall-mounted stadiometer. BMI was then calculated using the standard formula: BMI = weight (kg) / [height (m)]$^2$.

*Serum SOD Measurement*

The serum SOD levels of stroke patients were measured both before and after the 4-week tDCS intervention using the Superoxide Dismutase (SOD) Activity Assay Kit. The microplate reader was preheated to 560 nm for 30 minutes to ensure stable wavelength detection. Working reagents (Reagents I, II, and V) were equilibrated in a water bath at 37°C for 5 minutes before use. Additionally, Reagent IV was mixed with Reagent V by oscillation to ensure proper dissolution immediately before application.

The assay utilized four types of tubes: two blank tubes (B1 and B2), a test tube (T), and a control tube (C). Reagents were added to the respective tubes according to the manufacturer's instructions, and the mixtures were thoroughly combined. The tubes were then incubated at 37°C for 30 minutes. After incubation, the solutions were transferred into an ultra-micro cuvette or a 96-well flat-bottom plate to measure absorbance at 560 nm using the microplate reader. Serum SOD concentrations were calculated based on the absorbance values using the manufacturer's formula:

SOD (U/mL) = [P÷ (1-P) ×Vrv] ÷Vs×F=11.11×P÷ (1-P) ×F

compared to only 20% in the control group within the same age category. Gender distribution showed a higher proportion of males in the tDCS group (73.3%) compared to the control group (53.3%).

Educational attainment among participants varied, with most having received non-formal, primary, secondary, or post-secondary education. Secondary school education was the most common, with 46.7% and 33.3% in the combined intervention and control groups, respectively. A large proportion of participants resided in rural areas (53.3% in the combined intervention group, 60% in the control group) and were married (80% in the combined intervention group, 60% in the control group). Small-scale trading was the primary occupation for most participants, comprising 66.7% in the combined intervention group and 53.3% in the control group.

The baseline serum SOD levels were comparable between the groups, with mean values of 215.58 ± 53.60 U/L for the combined intervention group and 198.04 ± 40.74 U/L for the control group ($p>0.05$). Most participants presented with right-sided hemiparesis, indicative of left hemispheric cerebral involvement. Elevated SBP was more common in the combined intervention group (52.7%) than in the control group (33.3%), while DBP was within the normal range (<90 mmHg) for most participants. The mean BMI was 24.23 kg/m² in the combined intervention group and 25.11 kg/m² in the control group. These baseline characteristics were recorded to ensure group comparability and to explore potential confounding influences on the primary outcome (serum SOD). Table I summarises the socio-demographic and clinical characteristics of participants of both groups.

Within the combined intervention group, serum SOD levels increased significantly from 215.58 ± 53.65 U/L at baseline to 289.02 ± 64.94 U/L post-intervention ($p<0.001$). Similarly, the control group showed a smaller but significant increase, with serum SOD levels rising from 198.04 ± 40.74 U/L to 216.67±45.02 U/L post-intervention ($p<0.001$) (Table II). A statistically significant increase in serum SOD levels was observed in the combined intervention group compared to the control group (mean increase of 73.44 U/L vs. 18.63 U/L, $p<0.05$) (Table III). No adverse events related to the tDCS or physiotherapy interventions were reported throughout the study period.

No statistically significant effects were observed for the studied risk factors, including age, SBP, DBP, stroke laterality, or BMI, on serum SOD levels ($p>0.05$). Younger participants (aged 35–49 years) had slightly higher serum SOD levels (56.99 U/L) than those aged 50–65 years (43.85 U/L). Participants with elevated SBP and DBP demonstrated slightly higher serum SOD levels (51.69 U/L and 65.87 U/L, respectively) compared to those with normal SBP and DBP (41.72 U/L and 42.99 U/L, respectively). Additionally, participants with normal BMI showed higher serum SOD levels (53.23 U/L) compared to those in the pre-obesity category (40.54 U/L) (Table IV).

## DISCUSSION

This pilot study demonstrated a statistically significant increase in serum SOD levels following a four-week intervention of combined tDCS and CIP, with a markedly greater improvement compared to CIP alone. The observed increase in serum SOD levels, approximately a 34% rise in the tDCS+CIP group compared to a 9.4% increase in the CIP group, may have substantial clinical relevance. Higher antioxidant capacity is theorised to mitigate neuronal damage in the ischemic penumbra, potentially slowing infarct expansion and creating a more favourable environment for neuroplasticity. This biochemical improvement could translate to enhanced recovery of cognitive and motor functions,[9,10] although direct correlation between SOD levels and functional outcomes requires larger study trials.

The synergistic effect observed in the tDCS+CIP group may be attributed to the complementary mechanisms of both therapies. While conventional physiotherapy promotes use-dependent plasticity through repetitive task training, anodal tDCS primes the motor cortex by increasing neuronal excitability and facilitating long-term potentiation (LTP)-like mechanisms, thereby enhancing the effects of subsequent physiotherapy.[12,20] This heightened cortical responsiveness may not only improve motor learning but also upregulate endogenous neuroprotective pathways, including the expression of antioxidant enzymes such as SOD. These findings are consistent with previous research demonstrating that tDCS reduces proinflammatory cytokines and promotes neurotrophic factor expression.[17,26,27] In our study, the significant increment in serum SOD after the intervention suggests that tDCS amplifies rehabilitation benefits at a biochemical level.[20,21,26] Despite challenges in comparing studies due to variations in stimulation protocols (such as electrode montage, current intensity, frequency, and session number), the consistent findings that neuroplasticity enhancement by tDCS through increased cortical excitability, synaptic plasticity modulation, current calcium increments, and oxidative stress regulation support its clinical relevance in neurological recovery.

There are no identified risk factors demonstrating a statistically significant association with changes in SOD levels. The gender distribution and BMI range in this study are consistent with previous research on stroke populations.[29] The slightly higher mean increase in SOD among younger participants (35-49 years) may reflect age-related declines in neuroplasticity or antioxidant regulatory mechanisms in older cohorts.[12] Similarly, participants with a normal BMI showed a greater, though non-significant, increase than those in the pre-obesity category, which aligns with literature suggesting obesity is associated with chronic inflammation and oxidative stress that may blunt intervention efficacy.[11] However, this contrasts with prior findings reporting increased levels of SOD isoenzymes (manganese (Mn) SOD and copper-zinc (CuZn) SOD) in obese individuals.[30] Regarding blood pressure, previous studies have reported significantly lower SOD activity in hypertensive patients compared to normotensive controls.[31,32] In contrast, our study found no association between blood pressure or stroke laterality and changes in SOD levels, which may be due to

the small sample size. The absence of significant effects from blood pressure and laterality suggests that the combined tDCS and physiotherapy intervention benefits were consistent across these clinical subgroups, indicating its potential broad applicability. Nonetheless, these trends should be interpreted cautiously and require validation in larger, more diverse cohorts.

This pilot study provides preliminary evidence supporting the potential benefits of tDCS in reducing oxidative stress among stroke patients. However, several limitations should be acknowledged. The small sample size and single-centre design may restrict the generalisability of the findings. Although assessor blinding was maintained for the primary outcome (serum SOD levels), the absence of participant blinding introduces the possibility of performance bias. The fixed stimulation parameters used may not reflect the full therapeutic potential achievable with varied intensities, session frequencies, or electrode placements. Furthermore, outcome assessment was limited to the four-week intervention, with no extended follow-up to evaluate the durability of effects. The study did not correlate serum SOD levels with cognitive function measures, and the variability in stroke subtypes lack systematic control, which may have confounded the findings. While no adverse events were reported, future studies should incorporate standardised monitoring tools to comprehensively assess safety.

To build on these findings, future studies should recruit larger and more diverse populations to enhance generalisability. An extended intervention period and long-term follow-ups would help elucidate the sustainability of the tDCS effects. Combining tDCS with other neuroplasticity-focused rehabilitation strategies may reveal synergistic benefits. Importantly, subsequent studies should integrate functional, cognitive, and quality-of-life assessments alongside biomarker analysis to better determine the clinical relevance of biochemical changes observed.

## CONCLUSION

This pilot study demonstrated that anodal tDCS, when combined with conventional therapy, increases SOD levels in ambulatory stroke patients. The findings highlight the potential of tDCS as a non-invasive and adjunctive therapeutic approach to reduce oxidative stress and support recovery in stroke rehabilitation. While these results are promising, further research with larger sample sizes, incorporating functional outcomes, and employing longer-term follow-up is necessary to validate the findings and optimise the clinical application of tDCS in managing post-stroke complications.

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# Comparing the risk of adverse effects of intrapartum and postpartum versus antepartum preeclampsia/eclampsia among women admitted at federal teaching hospital, Birnin Kebbi, northwest Nigeria

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## ABSTRACT

**Introduction: Preeclampsia is a multi-organ system disorder that is responsible for a significant rate of maternal and perinatal morbidity and mortality worldwide. Though many studies have worked on the risk factors, there is a limited study on the adverse maternal outcomes highlighting the comparison between antepartum preeclampsia/eclampsia (APE/E), intrapartum preeclampsia/eclampsia (IPE/E) and postpartum preeclampsia/eclampsia (PPE).**

**Materials and Methods: A retrospective cohort study was conducted by reviewing records of gestational induced hypertension patients who progressed to having preeclampsia/eclampsia at admitted at the Federal Teaching Hospital, Birnin Kebbi, between January 2009 and December 2019.**

**Results: The multinomial logistic final model identified six maternal adverse outcomes of preeclampsia/eclampsia: stillbirth, (ARR 0.86 and 0.31, respectively), preterm delivery (ARR 8.55 and 3.10, respectively), induction of labour (ARR 0.39 and 0.36, respectively), maternal death (ARR 12.28 and 8.75, respectively), low birth weight (ARR 0.09 and 0.11, respectively) and convulsion (ARR 8.17 and 8.22, respectively).**

**Conclusion: These study findings offer valuable insights into the adverse maternal outcomes of preeclampsia and eclampsia within the Nigerian context. It serves as a potential research project that can be applied to the clinical setting to help clinicians manage preeclamptic and eclamptic patients better.**

**KEYWORDS:**
*Antepartum preeclampsia/eclampsia, intrapartum preeclampsia/eclampsia, postpartum preeclampsia/eclampsia, multinomial logistic regression, adverse maternal outcome*

## INTRODUCTION

Pre-eclampsia is a pregnancy disorder; it is one of the most serious acute complications of pregnancy, which is the most serious of the hypertensive disorders of pregnancy.[1-3]

Pre-eclampsia (PE) is a common disorder that particularly affects first pregnancies, though the causes, placental and maternal factors, vary among individuals. It has various types of clinical presentation, and most women are asymptomatic. The disease is often diagnosed during routine antenatal care; it is also one of the complications of pregnancy that results in foetal and maternal morbidity and mortality.[4-7]

Preeclampsia is characterised by new onset of hypertension and proteinuria or by hypertension accompanied by significant end–organ dysfunction with or without proteinuria after 20 weeks of gestation or postpartum in a previously normotensive patient. However, it is important to note that a diagnosis can still be made in the absence of proteinuria if the new-onset hypertension presents with specific signs or symptoms of significant end-organ dysfunction.[8]

The International Society for the Study of Hypertension in Pregnancy (ISSHP) in 2021 defined Pre-eclampsia (de novo) as gestational hypertension accompanied by one or more of the following new onset conditions at ≥ 20 weeks of gestation: (i) Proteinuria, (ii). Other maternal end-organ dysfunction, including: a) Neurological complications (e.g., eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, or persistent visual scotomata), (b) Pulmonary oedema, (c) Haematological complications (e.g., platelet count < 150,000/μL, DIC, haemolysis), (d) AKI (such as creatinine ≥ 90 μmol/L or 1 mg/dL), (e) Liver involvement (e.g., elevated transaminases such as ALT or AST > 40 IU/L) with or without right upper quadrant or epigastric abdominal pain).[9]

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Preeclampsia could progress to eclampsia, characterised by seizures, and other complications such as kidney or liver damage and ultimately death of the mother and/or foetus if left untreated, and it is one of the serious obstetric emergencies. [10,11]

[2,10,12,13] observed that 10% of all pregnancies globally are complicated by hypertension, with preeclampsia and eclampsia (PE and E) being the major causes of maternal and perinatal morbidity and mortality, with an expected 62,000–77,000 deaths per year.[3] also noted that the global prevalence of all pre-eclampsia in the years 2002–2010 was approximated at 4.6% of deliveries. Preeclampsia and eclampsia have an incidence ranging from 2-5% of pregnancies in Western Europe and North America to as high as 18% in parts of Africa.[14,15] With a prevalence range of 1.8 to 16.7% in developing countries, and an occurrence of 10% of pregnancies in African women.[3]

Furthermore [16] acknowledged that PE is a major cause of almost a third of a million maternal deaths in low and middle-income settings and also causes more than six million perinatal deaths, approximately eight million preterm births and almost 20 million low birthweight infants in developing nations; Additionally, pre-eclampsia and its adverse outcomes have been linked to higher risks of chronic non-communicable diseases (NCDs) in later life, thus creating a huge challenge within the context of double burden and limited resources in the developing world.

In West Africa, PE and E account for 15-25% of maternal deaths in Ghana, while in Nigeria, they account for a high maternal mortality ratio of 512 per 100,000 live births and a high infant mortality rate (67 deaths per 1000 live births).[1]

Despite the high rate of pre-eclampsia and eclampsia-related maternal and perinatal morbidity and mortality in developing countries like Nigeria, there are limited studies on its maternal outcome, especially in the northern part of Nigeria. Researchers have attempted to examine the global trend, severity and risk factors of PE and E, but almost all these studies used binary logistic regression and other statistical analyses.

In this study, preeclampsia/eclampsia (PE/E) was classified according to the study by [17], which stated that PE/E has been traditionally divided into three groups: antepartum preeclampsia/eclampsia (APE/E), intrapartum preeclampsia/eclampsia (IPE/E), and postpartum preeclampsia/eclampsia (PPE/E). APE/E occurs during pregnancy. Intrapartum PE begins when labour starts and ends following the third stage of labour, while postpartum PE, which has traditionally been defined as new-onset PE 48 hours after delivery to 6 weeks postpartum

Thus, the multinomial logistic regression analysis is used in this study to investigate maternal outcomes comparing IPE/E and PPE/E versus APE/E.

## MATERIALS AND METHODS

A retrospective cohort study research design was used to comprehensively review 131 preeclampsia/eclampsia (PE/E) patients in Federal Teaching Hospital (FTH), formerly known as the Federal Medical Centre (FMC), Birnin Kebbi, between January 2009 and December 2019. Data analysed included demographic data, clinical presentation, medical treatment received, method and outcome of delivery, maternal outcome and discharge data. Data was filled in a data proforma anonymously. All confirmed preeclampsia and eclampsia patients who had no previous history of hypertension were included in the study. Gestational Induced Hypertension (GIH) patients who did not have PE/E and PE/E patients who died before they got to the hospital were excluded from the study. All the patients included in the study were not less than 17 years old at diagnosis.

Data was collected from the Health Information Management department based on the patient's demographics, booking status, parity and adverse outcomes of preeclampsia/eclampsia. FTH is the main tertiary health centre in Kebbi State, and it serves as a referral centre to all primary and secondary health centres in the state. The calculation of sample size was done using G Power Sample size calculation software, with a significance level (alpha) of 0.05 and a power of the study (1-β) of 90%. An estimated 10% was added in anticipation of missing data or loss to follow-up. The final sample size was calculated as 131 patients. The Multinomial logistic regression is one of the generalised linear models, which shows an extension of the binary logistic regression model. It is generally used in cases where response variables have more than two categories or levels. The explanatory variable (s) of the regression model can be either continuous, categorical or both.

*Statistical Analysis*

The statistical analysis was done using STATA SE version 14.0.[18] Descriptive statistics were used to examine the distribution of patients' demographic characteristics. The multinomial logistic regression (MLOGIT) model was applied to estimate the relationship between the adverse maternal outcomes and preeclampsia/eclampsia (antepartum, intrapartum and postpartum), the univariable and multivariable analyses, respectively. For the univariable analysis step of (MLOGIT), simple MLOGIT was used to identify potential adverse maternal outcomes. Covariates with a significant level (p-value) ≤0.25 were selected for inclusion in subsequent multivariable modelling.[19]

The multivariable MLOGIT model was applied to estimate the relationship between adverse maternal outcomes and preeclampsia/eclampsia (antepartum, intrapartum and postpartum). The parsimonious model, including the statistically significant and clinically plausible variables, was chosen in this study. Linearity was not done due to the absence of numerical variables, though multicollinearity was done by evaluating the correlation matrix, standard errors, p-value and confidence interval. Omission of correlated variables (PPH and placenta abruptio) with high confidence intervals was done. Subsequently, potential two-way biologically or clinically meaningful interaction terms between significant independent variables were evaluated,

**Table I: Demographic characteristics of preeclamptic/eclamptic patients in FTH Birnin Kebbi for multinomial regression analysis**

| Demographic variables | Frequency (n) | Percentage (%) | Mean (SD) |
|---|---|---|---|
| Age* | | | 23.45 (5.42) |
| Tribe / Ethnicity | | | |
| HAUSA | 78 | 59.54 | |
| IBO | 17 | 12.98 | |
| YORUBA | 11 | 8.40 | |
| OTHERS | 25 | 19.08 | |
| SOCIO-ECONOMIC STATUS | | | |
| Low | 98 | 74.81 | |
| Medium | 33 | 25.19 | |
| EDUCATION LEVEL | | | |
| Illiterate | 95 | 73.28 | |
| Literate | 35 | 26.72 | |
| BOOKING STATUS | | | |
| Booked | 44 | 33.59 | |
| Unbooked | 87 | 66.41 | |
| PARITY | | | |
| Nulliparous | 89 | 67.94 | |
| Multiparous | 42 | 32.06 | |
| MULTIPLE PREGNANCY | | | |
| Yes | 11 | 8.40 | |
| no | 120 | 91.60 | |
| PE/E DIAGNOSIS | | | |
| APE | 88 | 67.18 | |
| IPE | 15 | 11.45 | |
| PPE | 28 | 21.37 | |

SD = standard deviation, *normally distributed

and no interaction was found. The overall fitness of the model was assessed, and the model was found to be fit.

Model adequacy was done using the Hosmer-Lemeshow test, Pearson chi-square, classification table and Area under ROC curve. Statistical results were reported as adjusted Relative Risk Ratios (ARRs) and corresponding 95% confidence intervals (CIs). A p-value of <0.05 was regarded as significant.[19]

*Ethics Approval*

This study was approved by the ethics committee of the Federal Medical Centre (FMC), now known as Federal University Teaching Hospital (FTH), Birnin Kebbi and the Universiti Zainal Abidin Human Research Ethics Committee (UHREC). The patient consent form was not mandatory for the ethics approval from FTH Birnin Kebbi, due to the non-interventional nature of the study and secondary data collection, with all data being anonymous.

## RESULTS

The results from Table I, which presents the characteristics of PE/E patients in FTH, Birnin Kebbi, show that respondents numbering 131 had a mean (standard deviation) age of 23.45 (5.42) years. 78(59.54%) were Hausas, 98(74.81%) had low socioeconomic status, 35(26.72%) were literate, 89(67.94%) were nulliparous, 11 (8.4%) had multiple pregnancies, and only 44 (33.59%) were booked in the antenatal clinic (ANC). 88 (67.94%) had APE, 15 (11.45%) had IPE, and 28 (21.37%) had PPE.

The univariable analysis of the multinomial regression model of maternal adverse outcomes of preeclampsia/eclampsia in FTH Birnin Kebbi, Kebbi State, results show the adverse outcomes of preeclampsia/eclampsia: logit one (intrapartum preeclampsia/eclampsia versus antepartum preeclampsia/eclampsia) and logit two (postpartum preeclampsia/eclampsia versus antepartum preeclampsia/eclampsia). The adverse outcomes of preeclampsia/eclampsia included in the univariable analysis are: Blurring of vision, PPH, stillbirth, preterm delivery, induced labour, maternal death, placenta abruptio, low birth weight and convulsion. All the factors that had a p-value ≤ 0.25 in both logit one and logit two were included in the multivariable analysis.

Results from the fitted model of multinomial regression for adverse maternal outcomes of PE/E in the FTH Birnin Kebbi are presented in Table II. The table reports adjusted relative risk ratios (ARRs), associated 95% confidence intervals (CIs) and p-values. APE was used for the baseline or reference category in the logit models one and two. IPE and APE were paired in the first logit, and PPE and APE were paired in the second logit; this resulted in two equations in one model fit. Six statistically significant variables from the multinomial regression univariable model were analysed, and all were found to be statistically significant based on p-value <0.05 and have been reported in the paper.

**Table II: Adverse maternal outcome of Preeclampsia/eclampsia (intrapartum and postpartum preeclampsia/eclampsia) logit function 1 and 2**

| Logit function | Variable | Regression coefficient (β) | Adjusted rrr (95% CI) | P value |
|---|---|---|---|---|
| 1 | PPH | | | |
| | no | 1 | 0.00 | |
| | yes | 3.07 | 0.05 (0.01, 0.44) | 0.007 |
| | STILLBIRTH | | | |
| | no | 1 | 0.00 | |
| | yes | 0.15 | 0.86 (0.29, 2.55) | 0.791 |
| | PRETERM | | | |
| | no | 1 | 0.00 | |
| | yes | 2.14 | 8.55 (2.93, 24.96) | <0.001 |
| | INDUCED LABOUR | | | |
| | no | 1 | 0.00 | |
| | yes | 0.95 | 0.39 (0.18, 0.85) | 0.018 |
| | MATERNAL DEATH | | | |
| | no | 1 | 0.00 | |
| | yes | 2.51 | 12.28 (3.72, 40.46) | <0.001 |
| | LOWBIRTH WEIGHT | | | |
| | no | | | |
| | yes | 2.46 | 0.09(0.03, 0.29) | <0.001 |
| | CONVULSION | | | |
| | no | 1 | 0.00 | |
| | yes | 2.10 | 8.17 (3.24, 20.62) | <0.001 |
| 2 | PPH | | | |
| | no | 1 | 0.00 | |
| | yes | 2.64 | 0.07 (0.01, 0.67) | 0.02 |
| | STILLBIRTH | | | |
| | no | 1 | 0.00 | |
| | yes | 1.16 | 0.31 (0.10, 0.93) | 0.04 |
| | PRETERM DELIVERY | | | |
| | no | 1 | 0.00 | |
| | yes | 1.13 | 3.10 (1.35, 7.15) | 0.008 |
| | INDUCED LABOUR | | | |
| | no | 1 | 0.00 | |
| | yes | 1.00 | 0.36 (0.17, 0.80) | 0.012 |
| | MATERNAL DEATH | | | |
| | no | 1 | 0.00 | |
| | yes | 2.17 | 8.75 (2.61, 29.32) | <0.001 |
| | LOW BIRTH WEIGHT | | | |
| | no | 1 | 0.00 | |
| | yes | 2.23 | 0.11(.03, 0.37) | <0.001 |
| | CONVULSION | | | |
| | no | 1 | 0.00 | |
| | yes | 2.11 | 8.22 (2.91, 23.17) | <0.001 |

- Multinomial logistic regression was applied
- Interaction was not reported
- RRR= relative risk ratio, CI = confidence interval, b: Regression coefficient; Adjusted RRR= Adjusted relative risk ratio.
- Overall fitness of the model was checked and reported to be Hosmer-Lemeshow test (logit function 1=P = 0.783; logit function 2 = P = 0.827), Pearson chi square test (logit function 1: P = 0.886; logit function 2: P = P = 0.884), overall correctly classified percentage (logit function 1: 82.01%; logit function 2; 82.76%), Area under the ROC curve (logit function 1: 0.879; logit function 2: 0.837).

**Table III: Overall Fit of the Model**

| | Logit function 1 | Logit function 2 |
|---|---|---|
| Hosmer –Lemeshow test | P = 0.783 | P = 0.827 |
| Pearson chi-square test | P = 0.886 | P = 0.884 |
| Classification table correctly classified | 82.01% | 82.76% |
| Area under the ROC curve | 0.879 | 0.837 |

## DISCUSSION

Pre-eclampsia is the most severe form of hypertensive disorders in pregnancy and is a major contributor to maternal and fetal morbidity and mortality worldwide.[3] It remains one of the most serious causes of adverse pregnancy outcomes, particularly in developing countries where access to timely and adequate healthcare is often limited.[1,2]

This hospital-based retrospective study was performed to identify the adverse maternal outcomes among Preeclamptic/eclamptic patients, and the results obtained above show that most patients (82.25%) were Hausa by tribe. The majority of the women (88.75%) had low socioeconomic status, 85.4% were illiterate, and 81.7% of the women had no antenatal care (ANC) booking. This may be because most of the patients are from low-resource settings with financial constraints. This gives an indication that most women having a high risk of the adverse outcome of preeclampsia/eclampsia in Kebbi State are Indigenous young, uneducated women, mostly from rural areas, on referral to FTH Birnin Kebbi.[20,21]

Adverse maternal outcomes of preeclampsia/eclampsia can be severe, leading to maternal and perinatal morbidity and mortality.[4,22] This study identified stillbirth as one of the adverse maternal outcomes of PE/E. Similarly, studies by [12,23-26] have all confirmed stillbirth as an adverse maternal outcome of PE/E. The findings of this study also noted preterm delivery as another adverse maternal outcome of PE/E. This result was corroborated by [24], which attests that the high prenatal mortality of PE/E mothers was a result of preterm delivery.[26] also affirmed preterm delivery as an adverse outcome of PE/E. The following were other adverse maternal outcomes of PE/E as found out in this study, which were consistent with the results of previous works: Induction of labour [26,27], maternal death [12, 24, 25, 27], low birth weight 26 and convulsion.[12,26]

*Comparison of IPE and PPE VERSUS APE*

Studies have consistently shown that stillbirth is a major adverse outcome associated with Preeclampsia/eclampsia globally.[28-30] In line with these inclinations and previous local research in Nigeria.[31,32] This study finds that IPE and PPE have a lower risk of stillbirth compared to APE (RRR 0.86 and 0.31, respectively). This comparison indicates that pregnant women with APE face a higher risk of stillbirth than those with IPE and PPE.

This study compared the results of IPE and PPE versus APE as it relates to the outcome of preterm delivery. Globally, most studies emphasised preterm delivery or prematurity as an adverse outcome of preeclampsia/eclampsia.[33] Consistent with these trends and previous local studies in Nigeria and other Sub-Saharan countries [4,24,26], our study found that women with IPE and PPE had a higher risk of preterm than women with APE (ARRR 8.55 and 3.10, respectively). Thus, the outcome of this research is emphasising preterm delivery as an adverse maternal outcome of PE/E

There is increasing evidence suggesting that most eclamptic mothers undergo induction of labour to terminate their pregnancy due to foetal distress.[26,27] In this study, women with IPE and PPE who experienced induced labour had a lower risk of induction compared to women with APE (ARR 0.39 and 0.36, respectively). This indicates that pregnant women with APE face a higher risk of requiring induction of labour compared to those with IPE and PPE. The difference in the likelihood of undergoing induction of labour as an IPE or PPE mother is 3% according to this study.

In our study, women with IPE and PPE had a higher risk of maternal death than women with APE (ARRR 12.28 and 8.75, respectively). Though the direct risk comparison of APE, IPE, and PPE has not been observed in literature, a study conducted in Canada revealed that PPE was strongly associated with pregnancy complications, which could lead to maternal mortality.[33] Other studies from East and Northern Africa also noted maternal death as an outcome of PE/E, a Nigerian study indicated that maternal death could be due to lack of early presentation at the hospital. [10,21,27]

The issue of low birth weight in babies is a global phenomenon. It was noted in a study that 50% of the newborns have a low birth weight (1.5 - 2.5 kg), which was consequent with their premature birth and intrauterine growth retardation (IUGR).[34] This suggests that low birth weight in PE/E mothers is not only subsequent to the disease as a primary cause, but also other associated factors like prematurity/preterm delivery and IUGR. It was observed in this study that women with IPE and PPE had a lower risk of low-birth-weight babies compared to APE (ARRR 0.09 and 0.11, respectively). Although no comparison of IPE and PPE mothers has been observed in the literature, a study reported that nearly half (40.1%) of babies born to eclamptic mothers had low birth weight.[26]

Convulsion in a non-epileptic pregnant woman is a sign of eclampsia, as observed by.[8] This study observed that IPE and PPE had a higher risk of convulsion compared to APE (ARR 8.17 and 8.22, respectively). This reflects that eclampsia could be antepartum, intrapartum or postpartum and the difference in the risk of convulsing as an IPE mother and a PPE mother in FTH Birnin Kebbi is 0.05 times, which is a small difference, emphasising that there are almost equal chances of having eclampsia at any stage of pregnancy. Though a study observed different findings from this study, among 250 patients diagnosed with eclampsia, 148 patients had the seizures antenatally, 2 patients had the seizures intra-partum, and 100 patients had the seizures after delivery.[27] Another study from Tanzania, which differs from our findings, recorded that Convulsions in patients with eclampsia were classified as antepartum (44%), intrapartum (42%) and postpartum (15%) convulsions most commonly occurred after the onset of labour or within 24 hours after birth of the eight women (15%) with postpartum convulsions, seven women (88%) developed eclampsia within 24 hours, one woman (1%) had convulsions 1 week after delivery (late post-partum eclampsia).[35]

## CONCLUSION

This retrospective cohort study reported adverse maternal outcomes of preeclampsia/eclampsia patients in FTH, Birnin Kebbi, comparing the risk of IPE and PPE versus APE. It was

observed that women with IPE and PPE had a lower risk of stillbirth, induction of labour and low birth weight and a higher risk of preterm delivery, maternal death and convulsion than women with APE. The findings represent Kebbi state, as FTH, Birnin Kebbi, is the major tertiary hospital in Kebbi state, North West, Nigeria. This study suggests policy interventions to enhance health and medical interventions for women in the childbearing age group in the less developed areas of the northern states and the country at large. Awareness of preeclampsia should be raised by healthcare providers and policy makers in the antenatal clinics and through the mass media, focusing on younger women, low wealth status, and those living in rural areas, emphasising on preceding symptoms, high complication and mortality rate.

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ORIGINAL ARTICLE

# The effects of quercetin on lipid profile, inflammatory biomarkers and ACE2 in a dyslipidaemic rabbit model

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## ABSTRACT

**Introduction: Dyslipidaemia is a major cardiovascular risk factor associated with elevated low-density lipoprotein (LDL) cholesterol and triglyceride levels. While statins are the primary treatment, inflammation remains a significant predictor of cardiovascular events, highlighting the need for adjunctive therapies targeting both lipids and inflammation. Quercetin, a flavonoid with antioxidant and anti-inflammatory effects, has shown promise in preclinical models, although clinical results are mixed. This study investigates the effects of quercetin on lipid profiles, inflammatory biomarkers (bradykinin, C-reactive protein [CRP], interleukin-6 [IL-6]) and angiotensin-converting enzyme 2 (ACE2) levels in a rabbit model of dyslipidaemia, exploring its potential as an adjunctive therapy for dyslipidaemia.**

**Materials and Methods: Twenty male New Zealand White rabbits were randomly assigned to four groups (n=4): control, high-fat diet (HFD), HFD with quercetin (20 mg/kg/day), and HFD with atorvastatin (0.43 mg/kg/day, positive control). Rabbits received either a standard or HFD (1.2% cholesterol, 10% coconut oil) alone or HFD with quercetin or atorvastatin co-administered orally for 12 weeks. Blood samples were collected pre- and post-treatment for serum analysis. Body weight was measured weekly, and serum lipid profiles (total cholesterol, triglycerides, high-density lipoprotein [HDL], and LDL) were measured post-treatment. Serum levels of bradykinin, IL-6, CRP, and ACE2 pre- and post-treatment were quantified using enzyme-linked immunosorbent assay.**

**Results: The HFD significantly increased body weight, total cholesterol, triglycerides, and LDL cholesterol in rabbits, while atorvastatin and quercetin co-treatments effectively reduced total and LDL cholesterol levels ($p < 0.05$) but had no impact on body weight. Neither HFD nor treatments significantly altered HDL cholesterol, bradykinin, IL-6, CRP, or ACE2 levels after 12 weeks. Changes in these inflammatory and enzymatic markers from pre-treatment to post-treatment were also not significant across groups.**

**Conclusion: Quercetin demonstrated lipid-lowering effects, particularly on total and LDL cholesterol, in a rabbit model of dyslipidaemia. However, it did not significantly affect systemic inflammatory or enzymatic markers. These findings suggest potential lipid-lowering effects independent of inflammation. While quercetin was not superior to atorvastatin, it shows promise as a natural adjunct or alternative therapy.**

**KEYWORDS:**
*Quercetin, hyperlipidaemia, bradykinin, inflammation, flavonoid*

## INTRODUCTION

Dyslipidaemia is a well-established risk factor for cardiovascular disease, often identified only after patients experience acute vascular complications such as ischaemic heart disease or stroke. The association between high cholesterol levels and an increased risk of cardiovascular events is primarily attributed to elevated low-density lipoprotein (LDL) cholesterol.[1] High-density lipoprotein (HDL) cholesterol, initially thought to be inversely related to coronary heart disease (CHD) mortality[2], has since been shown to have more complex associations.[3,4] Furthermore, triglyceride (TG) reduction is linked to a decreased risk of major vascular events in randomised controlled trials; however, this effect is weaker than that of LDL cholesterol.[5]

Current guidelines for managing dyslipidaemia emphasise lowering LDL cholesterol levels as a central strategy, supported by the introduction of new therapeutic agents. Statins remain the first-line treatment for LDL cholesterol reduction in a broad population, including those undergoing primary or secondary prevention. However, for some patients, achieving LDL cholesterol targets may necessitate combination therapy.[6]

While the link between inflammation and atherosclerosis is well-documented[7], the association between systemic inflammatory indices and hyperlipidaemia has been observed but remains insufficiently supported by evidence

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(8). Recent analyses of randomised controlled trials revealed that, among statin-treated patients, inflammation—as measured by high-sensitivity C-reactive protein (CRP)—is a stronger predictor of future cardiovascular events and mortality than cholesterol levels assessed by LDL cholesterol. These findings underscore the importance of exploring adjunctive therapies beyond statins and suggest that combined lipid-lowering and anti-inflammatory treatments may further reduce atherosclerotic risk.[9]

Flavonoids, plant-derived polyphenolic compounds, have gained attention due to their dual antioxidant and anti-inflammatory properties. Quercetin, a flavonoid-rich phytochemical found in foods such as tea, broccoli, onions, papaya and Parkia speciosa,[10-12] has shown promise in preclinical studies. While preclinical studies in rat models of metabolic disease have demonstrated the beneficial effects of synthetic quercetin on diabetes and dyslipidaemia,[13-15] clinical findings remain inconsistent. For instance, a double-blind randomised trial involving women with type 2 diabetes demonstrated that ten weeks of quercetin supplementation significantly reduced systolic blood pressure but had no significant effect on lipid profiles or inflammatory markers (TNF-α, hs-CRP, interleukin-6 [IL-6]).[16] Similarly, another randomised trial in post-myocardial infarction patients found that eight weeks of quercetin supplementation increased total antioxidant capacity but failed to impact inflammatory markers or blood pressure.[17] The limitations of previous clinical studies such as small sample sizes and varying disease contexts underscore the need for more targeted research specifically focused on dyslipidaemia. Animal models provide a standardised disease context, allowing greater experimental control and reproducibility. Among these, rabbits are considered the most suitable model for human dyslipidemia and atherosclerotic disease because they possess cholesteryl ester transfer protein, which is absent in rats and mice. Moreover, rabbits share key similarities with humans in lipoprotein metabolism and demonstrate heightened sensitivity to high-cholesterol diets, making them invaluable for translational research on dyslipidaemia and atherosclerosis.[18] Therefore, the use of rabbit models offers distinct advantages for investigating lipid-related disorders. Such research could yield important insights into the therapeutic potential of quercetin as an adjunctive treatment for dyslipidaemia and its associated inflammatory processes.

This study aimed to evaluate the effects of quercetin on serum lipid profile as well as the inflammatory biomarkers specifically bradykinin, CRP, and IL-6 in a rabbit model of dyslipidemia. Additionally, this study investigates the levels of angiotensin-converting enzyme 2 (ACE2), a cardiovascular protective enzyme known to metabolise the bradykinin derivative des-arg9-bradykinin, within this dyslipidaemic model. It was hypothesised that quercetin could potentially serve as a natural adjunct to conventional lipid-lowering therapy due to its anti-inflammatory properties.

## MATERIALS AND METHODS

Quercetin hydrate was purchased from the manufacturer (Merck KGaA, Germany). Atorvastatin (Lipitor®) was purchased from pharmacy, marketed by the pharmaceutical company (Pfizer Inc., USA). A commercial rabbit diet pellet (Gold coin, Malaysia), cholesterol (Pharmaceutical Secondary STD) and hydrogenated coconut oil (MELBIO, Malaysia) were used in this study.

*Experimental Animals and Study Design*

The sample size was determined using the 'resource equation' method, where the degree of freedom (E) in the analysis of variance (ANOVA) should range from 10 to 20.[19] E = (Total number of animals) – (Total number of groups) = (4 animals per group x 4) - 4 = 12. Since E falls within the acceptable range, and given the use of a medium-sized animal with no mortality observed in this study, a sample size of n = 4 is considered justified. After one week of acclimatization, 20 male New Zealand White rabbits weighing 2.2 kg to 2.4 kg were randomly allocated to four groups, each containing four rabbits (n=4): control, high fat diet (HFD), HFD with concurrent treatment of 20 mg/kg body weight/day quercetin (HFDQ), and HFD with concurrent treatment of 0.43 mg/kg body weight/day atorvastatin (HFDA). Atorvastatin served as the positive control. Except for the control group, which received a standard rabbit diet, the rabbits in the other three groups were fed an HFD consisting of a standard diet supplemented with 1.2% cholesterol and 10% coconut oil for 12 weeks. This dietary regimen and its duration fall within the range of HFDs employed in many studies using hamsters—another well-established hyperlipidaemic animal model with lipid metabolism comparable to that of rabbits and humans.[20] Quercetin and atorvastatin were administered orally, directly via a 1-mL syringe to their respective groups as co-treatments alongside the HFD (abbreviated as HFDQ and HFDA, respectively) during this period. The quercetin dose was selected based on a previous study by Milenković et al.,[21] while the atorvastatin dose was calculated using the animal equivalent dose from a standard human dosage for atorvastatin. Normal saline and corn oil were used as vehicles for atorvastatin and quercetin, respectively. All the animals were provided with 120 g of diet per day and kept under a circadian rhythm of 12 hours of light and 12 hours of dark with free access to drinking water. This study was approved by the Universiti Sultan Zainal Abidin (UniSZA) Animal and Plant Research Ethics Committee (UAPREC/07/011).

*Blood Collection and Serum Preparation*

Blood samples were withdrawn from the ears or legs after overnight fasting, both before the treatment period (pre-treatment) and at the end of the experiment (post-treatment). All blood samples were centrifuged at 4 °C for 10 minutes at 3000 g. Serum (the supernatants) was collected and stored at –80 °C until further biochemical analysis (lipid profile, inflammatory biomarkers and ACE2). At the end of the experiment, the rabbits were euthanised via carbon dioxide inhalation.

*Body Weight Monitoring*

Body weight was measured weekly using a calibrated digital weighing machine (Tanita, Japan).

*Serum Lipid Profile*

Serum was sent to the Innoquest Pathology (formerly Gribbles Pathology) laboratory for analysis of serum total cholesterol (TC), TGs, HDL, LDL, and non-HDL cholesterol using the colourimetric test method principle. The technical

personnel at Innoquest Pathology were blinded to the group allocations.

*Serum Inflammatory Biomarkers and ACE2 Quantification*
Serum was sent to the Biomics Solution laboratory for analysis. The levels of serum bradykinin, IL-6, CRP and ACE2 were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Houston, TX, United States) as per the manufacturer's protocol. Each sample was run in duplicate for technical replication. The technical personnel at Biomics Solution were blinded to the group allocations.

*Statistical Analysis*
Data were tested for normality using the Shapiro–Wilk test. Normally distributed data were analysed using parametric tests, while non-normally distributed data were analysed using non-parametric tests. For the body weight measurements, which were normally distributed, one-way ANOVA was used to assess differences among groups across time points, followed by the Tukey HSD post-hoc test for multiple comparisons. Data were presented as mean ± SD for parametric variables. The blood parameters were not normally distributed and therefore were analysed using non-parametric tests. For lipid profile parameters—TC, TG, HDL, LDL and non-HDL cholesterol—comparisons among the four experimental groups (Control, HFD, HFDQ and HFDA) were made using the Kruskal–Wallis H test. When significant differences were found ($p<0.05$), pairwise comparisons were conducted using the Mann–Whitney U test. For inflammatory and vasoactive markers—IL-6, CRP, bradykinin, and ACE2—within-group (pre vs. post) changes were analysed using the Wilcoxon signed-rank test, while between-group differences in change values (Δ = post – pre) were analysed using the Kruskal–Wallis test. Data were presented as median (interquartile range [Q1–Q3]) for non-parametric variables. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 24.0 (SPSS Inc, Chicago, IL, USA).

## RESULTS

*Body weight*
There was an increase in body weight among rabbits in all groups throughout the study period (Figure 1). The HFD group showed a significantly higher body weight compared to the control group from week 4 to week 9 ($p = 0.004–0.048$). Co-treatment with atorvastatin or quercetin did not prevent this weight gain. However, the difference in body weight between the HFDA and the control group was not significant. Body weight was significantly higher in the HFDQ group compared to the control group. There was no significant difference between the HFDA and HFDQ groups.

*Serum Lipid Profile*
Descriptive analysis, presented as median (interquartile range [Q1–Q3]) values of serum TC, LDL, and non-HDL cholesterol across treatment groups, together with inferential statistics for group comparisons, are shown in Table I. The Kruskal–Wallis test showed significant group differences for TC (p=0.005), LDL (p=0.004), and non-HDL cholesterol (p=0.004), while TG (p=0.068) and HDL (p=0.254) were not significantly different among groups (Table I).

Post-hoc pairwise comparisons using the Mann–Whitney U test showed that the HFD group had significantly higher TC (p=0.021), LDL (p=0.021), and non-HDL (p=0.020) levels compared with the Control group. Both HFDQ and HFDA groups demonstrated significantly lower TC (p=0.021), LDL (p=0.021), and non-HDL (p=0.020) levels compared with the HFD group. No significant difference was observed between HFDQ and HFDA in TC (p=0.248), LDL (p=0.083) and non-HDL ($p = 0.083$) (Table II).

*Serum Inflammatory Markers and Vasoactive Markers (IL-6, CRP, Bradykinin, ACE2)*
Within-group comparisons using the Wilcoxon signed-rank test showed no significant pre–post changes in serum bradykinin, ACE2, CRP, or IL-6 levels in any of the groups ($p = 0.068–1.44$). Descriptive data for each group is presented as median (interquartile range [Q1–Q3]) (Table III).

Between-group comparisons of the change values (Δ = post – pre) using the Kruskal–Wallis H test also showed no significant differences for bradykinin ($p = 0.362$), ACE2 ($p = 0.803$), CRP ($p = 0.750$), or IL-6 ($p = 0.907$). Descriptive statistics for the change values are expressed as median (interquartile range [Q1–Q3]) (Table IV).

## DISCUSSION

In this study, a dyslipidaemic model was successfully established, evidenced by elevated TC and LDL levels induced by an HFD. Elevated LDL cholesterol is an established key driver of cardiovascular risk.[1] Meanwhile, HDL-C cholesterol, once thought to lower CHD mortality [2], has more complex effects. For example, specific HDL subfractions have been linked to a higher risk of CHD [3], and genetic polymorphisms associated with LDL cholesterol levels have been implicated in the risk of myocardial infarction.[4] These findings challenge the simplistic notion that uniformly raising HDL cholesterol levels will consistently reduce CHD risk. Our findings on HDL cholesterol levels also did not demonstrate any significant differences among the groups. Although elevated TG levels are associated with high carbohydrate intake, any dietary practices or factors that lead to excess body weight are associated with elevated TG levels.[22] Hence, the elevated TG levels after HFD in rabbits may be linked to their excess body weight.

Previous research has shown that New Zealand White rabbits can develop hyperlipidaemia within five weeks of HFD exposure and progress to hyperlipidaemia with insulin resistance after ten weeks. Concurrently, HFD has been reported to activate inflammatory pathways in peripheral blood mononuclear cells (PBMCs) as early as five weeks, marked by increased expression of IL-1β and mitogen-activated protein kinase pathways. After prolonged HFD exposure, altered autophagy was observed, indicated by increased biomarkers of autophagy in PBMCs.[23] Autophagy plays a critical role in clearing misfolded proteins and damaged organelles and is known to regulate excessive inflammation.[24] It is plausible that the rabbits in this study

**Table I: Serum lipid profile of all groups (median) and differences among the groups**

| Parameter | Group values (Median [Q1–Q3]) | | | | Kruskal–Wallis H test | | |
|---|---|---|---|---|---|---|---|
| | Control | HFD | HFDQ | HFDA | $\chi^2$ (Chi-square) | df | p-value |
| TC (mmol/L) | 2.75 (2.37–3.12) | 4.92 (4.75–5.13) | 3.90 (3.52–4.27) | 3.50 (3.15–3.77) | 12.640 | 3 | 0.005* |
| TG (mmol/L) | 0.56 (0.41–0.76) | 1.03 (0.84–1.21) | 0.74 (0.63–0.78) | 0.53 (0.42–0.87) | 7.123 | 3 | 0.068 |
| HDL (mmol/L) | 0.71 (0.63–0.86) | 0.79 (0.71–0.89) | 0.85 (0.75–0.93) | 0.88 (0.81–1.03) | 4.066 | 3 | 0.254 |
| LDL (mmol/L) | 1.73 (1.41–1.79) | 3.79 (3.71–4.00) | 2.72 (2.46–2.98) | 2.27 (2.08–2.51) | 13.500 | 3 | 0.004* |
| Non-HDL (mmol/L) | 1.93 (1.72–2.12) | 4.25 (4.14–4.27) | 3.06 (2.76–3.35) | 2.51 (2.28–2.91) | 13.520 | 3 | 0.004* |

Descriptive analysis was presented as median (interquartile range [Q1–Q3]). Statistical comparisons were performed using the Kruskal–Wallis H test. *Significant at $p < 0.05$.

**Table II: Pairwise comparison of serum lipid profile between groups**

| Parameter | Pairwise Comparison | Mann-Whitney U test | Z | p-value |
|---|---|---|---|---|
| TC | Control vs HFD | 0.000 | -2.309 | 0.021* |
| | Control vs HFDQ | 0.000 | -2.309 | 0.021* |
| | Control vs HFDA | 0.000 | -2.021 | 0.043* |
| | HFD vs HFDQ | 0.000 | -2.309 | 0.021* |
| | HFD vs HFDA | 0.000 | -2.309 | 0.021* |
| | HFDQ vs HFDA | 4.000 | -1.155 | 0.248 |
| LDL | Control vs HFD | 0.000 | -2.309 | 0.021* |
| | Control vs HFDQ | 0.000 | -2.309 | 0.021* |
| | Control vs HFDA | 0.000 | -2.309 | 0.021* |
| | HFD vs HFDQ | 0.000 | -2.309 | 0.021* |
| | HFD vs HFDA | 0.000 | -2.309 | 0.021* |
| | HFDQ vs HFDA | 2.000 | -1.732 | 0.083 |
| Non-HDL | Control vs HFD | 0.000 | -2.323 | 0.020* |
| | Control vs HFDQ | 0.000 | -2.309 | 0.021* |
| | Control vs HFDA | 0.000 | -2.309 | 0.021* |
| | HFD vs HFDQ | 0.000 | -2.323 | 0.020* |
| | HFD vs HFDA | 0.000 | -2.323 | 0.020* |
| | HFDQ vs HFDA | 2.000 | -1.732 | 0.083 |

Pairwise comparisons were conducted using the Mann–Whitney U test (two-tailed). *Significant at $p < 0.05$. HFD = high-fat-diet group; HFDQ = high-fat-diet + quercetin; HFDA = high-fat-diet + atorvastatin.

**Table III: Within-group comparisons in serum bradykinin, ACE2, CRP and IL-6 between pre- and post- treatment**

| Parameter | Group | Group values (Median [Q1–Q3]) | | Wilcoxon signed-rank test | |
|---|---|---|---|---|---|
| | | Pre-treatment | Post-treatment | Z | p-value |
| Bradykinin (ng/mL) | Control | 6.45 (5.99–7.40) | 7.06 (6.02–9.25) | 0.000 | 1.000 |
| | HFD | 7.46 (5.03–9.24) | 11.64 (5.18–18.40) | 1.095 | 0.273 |
| | HFDQ | 5.92 (4.54–7.68) | 9.49 (6.21–14.32) | -1.826 | 0.068 |
| | HFDA | 7.37 (6.28–9.04) | 9.22 (8.29–12.68) | -1.826 | 0.068 |
| ACE2 (ng/mL) | Control | 46.06 (42.57–49.26) | 46.12 (41.07–51.08) | -0.365 | 0.715 |
| | HFD | 44.88 (37.72–49.32) | 44.77 (36.36–60.56) | -0.365 | 0.715 |
| | HFDQ | 41.74 (35.00–50.75) | 48.97 (35.41–56.85) | -1.461 | 1.44 |
| | HFDA | 50.61 (45.17–52.34) | 50.00 (40.20–54.73) | 0.000 | 1.00 |
| CRP (ng/mL) | Control | 1167.33 (1144.83–1342.33) | 1156.50 (1026.50–1444.00) | 0.000 | 0.715 |
| | HFD | 1225.66 (1170.66–1353.16) | 1391.50 (1252.75–1504.00) | -1.095 | 0.273 |
| | HFDQ | 1184.00 (1005.66–1289.83) | 1184.83 (990.25–1335.66) | 0.000 | 1.000 |
| | HFDA | 1212.33 (1118.58–1409.83) | 1217.33 (1088.16–1341.50) | 0.000 | 1.000 |
| IL-6 (ng/mL) | Control | 266.07 (204.12–315.36) | 205.82 (182.29–245.13) | -1.461 | 1.44 |
| | HFD | 263.34 (182.99–296.27) | 218.47 (185.13–226.01) | -1.461 | 1.44 |
| | HFDQ | 249.91 (203.32–316.95) | 190.36 (164.68–233.77) | -1.826 | 0.068 |
| | HFDA | 249.00 (215.13–299.91) | 192.18 (180.82–233.54) | -1.461 | 1.44 |

Values are expressed as median (interquartile range [Q1–Q3]). Statistical comparisons between pre- and post-treatment were performed using the Wilcoxon signed-rank test (two-related samples). Significant at $p < 0.05$. HFD = high-fat-diet group; HFDQ = high-fat-diet + quercetin; HFDA = high-fat-diet + atorvastatin.

**Table IV: Between-group comparisons of the change values (Δ = post – pre)**

| Parameter | Group values (Median [Q1–Q3]) | | | | Kruskal–Wallis H test | | |
|---|---|---|---|---|---|---|---|
| | Control | HFD | HFDQ | HFDA | $\chi^2$ (Chi-square) | df | p-value |
| Δ Bradykinin (ng/mL) | -0.08 (-0.50–3.06) | 4.33 (-1.09–10.25) | 3.57 (1.66–6.63) | 2.03 (1.63–3.83) | 3.199 | 3 | 0.362 |
| Δ ACE2 (ng/mL) | 1.69 (-6.68–5.36) | 0.48 (-1.96–11.23) | 3.82 (0.41–9.50) | 0.22 (-12.14–8.7) | 0.993 | 3 | 0.803 |
| Δ CRP (ng/mL) | -27.50 (-276.66–276.66) | 165.83 (-77.91–310.83) | 48.33 (-183.75–166.66) | 37.50 (-321.66–190.41) | 1.213 | 3 | 0.750 |
| Δ IL-6 (ng/mL) | -49.72 (-103.37–0.78) | -44.87 (-70.26–2.14) | -52.73 (-123.41– [-5.22]) | -44.54 (-116.13–3.18) | 0.551 | 3 | 0.907 |

Values are expressed as median (interquartile range [Q1–Q3]). Statistical comparisons among groups were performed using the Kruskal–Wallis H test. Significant at $p < 0.05$.

experienced early-stage weight gain and dyslipidaemia from the 4th to the 9th weeks. From the 10th week onwards, their weight was not significantly higher than that of the control group, suggesting a possible progression to hyperlipidaemia with insulin resistance. However, this remains speculative as insulin resistance was not assessed. Quercetin did not significantly reduce body weight or serum TG levels in this study, aligning with previous findings that demonstrate variable effects of quercetin on TGs.[14-16] This variability underscores the complexity of lipid metabolism and suggests that quercetin may primarily target LDL cholesterol metabolism rather than TGs. Further investigation is needed to elucidate the specific mechanisms by which quercetin differentially affects lipid fractions.

In contrast, atorvastatin, a well-known statin, demonstrated superior efficacy in lowering both serum TGs and LDL cholesterol levels, as anticipated. Statins achieve their potent lipid-lowering effects by inhibiting HMG-CoA reductase, a key enzyme in cholesterol synthesis.[25] While quercetin showed notable LDL-lowering effects, its comparatively lesser efficacy highlights its potential as an adjunct therapy rather than a standalone treatment for dyslipidaemia. Exploring the potential synergy between quercetin and statins could be a promising avenue for future research, particularly for patients intolerant to high-dose statins or those seeking complementary approaches to enhance lipid control. Additionally, quercetin's natural origin and favourable side-effect profile make it an attractive option for long-term management of dyslipidaemia.

The LDL-lowering effects of unformulated quercetin have been demonstrated in metabolic disease models using rats, typically at doses of 10–100 mg/kg/day [13-15], which corresponds to 5–50 mg/kg/day in rabbits. Studies using rabbits have shown wide range of dosing, from 0.05 mg/kg/day for 12 weeks to 25 mg/kg/day for 90 days, ameliorating hypercholesterolemia.[26,27] Our findings align with these studies.

Moreover, quercetin formulations, such as quercetin: β-cyclodextrin complexes and liposomal encapsulations, have shown enhanced lipid-lowering effects in diabetic rats.[28,29] However, clinical trials have yielded mixed results. For example, in a double-blind randomised trial involving 72 women with type 2 diabetes, 500 mg/day of quercetin capsules for ten weeks significantly reduced systolic blood pressure but had no significant impact on lipid profiles.[16] Several factors may explain quercetin's inconsistent efficacy in clinical studies. Differences in disease stage, such as progression to insulin resistance and the relatively short treatment durations of quercetin treatment, may limit the observed benefits. These findings emphasise the need for further research into optimising quercetin dosage, formulation and timing to enhance its therapeutic efficacy in lipid management.

There are several potential explanations for the lack of elevation in inflammatory markers (IL-6 and CRP) observed in our dyslipidaemia model, despite the presence of metabolic changes. First, it remains unclear whether the activation of inflammatory pathways precedes the development of dyslipidaemia or manifests later in the disease progression following inflammation of the adipose tissue.[30] Second, the inflammatory state in our model might be subtle and subclinical, rendering it undetectable at the protein level. While the interplay between inflammation and atherosclerosis is well-documented [7], evidence linking systemic inflammatory marker levels directly to dyslipidaemia remains limited and inconclusive.[8] Consequently, our study was unable to demonstrate a clear association between systemic inflammation and dyslipidaemia.

Our findings also indicate that quercetin did not significantly influence inflammatory markers in this model. This is consistent with previous clinical studies. For instance, in a double-blind randomised clinical trial involving 72 women with type 2 diabetes, ten weeks of quercetin supplementation (500 mg/day) significantly reduced systolic blood pressure but showed no significant effect on inflammatory biomarkers such as TNF-α, hs-CRP, and IL-6.[16] Similarly, another randomised double-blind, placebo-controlled trial in 88 post-myocardial infarction patients found that eight weeks of quercetin supplementation significantly improved total antioxidant capacity but failed to show any significant effects on inflammatory markers or blood pressure.[17] In contrast, quercetin has been shown to have anti-inflammatory properties.[12] Several factors could explain why quercetin had no significant effect on inflammation in these clinical studies as well as in our study. First, the anti-inflammatory activity of quercetin may not be exerted in the absence of a significant inflammatory state, as observed in our study. Second, differences in disease stages, such as insulin

resistance or post-myocardial infarction in the aforementioned clinical studies, likely influenced the outcomes, as these conditions involve advanced metabolic and inflammatory complications. Thirdly, this model is optimal for dyslipidaemia but may have limitations in representing inflammation. The lack of systemic inflammation in the model could be a key limitation in testing anti-inflammatory potential. Moreover, the relatively short durations of supplementation (8–10 weeks) may have been insufficient to produce measurable changes in inflammatory markers. These findings underscore the complexity of quercetin's effects on inflammation and highlight the need for further studies to optimise its dosage, formulation, and treatment duration for effectively targeting inflammation in dyslipidaemia and related conditions.

This study investigates the effects of quercetin on bradykinin, a relatively understudied inflammatory biomarker. Bradykinin, often referred to as a 'proinflammatory kinin', plays a pivotal role in various inflammatory conditions (31). Bradykinin is a nonapeptide derived from high-molecular-weight kininogen, and elevated levels beyond physiological thresholds in cardiometabolic diseases can have harmful effects. For instance, Perhal et al.[32] demonstrated that transgenic overexpression of B2R in a hypercholesterolemic atherosclerotic mouse model exacerbated atherosclerotic plaque formation. In this study, bradykinin levels did not significantly differ between pre- and post-treatment or across groups, possibly because bradykinin plays a limited role in subclinical, non-infectious inflammation and its short half-life leads to rapid degradation, reducing measurable changes. It has been suggested that in a physiological state, an increase in des-Arg9-bradykinin (DABK) in cardiometabolic patients acts as a compensatory response to elevated blood pressure.[7]

Bradykinin is closely linked to ACE2, which degrades DABK into inactive metabolites.[33] Clinical and in vivo studies have reported that hypertension and metabolic syndrome are associated with elevated ACE2 levels.[34,35] As part of the protective arm of the renin-angiotensin system (RAS), ACE2 has demonstrated anti-atherosclerotic effects in numerous in vivo studies.[36,37] Elevated ACE2 levels in patients with cardiometabolic diseases and risk factors suggest a compensatory role in CHD [38] and indicate its potential as a biomarker for cardiovascular risk assessment before disease onset. In this study, ACE2 levels showed no significant changes across groups or between pre- and post-treatment. The lack of significant changes in ACE2 levels may also be attributed to factors such as the treatment duration, or the change might be subtle, rendering it undetectable at the protein level.

There are limited primary study investigating the effect of quercetin on bradykinin. While studies are reporting on the inhibitory effect of quercetin on rhACE2 activity in assay conditions [39] and expression of ACE2 in SARS-CoV-2 infection models [40], it has never been substantially investigated in cardiovascular disease models. This study may represent one of the first to investigate ACE2 and bradykinin in a dyslipidaemic rabbit model.

This study has some limitations that should be acknowledged. The small sample size may limit the generalisability of the findings, and larger studies are needed to confirm the results. Additionally, the duration of the study may not have been sufficient to observe the long-term effects of quercetin on ACE2 expression and other cardiovascular outcomes. Finally, the dosage of quercetin used in this study may not have been optimal for eliciting maximal therapeutic effects, and future studies should explore different dosages, formulations and treatment regimens.

## CONCLUSION

In summary, this study demonstrated that quercetin significantly improved serum TC and LDL levels, albeit without significant changes in serum IL-6, CRP, bradykinin and ACE2 levels, in a rabbit model of diet-induced dyslipidaemia. While it did not outperform atorvastatin in all measured parameters, quercetin showed potential as a natural alternative or adjunct therapy to conventional lipid-lowering drugs. This further suggests that its lipid-lowering effects may be independent of its anti-inflammatory effects.

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# Development and validation of a group counselling resilience module intervention (GC-ReMI) among breast cancer patients

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## ABSTRACT

**Introduction: Resilience is crucial for breast cancer patients. It can enhance mental health and treatment outcomes. Group counselling intervention supports these patients by fostering their inner strength and helping them cope with the psychological impacts of their diagnosis and treatment. This intervention has improved their quality of life.**

**Materials and Methods: This study used a mixed design. It was conducted in two stages: i) Phase I: literature search, interviews, and focus group discussions (FGDs); ii) Phase II: module development, content, and face validation for the Group Counselling Resilience Module Intervention (GC-ReMI). Interviews and FGDs were used to gather information from patients and healthcare workers. Ten independent experts assessed the content validity, while ten counsellors and breast cancer patients evaluated the face validity.**

**Results: Twenty-three participants took part in the interviews and FGDs. Data saturation was reached at the third FGD and the fourteenth in-depth interview. Two themes emerged from the qualitative analysis and were integrated into the module. The final GC-ReMI module included nine subthemes. The content validity index (CVI) was 0.84, and the face validity index (FVI) was 1.0 for counsellors and 0.97 for breast cancer patients, all of which meet satisfactory levels.**

**Conclusion: The GC-ReMI module has satisfactory and acceptable content and face validity, suggesting its potential as a valuable psychoeducational tool for enhancing resilience among breast cancer patients.**

**KEYWORDS:**
*Module development and validation, psychoeducation module, resilience, breast cancer, group counselling*

## INTRODUCTION

Breast cancer diagnosis often induces stress and leads to significant changes in life routine among patients. Psychological resilience became a protective factor in dealing with stressful clinical situations. Resilience refers to the ability to adapt to and recover from adversity. For breast cancer patients, resilience is crucial in managing the disease's psychological and emotional impact. Higher levels of resilience have been associated with better psychological outcomes, including lower levels of distress, improved mental health, and a greater sense of control over one's life.[1]

Resilience is crucial in chronic illnesses such as cancer, where patients face substantial psychological challenges. Group counselling modules have gained recognition for enhancing resilience through shared experiences and peer support.[2] Group counselling is particularly beneficial for breast cancer patients, as it fosters a sense of community and reduces feelings of isolation that are common in cancer patients, and can offer opportunities for social connection, shared coping strategies, and emotional support among breast cancer patients.[3] In fact, resilience-focused interventions promote shared experiences and collective coping, which in turn strengthen individual recovery.[4]

Existing interventions aimed to enhance resilience among breast cancer patients fall short of addressing the comprehensive psychosocial needs of this population, leading to gaps in efficacy and applicability. Most resilience-building programs emphasise cognitive restructuring and individual coping strategies without adequately recognising the crucial role of social support and the collective experience of peers.[1,5] In addition, other research indicates that resilience is frequently bolstered by social connections and shared experiences, suggesting that a strictly individual-focused approach may not fully harness the benefits derived from group dynamics.6 In fact, fewer studies have focused on group intervention for resilience aspects among survivors.

Developing and validating interventions in module form offers a structured approach to enhancing resilience, thereby improving quality of life and psychological well-being. The GC-ReMI module is tailored to the specific needs of breast cancer patients, addressing holistic domains identified in Phase I to foster emotional regulation, social support, and adaptive coping. Phase II validation involved rigorous

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testing to ensure its effectiveness in strengthening resilience and related psychological outcomes.

This study was conducted in two phases. Phase I identified resilience domains among Malaysian breast cancer survivors, and Phase II developed and validated the Group Counselling Resilience Module Intervention (GC-ReMI) using the Three-Round Modified Delphi Technique and content and face validity testing. A mixed-methods design was employed to triangulate data, enhance validity, and provide comprehensive insights.[7]

## MATERIALS AND METHODS

The study used a mixed-methods design, combining qualitative and quantitative research methods. The study is conducted in two phases: i) Phase I: literature search, interviews, and focus group discussions (FGDs); ii) Phase II: module development, the three-round modified Delphi technique, content validation, and face validation for the GC-ReMI module. All the data was gathered after completing the interview and the focus group discussion session. Then, the data were analysed using thematic analysis using ATLAS.ti software version 7.9.

Thematic analysis was employed to capture the nuances of participants' experiences following interviews and discussions. Guided by Braun and Clarke's six-step framework (2006), the process involved data familiarisation, coding, theme development, review, definition, and reporting. This structured approach ensured transparency and rigour, with themes emerging inductively through iterative coding.[8,9]

Ten independent experts assessed the content validity, while ten counsellors and breast cancer patients evaluated the face validity. Only patients who provided written informed consent and those who were willing to participate in the intervention were recruited.

*Phase I: Module Development (Literature Search, Interview, and FGDs)*

A systematic literature search was conducted across PubMed, PsycINFO, Scopus, Web of Science, Google Scholar, and ScienceDirect for studies published between 2014 and 2024 to identify relevant modules. The extracted information was summarised, and draft domains were constructed accordingly.

In-depth semi-structured interviews and FGDs were then conducted to gather qualitative data informing the development of the GC-ReMI module. Interviews explored potential domains, resilience profiles, and participant preferences, with questions aligned with study objectives. Participants were recruited during clinic appointments, and sessions were conducted in private settings to ensure comfort. Open-ended and probing questions were used to elicit rich insights, while building rapport encouraged open sharing of experiences.

Interviews and FGDs were audio-recorded with participants' consent to ensure accuracy, while detailed field notes captured non-verbal cues and contextual information. Fourteen participants were interviewed individually, and three FGDs were conducted with groups of four, three, and two participants, using similar guiding questions. The sample size was determined based on the principle of data saturation, where no new themes emerge, typically achieved with 12 to 30 participants.[10] A total of 23 participants were included, which is consistent with qualitative research standards emphasising depth over statistical generalizability. For the Delphi rounds, expert panels were purposively selected from diverse disciplines to enhance the breadth and depth of insights.[11] Ten experts were considered sufficient to reach consensus and achieve saturation, balancing methodological rigour, expert quality, and practical feasibility.[12]

Audio recordings were transcribed verbatim and analysed using thematic analysis. Transcripts were thoroughly reviewed for familiarisation, followed by open coding where meaningful segments were assigned descriptive codes. Two researchers independently generated initial codes, which were then organised into categories and refined into overarching themes. NVivo 12 (QSR International Pty. Ltd., Doncaster, Victoria, Australia) was used to support data management and analysis. Triangulation of interviews, FGDs, and literature ensured the rigour and trustworthiness of the findings.

*Phase II: Module Validation (the Three-Round Delphi Technique, Content Validation, and Face Validation of GC-ReMI Module)*

*Consensus Process (the Three-Round Delphi Technique)*

Module development followed an iterative triangulation and review process through online and face-to-face meetings with ten experts, including psychiatrists, a pathologist, counsellors, religious leaders, a matron, a clinical psychologist, and an educator. The content was refined from dominant to less dominant themes, with careful assessment of wording, activities, and visual materials (tables, pictures, and infographics) to ensure cultural appropriateness, clarity, and accessibility for the target population.

The Three-Round Delphi technique is a structured communication method used to achieve consensus among the expert panels through rounds of questionnaires.13 Phase II involved content and face validation using self-administered inventories and guided interviews, followed by a three-round Delphi technique with ten experts. Structured feedback forms on potential GC-ReMI domains were distributed via email, with each round lasting two weeks. The structured feedback forms regarding all items with potential domains of the GC-ReMI were emailed to all ten expert panels with an official letter of invitation for 2 weeks. In Round 1, experts provided open-ended responses; in Round 2, they rated the relevance of items based on the summary from Round 1; and in Round 3, they reviewed statistical feedback and reconsidered their ratings. Consensus was defined as a mean score ≥4.0 and ≥75% agreement.[14] This process established agreement on the module's components and subcomponents. Expert panel selection was based on relevant expertise, willingness, time commitment, and communication skills.[12]

*Content Validation by Expert Panels*

The Content Validation Index (CVI) is used for the study, which is a statistical measure to quantify the content validity of items in a scale measurement tool. The CVI has assessed the degree of relevance of each item used in the module. There were six steps of the CVI procedure using a Likert scale rating, usually from 1 to 4.[15,16] The content validation process followed a six-step procedure. First, 23 items were developed based on the draft module to ensure comprehensive coverage of the content domain. Second, six experts were purposively selected, representing psychiatry (n=2), surgery (n=1), medicine (n=1), clinical psychology (n=1), and counselling (n=1). Each expert independently evaluated item relevance using a four-point Likert scale (1 = not relevant to 4 = highly relevant). Third, the CVI was calculated, with items rated 3 or 4 considered relevant. Fourth, items with lower scores were revised, replaced, or removed. Fifth, the scale was refined and retested where necessary. Finally, both numerical ratings and qualitative feedback from the experts were incorporated to improve the overall validity of the module. It has been proposed that an index of 0.83 or higher is required to achieve satisfactory CVI values if six experts are involved.[15-17]

*Face Validation by the raters (ten counsellors and ten breast cancer patients)*

The Face Validation Index (FVI) is used as a systematic approach to evaluate face validity, similar to how CVI is calculated. The FVI has assessed the clarity and comprehension of each item used in the module. There are six steps of the FVI procedure using a Likert scale rating, usually from 1 to 4.[18] Face validity was assessed by ten counsellors and ten breast cancer patients, who independently reviewed the full module and completed a structured face validity inventory form. The evaluation focused on clarity, comprehensibility, and overall presentation of each item. Participants were also invited to provide written suggestions for refinement. Feedback from both groups informed revisions to enhance the module's readability and acceptability.

## RESULTS

*PHASE I: Module Development (Literature Search, Interview, and FGDs)*

Phase I involved the design and development of the module, which consisted of the literature search, in-depth interviews, and FGDs.

*Sociodemographic Characteristics*

A total of 23 participants were recruited, comprising 14 for individual interviews (11 breast cancer patients, a surgeon, a radiologist, and a matron) and 9 for FGDs (5 breast cancer patients, 2 medical officers, and 2 staff nurses). All participants were Malay females; 78% were employed and 22% unemployed. Overall, 82% were breast cancer patients and 18% were clinic staff. In terms of age, 18% were below 18 years, 52% were adults (19–59 years), and 30% were elderly (>60 years). Among breast cancer patients, only 25% were employed.

*Emergent Themes*

Quotations from transcripts were coded into sub-themes, which were then consolidated into themes and two main domains. Data saturation was reached at the third FGD and the fourteenth in-depth individual interview. The emergent themes are summarised in Table I.

The results generally found two main domains with eleven themes and twenty-three subthemes. The domains are classified into internal (6) and external (5). The internal domains were knowledge, cognitive, religion and spirituality, self-efficacy, emotion, and physical, whereas the external domains were family support, social support, cancer stages, financial, and mental health.

*PHASE II: Module Validation (Three-Round Modified Delphi Technique, Content Validation, and Face Validation of GC-ReMI Module)*

Whereas, phase II involved the validation of the module, which consisted Three-Round Modified Delphi technique, content, and face validation.

*Consensus in Module Development Process using the Three-Round Delphi Technique*

The Delphi process was conducted in three rounds with expert anonymity maintained. Experts rated, revised, and commented on items across rounds. Consensus was achieved for 27 subthemes, except for subthemes 8 and 27, which initially showed low consensus (QD ≥ 1.0) in Round 1. Following modifications, subtheme 27 reached medium consensus in Round 2, while subtheme 8 remained with no consensus. In Round 3, both subthemes achieved high consensus. The results are summarised in Table II.

*Content and Face Validation*

CVI and FVI emerge as a critical metric in evaluating validity, and it is subdivided into several key components: item level (I-CVI/FVI), the scale level (S-CVI/FVI) and the average scale level (S-CVI/Ave; S-FVI/Ave).15,16 I-CVI is the percentage of content experts who rate an item as relevant (3 or 4), while S-CVI is the average of these item scores. The percentage of raters that rate an item as clear and understandable (a rating of 3 or 4) is known as I-FVI for FVI, and the average of these ratings is known as S-FVI. Furthermore, a universal agreement-based scale-level index (S-CVI/UA and S-FVI/UA) was computed, which shows the percentage of items that received the highest relevance/clarity rating (3 or 4) from all experts/raters. A universal agreement score of 1 denotes 100% agreement.

*The CVI for Six Panels*

The CVI was calculated based on six expert ratings. Items 1, 8, 20, and 27 did not reach the acceptable threshold of 0.84, each scoring below 0.83, and were therefore excluded from the module, as they did not achieve a satisfactory level of content validity. Overall, I-CVI, S-CVI/Ave, and S-CVI/UA met the satisfactory level, 0.84 (>0.83), when six experts were involved. Thus, the module items have achieved satisfactory content validity.[15,17] The result for CVI is shown in Table III below.

**Table I: Themes emerged from individual interviews and FGDs (Internal Domain & External Domain)**

| DOMAIN | THEMES | SUBTHEMES | EVIDENCE |
|---|---|---|---|
| Internal | Emotion | Emotional flexibility | "As for breast cancer cases, we, as medical doctors, play an important role in dealing with patients' emotions. We need to look for the emotional aspect when handling them. Treat not only their disease but also their emotions as well." (IDPT 3/48-49/050123). |
| | | Emotional regulation skills | "I strengthened myself to be happy. Self-talk to myself by saying, 'It's okay (you can cry, can do good to yourself).' "(FGD1/185/041022) |
| | Knowledge | Knowledge of cancer stages | "Yes. I have to be strong. If I feel it a little bit, I divert attention by pretending not to know." (IDPT 5/22/301122) |
| | Cognitive | Mental flexibility | "I often talk nicely to myself(self-talk). It doesn't matter if I die now, that's enough. I'm satisfied." (IDPT 8/105-107/010123) |
| | | Positive thought | "After getting sick, I try to accept it well and try not to stress. Now, I can't think negatively. I used to think a lot. "I didn't feel happy when I couldn't finish my work. I can't. I only need to do as much as I can." (FGD3/20-22/291122) |
| | | Optimistic view | "When I visit my cousin with the same diagnosis, I feel grateful that I can still walk while she cannot. I thank God for my health and remind myself by looking at others who face greater challenges."(FGD3/22-25/301122) |
| | | Gratitude | "Yes. I have to be strong. If I feel it a little bit, I divert attention by pretending not to know." (IDPT 5/22/301122) |
| | Physical | Physical fitness – functional ability | Yes, I'm fit after gardening. (IDPT 3/80/041022) |
| | | Physical health – good nutrition intake | Since I got this disease, I started abstinence and haven't eaten meat anymore. (FGD1/133/041022)<br>But there's only one thing I'm advised not to eat: soybeans. (FGD3/27/291122) |
| | | Physical activities | I do, too. Just a normal, brisk walk. (FGD2/40/301122)<br>Then we do some gardening together. (IDPT 3/72-73/041022) |
| | Religion and spirituality | Religious Coping – religious belief | Because God chose me, God is close to me, hear me. (FGD3/13/301122) |
| | | Religious practice | Another effort, an effort to pray well. Immerse me in religion. Go and listen to religious talk. Yes, that's it. Aah, pray for Solat hajat and Qiamullail. (FGD1/135-139/041022) |
| | | Spiritual coping – spiritual activity | My strength. I want to keep healthy and live longer. It is all in yourself. I fight, too. I feel like I want to be sick. I fight for it. (FGD2/33-34/041022) |
| | | Flexibility and tolerance of others' values and beliefs | "What I want most is to have my husband and children with me. I try to appreciate every day and remind myself to stay strong and get well, especially for my children's sake." (IDPT 2/41-47/010123) |
| | Self-efficacy | Commitment to core values | I was sick before, and I had a cyst. Maybe that's where my strength comes from. From the beginning, the strength was always in me. (IDPT 1/32-33/291122) |
| External | Social support | Sources of social support | We socialise by joining a cancer association club or Dr Dato Ili's cycling club. There are lots of activities. He is also the doctor who listens to us. Whatever he does, he remembers the patient. (IDPT 7/78-82/231122) |
| | | Medical support | So, I joined the group. There is also a group with USM staff who always share important information about breast cancer. (FGD7/45-46/041022) |
| | | Group of cancer survivors supports | "Mrs.Z is very positive, and I followed her example. She disagreed with people who said cancer patients shouldn't eat eggs, as she ate them for energy and recovery." (FGD7/87-90/041022) |
| | | Resilient role-model | Ha, always follow them as role models. People who have just been diagnosed with cancer and follow the group, I always share my personal opinions about my journey (cancer). (IDPT 3/56-57/041122) |
| | Family support | Nucleus family support | "It is because of my husband's support. He takes me to the hospital for treatments, and my children support me as well." (FGD7/12-14/041022) |
| | | Extended family support | Yes. After that, I got strong support from my siblings. (FGD5/20/241122)<br>"My mother also gives me strength and support." (FGD5/22/241122) |
| | Mental health | Mental health status/well-being | Patients and families have misunderstood. If we offer a referral to a psychiatric clinic, they think they have mental problems. (FGD11/56/050123) |
| | Financial support | Financial status | "The hospital can assist by applying for reduced payment rates, and we will contact the management to request a possible reduction, perhaps by half." (FGD11/67-69/050223) |
| | Cancer stages | | And one more thing, I was diagnosed with early breast cancer, stage 1A. I felt thankful. It means the treatment is early. So far, thank you to Allah. (FGD6/34-36/231122) |

**Table II: Consensus from Three-Round Delphi**

| Bil | Domain (theme) | Item (subtheme) | Round of Delphi | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Round 1 | | | Round 2 | | | Round 3 | | |
| | | | Median | Mean | QD | Median | Mean | QD | Median | Mean | QD |
| 1 | Emotional | Item 1 | 5 | 4 | 0 | 5 | 4 | 0 | 5 | 4 | 0.375 |
| | | Item 2 | 5 | 4 | 0.38 | 5 | 4 | 0.375 | 5 | 4 | 0 |
| 2 | Cognitive | Item 3 | 5 | 4 | 0 | 5 | 4 | 0 | 5 | 4 | 0 |
| | | Item 4 | 5 | 4 | 0 | 5 | 3 | 0.5 | 4.5 | 3 | 0.5 |
| | | Item 5 | 5 | 2 | 0 | 4.5 | 3 | 0.5 | 4 | 3 | 0.5 |
| | | Item 6 | 5 | 4 | 0.38 | 5 | 4 | 0 | 5 | 4 | 0.375 |
| 3 | Knowledge | Item 7 | 5 | 3 | 0.5 | 5 | 3 | 0.375 | 5 | 3 | 0.875 |
| | | Item 8 | 4 | 1 | 1.38 | 3.5 | 1 | 1.375 | 3 | 1 | 0.375 |
| 4 | Physical | Item 9 | 4 | 1 | 1 | 5 | 4 | 0 | 5 | 3 | 0.5 |
| | | Item 10 | 5 | 4 | 0 | 5 | 5 | 0 | 5 | 4 | 0 |
| | | Item 11 | 5 | 3 | 0.38 | 5 | 4 | 0 | 5 | 4 | 0.375 |
| 5 | Religion and spirituality | Item 12 | 5 | 5 | 0 | 5 | 5 | 0 | 5 | 5 | 0 |
| | | Item 13 | 5 | 1 | 0 | 5 | 4 | 0.38 | 5 | 5 | 0 |
| | | Item 14 | 4 | 1 | 0.5 | 5 | 3 | 0.375 | 5 | 3 | 0.375 |
| | | Item 15 | 5 | 2 | 0 | 5 | 4 | 0 | 5 | 2 | 0.375 |
| | | Item 16 | 4 | 3 | 0.38 | 4 | 3 | 0.5 | 4 | 3 | 0 |
| 6 | Social support | Item 17 | 5 | 4 | 0 | 5 | 5 | 0 | 5 | 5 | 0 |
| | | Item 18 | 5 | 3 | 0 | 5 | 3 | 0.5 | 5 | 4 | 0 |
| | | Item 19 | 5 | 4 | 0.5 | 5 | 4 | 0 | 5 | 3 | 0 |
| | | Item 20 | 4 | 1 | 0.38 | 4.5 | 4 | 0.5 | 4 | 4 | 0.5 |
| 7 | Family support | Item 21 | 5 | 3 | 0.88 | 5 | 3 | 0 | 5 | 3 | 0 |
| | | Item 22 | 5 | 2 | 0 | 4 | 2 | 1 | 4.5 | 2 | 0.875 |
| 8 | Mental health | Item 23 | 5 | 2 | 0 | 5 | 4 | 0 | 5 | 4 | 0 |
| 9 | Financial | Item 24 | 5 | 2 | 0 | 5 | 4 | 0.375 | 5 | 4 | 0 |
| 10 | Self-efficacy | Item 25 | 5 | 3 | 0 | 5 | 4 | 0 | 5 | 3 | 0 |
| 11 | Cancer stages | Item 26 | 5 | 3 | 0.38 | 4.5 | 1 | 1 | 5 | 3 | 0.5 |
| | | Item 27 | 5 | 1 | 1.25 | 3 | 1 | 1 | 3 | 2 | 0 |

**Table III: The relevance rating on the item (theme) scale by six expert panels**

| ITEMS | EXPERT 1 | EXPERT 2 | EXPERT 3 | EXPERT 4 | EXPERT 5 | EXPERT 6 | | Expert in Agreement | I-CVI | UA |
|---|---|---|---|---|---|---|---|---|---|---|
| Item 1 | 1 | 1 | 1 | 1 | 0 | 1 | | 5 | 0.71 | 0 |
| Item 2 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 3 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 4 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 5 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 6 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 7 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 8 | 1 | 1 | 1 | 1 | 0 | 1 | | 5 | 0.71 | 0 |
| Item 9 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 10 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 11 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 12 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 13 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 14 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 15 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 16 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 17 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 18 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 19 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 20 | 1 | 1 | 1 | 1 | 0 | 1 | | 6 | 0.71 | 0 |
| Item 21 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 22 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 23 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 24 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 25 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 26 | 1 | 1 | 1 | 1 | 1 | 1 | | 6 | 0.86 | 1 |
| Item 27 | 0 | 0 | 1 | 1 | 1 | 1 | | 4 | 0.57 | 0 |
| | | | | | | | | S-CVI/Ave | 0.84 | |
| Proportion relevance | 0.96 | 0.96 | 1.00 | 1.00 | 0.93 | 1.00 | | S-CVI/UA | | 0.89 |
| The average proportion of items judged as relevant across the six experts | | | | | | | 0.98 | | | |

**Table IV: Face validation for Counsellors**

| Item | Rater 1 | Rater 2 | Rater 3 | Rater 4 | Rater 5 | Rater 6 | Rater 7 | Rater 8 | Rater 9 | Rater 10 | | Raters in agreement | I-FVI | UA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 21 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 22 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 23 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| | | | | | | | | | | | | S-FVI/Ave | 1 | |
| Proportion relevance | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | S-FVU/A | | 1 |
| The average proportion of items judged as clarity and comprehension across the ten raters | | | | | | | | | | | 1 | | | |

**Table V: Face Validation among 10 Breast Cancer Patients**

| Item (subtheme) | Rater 1 | Rater 2 | Rater 3 | Rater 4 | Rater 5 | Rater 6 | Rater 7 | Rater 8 | Rater 9 | Rater 10 | | Raters in agreement | I-FVI | UA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 7 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | | 8 | 0.8 | 0.8 |
| Item 8 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | | 8 | 0.8 | 0.8 |
| Item 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | | 9 | 0.9 | 0.9 |
| Item 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | | 9 | 0.9 | 0.9 |
| Item 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | | 9 | 0.9 | 0.9 |
| Item 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 21 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 22 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| Item 23 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 10 | 1 | 1 |
| | | | | | | | | | | | | S-FVI/Ave | 0.969565 | |
| Proportion relevance | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | | S-FVIU/A | | 0.969565 |
| The average proportion of items judged as relevant across the ten raters | | | | | | | | | | | 0 | | | |

*The FVI for Ten Counsellors*
The FVI reflected the clarity and comprehensibility of the items. All twenty domains I-FVI is 1.0 (Accepted ≥ 0.8) and S-FVI/Ave 1.0 (Accepted ≥ 0.9) for clarity and comprehension. Face validity for counsellors has achieved a satisfactory level of face validity. The result for FVI is shown in Table IV below:

*The FVI among 10 Breast Cancer Patients*
The face validation was done twice. After modification, the face validation was accepted. Three domains, I-FVI 3,4, and 6, score lower than ≤ 0.8. and S-FVI/Ave was 0.77. All the activities in the module with low I-FVI were changed from subjective responses (fill-in-the-blank activities) to objective responses (some give an option to additional subjective responses).

FVI was repeated, and all domains I-FVI (I-FVI ≥ 0.8) and S-FVI/Ave (S-FVI-Ave ≥ 0.9) were satisfactory (Table V). The response anchors were simplified from subjective to more objective formats to accommodate the difficulties elderly patients experienced in completing the activities in the module.

All 23 domains of I-FVI ranged from 0.8 to 1.0 (Accepted ≥ 0.8), and S-FVI/Ave was 0.97 (Accepted ≥ 0.9) for clarity and comprehension. Face validity for patients has been achieved at a satisfactory level.

## DISCUSSION

*Phase I: Module Development (Literature Search, Interview, and FGDs)*
The present study has successfully developed a validated GC-ReMI Module among breast cancer patients. The module was developed for psychoeducational purposes, specifically for breast cancer patients, since there was no available psychological module as a referral in Malaysia.

Resilience is closely linked to mental well-being, particularly in populations facing significant health challenges such as breast cancer. Addressing important resilience domains is critical to ensuring a high quality of life among breast cancer patients. The resilience domain on mental health outcomes across various cancer types illustrates its broader implications on quality of life and psychological health.[19] Psychological resilience is instrumental in lessening the emotional burden of breast cancer, which directly correlates with improved mental well-being and life satisfaction among patients.[20]

Resilience plays a pivotal role in managing the psychological challenges of breast cancer and contributes to improved mental well-being. The findings of this study highlight both internal domains (e.g., knowledge, spirituality, and self-efficacy) and external domains (e.g., family and social support) as essential attributes fostering resilience among breast cancer patients.[1]

The core areas of resilience among cancer survivors were captured within internal and external domains, comprising eleven themes. Within the internal domain, religion and spirituality emerged as the most dominant theme. Spiritual well-being has been consistently linked to improved mental health outcomes, as it enables individuals to find meaning and purpose in their experiences, thereby enhancing psychological resilience. Evidence indicates that higher levels of spiritual well-being are associated with reduced depression and anxiety among breast cancer patients. According to Koenig[21], many studies have shown how spiritual well-being is closely linked to mental health, particularly in enhancing resilience and finding purpose through difficult experiences like illness. Other studies also confirm that spiritual well-being helps individuals cope with stressful life experiences by enhancing psychological resilience and improving mental health outcomes.[22] Spiritual well-being positively affected psychological resilience among breast cancer patients.[23] In Canada, both spirituality and religion connect the individual to themselves or others.[24] Previous literature has shown that both can be protective factors for mental health conditions and significantly improve overall well-being.[25] In fact, in a similar study shown both Western and Eastern cultural contexts have shaped results in nuanced ways about spirituality and religion in which being a protective effect on mental health status.

For the external domain, family support themes became the most dominant theme. The role of strong family support systems in boosting subjective well-being and resilience. Family resilience acts as a protective factor, which then enhances the patients' ability to manage stress and recover from treatment.[23] Previous studies have provided insight into the role of family support in enhancing resilience among breast cancer patients. Like in China, family support became the mechanism to enhance resilience and overall well-being among breast cancer patients.[26] In addition, more involvement in family support contributes to more coping strategies and resilience among Chinese breast cancer patients.[27] This evidence shows that family support influences psychological resilience.

*Phase II: Module Validation (the Three-Round Modified Delphi Technique, Content Validation, and Face Validation of GC-ReMI Module)*
In research, the Three Round Modified Delphi technique is applied properly before the validation process. It is critical to ensure that the rigour and quality of the final instrument or framework have been achieved. It provides the foundational knowledge required for consensus. According to Jones and Smith et al.'s study, well-chosen experts should improve the robustness of the results.[28] The appropriate panel selection in the Delphi technique directly impacts the credibility of the data used for validation. Then, the experts provide feedback after each round to ensure they are informed of the collective responses and can adjust their opinions accordingly. Other studies have stated that effective management of this feedback process ensures that the most relevant and agreed-upon elements are carried forward into the validation process.[29]

Sample size considerations for the Delphi process vary widely in the literature, ranging from a few experts to several hundred.[30-32] For homogeneous groups, panels of 10 to 15 participants are often recommended as sufficient to yield reliable outcomes.[33,34] Consistent with these guidelines, this study employed a panel of ten experts. Inclusion criteria

required a minimum of three years of professional experience in relevant fields and direct clinical engagement with breast cancer cases in Malaysia. Candidate expertise was carefully assessed, with final selection independently verified by two researchers.

Addressing bias in the selection process is essential in qualitative research, particularly with small sample sizes, to ensure validity and credibility. Coding software such as NVivo or ATLAS.ti was used to organise data and enhance analytical consistency.[35] Collaborative coding, where multiple team members independently coded transcripts before reaching consensus, further strengthened rigour. A secondary reviewer cross-checked coding to minimise individual bias and misinterpretation.[36] Codes and themes were iteratively refined through team discussions and member checking, whereby participants reviewed and confirmed interpretations to ensure accurate representation of their experiences. Iterative review processes have been shown to enhance reliability in small-sample qualitative studies.[37] Furthermore, strategic recruitment of experts from diverse professional backgrounds enriched the data and reduced selection bias.[14] Collectively, these strategies ensured methodological rigour and trustworthiness of the findings.

Handling the content and face validation process is crucial in research to ensure the module's quality, relevance, clarity, and comprehension. The I-CVI/Ave was 0.84, thus achieving the content validity for the module. The I-FVI/Ave for the counsellors was 1.0, and the FVI for breast cancer patients was 0.77 after adjusting activities in the module. Both achieved acceptable values for the validation index. Content validation can ensure that the items in a measurement tool accurately represent the construct being measured.38 This is crucial for establishing the credibility of the research findings. A well-validated tool has reflected the theoretical framework and intended constructs, enhancing the study's validity. Besides, the content validation process provides a framework for developing relevant and representative items of the target domain.[39] It ensures that all necessary aspects of the construct are considered, which can lead to more comprehensive and effective measurement tools. Thus, the content validation process is significant for the study.

Unlike content validation, which is systematic and expert-driven, face validity is more subjective and relies on judgments from target participants or non-experts. Despite its simplicity, it remains important, as it can enhance participant acceptance and engagement.[40] When participants perceive the module activities as appropriate for the intended construct, they are more likely to respond seriously and accurately. In fact, high face validity also improves user-friendliness, reducing response errors and facilitating a smoother, more efficient data collection process.[41]

The integration of resilience domains into clinical practice offers a pathway to enhance psychological well-being and overall quality of life among breast cancer patients. Tailored interventions, whether individual or group-based, can strengthen coping strategies, social support, and emotional adjustment among patients. By equipping healthcare providers to apply resilience-oriented approaches, patient outcomes during and after treatment can be significantly improved.

While this study offers valuable insights, certain limitations must be acknowledged. The use of mixed methods provided a robust framework for examining both clinical outcomes and participant experiences; however, the absence of a control group may limit the causal inferences. Nonetheless, triangulation strengthened the findings, as qualitative data enriched the quantitative results and enhanced interpretative validity.[42] The generalisability of this mixed-method, module development study is limited by the small sample size and single-centre setting. Conducting the study within one institution reduces the extent to which the findings can be applied to broader populations or different organisational contexts.

## CONCLUSION

In conclusion, the GC-ReMI module was designed to strengthen resilience among breast cancer patients, thereby enhancing psychological well-being and survivorship care within the cultural context. The module addresses the specific internal and external domains, such as emotional, social, and cultural challenges faced by Malaysian breast cancer patients undergoing breast cancer treatment and recovery. The module demonstrates effectiveness in preliminary validation through these systematic content and face validation approaches. This module was tailored to the cultural, linguistic, and healthcare needs of Malaysian breast cancer patients. This was making the module reliable and culturally sensitive. It also addresses clinical aspects and the psychosocial and emotional challenges patients face in promoting resilience. The validated module holds promise as a valuable resource for integrating mental health support in the form of psychoeducational resources into breast cancer care in Malaysia, indicating its utility as a validated psychoeducational tool and helping patients better navigate the psychological challenges of their cancer journey. The findings underscore the potential for this module to be integrated into standard clinical practice for clinicians, and future research should examine its implementation across various clinical populations and service environments.

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## ETHICAL APPROVAL

The study protocol was approved by the Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/22020138).

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ORIGINAL ARTICLE

# Interrupted sitting at work can alter postprandial glucose, triglycerides and non-esterified fatty acids (NEFA)

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## ABSTRACT

**Introduction: Prolonged sitting at work is a major health issue as sedentary behaviour has been linked to cardio-metabolic risks. Workplace interventions have trialled intermittent interruption to sitting throughout the working day to mitigate effects on employees' health. This study tested effects of the intensity of the interruptions to sitting on postprandial glucose, triglycerides and NEFA in a laboratory setting.**

**Materials and Methods: Overweight and obese adults (n=24) were recruited for a randomised cross over trial comparing, 1) uninterrupted sitting, 2) sitting interrupted by light intensity walking at 3 km.hr-1, and 3) vigorous intensity stair climbing at 60 steps.min-1. The interruptions to sitting were performed for two-minute bouts every 20 minutes following a meal. The moderate fat meal was provided two hours after the trial started and blood samples were collected each hour throughout the seven-hour sessions.**

**Results: There was a lower postprandial peak in plasma glucose and attenuation of the NEFA reductions for stair climbing compared to uninterrupted sitting. In contrast, triglycerides were reduced postprandially following light intensity walking compared to uninterrupted sitting.**

**Conclusion: Interrupted sitting with higher intensity demonstrates greater improvements postprandially.**

**KEYWORDS:**
*Interrupted sitting, light intensity walking, stair climbing, postprandial glucose, lipids*

## INTRODUCTION

Adults can spend up to half of their waking hour sitting with sitting in the workplace making a major contribution to this total.[1,2] Prolonged sitting is associated with increased cardio-metabolic risk; increases in BMI and waist circumference, impaired levels of high density lipoprotein cholesterol (HDL-C), elevated triglycerides and postprandial glucose in the circulation are associated with sedentary behaviour.[3-5] Many public health agencies have developed physical activity and sedentary behaviour guidelines to discourage prolonged sitting.[6,7] The recommendations are meant not only to reduce the total amount of sitting but also to regularly interrupt the prolonged sitting.[8] In epidemiological data, the duration of objectively measured sitting and the number of interruptions have been independently associated with cardio-metabolic risk.[9,10]

Physical activity performed in short bouts to interrupt sitting, regularly incorporated into the daily routine, has been shown to be more effective than a single equivalent session of continuous physical activity.[11-13] Since the main barrier to promoting sedentary breaks among employees may be a lack of work flexibility,[14] frequent short sedentary breaks might avoid disruption of office tasks and increase the feasibility of interventions while at work. Interrupted sitting interventions have been conducted in adults who were healthy and of healthy weight,[13,15-18] those who were sedentary and inactive[19,20] and those who were overweight and obese.[5, 21-22] Individuals receive a standard 'meal' in liquid form after two hours of interrupted sitting, with any physical activity interruptions beginning in sometime after the meal. As a result, these studies aim to assess the effects of interruptions by physical activity with differing levels of intensity on postprandial metabolism of glucose, triglycerides and NEFAs; since the postprandial increases in the concentrations of glucose, triglycerides and NEFAs in the bloodstream will contribute the most predictive power towards cardiovascular disease risk.[23]

For standing, the activity trialled with public health interventions that use 'sit-to-stand desks', Henson et al. reported reduced AUC following the meal for glucose and insulin[22] whereas neither Pulsford et al. nor Bailey & Locke reported any differences from sitting for glucose and insulin or glucose respectively.[16,20] In a 27-hour protocol, effects of intermittent standing after the first meal on glucose but not insulin were reported whereas an interruption of 30 minutes of moderate intensity walking reduced both glucose and insulin in the same period.[24] It is noteworthy that the Henson and co-workers sample were overweight and obese women with impaired glucose metabolism at baseline whereas the null findings for standing employed healthy individuals.[22] Consistent with this potential effects of the sample, Thorp et al. reported reductions in glucose AUC after five days of intermittent standing in a simulated office environment for overweight and obese participants on the fifth day, though there were no effects on insulin.[25] For light intensity walking, Bailey and Locke (3.2 km.hr-1) reported reductions in glucose whereas Pulsford et al. (3.6 km.hr-1), McCarthy et al. (3.0 km.hr-1) and Henson et al. (individually selected light

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intensity) reported reductions in both glucose and insulin consistent with the results in Dunstan and co-workers' original study.[5,16,19,20,22]

Exploratory analyses of the Australian group's overweight samples with consistent methodology revealed that greater intensity of physical activity performed during the interrupted would produce greater effects in the reduction of cardio-metabolic risk.[28] Such a result was consistent with the greater effects on the reduction of cardio-metabolic risk by more vigorous physical activity when compared to that by less intense physical activity, as shown by most of the available epidemiological data.[29-31]

Stair climbing requires more energy per minute than jogging, having been estimated as 9.6 metabolic equivalents (METs) of the sedentary state in the field[32] and 8.6 METs under laboratory conditions.[33] As such stair climbing is a vigorous physical activity. Experimental studies reveal that increased stair climbing can have effects on some cardio-metabolic risk factors.[34-39] To date, however, the effects of stair climbing have not been investigated as an interruption to sitting. The current study aimed to compare the acute effects of prolonged uninterrupted sitting with light intensity walking and the vigorous intensity physical activity of stair climbing on postprandial glucose, triglycerides (TG) and non-esterified fatty acid (NEFA). NEFA is free fatty acid (FFA) derived from the hydrolysis of fat or triglyceride in adipose tissue.[40]

## MATERIALS AND METHODS

*Study site*

The intervention was conducted in the Stair Laboratory of the School of Sports, Exercise & Rehabilitation Sciences at the University of Birmingham.

*Study population*

Volunteers were recruited from a population within one-mile radius of the university. Posters were placed on the notice boards in the university buildings as well as distributed outside the campus adjacent to the university entrance to the pedestrians. Inclusion criteria involved: 1) overweight/obese, 2) healthy and not on any medication, 3) no knee or joint problem, 4) no restrictions on movement, and 5) sedentary (less than 60 min.wk-1 of MVPA). The exclusion criteria were; 1) pregnant, and 2) physically active.

*Study design*

This intervention was a randomised, counterbalanced cross-over design. The total number of participants was 24.

*Sample Size Calculation*

The required sample size was calculated using the two-means formula, with a power of 80% and a significance level (α) set at $p < 0.05$. This calculation considered several independent variables identified from previous literature, and after accounting for a 10% non-response rate, the final sample size was determined to be 24 participants.

*Study protocol*

The information sheet was emailed to the volunteers to allow them to fully understand the details of the study. They were also asked to complete physical activity readiness (PAR-Q) and international physical activity (IPA-Q) questionnaires to ensure eligibility and safety for participation. Those who were found eligible and agreed to join this study were given a consent form. Prior to the starting of the trials, participants were randomly assigned into one of eight groups. Each group was arranged to complete three sessions in a counterbalanced order involving the three different physical activities; uninterrupted sitting, walking and stair climbing. A minimum of six days of wash out period was kept between the sessions. Participants fasted overnight and refrained from drinks containing caffeine on the day of testing. Prior to the testing protocol, a baseline session was conducted to measure weight, height and BMI of participants.

*a) Testing session*

The testing session started at 9 a.m. with hourly withdrawal of 5 ml of fasting blood sample (-2h, -1h & 0h). After sitting for two hours, participants were provided with a moderate fat meal containing 35% fat, 47% carbohydrate and 18% protein providing 68.6 kJ energy per kg body mass as the British diet is moderate in fat. Calculation of total energy was based on participant's weight. The moderate fat meal comprised of a drink containing skimmed milk, single cream, sugar and flavoured yogurt, along with an energy bar, veggie snacks and fruit. The meal was consumed within 20 minutes before starting the first interruption with physical activity. Blood was collected every hour after the meal and the catheter was flushed every 15 minutes to avoid coagulation. Blood was transferred into red top vacutainer containing clot activator and left 30 minutes in room temperature to allow clotting. Samples were then centrifuged at 3000 rpm for 15 minutes at 4˚ C and kept in a -20˚ C freezer prior to analysis.

Each day's session involved three participants. One participant of the group completed sitting uninterrupted while the others either walked on the treadmill or completed stair climbing as interruptions to sitting. The uninterrupted sitting participant remained seated throughout the seven-hour period. The participant for interrupted sitting with light intensity activity walked on a treadmill at 3.0 km.hr-1 for two minutes and for the interrupted sitting by MVPA, participant performed stair climbing using the StairMaster 7000 at 60 steps.min-1 for two minutes. Walking at this speed requires approximately 2 METs. Climbing at this speed required 8.7 ± 0.6 METs in a young, aerobically fit sample, similar to the expenditure of 8.6 and 8.8 METs reported by Bassett et al. and Jetté et al. respectively.[33]

*b) Blood samples analyses*

Serum analyses were performed in duplicate using semi-automatic spectrophotometric clinical chemistry analyser, Instrumentation Laboratory (ILab 650, Cheshire, UK) to measure the concentrated glucose, triglycerides (TG) and non-esterified fatty acid (NEFA).

*Ethical approval*

Ethics for this study was approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham with ethics reference number ERN_16_0472.

*Data deduction and statistical analysis*
To evaluate the changes of the variables in response to the test meal, the area under the curve (AUC) was calculated using the trapezoidal formula.

To allow comparison with previous research,5 AUC for two hours postprandial as well as AUC for the five-hour period were calculated. In addition, many metabolic processes when plotted over time are characterised by relatively simple shapes. To test for differences in these shapes between conditions, orthogonal polynomial trends across time were computed for the linear, quadratic, and cubic components, and these polynomial trends were analysed.

Data were analysed using the SPSS statistical package for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). All participants provided complete data for the variables of interest; no missing data were observed. Shapiro-wilk's test for normality showed p>0.05 and visual judgment of the histogram, Q-Q plots and boxplots showed that the data were approximately normally distributed with skewness within the acceptable range of ± 1.96. A natural log transformation was applied to TG levels to improve the normality of their distribution. Repeated measures ANOVA with orthogonal polynomial contrasts was used to examine condition differences.

## RESULTS

Total of 24 participants involved in the study had a BMI of 31.06 (SD=3.96) in obese class I (WHO, 2000). All participants would be classified as overweight (BMI > 25).

*Glucose*
Figure 1 below displays the pattern of glucose concentrations at one hour intervals throughout the seven-hour sessions. As can be seen, glucose increased immediately after meal and reached its peak at two hours postprandially.

To reiterate, an ordered relationship across conditions was predicted such that uninterrupted sitting > light walking > stair climbing based on the different intensities of the activities. The test of this ordered relationship would be the linear polynomial across conditions.

Analysis of the data from the immediate pre-meal values to five hours later, revealed a main effect of time ($F(3, 60.8) = 51.92$, $p<0.001$) that was described primarily by significant quadratic ($F(1, 23) = 103.80$, $p<0.001$; 63.6% of the variance) and cubic components ($F(1, 23) = 65.74$, $p<0.001$; 32.3% of the variance), with a significant interaction between the quadratic component and condition ($F(1, 23) = 5.05$, $p =0.04$). Follow-up analyses revealed differences in the quadratic component between uninterrupted sitting and stair climbing ($p = .04$) reflecting the lower peak response with stair climbing. There were no differences between the conditions for the cubic component over time ($F(1, 23) = 0, 94$ $p=0.34$). In addition, the omnibus ANOVA contained a significant linear component over time ($F(1, 23) = 6.97$, $p=0.02$; 2.7% of the variance) which did not differ between conditions ($F(1, 23) = 2.13$, $p=0.16$).

Table II summarises the AUC for glucose, NEFA and TG measured for the conditions of uninterrupted sitting, walking and stair climbing. As NEFA yielded a negative AUC due to the postprandial decrease, a change in concentration was calculated and subtracted from the level just before the meal to test the reductions postprandially. The actual level is shown in the table II.

For analyses of glucose AUC, a marginally significant linear polynomial over conditions ($F(1, 23) = 3.22$, $p=0.086$) was suggestive of an ordered relationship between the means for the five-hour period. Follow-up analyses suggested a smaller AUC for stair climbing than uninterrupted sitting ($p=0.09$) which would be significant with one tailed probability for the predicted effect. Similarly, a marginally significant linear polynomial for the two-hour AUC ($F(1, 23) = 4.14$, $p = .054$) was consistent with a smaller AUC for stair climbing than uninterrupted sitting ($p = .06$).

*Triglycerides*
Figure 2 displays the pattern for TG concentrations at one hour intervals throughout the seven-hour sessions. As can be seen, there was a steady increase postprandially in all three conditions.

Analysis of the immediate pre-meal and post-meal values revealed a main effect of time ($F(2, 51.5) = 177.65$, $p<0.001$) that was described primarily by a significant linear component ($F(1, 23) = 297.62$, $p<0.001$; 93.4% of the variance), with a suggestion that this differed between conditions ($F(1, 23) = 3.13$, $p=0.09$). The response also contained significant quadratic ($F(1, 23) = 40.00$, $p<0.001$; 5.1% of the variance) and cubic components ($F(1, 23) = 16.95$, $p<0.001$; 1.0% of the variance) which did not differ between conditions (both $p>0.37$). In addition, the ANOVA contained a main effect of condition ($F(2, 41.6) = 3.99$, $p=0.03$). In follow-up analyses, there was a smaller response for light walking than uninterrupted sitting ($p=0.02$), with a suggestion of a comparable difference for stair climbing ($p=0.09$).

For analyses of the TG AUC, a significant main effect of condition ($F(2, 46) = 4.08$, $p=0.024$) was accompanied by a marginally significant linear polynomial ($F(1, 23) = 3.28$, $p=0.083$), suggestive of an ordered relationship between the means for the five-hour period. Follow-up analyses revealed smaller magnitude AUC for light walking than sitting ($p=0.02$) with a suggestion of a similar difference with stair climbing ($p=0.08$). For the two-hour AUC, a significant main effect of condition ($F(2, 43.5) = 3.59$, $p=0.04$) was accompanied by a significant linear polynomial across conditions ($F(1, 23) = 7.16$, $p=0.01$). Follow-up of the main effect of condition revealed that light walking differed from uninterrupted sitting ($p=0.04$).

*NEFA*
Figure 3 displays the pattern for NEFA concentrations at one hour intervals throughout the seven-hour sessions. For NEFA, the level dropped postprandially as expected, with a nadir reached two hours after the meal.

**Table I: Participants' characteristics and values of metabolites prior to the test meal (n=24)**

| Variable | Mean ± SD | Mean ± SD (mmol.L-1.hr-1) | | |
|---|---|---|---|---|
| | | Sitting uninterrupted | Walking | Stair climbing |
| Age | 32.17 (5.57) | | | |
| Weight (kg) | 85.34 (14.52) | | | |
| Height (cm) | 165.05 (9.09) | | | |
| BMI | 31.06 (3.96) | | | |
| Glucose | | 4.38 (0.55) | 4.26 (0.38) | 4.23 (0.32) |
| TG | | 1.32 (0.69) | 1.19 (0.78) | 1.22 (0.58) |
| NEFA | | 0.84 (0.33) | 0.79 (0.21) | 0.78 (0.23) |

As shown in Table I above, there were no differences found between the conditions pre-meal (all p > .12). The accepted statistically significant p-value was <0.05 (two-tailed).

**Table II: Area under the curve for glucose, NEFA and TG (n=24)**

| Variable | Mean ± SD (mmol.L-1) | | |
|---|---|---|---|
| | Sitting uninterrupted | Walking | Stair climbing |
| 2-hour test | | | |
| Glucose | 5.65 (1.40) | 5.44 (0.82) | 5.30 (0.52) |
| TG | 1.64 (0.87) | 1.45 a (0.89) | 1.46 (0.63) |
| NEFA | 0.42 (0.15) | 0.44 (0.017 | 0.38 (0.11) |
| 5-h test | | | |
| Glucose | 5.41 (0.99) | 5.24 (0.52) | 5.12 (0.44) |
| TG | 2.25 (1.11) | 1.95 [a] (1.21) | 2.01 (0.94) |
| NEFA | 0.28 (0.10) | 0.32 (0.12) | 0.30 (0.09) |

[a] significantly different from uninterrupted sitting.

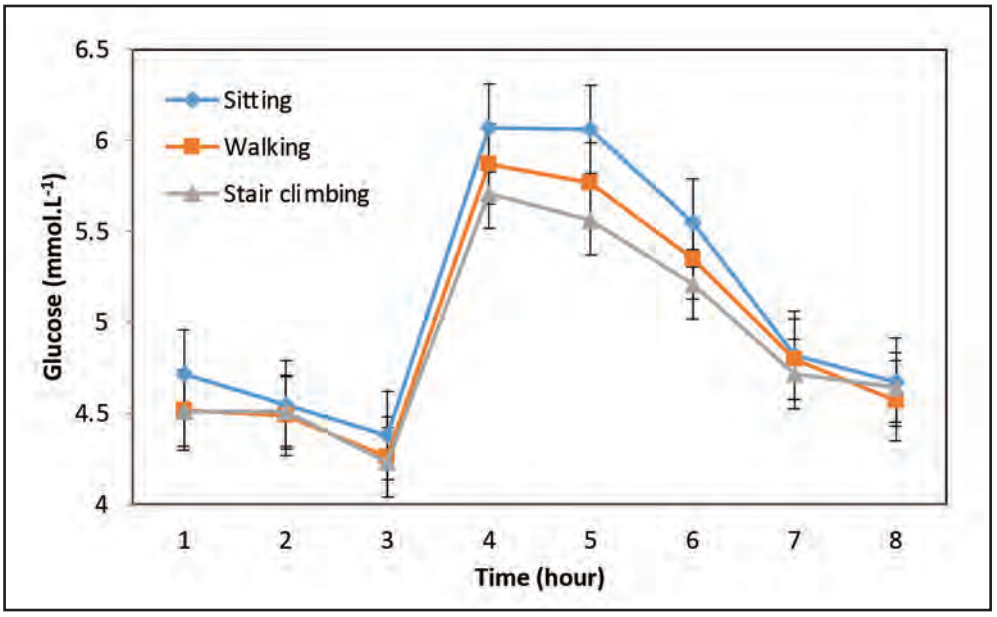


**Fig. 1:** Glucose concentrations throughout the sessions (n=24)

Analysis of the immediate pre-meal value to five hours later, revealed a main effect of time ($F(2, 48.6) = 105.62$, $p<0.001$) that was described primarily by linear ($F(1, 23) = 81.29$, $p<0.001$; 31.6% of the variance) and quadratic components ($F(1, 23) = 163.23$, $p <0.001$; 63.2% of the variance), with a cubic component that explained less variance ($F(1, 23) = 49.09$, $p<0.001$; 4.8% of the variance). There were significant interactions between condition and both the linear ($F(1, 23) = 17.86$, $p<0.001$) quadratic components ($F(1, 23) = 4.71$, $p=0.04$). Follow-up analyses for the linear component revealed differences such that stair climbing was of smaller magnitude than uninterrupted sitting ($p<0.001$) and light walking ($p=0.02$). Similar differences between conditions occurred for the quadratic component but in this case stair climbing was of greater magnitude than uninterrupted sitting ($p=0.04$) and light walking ($p=0.04$). There were no differences between the conditions for the cubic component over time ($F(1, 23) = 0.24$ $p=0.63$). Inspection of the graph suggests that the NEFA response with stair climbing returned more rapidly towards pre-meal levels after the nadir at two hours.

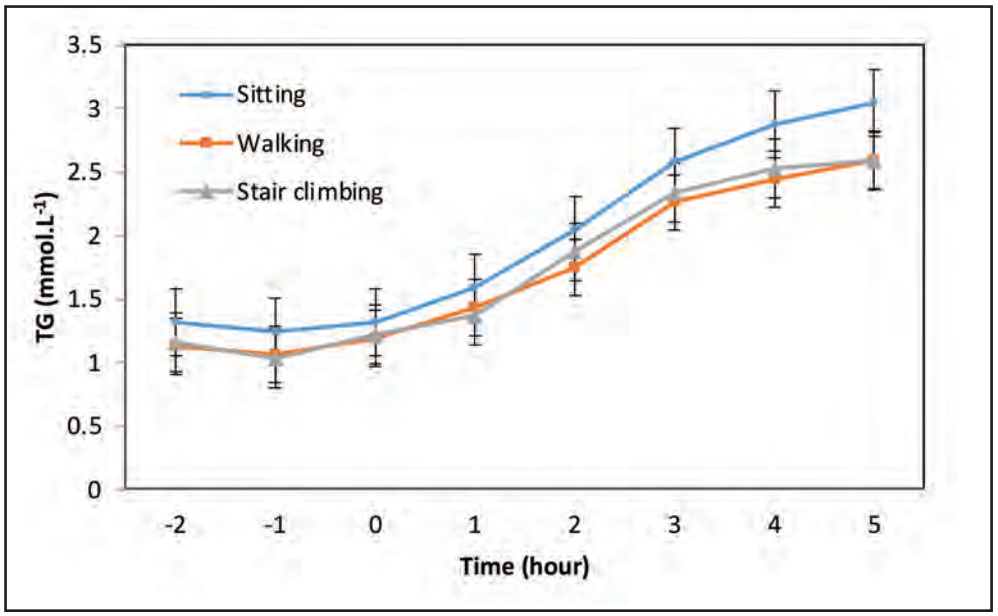


**Fig. 2:** Triglycerides concentrations throughout the sessions (n=24)

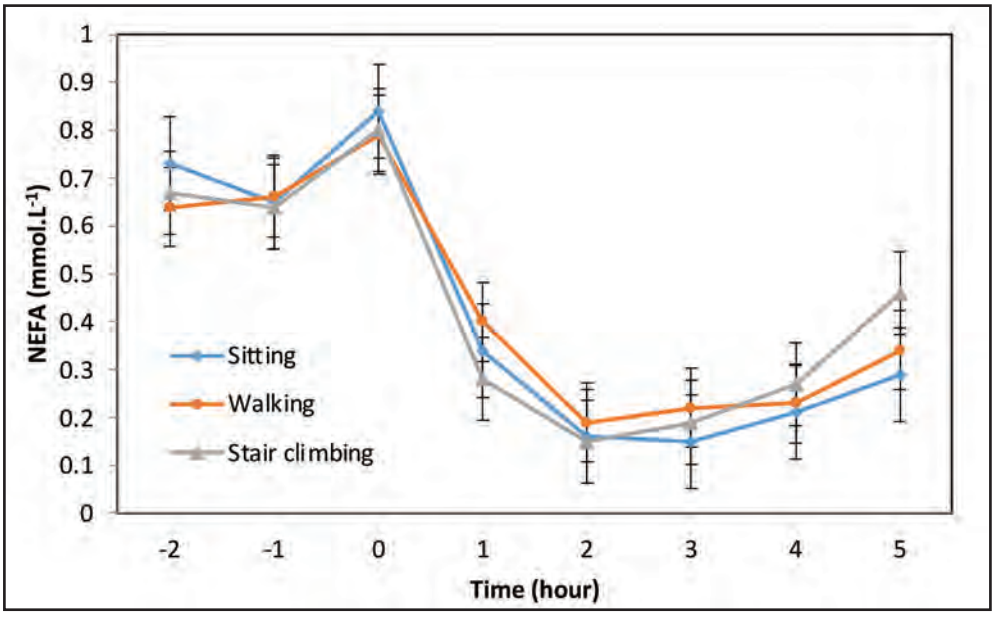


**Fig. 3:** NEFA concentrations throughout the sessions (n=24)

For the NEFA five-hour AUC, there were no significant differences between conditions (condition linear F(1, 23) =2.86, p=0.104). Similarly, for the two-hour AUC, there was only a marginally significant main effect of condition (F(2, 40.7) = 2.60, p=0.09) suggesting equivalent reductions over two hours postprandially.

## DISCUSSION

A mixed pattern was seen in this study. For triglycerides, light intensity walking reduced concentrations postprandially, with only suggestive evidence of a similar effect for the more intense activity of climbing stairs. For glucose, the evidence was reversed. Stair climbing lowered the postprandial peak compared to sitting, with no significant effects of light intensity walking, contrary to Dunstan et al.[5] Nonetheless, stair climbing did not have a significantly greater effects than light walking as predicted. For NEFA, stair climbing resulted in a more rapid return towards pre-meal values than uninterrupted sitting and light walking but unlike Henson et al.,[22] there was no attenuation of NEFA reductions postprandially with light walking. Overall, the data do not confirm the hypothesis of greater effects, the more intense the physical activity used to interrupt sitting.[28]

As outlined in the introduction, light intensity walking has been shown to reduce postprandial glucose in healthy individuals[16,19,20] and in those who were overweight/obese.[5,22]

Mechanically, even light ambulation enhances skeletal muscle glucose uptake through contraction-mediated GLUT4 translocation, independent of insulin which helps dispose of circulating glucose after meals. This effect is particularly relevant postprandially, when skeletal muscle accounts for the majority of glucose disposal. Moreover, breaking prolonged sitting with light walking attenuates the suppression of skeletal muscle lipoprotein lipase (LPL) activity, thereby supporting both lipid and glucose metabolism. In contrast, light intensity walking here had no effect on glucose in a sedentary overweight/obese sample of comparable size to Dunstan and co-workers original study. One possible explanation is that the relative young, lower risk sample here (32 years; fasting glucose 4.29 mmol.L-1) compared to Dunstan and co-workers (54 years; fasting glucose 5.03 mmol.L-1).[5] Younger adults exhibit higher baseline insulin sensitivity and greater cardiorespiratory fitness which can buffer the impact of low-intensity activity. In aging populations by contrast, declines in mitochondrial function, vascular health and insulin sensitivity mean that even light activity provides a proportionally greater benefit for glucose disposal. Typically, cardio-respiratory fitness declines with age as does glucose control. Importantly, BMI and cardio-respiratory fitness exert independent and sometimes opposing effects on glucose homeostasis. McCarthy and co-workers have demonstrated reduced effects of light intensity walking in fitter individuals relative to less fit participants.[19] In contrast, the vigorous activity of stair climbing reduced postprandial glucose, consistent with the effects of walking at moderate intensity in both Peddie et al. and Dunstan et al.[5,13]

As with other metabolic variables, results for the effects of interruptions to sitting on triglycerides postprandially have been mixed. Neither Thorp et al. nor Benatti et al. reported any effects of standing on triglycerides in overweight/obese and healthy samples respectively.[24,25] In contrast, a combination of light walking and standing under free living conditions reduced triglycerides in elderly diabetic individuals the next day.[11] Consistent with the latter study, walking at light intensity and moderate intensity[12] reduced postprandial triglycerides in healthy men and an older sample of healthy post-menopausal women respectively. The reductions in triglycerides with light walking are consistent with two previous studies. What is surprising is that the more intense activity of stair climbing did not have a similar effect. Nonetheless,

Analyses based on orthogonal polynomials were more sensitive to changes than those using the AUC. It is possible that the failure to find consistent effects of the intensity of the interruption reflected a lower risk sample here than in previous research.[5,28] As noted in the methods, changes in biochemical variables have characteristic shapes over time. An overall increase would result in a linear component whereas a peak within the time window would be reflected by a quadratic component. These separable aspects of change over time with polynomials allow independent tests of each aspect of shape in the response. For triglycerides, the consistent increase postprandially was reflected in a substantial linear trend (93% of variance). Both AUC and the linear polynomial analyses concurred that light intensity walking reduced triglycerides postprandially in this data set. In contrast, the glucose response was primarily quadratic in shape (63.6% of the variance). The peak response in the quadratic polynomial was smaller with stair climbing than uninterrupted sitting; there were no AUC differences. For NEFA, statistically similar AUCs contrasted with significant polynomial differences between stair climbing and the other conditions. An AUC provides a single estimate of change over time yet for the glucose polynomials, a substantial cubic component (32.3% of variance) reflected the asymmetrical change as glucose peaked at two hours within a five-hour window. Neither this cubic component nor the minor linear one (2.7% of the variance) differed between conditions. In essence, the glucose AUC included 35% of the variance for glucose change which did not differ between conditions. These two components simply added noise to the glucose AUC. In this study, polynomials were more sensitive to visible differences over time for glucose and NEFA.

## CONCLUSION

This study had tested the effects of the intensity of short bouts of physical activity as intermittent interruptions to sitting. Light intensity walking improved triglycerides whereas vigorous intensity stair climbing improved glucose and NEFA when compared to sitting uninterrupted. The results of both AUC and the linear polynomial analyses concurred that light intensity walking reduced triglycerides postprandially in this data set. As it is already known that analyses based on orthogonal polynomials were more sensitive to changes than those using the AUC; hence it is possible that the failure to find consistent effects of the intensity of the interruption reflected a lower risk sample here than those found in previous research.

## ACKNOWLEDGMENTS

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# Unlocking the potential of augmented reality (AR) in education: Insights from a systematic review of AR-enhanced instructional approaches

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## ABSTRACT

**Introduction: This review explores instructional approaches for AR-enhanced learning to improve student motivation, engagement, and learning outcomes. With AR technology gaining momentum, educators aim to implement best practices that leverage its benefits in diverse subject areas.**

**Materials and Methods: Using a systematic review approach and following PRISMA guidelines, this study analyzed 26 peer-reviewed articles published from 2014 to 2024.**

**Results: Findings were grouped around three central themes: AR's impact on learning and motivation, instructional approaches that support AR integration, and barriers to AR adoption in educational contexts. The analysis suggests that inquiry-based and collaborative approaches with AR improve student engagement, understanding, and academic performance. These results underscore the need for thoughtfully designed AR activities that provide a balance between student autonomy and guided instruction to avoid cognitive overload. However, challenges like accessibility, inclusivity, and limited resources remain obstacles to broader implementation, especially in under-resourced areas.**

**Conclusion: Future research should concentrate on developing standardized frameworks for AR in education, improving inclusivity, and assessing AR's long-term impact on learning outcomes across various educational settings.**

**KEYWORDS:**
*Instructional strategies, augmented reality, educational approaches, education*

## INTRODUCTION

Augmented reality (AR) has the capability to transform the learning experience by introducing an interactive and immersive dimension that extends beyond traditional educational practices. Integrating AR in educational settings helps learners see abstract ideas, experience through hands-on activities, and interact with three-dimensional models that enhance their learning.[1] These technologies are especially promising in disciplines such as science, medicine, and engineering, where spatial visualization is essential. Their potential motivates educators to explore how AR can enhance comprehension and engagement in such contexts.[2] The rapid rise of digital tools and the global shift toward technology-enhanced learning indicate a need to investigate effective instructional strategies for optimizing AR use in education.

Research in this area has shown that AR effectively boosts learning outcomes, focusing on increasing student engagement and long-term memory of complex information. For example, research has demonstrated AR can develop students' spatial ability and foster deeper understanding because they can manipulate 3D models in real time.[3] AR has additionally been proven to enhance motivation, specifically in health sciences and engineering disciplines that require an abstract concept visualization. AR-based interventions are only as effective as how well that technology is integrated into the instructional design. Studies comparing self-directed exploration with instructor-guided AR experiences have produced mixed results regarding which approach yields better learning benefits.[4]

Despite encouraging results, many aspects of AR integration remain unexplored and warrant further investigation. One of the main struggles is figuring out which type works in what educational setting and learning objectives. AR technologies vary widely—from mobile applications to fully immersive environments—making it challenging to compile a comprehensive classification of instructional approaches. Additionally, studies have found that AR technologies lead to better engagement and understanding; however, there is a lack of consensus regarding the balance between self-directed experiences and guided learning approaches. While some studies indicate that too much AR-based learning freedom might cause cognitive overload, others argue that guided approaches could restrain creativity and underuse technology features.[5] These issues are unresolved.

To maximize the educational benefits of AR, it is crucial to identify the instructional strategies that most effectively support its implementation. This study addresses that need by systematically reviewing existing research to determine effective instructional approaches for AR-based learning. Specifically, it seeks to answer the research question: What are the most effective instructional strategies for optimizing AR to enhance student performance, engagement, and comprehension across diverse educational contexts? The

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findings aim to synthesize current evidence, highlight pedagogical implications, and provide practical recommendations for educators and instructional designers seeking to integrate AR meaningfully into teaching and learning.

## MATERIALS AND METHODS

*Identification*

This study followed established systematic review protocols to identify relevant studies. After selecting the main keywords, related synonyms were identified using dictionaries, encyclopaedias, and prior studies. These were used to develop search strings for the Scopus, Web of Science, and ERIC databases (see Table I). The systematic literature review was built on three databases: Scopus, Web of Science, and ERIC, which provide a solid basis to identify the main high-quality research in augmented reality (AR) applications in education.

The systematic search was conducted across three major databases: Scopus, Web of Science, and ERIC. Scopus provided extensive coverage of peer-reviewed interdisciplinary studies, Web of Science ensured high-quality indexed research, and ERIC contributed depth in educational and pedagogical studies.[6] Using these databases ensured wide disciplinary coverage and enhanced the comprehensiveness of the review. This process substantiates the credibility of the review by providing a broad range of studies, that are statistically significant and representative of the present orientation on AR in education. A total of 776 papers relevant to the study question in the first review stage from all three databases' references.

*Screening*

In the screening phase, relevant studies are assessed to confirm they meet the predefined research question(s). Studies focusing on instructional strategies and best approaches for AR in education while removing duplicate entries. Initially, 573 publications were excluded, leaving 203 papers for further review. (as shown in Table II). The initial criterion focused on peer-reviewed journal literature to ensure the inclusion of studies with rigorous methodological quality, transparent reporting, and replicability. Conference papers, book chapters, and dissertations were excluded because they often lack detailed methodology, may not be peer-reviewed, and vary in academic rigor. These exclusions-maintained consistency and reliability across the studies reviewed, thereby strengthening the validity of the systematic synthesis. In this review, only English-language papers published between 2014 and 2024 were included. All identified references were imported into Mendeley to organize citations, remove duplicates efficiently and ensure a systematic screening workflow. Ultimately, 59 papers were excluded for duplication.

*Eligibility*

In the third phase, the eligibility stage, 144 articles were selected. Titles and abstracts of the retrieved articles were screened to determine if they met inclusion criteria as per research objectives. As a result, 118 articles were excluded due to being out of the relevant field, having irrelevant titles and abstracts unrelated to the objectives, or lacking full access even with empirical evidence available. In total, 26 articles were included and finally reviewed.

*Quality Appraisal*

To ensure external validity, the inclusion of all 26 papers was independently reviewed by three reviewers. The reviewers applied the Critical Appraisal Skills Program (CASP) checklist containing eight criteria (Table III). It was assessed using a quality appraisal by experts with over 10 years of experience in educational technology within academic institutions. It was used as a checklist to appraise the quality of studies derived from different types of evidence.[7] Quality appraisal was rated on three levels: excellent, sound, and moderate. Each article was evaluated based on clarity of research aims, methodological appropriateness, data collection relevance, and suitability of analysis techniques. In cases where there were discrepancies in scoring, the reviewers engaged in discussion to reach a consensus. If disagreement remained unresolved, a fourth independent reviewer was consulted to make the final decision. This consensus process ensured consistency and minimized bias in the quality evaluation. Following the quality appraisal, all 26 articles were reviewed, as summarized in Table III.

*Data Abstraction and Analysis*

An integrative analysis approach was used to synthesise studies with diverse research designs, particularly quantitative methods, to identify main themes and subthemes. The first step in theme development was data collection. Following this, the authors reviewed significant existing studies on instructional strategies and best approaches for augmented reality in education, examining the methodologies and research outcomes. The authors then worked collaboratively to create themes based on the evidence within the study's context. A reflective log was maintained to document analytical decisions, challenges, and interpretations. Results were then compared to identify any discrepancies in theme development. When there were conceptual disagreements, the authors discussed them to a mutual consensus.

Additionally, the authors discussed and resolved differences in the coding process by comparing these results during theme development. The authors discussed any discrepancies. In the end, themes were refined for consistency. Three experts—two in educational technology and one in instructional design—validated the identified themes to ensure clarity, relevance, and domain validity. Adjustments were made based on expert feedback.

The questions are as follows below:

1. How does the use of augmented reality (AR) technology influence learning outcomes and motivation among health professional students compared to traditional learning methods?
2. What are the most effective instructional approaches for implementing augmented reality (AR) to enhance students' comprehension and engagement in anatomy education?
3. What are the primary challenges and limitations of using augmented reality (AR) in higher education, and how do these factors affect its effectiveness in enhancing learning outcomes?

**Table I: The Search String**

| | |
|---|---|
| Scopus | TITLE-ABS-KEY ( ("learn* approach*" OR "learn* technique*" OR "instructional technique*" OR "teach* strategie*" OR "instructional strategie*") AND ("augmented reality") AND ("education") ) AND (LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2016 ) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2019 ) OR LIMIT-TO ( PUBYEAR , 2020 ) OR LIMIT-TO (PUBYEAR , 2021 ) OR LIMIT-TO (PUBYEAR , 2022 ) OR LIMIT-TO ( PUBYEAR , 2023 ) OR LIMIT-TO ( PUBYEAR , 2024)) AND (LIMIT-TO (DOCTYPE , "ar") ) AND (LIMIT-TO (LANGUAGE, "English") ) |
| Wos | ("learn* approach*" OR "learn* technique*" OR "instructional technique*" OR "teach* strategie*" OR "instructional strategie*") AND ("augmented reality") AND ("education") (Topic) and 2024 or 2023 or 2022 or 2021 or 2020 or 2019 or 2018 or 2017 or 2016 or 2015 or 2014 (Final Publication Year) and Article (Document Types) and English (Languages) |
| ERIC | augmented reality AND ("learning approaches" OR "instructional techniques" OR "learning techniques" OR "teaching strategies" OR "instructional strategies") AND education |

**Table II: The Selection Criterion is Searching**

| Criterion | Inclusion | Exclusion |
|---|---|---|
| Language | English | Non-English |
| Timeline | 2014–2024 | < 2014 |
| Literature type | Journal (Article) | Conference, Book, Review |

**Table III: The Quality Appraisal**

| | | Yes Expert | | | No Expert | | | Total agreement | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | (%) | |
| Section A: Are the results valid? | 1. Was there a clear statement of the aims of the research? | / | / | / | | | | 100 | Excellent |
| | | / | / | / | | | | 100 | Excellent |
| | 2. Is a qualitative, quantitative and mixed-method research approach appropriate? | / | / | / | | | | 100 | Excellent |
| | | / | / | / | | | | 100 | Excellent |
| | 3. Was the research design appropriate to address the aims of the research? | / | / | / | | | | 100 | Excellent |
| | | / | / | / | | | | 100 | Excellent |
| | 4. Was the recruitment strategy appropriate to the aims of the research? | / | / | / | | | | 100 | Excellent |
| | | / | / | / | | | | 100 | Excellent |
| | 5. Was the data collected in a way that addressed the research issue? | | | | | | | | |
| Section B: What are the results | 6. Was the data analysis sufficiently rigorous? | | | | | | | | |
| | 7. Is there a clear statement of findings? | | | | | | | | |
| Section C: How valuable is the research? | 8. How valuable is the research? | | | | | | | | |

## RESULTS

Table IV outlines how the authors thoroughly analyzed 26 publications to extract assertions or information pertinent to the study's focus and Figure 1 shows a PRISMA flowchart detailing the search and selection processes.

This systematic review synthesized evidence from 26 eligible studies examining the integration of augmented reality (AR) in educational contexts. The results were organized according to three main themes: (i) the impact of AR on learning outcomes and motivation, (ii) instructional approaches for effective AR implementation, and (iii) challenges and limitations influencing AR adoption.

*Impact of AR on Learning Outcomes and Motivation*

Across the included studies, AR was consistently found to improve students' academic performance, conceptual understanding, and overall engagement. Several investigations reported that AR-based instruction led to higher post-test scores and improved retention compared to conventional methods. [8,9,13,18,19,23,26,30] These effects were particularly evident in science, medical, and engineering subjects that require spatial reasoning and visualization. The interactive manipulation of virtual models allowed learners to grasp abstract or dynamic phenomena more effectively than when using static resources.

In addition to cognitive benefits, motivation and attention levels were positively influenced. Multiple studies highlighted that learners exposed to AR activities displayed stronger curiosity, emotional involvement, and satisfaction during lessons. [9,11,13,18,20,25] The immersive and hedonic qualities of AR promoted intrinsic motivation by increasing enjoyment and reducing boredom. Learners reported feeling more confident and focused, suggesting that the technology supports sustained participation.

Collectively, the results indicate that AR enhances both cognitive and affective learning domains. When aligned with learning objectives, AR stimulates interest, maintains concentration, and supports meaningful knowledge construction through active engagement.

**Table IV: Summary of Selected Article**

| No | Authors | Title | Journal | Findings |
|---|---|---|---|---|
| 1 | Huang et al.[8] | Animating eco-education: To see, feel, and discover in an augmented reality-based experiential learning environment | Computers & Education (2016) | - AR system improved learners' emotional engagement and learning effectiveness compared to traditional methods<br>- increased curiosity and interest |
| 2 | Chiang et al.[9] | An Augmented Reality-based Mobile Learning System to Improve Students' Learning Achievements and Motivations in Natural Science Inquiry Activities | Educational Technology & Society (2014) | - augmented reality-based mobile learning system significantly improved students' learning achievements in inquiry-based activities<br>- higher motivation levels in attention, confidence, and relevance dimensions<br>- enhancing learning performance by reducing cognitive load |
| 3 | Cai et al. [10] | Tablet-based AR technology: Impacts on students' conceptions and approaches to learning mathematics according to their self-efficacy | British Journal of Educational Technology (2019) | - enhance students' conceptions and learning approaches<br>- enhance learning experiences, particularly for students with higher self-efficacy, by fostering deeper understanding and engagement |
| 4 | Hsieh [11] | Development and Application of an Augmented Reality Oyster Learning System for Primary Marine Education | Electronics (2021) | - improved students' learning achievements<br>- increased interest and motivation in learning<br>- more engaging and interactive learning experience |
| 5 | Hsu [12] | Learning English with Augmented Reality: Do learning styles matter? | Computers & Education (2017) | - self-directed and task-based Augmented Reality (AR) educational game systems resulted in similar high learning effectiveness |
| 6 | Czok et al. [13] | Learning Effects of Augmented Reality and Game-Based Learning for Science Teaching in Higher Education in the Context of Education for Sustainable Development | Sustainability (2023)<br><br><br>International | - enhances motivation and user engagement<br>- AR does not negatively affect knowledge acquisition compared to traditional learning environments |
| 7 | Kamal and Junaini [14] | The Effects of Design-Based Learning in Teaching Augmented Reality for Pre-University Students in The ICT Competency Course | Journal of Scientific & Technology Research (2019) | - design-based learning (DBL) approach significantly enhances students' academic performance<br>- increase in both lower-order thinking skills (LOTS) and higher-order thinking skills (HOTS) after the implementation of DBL |
| 8 | Nasir and Fakhruddin [5] | Design and Analysis of Multimedia Mobile Learning Based on Augmented Reality to Improve Achievement in Physics Learning | International Journal of Information and Education Technology (2023) | - multimedia mobile learning based on Augmented Reality (AR) significantly improves students' achievement |
| 9 | Chiang et al. [16] | Students' online interactive patterns in augmented reality-based inquiry activities | Computers & Education (2014) | - AR-based inquiry learning activity significantly increased student interactions for knowledge construction<br>- immersive learning experiences, enhancing students' concentration and enabling them to achieve high-level thinking |
| 10 | Wen et al. [17] | Integrating augmented reality into inquiry-based learning approach in primary science classrooms | Education Tech Research Dev (2023) | - emphasize the importance of providing autonomy in learning and encouraging diverse knowledge representation to enhance creative thinking |
| 11 | Cercenelli et al. [18] | AEducaAR, Anatomical Education in Augmented Reality: A Pilot Experience of an Innovative Educational Tool Combining AR Technology and 3D Printing | International Journal of Environmental Research and Public Health (2022) | - enhance learning, motivation, and confidence with future medical technologies |

**Table IV: Summary of Selected Article**

| No | Authors | Title | Journal | Findings |
|---|---|---|---|---|
| 12 | Rodríguez-Abad et al.[19] | Online (versus face-to-face) augmented reality experience on nursing students' leg ulcer competency: Two quasi-experimental studies | Nurse Education in Practice (2023) | - (AR)-based online educational experience significantly improved academic performance<br>- High scores were reported in motivation, satisfaction, and other learning determinants, indicating that AR enhances the teaching-learning process |
| 13 | Li et al. [20] | From motivational experience to creative writing: A motivational AR-based learning approach to promoting Chinese writing performance and positive writing behaviours | Computers & Education (2023) | - motivational AR-based (MAR) learning approach significantly improved students' performance<br>- greater immersion and interaction |
| 14 | Lin et al. [21] | Mitigating the Urban-rural Digital Divide: A Dual Scaffolding-embedded Mobile Augmented Reality Learning Approach in the Post-COVID-19 Pandemic | Educational Technology & Society (2023) | - dual scaffolding-embedded mobile AR learning approach significantly improved learning achievement, higher-order cognition, and self-efficacy |
| 15 | Lee and Hsu[22] | Sustainable Education Using Augmented Reality in Vocational Certification Courses | Sustainability (2021) | - AR learning approach significantly improved students' learning effectiveness<br>- reduced cognitive load for both active and reflective learners, making the learning process less burdensome |
| 16 | Küçük et al.[23] | Learning Anatomy via Mobile Augmented Reality: Effects on Achievement and Cognitive Load | Anatomical Sciences Education (2016) | - using mobile augmented reality (mAR) applications for learning anatomy achieved higher academic success<br>- mAR applications effectively reduced the mental effort required to learn complex anatomical structures |
| 17 | Rizki et al. [24] | Cooperative model, digital game, and augmented reality-based learning to enhance students' critical thinking skills and learning motivation | Journal of Pedagogical Research (2024) | - Cooperative Model, Digital Game, and Augmented Reality (CAP)-based learning approach showed a significant improvement, achieving a high level of critical thinking skills |
| 18 | Harncharnchai and Saeheaw [25] | Context-aware learning using augmented reality and WebQuest to improve students' learning outcomes in history | Int. J. Innovation and Learning (2018) | - improved learning outcomes<br>- expressed high satisfaction with the use of AR and WebQuest, enhancing their engagement and motivation in learning |
| 19 | Weng et al.[26] | Can an augmented reality-integrated gamification approach enhance vocational high school students' learning outcomes and motivation in an electronics course? | Education and Information Technologies (2024) | - AR-integrated gamification approach significantly improved students' learning outcomes across all domains (cognitive, affective, and psychomotor) |
| 20 | Cascales-Martínez et al. [27] | Using an Augmented Reality Enhanced Tabletop System to Promote Learning of Mathematics: A Case Study with Students with Special Educational Needs | EURASIA Journal of Mathematics Science and Technology Education (2017) | - increase in knowledge among students with learning disabilities, learning disorders, and attention deficit hyperactivity disorder<br>- improved student motivation and collaboration during the learning process |
| 21 | Hsu [28] | Effects of gender and different augmented reality learning systems on English vocabulary learning of elementary school students | Univ Access Inf Soc (2019) | - Collective Game-Based (CGB) and Sequential-Mission Gaming (SMG) systems resulted in high learning effectiveness with no significant gender differences in performance |
| 22 | Cheng et al. [29] | An in-depth analysis of the interaction transitions in a collaborative Augmented Reality-based mathematic game | Interactive Learning Environments (2019) | - interaction transitions in a digital game-based learning environment are dynamic and non-linear |
| 23 | Ruiz-Ariza et al. [30] | Effect of augmented reality game Pokemon GO on cognitive performance and emotional intelligence in adolescent young | Computers & Education (2018) | - significant improvements in selective attention, concentration, and sociability<br>- no significant improvements were observed in memory, mathematical calculation, or linguistic reasoning |

**Table IV: Summary of Selected Article**

| No | Authors | Title | Journal | Findings |
|---|---|---|---|---|
| 24 | Bos et al. [31] | Educational Technology and Its Contributions in Students' Focus and Attention Regarding Augmented Reality Environments and the Use of Sensors | Journal of Educational Computing Research (2019) | - increased student attention compared to traditional educational technologies like the Moodle virtual learning environment (VLE)<br>- higher concentration level while interacting with the AR application |
| 25 | Giancaspro et al. [32] | An active learning approach to teach distributed forces using augmented reality with guided inquiry | Computer Applications in Engineering Education (2024) | - augmented reality (AR) as a guided instructional tool significantly improved students' understanding of distributed forces<br>- AR technology also helped address misconceptions |
| 26 | Chen et al. [33] | Supporting informal science learning with metacognitive scaffolding and augmented reality: effects on science knowledge, intrinsic motivation, and cognitive load | Research in Science & Technological Education (2023) | - combination of metacognitive scaffolding and augmented reality (AR) significantly improved the science knowledge post-test scores<br>- No significant differences were observed in cognitive load |

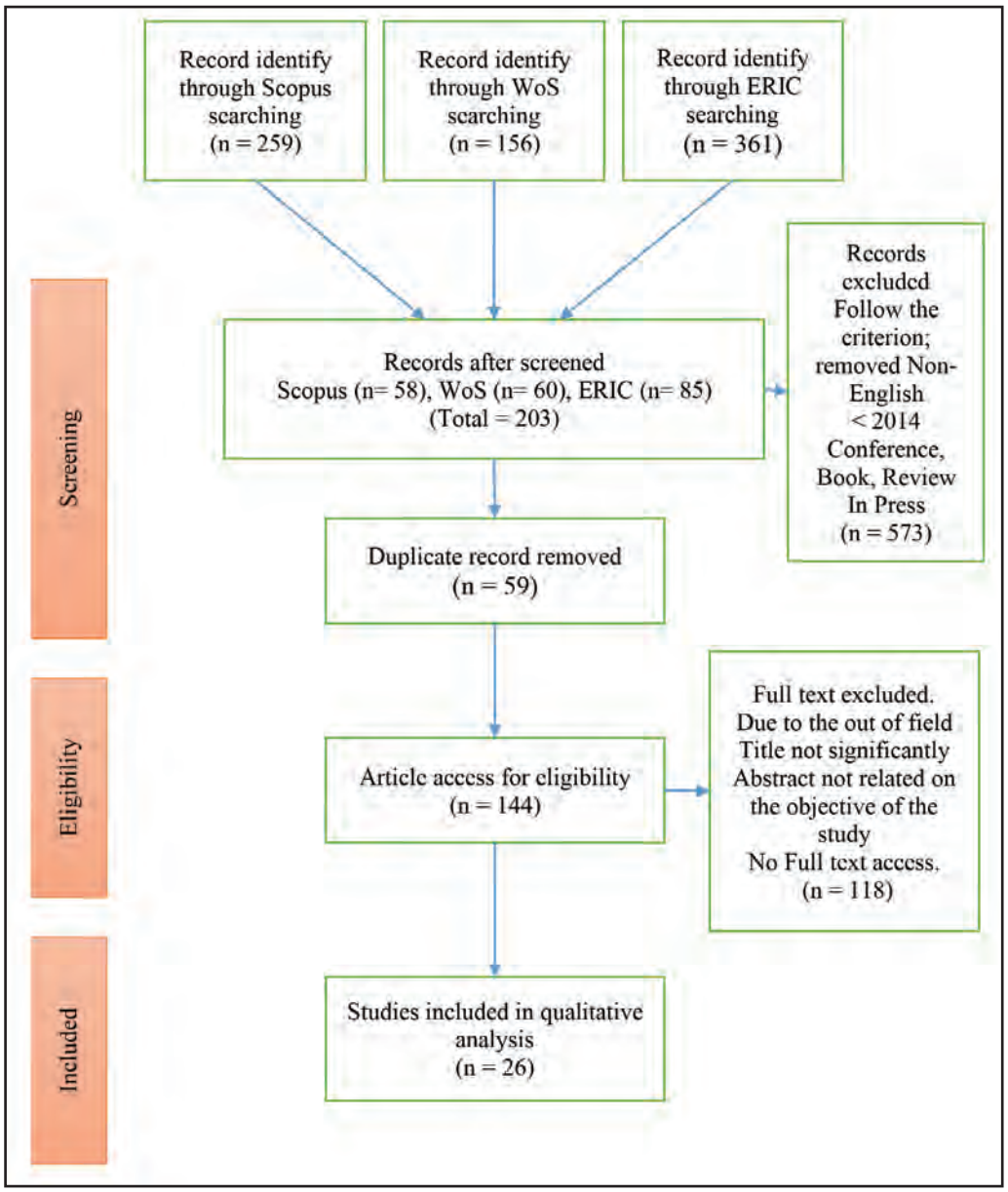


**Fig. 1:** Flow Diagram of the Proposed Searching Study [34]

*Instructional Approaches for Effective AR Implementation*
The majority of studies emphasized that AR's educational effectiveness depends greatly on the instructional framework used to deliver it. Inquiry-based learning emerged as a dominant approach, with several studies reporting improvements in students' scientific inquiry skills, problem-solving ability, and higher-order thinking when AR was integrated within structured investigations. [9,16,17,21,24,32] These designs encouraged exploration and hypothesis testing, allowing students to connect virtual observations with real-world phenomena.

Another recurring theme was the application of collaborative and game-based learning. Studies that combined AR with teamwork or gamified activities found that learners were more engaged, communicative, and cooperative in achieving shared objectives. [14,19,27,29] Collaborative AR environments fostered peer interaction and social learning, which contributed to greater conceptual understanding and skill development.

Additionally, a number of studies incorporated systematic design models such as ADDIE and design-based learning (DBL) to guide the creation and evaluation of AR modules. [15,17,22,28,30] The sequential phases of analysis, design, development, implementation, and evaluation ensured instructional alignment and minimized extraneous cognitive load. When AR content followed these structured processes, students demonstrated better comprehension, creativity, and application of learned concepts.

Overall, the reviewed studies suggest that AR integrated within structured instructional models—particularly inquiry-based, collaborative, and design-model approaches—produces more durable learning gains by aligning technological affordances with sound pedagogical principles.

*Challenges and Limitations of AR in Education*
Despite its promising outcomes, the reviewed evidence also revealed several limitations that constrain widespread AR implementation. Technical barriers such as limited device availability, unstable internet connectivity, and insufficient institutional support were frequently cited. [15,21,26,31] These issues reduced accessibility and created disparities between schools with different resource capacities.

Pedagogical challenges were also evident. Some studies warned that poorly aligned or overly complex AR content could overwhelm learners, leading to cognitive overload and confusion about learning objectives. [15,18,23,27] The absence of appropriate scaffolding or integration with curriculum goals diminished the educational value of AR activities.

Finally, social and contextual constraints emerged as critical barriers. Teacher readiness and professional development remained inadequate, and some educators expressed uncertainty or reluctance to adopt emerging technologies.[21,24,30] Cultural factors and accessibility concerns, particularly for students with disabilities or in under-resourced regions, further limited equitable use of AR.

In summary, the main constraints identified were technological limitations, instructional misalignment, and insufficient teacher preparedness. Addressing these issues through improved training, infrastructure, and inclusive design will be essential to optimize the pedagogical potential of AR in education.

In summary, evidence from studies 24–49 demonstrates that AR can significantly enhance learning outcomes, engagement, and motivation when embedded within well-structured pedagogical frameworks. However, its effectiveness depends on the availability of resources, the quality of instructional design, and the readiness of educators to implement it. Overcoming technical and contextual barriers remains critical to realizing the full educational potential of AR across disciplines.

## DISCUSSION

This systematic review synthesized evidence from 26 studies examining the use of augmented reality (AR) within instructional frameworks. Overall, the findings indicate that AR-enhanced learning approaches are associated with improved learning outcomes, stronger learner engagement, and higher motivation. These results are consistent across a wide range of subjects, though the impact appears particularly significant in the sciences and health professions, where visual-spatial reasoning and procedural understanding are crucial. Prominent pedagogical strategies identified include inquiry-based, collaborative, and design-model-based learning approaches. The variation in study quality, design, and learner population, however, suggests that these outcomes should be interpreted with care. [35,36]

In medical and health-science education, the benefits of AR integration are clearly evident. AR has been applied successfully in teaching anatomy, clinical skills, and radiology, where complex spatial and procedural content often limits traditional instruction. Platforms such as AEducaAR, which combine 3D printing with AR, have been shown to enhance anatomical comprehension, motivation, and learner confidence among medical students. [18] Similarly, AR-based nursing and radiology modules have improved procedural performance and satisfaction, while reducing the cognitive strain associated with interpreting static two-dimensional materials. [37] By overlaying dynamic 3D anatomical structures onto real-world references, AR helps learners visualise spatial relationships that are otherwise difficult to grasp, thereby supporting both cognitive and psychomotor learning. [38]

When embedded within structured instructional models, AR appears to promote more meaningful and sustained learning. Inquiry-based frameworks encourage students to explore, test, and construct knowledge through guided discovery, whereas collaborative and game-based approaches create active learning environments that foster teamwork and communication. The ADDIE model offers a systematic process—analysis, design, development, implementation, and evaluation—that ensures AR activities remain aligned with learning outcomes and reduce unnecessary cognitive load.[39,40] Through such structured

integration, AR can bridge the gap between theoretical knowledge and practical application, helping students connect classroom instruction to real-world clinical contexts.[41]

Despite its advantages, several limitations remain. Technical issues such as high hardware costs, unstable connectivity, and the limited availability of technical support continue to hinder implementation, particularly in low-resource or rural settings. [39] Pedagogical challenges also persist, as poorly aligned AR content can overwhelm learners and dilute intended learning objectives. Furthermore, educators often report insufficient training and support to incorporate AR effectively into their courses. Socio-cultural barriers—including resistance to new technologies and limited accessibility for students with disabilities—further restrict widespread adoption. [42,43]

Addressing these limitations requires a balanced approach that combines infrastructure investment with thoughtful instructional design and inclusive practice. Educators need ongoing professional development to ensure that AR integration is both purposeful and pedagogically sound. As AR continues to evolve, future research should examine its long-term effects on learning retention, clinical competence, and cost-effectiveness across different healthcare disciplines.

*Review Strengths and Weaknesses*

A key strength of this review lies in its methodological rigour and the breadth of educational settings examined. The systematic search strategy, which identified 26 peer-reviewed studies (references 8–33) from multiple databases, ensured that the evidence base was comprehensive and reflective of diverse instructional contexts. This methodological consistency enhanced the credibility of the synthesis and provided a balanced overview of AR integration across disciplines, including health sciences and STEM education.

However, the review was limited to peer-reviewed English-language publications and did not include grey literature, which may introduce a degree of reporting bias. The diversity of AR applications and instructional models also precluded a meta-analytic approach, as the data were too heterogeneous for quantitative synthesis.

## CONCLUSION

Augmented reality (AR) holds strong transformative potential in education, particularly when carefully integrated with structured instructional approaches such as inquiry-based and collaborative learning. Its effectiveness depends on thoughtful instructional design, continuous educator support, and adequate infrastructure.

To fully realise this potential, technical, pedagogical, and social barriers must be addressed through coordinated institutional and policy efforts. Establishing standardised frameworks for AR implementation, ensuring inclusive access for diverse learners, and evaluating its long-term impact across educational settings are essential next steps. With these foundations, AR can evolve into a sustainable and equitable tool that fosters engagement, motivation, and deep learning across disciplines, especially in health sciences education.

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