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Systematic / Narrative Review Article

Case Report

Prognostic factors for five-year survival in children with biliary atresia after the Kasai procedure

Dyah Utami Nugraheni, MD[1], Agnes Treyssia Sandewa, MD[1], Dennis, MD[1], Pramana Adhityo, MD[1], Raffi Gani, MD[1], Petrus Gandi Purwosatrio, MD[1], Maulida Maharani, MD[1], Ira Puspitawati, MD[2], Akhmad Makhmudi, PhD[1], Gunadi, PhD[1]

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ABSTRACT

Introduction: Biliary atresia (BA) is an idiopathic disease characterized by progressive fibro-obliteration of extra- and intrahepatic biliary ducts manifesting jaundice >2 weeks. The primary treatment for BA is the Kasai procedure. However, patient survival in BA is influenced by several prognostic factors. We aimed to identify the prognostic factors for 5-year survival of BA patients following the Kasai procedure at Dr. Sardjito Hospital, Yogyakarta, Indonesia.

Materials and Methods: This observational analytic study employed a retrospective cohort design, included BA patients who underwent Kasai procedures at our hospital between January 2012 and December 2018.

Results: There was no association between age at surgery (p=0.408), TB7 (p=0.973), operator experience (p=0.649), AST0 (p=0.973), AST7 (p=1), the AST7/AST0 ratio (p=0.682), ALT0 (p=0.682), ALT7 (p=0.697), and the ALT7/ALT0 ratio (p=1) with 5-year survival in BA patients after Kasai procedure. A log-rank analysis showed no significant results: age at surgery (p=0.264), TB7 (p=0.961), operator experience (p=0.479), AST0 (p=0.993), AST7 (p=0.931), AST7/AST0 ratio (p=0.562), ALT0 (p=0.708), ALT7 (p=0.640), and ALT7/ALT0 ratio (p=0.963).

Conclusion: The timing of surgery, total bilirubin levels at 7 days post-surgery, surgeon experience, and both pre-and post-operative AST and ALT may not predict 5-year survival outcomes in BA patients following Kasai surgery. Further extensive cohort studies are necessary to confirm these preliminary findings.

KEYWORDS:
Biliary atresia, 5-year survival, Kasai procedure, prognostic factors

INTRODUCTION

Biliary atresia (BA) is an idiopathic condition in infants marked by the obliteration or discontinuity of the extrahepatic and intrahepatic bile ducts, resulting in bile flow obstruction.[1] Progressive fibro-obliteration of the biliary tract leads to jaundice lasting more than two weeks. The clinical features of BA include pathological jaundice, pale or clay-colored stools, dark urine, and hepatomegaly. BA is classified into two forms: syndromic BA (embryonic type) and non-syndromic BA (perinatal type).[2] Its incidence ranges from 1 in 12,000 in the US and the UK to 1 in 9,600 in Japan and 1 in 5,000-8,000 in China.[3] In Indonesia, the incidence of BA is also high, affecting 1 in 7,000 live births.[4]

Kasai portoenterostomy, which involves resecting the obstructed bile duct and forming an anastomosis between the hepatic portal and the jejunum using a Roux loop to enhance bile drainage from the intrahepatic ducts to the small intestine, remains the treatment of choice for BA.[5] When the Kasai procedure fails, liver transplantation becomes the secondary treatment, guided by post-Kasai liver transplantation scoring systems.[4] BA is the leading cause of end-stage liver disease in children and a primary indication for liver transplantation.[6] Due to the limited availability of liver transplants in Indonesia, the Kasai procedure serves as the first-line treatment for BA, enhancing survival with the native liver without the need for transplantation.[4]

The outcomes of the post-Kasai procedure are not consistently favorable.[5] Several prognostic factors that influence the success of the Kasai procedure include the age at the time of operation, total serum bilirubin levels seven days after surgery, and the experience of the surgeon.[7-10] However, studies on prognostic factors for 5-year survival of patients with BA post-Kasai surgery are still limited. Therefore, we aimed to identify the prognostic factors, including age at the Kasai operation, bilirubin levels 7 days post-Kasai (TB7), surgeon experience, preoperative AST levels (AST0), AST levels 7 days post-Kasai (AST7), AST7/AST0 ratio, preoperative ALT levels (ALT0), ALT levels seven days post-Kasai (ALT7), and ALT7/ALT0 ratio, for the 5-year survival of BA patients following the Kasai surgery.

MATERIALS AND METHODS

This observational analytic study employed a retrospective cohort design. The study population included BA patients treated at Dr. Sardjito Hospital, Yogyakarta, Indonesia. Inclusion criteria comprised BA patients who underwent Kasai procedures at our hospital between January 2012 and December 2018—exclusion criteria involved excluding patients with incomplete medical records. The Kasai portoenterostomy procedures performed at our institution

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adhered to the institutional guidelines for the surgical management of biliary atresia. These guidelines maintained a standardized approach of the Kasai procedure.[11-12] Following the Kasai procedure, patients also received standardized post-operative care at our institution. This included routine antibiotic prophylaxis, nutritional support, and a standardized high-dose corticosteroid regimen based on the principles of high-dose intravenous methylprednisolone, followed by a gradually tapered oral steroid regimen to improve bilirubin clearance.[13-14] Data collection involved reviewing the medical records of BA patients who underwent Kasai procedures during the specified period.

Prognostic variables included age at surgery, bilirubin levels 7 days post-surgery (TB7), surgeon experience, preoperative AST levels (AST0), AST levels 7 days post-surgery (AST7), AST7/AST0 ratio, preoperative ALT levels (ALT0), ALT levels 7 days post-surgery (ALT7), and ALT7/ALT0 ratio.

This research received ethical approval from the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada/Dr. Sardjito Hospital (Ref. #KE/FK/1252/EC/2018). Before participating in the study, all patients' parents or guardians provided informed consent.

Data Analysis

The chi-square or Fisher's exact test was used to examine the association between independent and dependent variables. The receiver operating characteristic (ROC) analysis was employed to determine cut-off values for laboratory parameters, and the Kaplan-Meier test was utilized to assess 5-year survival rates. Data was analyzed using IBM's Statistical Package for the Social Sciences (SPSS), version 23.

RESULTS

This study included 24 patients, the majority of whom were male (70.83%). Table I presents the subjects' baseline characteristics.

Next, we determined the cut-off values for the prognostic factors using ROC (Figure 1). For the age of the Kasai procedure, we defined the groups as <90 and ≥90 days. None of the prognostic factors were associated with the 5-year survival rate of BA patients following the Kasai procedure (Table II).

Subsequently, we performed the Kaplan-Meier analysis. None of the prognostic factors showed any significant association with the 5-year survival rate in BA patients following Kasai surgery (Figure 2).

DISCUSSION

Subject Characteristics

There was a male preponderance among the participants in our study, with 17 (70.83%) patients being male, while 7 (29.16%) were female. However, sex distribution in BA varies across different studies. Gunadi et al. (2018) reported a higher prevalence of BA among male patients[4], while Andrade et al. (2018) found a higher prevalence of female patients (60.31%) compared to males (39.69%).[15] Nonetheless, multiple reports demonstrated that sex does not significantly affect the prognosis of BA patients following the Kasai procedure, thus sex was not included in the further analysis.[16-17]

BA is often associated with syndromic conditions and is recognized as a factor influencing disease progression and surgical outcomes, including the success of the Kasai procedure, making the assessment of these factors valuable for understanding long-term survival.[18] Based on medical records, three patients died due to sepsis, one died from cholangitis, and others died from Disseminated Intravascular Coagulation (DIC), internal bleeding, and multiple organ failure. Additionally, one patient was identified as having Down syndrome.

Age of Surgery

Early age at the time of Kasai procedure has often been associated with better outcomes in BA patients. In our analysis, patients underwent surgery before 90 days of life showed a slightly higher survival rate than those operated on at or after 90 days (OR: 2.7, 95% CI: 0.51-14.37, p=0.408) indicating better survival with earlier surgery (Table II). However, this association was not statistically significant. Similarly, Gunadi et al. (2018) reported that patients younger than 60 days at the time of surgery had better outcomes compared to those aged ≥ 60 days, although not statistically significant.[4] Furthermore, Saragih (2019) also reported no statistically significant correlation between age at surgery and patient survival.[19] Hoshino et al. (2023) also found that patients who had Kasai procedure at a later timing (>30 days) have an earlier need for liver transplantation compared with patients with earlier KP (<=30 days).[20]

Based on our Kaplan-Meier analysis (Figure 1A), patients who underwent surgery at <90 days had better 5-year survival outcomes. However, according to the log-rank test, age at surgery was not associated with the 5-year survival of biliary atresia patients post-Kasai procedure (p = 0.264). This suggests that while early intervention may be beneficial, the age of surgery alone may not influence survival outcomes in BA patients who underwent Kasai surgery.

Total Bilirubin Seven Days Post-Surgery (TB7)

The cut-off value for TB7 was 9.635, with a sensitivity of 46.2% and a specificity of 54.5% (Figure 2A). Our findings found no significant correlation between total bilirubin (TB) levels seven days post-Kasai and five-year survival, although patients with TB7 < 9.635 demonstrated a slightly higher survival rate (Table II). Similarly, Chusilp et al. (2016) suggest that >20% decrease in serum TB7 is a reliable indicator of a favorable outcome as patients with TB7/TB0 ratios less than 0.8 have a significantly higher 5-year survival rate compared to those with ratios greater than 0.8.[21] Furthermore, previous report demonstrated that higher long-term (3 months) (more than 2.0 mg/dL) in the first 3 month following Kasai procedure had a higher risk of disease progression, leading to an earlier need of liver transplant.[22]

The Kaplan-Meier survival curve (Figure 1B) and the log-rank test indicated that the bilirubin levels 7 days post-surgery were not statistically significant in affecting the 5-year

Table I: Baseline characteristics of BA patients.

Characteristics	N (%)	Mean ± SD
Sex		
▪ Male	17 (70.83)	
▪ Female	7 (29.16)	
Age at Surgery		
▪ <90 days	10 (41.66)	
▪ ≥90 days	14 (58.33)	
TB7 (mg/dL)		9.42 ± 3.33
Surgeon		
▪ Consultant	18 (75)	
▪ Not-consultant	6 (25)	
AST (IU/L)		
▪ Pre-operative (AST0)		384.29 ± 495.37
▪ 7 days after surgery (AST7)		151.17 ± 101.37
ALT (IU/L)		
▪ Pre-operative (ALT0)		191.67 ± 165.08
▪ 7 days after surgery (ALT7)		186.58 ± 142.26
Survival		
▪ Alive	11 (45.83)	
▪ Deceased	13 (54.17)	

Table II: Association of prognostic factors with the 5-year survival rate in BA patients following Kasai surgery.

Prognostic Factor	Survived (N, %)	Deceased (N, %)	OR (95% CI)	p
Age at Surgery				
▪ < 90 days	6 (25)	4 (16.7)	2.7 (0.51-14.37)	0.408
▪ ≥ 90 days	5 (20.8)	9 (37.5)	Reference	
TB7				
▪ < 9.635	6 (25)	7 (29.2)	1.03 (0.21-5.15)	0.973
▪ ≥ 9.635	5 (20.8)	6 (25)	Reference	
Surgeon experience				
▪ Consultant	9 (37.5)	9 (37.5)	2 (0.29-13.81)	0.64
▪ Non-consultant	2 (8.3)	4 (16.7)	Reference	
AST0				
▪ < 265.5	6 (25)	7 (29.2)	1.03 (0.21-5.15)	0.973
▪ ≥ 265.5	5 (20.8)	6 (25)	Reference	
AST7				
▪ < 92.5	4 (16.7)	4 (16.7)	1.29 (0.23-7.05)	1.000
▪ ≥ 92.5	7 (29.2)	9 (37.5)	Reference	
AST7/AST0				
▪ < 0.473	5 (20.8)	7 (29.2)	0.71 (0.14-3.58)	0.682
▪ ≥ 0.473	6 (25)	6 (25)	Reference	
ALT0				
▪ < 162	5 (20.8)	7 (29.2)	0.71 (0.14-3.58)	0.682
▪ ≥ 162	6 (25)	6 (25)	Reference	
ALT7				
▪ < 135	4 (16.7)	6 (25)	0.67 (0.13-3.45)	0.697
▪ ≥ 135	7 (29.2)	7 (29.2)	Reference	
ALT7/ALT0				
▪ < 0.667	4 (16.7)	4 (16.7)	1.29 (0.23-7.05)	1.000
▪ ≥ 0.667	7 (29.2)	9 (37.5)	Reference	

OR, odds ratio; CI, confidence interval

survival of biliary atresia patients post-Kasai surgery (p=0.961). These suggest that although persistently elevated TB7 levels post-Kasai have been linked to poor bile drainage and increased risk of liver fibrosis or failure in previous research[21-23], our findings suggest that TB7 alone did not demonstrate the prognostic association for the survival of BA patients who underwent Kasai surgery.

Surgeon Experience

In this study, consultant surgeons resulted in a higher survival rate (37.5%) than non-consultants (8.3%). However, the odds ratio (2.0, 95% CI: 0.29-13.81, p=0.649) was not statistically significant, possibly due to the small sample size. Based on the Kaplan-Meier analysis (Figure 1C), senior surgeons demonstrated a better 5-year survival rate. However, according to the log-rank analysis, surgeon experience was not statistically significant (p=0.479) (Figure 2).

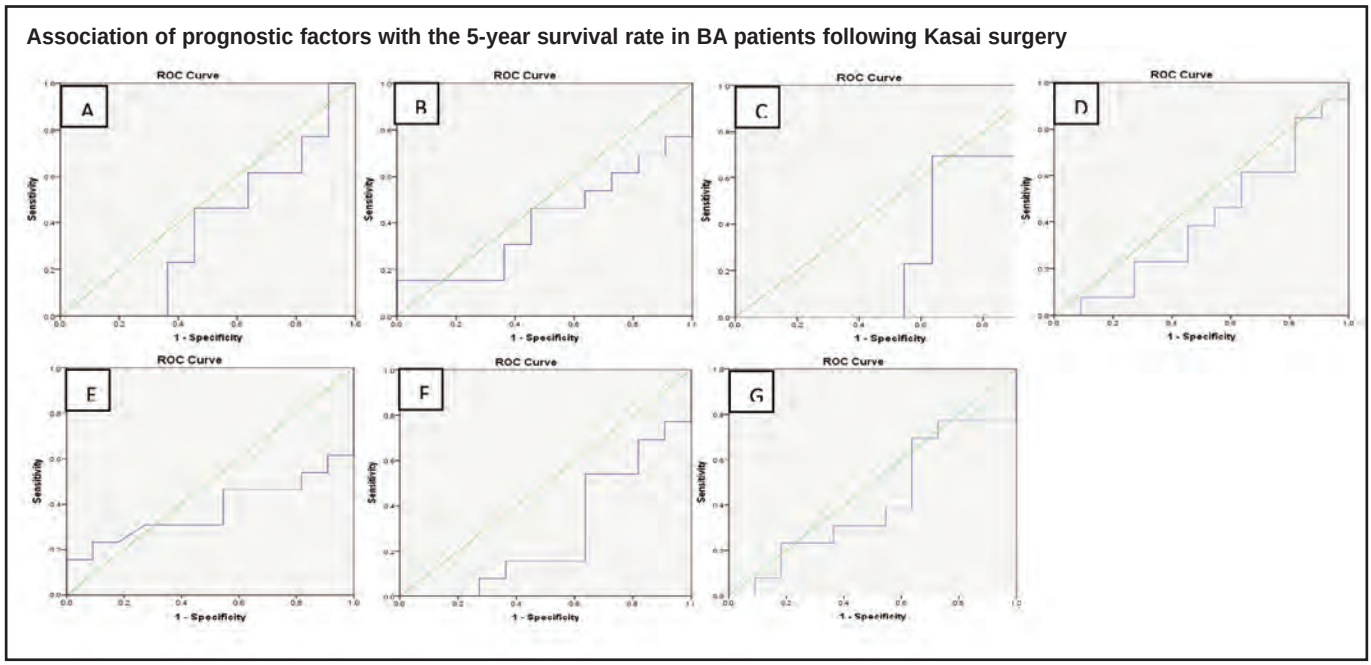

Fig. 1: ROC curve for: A) total bilirubin on postoperative day 7 (TB7); B) pre-operative AST; C) AST on postoperative day 7 (AST7); D) AST7/AST0 ratio; E) pre-operative ALT (ALT0); F) ALT on postoperative day 7 (ALT7); and G) ALT7/ALT0 ratio, with the AUC of 0.378 (95% CI=0.141-0.614), 0.406 (95% CI=0.174-0.637), 0.301 (95% CI=0.066-0.535), 0.406 (95% CI=0.172–0.639), 0.374 (95% CI=0.140-0.608), 0.280 (95% CI=0.069-0.49), and 0.413 (95% CI=0.178-0.647), respectively.

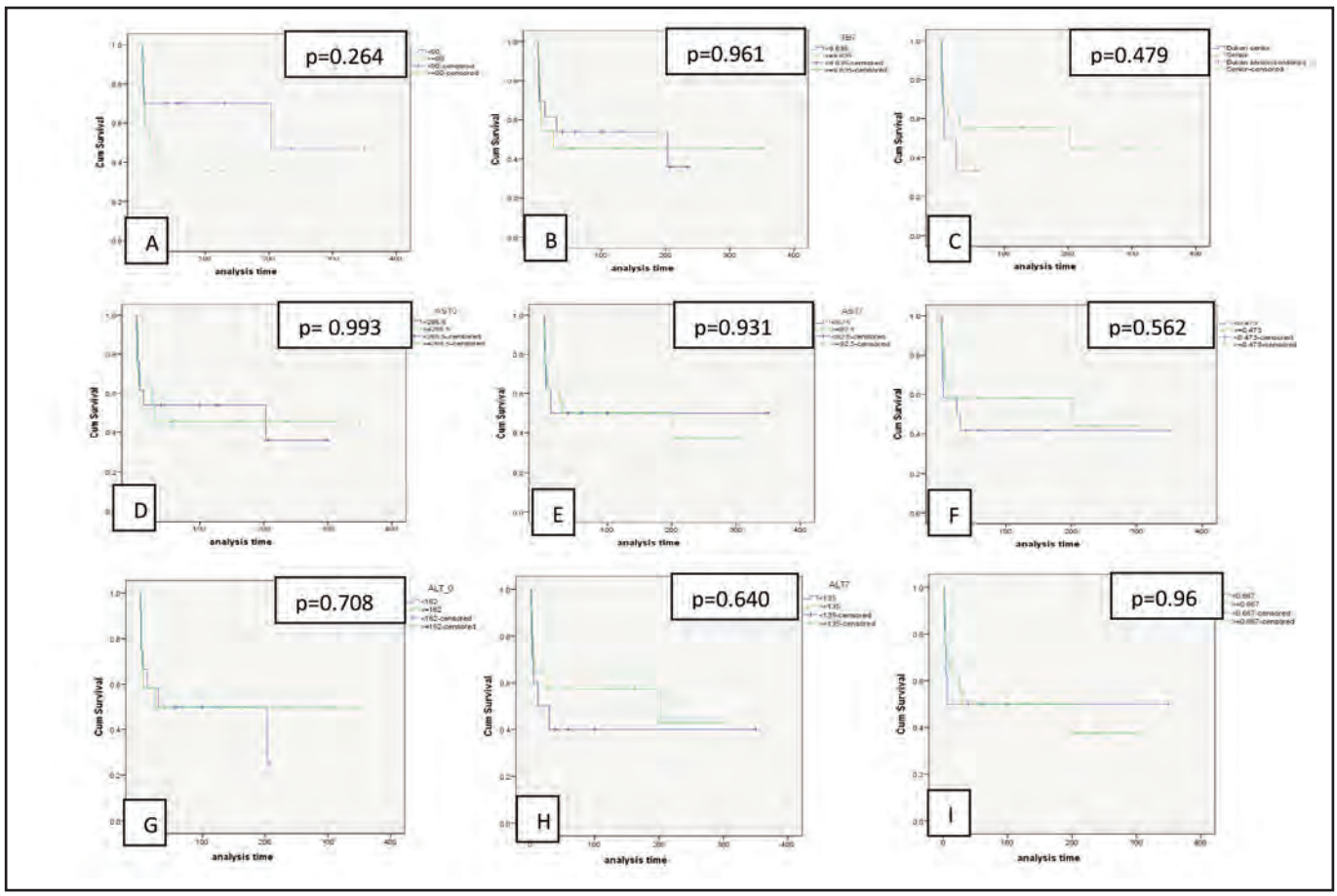

Fig. 2: Kaplan-Meier analysis of prognostic factors for the 5-year survival rate of BA patients after the Kasai procedure: A) age at the time of Kasai surgery; B) total bilirubin on post-operative day 7 (TB7); C) surgeon experience; D) pre-operative AST (AST0); E) AST on post-operative day 7 (AST7); F) AST7/AST0 ratio; G) pre-operative ALT (ALT0); H) ALT on post-operative day 7 (ALT7); I) ALT7/ALT0 ratio.

Nevertheless, studies have shown that surgeons with extensive experience in performing KPE achieve better outcomes, including higher rates of jaundice resolution and lower rates of complications such as cholangitis.24-25 Furthermore, a large multi-centre study from Germany highlighted that the outcomes of BA surgeries were significantly better in specialized centres, with a 2-year survival rate with the native liver of ~20% in less experienced settings.[26]

Pre-operative AST (AST0) and Post-operative AST Day 7 (AST7)

In our study, patients with AST0 levels below 265.5 had a survival rate of 25%, with an odds ratio of 1.03 (95% CI: 0.21-5.15, p=0.973). Likewise, patients with AST7 levels below 92.5 had a survival rate of 16.7%, with an odds ratio of 1.29 (95% CI: 0.23-7.05, p=1.000), indicating no profound prognostic association (Table II).

The pre-operative AST cut-off value was determined using ROC analysis (Figure 2B, 2C), with a cut-off of AST0 and AST7 were 265.5 and 92.5, respectively. Based on the Kaplan-Meier analysis (Figure 1D), AST0 ≥ 265.5 showed a better 5-year survival rate, but the log-rank test indicated that pre-operative AST levels were not statistically significant in affecting the 5-year survival of biliary atresia patients post-Kasai surgery (p =0.993). Similarly, while the Kaplan-Meier graph above shows that the AST7 < 92.5 group had better 5-year survival (Figure 1E). The log-rank test showed that AST levels 7 days post-operation were not statistically significant in influencing the 5-year survival of BA patients post-Kasai (p=0.931).

These findings suggest that pre- and post-operative AST levels alone may not be a strong predictor of survival. However, AST levels combined with serum direct bilirubin (DB) at 2 months after Kasai surgery were reported to be reliable for predicting long-term BA outcomes.[27] Moreover, Degtyareva et al. (2024) suggest that lower levels of postoperative AST were significantly associated with successful outcomes of Kasai surgery.[28] Additionally, Wang et al. (2019) revealed that AST, along with alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) levels tended to be increased during the first month before returning to normal levels within one year after Kasai surgery, which may explain a higher percentage of patients in AST7 ≥ 92.5 group.[29]

AST7/AST0 Ratio

The cut-off value for the AST7/AST0 ratio is 0.473, with a sensitivity of 46.2% and specificity of 45.5% (Figure 2D). A low ratio of AST7/AST0 (<0.473) was associated with slightly lower odds of survival (95% CI: 0.14-3.58, p=0.682), meaning that an AST7/AST0 ratio of ≥ 0.473 may act as a protective factor for patient survival. However, there was no statistically significant association between AST7/AST0 ratio and survival rates. Interestingly, Kaplan-Meier analysis shows that the group with an AST7/AST0 ratio cut-off ≥0.473 experienced a considerably increased mortality, with a mortality rate of 57.3% in week 203 (Figure 1F).

Altogether, our findings highlight that AST0, AST7 and AST7/AST0 ratio lacks significant prognostic value for AST levels alone in predicting long-term 5-years survival of BA patients who underwent Kasai surgery.

Pre-Operative ALT (ALT0) and Post-Operative ALT Day 7 (ALT7)

Patients with ALT0 levels below 162 had a survival rate of 20.8%, with an odds ratio of 0.71 (95% CI: 0.14-3.58, p=0.682). Similarly, ALT7 levels below 135 had a survival rate of 16.7%, with an odds ratio of 0.67 (95% CI: 0.13-3.45, p=0.697).

The preoperative ALT (ALT0) cut-off value was determined using ROC analysis (Figure 2E), with a result of 162. Based on the Kaplan-Meier analysis (Figure 1G), ALT0 ≥ 162 has a better 5-year survival rate, but the log-rank test revealed that preoperative ALT levels were not statistically significant in affecting the 5-year survival of BA patients after Kasai surgery (p=0.708). Furthermore, based on ROC analysis (Figure 2F). The cut-off value for post-operative ALT (ALT7) was determined to be 135. Based on the Kaplan-Meier analysis (Figure 1H), ALT7 ≥ 135 group had better 5-year survival but not statistically significant (log-rank test p=0.640)

Previous findings also revealed that pre-operative ALT levels were similar between the good outcome group (serum TB < 2 mg% or jaundice-free) and the poor outcome group (serum TB > 2 mg% or persistent jaundice), with a median of 162 IU/L.21 Another study also revealed no significant association, although patients with jaundice clearance had overall lower post-operative ALT levels (588.9 ± 288.7) compared to patients with impaired jaundice clearance (635.9 ± 273.2, p>0.05).[30]

ALT7/ALT0 Ratio

The cutoff value for the ALT7/ALT0 ratio is 0.667, determined through receiver operating characteristic (ROC) curve analysis, as it provided the best balance between sensitivity (69.2%) and specificity (36.4%) among the tested values. The corresponding AUC was 0.413 (95% CI: 0.178–0.647), indicating poor diagnostic performance; however, 0.667 remained the most appropriate cutoff based on the available data. (Figure 2G). Our study found no association between the ALT7/ALT0 ratio in predicting survival in BA patients following Kasai surgery. ALT7/ALT0 ratio of <0.667 had an odds ratio of 1.29 (95% CI: 0.23-7.05, p=1.000), showing no significant association between ALT ratio and survival. Previous findings also reported that BA patients post-Kasai surgery with an ALT7/ALT0 ratio ≥ 0.95 had a higher mortality rate, with mortality reaching 80% by day 43. However, no statistically significant findings were demonstrated.[31] These findings suggest that neither preoperative nor postoperative AST and ALT levels, nor their ratios, have significant prognostic value for AST levels alone in predicting long-term 5-years survival of BA patients who underwent Kasai surgery.

LIMITATIONS

The limited findings in our study may be attributed to several factors. Firstly, the sample size of this study is relatively small, and consequently, the variables examined were limited. To produce more robust outcomes regarding the 5-year survival rate and further elucidate prognostic factors, future research would significantly benefit from a larger sample size, ideally through a multi-center study design. The retrospective nature of the study may also introduce selection bias, and each patient's biochemical profiles were not measured at identical follow-up time points. Furthermore, reliance on biochemical markers measured only at Day 0 and Day 7 post-surgery may not fully capture the dynamic and longer-term post-operative changes critical for predicting native liver survival. Thus, further inclusion of relevant factors such as the types or regimens of post-operative medication (e.g., steroids, antibiotics) received, the presence and management of complications like cholangitis, and longer-term (e.g., 3-6 months) post-operative liver function biomarkers would strengthen future investigations.[32] Moreover, previous studies have shown that histologic factors such as scoring systems, fibrosis, and other common factors significantly affect the survivability of BA patients.[33] However, no data about histological factors were available at the time, and due to the nature of the retrospective cohort study design, the histological factors could not be analyzed. Future studies with a prospective design that provide more complete data (including histology data) need to be conducted to add perspective regarding the results. Further studies are also needed to analyze the potential relationship between these specific syndromes and the survival rate of BA patients after the Kasai procedure. This study also did not include an analysis of post-operative complications, such as cholangitis, which have been widely recognized as significant factors influencing outcomes in patients with biliary atresia following the Kasai procedure. Additionally, CMV serological status, which has been suggested as a potential prognostic factor in biliary atresia[34], was not included in our analysis due to the retrospective nature of the study and the lack of consistent CMV testing data in patient records. This study was primarily designed to evaluate clinical and biochemical parameters that are routinely available, with the objective of identifying early and accessible prognostic markers.

CONCLUSIONS

Our analysis indicates that several prognostic factors, including age at which Kasai surgery is performed, surgeon experience, total bilirubin on postoperative day 7, and AST and ALT levels on both preoperative and postoperative day 7, as well as the AST and ALT postoperative/preoperative ratios, lack association with 5-year survival outcomes in BA patients following Kasai surgery. Further extensive cohort studies are required to validate these preliminary findings due to the limited sample size.

List of abbreviations

BA: biliary atresia; AST: aspartate transaminase; ALT: alanine transaminase; AST7/AST0: AST postoperative day 7/preoperative ratio; ALT7/ALT0: ALT postoperative day 7/preoperative ratio; TB: total bilirubin; TB7: total bilirubin postoperative day 7; AUC: area under the curve; CI: confidence interval; OR: odds ratio; ROC: receiver operating characteristic curve; SD: standard deviation; IU/L: international units per liter.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

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ORIGINAL ARTICLE

Association between low cardiometabolic indexes and diastolic dysfunction in T2DM patients with HFpEF

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ABSTRACT

Introduction: Dyslipidemia in metabolic syndrome characterized by low cardiometabolic indexes (TG/FBG, TG/HDL-C, and LDL-C/HDL-C) pose inherent risks for the development of diabetic cardiomyopathy. The tangible association between type 2 diabetes mellitus (T2DM) and Heart Failure with Preserved Ejection Fraction (HFpEF) initiated by diastolic dysfunction also has been widely studied. However, whether cardiometabolic indexes may be independently associated with left ventricular (LV) diastolic dysfunction in T2DM patients with HFpEF remained elusive. The aim of this study is to investigate the association between cardiometabolic indexes (TG/FBG, TG/HDL-C, and LDL-C/HDL-C) and diastolic dysfunction in T2DM patients with HFpEF.

Materials and Methods: In this cross-sectional study, we analyzed electronic medical records from October 2021 to January 2022 from Dr. Sardjito Hospital. A total of 55 T2DM patients with clinical HFpEF were enrolled. Baseline characteristics, clinical and laboratory variables, medication, and echocardiography data were obtained. Cardiometabolic indexes are presented as numeric data (median with IQR). Meanwhile, the diastolic function is presented as categorical data based on the echocardiographic parameters. Mann-Whitney analysis was performed. P-value <0.05 represent significant associations.

Results: In a cohort of 55 T2DM patients with HFpEF, subjects with diastolic dysfunction demonstrated significantly lower median values for TG/FBG (p=0.015), TG/HDL-C (p=0.003), and LDL-C/HDL-C (p=0.044) compared to those with normal diastolic function. These findings suggest a potential, albeit paradoxical, link between these cardiometabolic markers and impaired ventricular relaxation in this population.

Conclusion: TG/FBG, TG/HDL-C, and LDL-C/HDL-C were significantly associated with diastolic dysfunction. Optimal dyslipidemia control represented by high TG/FBG, TG/HDL-C, and LDL-C/HDL-C may become an appealing approach to prevent HFpEF progression in T2DM patients.

KEYWORDS:
Cardiometabolic indexes, diastolic dysfunction, T2DM, HFpEF

INTRODUCTION

Heart Failure with preserved Ejection Fraction (HFpEF) is now responsible for almost 50% of all heart failure cases. HFpEF is known to have many comorbidities burden, such as hypertension, diabetes mellitus, obesity, chronic kidney disease, chronic.[1] HFpEF has distinctive phenotype, not limited to diastolic dysfunction but rather resemble a systemic metabolic disorder with characterized as inflammation and microvascular dysfunction. These characteristics are the same as type 2 diabetes mellitus (T2DM) pathophysiology. T2DM can cause unique changes in myocardium and play role as independent classical risk factors of cardiovascular disease.[2–4] Diabetic cardiomyopathy is characterized with structural and functional abnormality, include diastolic dysfunction, myocardial, hypertrophy, interstitial fibrosis.[4] T2DM conditions can increase risk of heart failure two times and worsened it prognosis. Uncontrol T2DM can increased HF patient mortality up to 30-50%.[5]

Cardiometabolic syndrome is a combination of metabolic disorders and insulin resistance. This combination can cause cardiovascular disease (CVD) and be the most common global morbidity and mortality. T2DM and dyslipidemia increase the complication of CVD exponentially. Meanwhile the cardiometabolic indexes that have a role as cardiovascular complication predictors remain inconclusive.[6] Several cardiometabolic parameters have been researched such as triglyceride-fasting blood glucose index (TG/FBG), triglyceride-high density lipoprotein (TG/HDL), and low-density lipoprotein-high density lipoprotein (LDL/HDL). Cardiometabolic index ratio is better cardiovascular complication predictor than single metabolic parameter.[7]

TG/FBG index significantly associated with heart failure, acute myocardium infarct, and stroke progressivity.[8–10] Meanwhile the TG/HDL index has been strongly associated with insulin resistance, central obesity, and increasing CVD risk.[11] TG/HDL index was independent variable of all-cause and CVD mortality in peritoneal dialysis patients.[12] On the other hand, LDL/HDL index associated with risk of coronary artery disease (CAD), diastolic dysfunction, and other cardiovascular complications.[13-14]

Cardiometabolic index (TG/FBG, TG/HDL, and LDL/HDL) represent dyslipidemia and glycemic control which are

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associated with cardiovascular complications.10 Meanwhile to our knowledge, there are just a few studies on the association between cardiometabolic index and left ventricular diastolic dysfunction as the initial sign of heart failure progressivity. Therefore, this study aimed to investigate the association between cardiometabolic index and diastolic dysfunction in T2DM patients with HFpEF.[1]

MATERIALS AND METHODS

This study is a cross-sectional study that was conducted from October 18, 2021, to January 31, 2022 at Dr. Sardjito Hopital. This study aimed to assess the association between cardiometabolic index and diastolic dysfunction in T2DM patients with HFpEF. All echocardiographic examinations were carried out using standard echocardiography tools (Vivid™) by the resident cardiologist on duty and the reading evaluation was carried out by a cardiology consultant specialist. Cardiometabolic parameters (TG, FBG, LDL, and HDL) examination was carried out using the standard method of blood venous which was checked when patients were routinely visit at the endocrinology clinic of Dr. Sardjito Hospital using a glucose one touch Point of Care Testing (POCT) examination so that it was recorded in laboratory symmetric data and medical records at Dr. Sardjito Hospital. The research subjects were patients who were routinely monitored at Dr. Sardjito Hospital. Inclusion criteria were all T2DM patients with clinical heart failure based on the Framingham criteria15 with LVEF > 50% . While the exclusion criteria were: (1) History of coronary heart disease; (2) History of heart surgery or congenital heart disease; (3) Severe renal impairment as indicated by estimated Glomerular Filtration Rate (eGFR) < 30 mL/min/1.73m^2 or undergoing renal replacement therapy; (4) Comorbid malignancy, chronic liver disease (5) Severe heart valve disorders; and (6) non-paroxysmal atrial fibrillation. Diagnosis of T2DM using the PERKENI criteria.[16]

This study received ethical approval from the Medical and Health Research Ethics Committee (MHREC) at Universitas Gadjah Mada – Dr. Sardjito General Hospital. Approval number KE/FK/0643/EC/2021 was issued on 11 June 2021, valid for one year, covering the study protocol, subject information, and informed consent forms. MHREC maintains oversight of the study in accordance with international and national guidelines.

Continuous variables were expressed as median and interquartile range (IQR), while categorical variables were presented as counts and percentages. Comparison of cardiometabolic indexes between the diastolic function groups was performed using the Mann–Whitney U test. The differences in medians along with p-values and 95% confidence intervals (CI) were reported to determine statistical significance. A p-value of less than 0.05 was considered statistically significant. Boxplots were used to illustrate the distribution of each cardiometabolic index (TG/FBG, TG/HDL-C, LDL-C/HDL-C) between groups. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, New York, USA).

RESULTS

A total of 55 eligible subjects with mean age 62.72 ± 8.27 years old are included in this study, which dominantly suffer T2DM for 5-10 years (62%). The subjects of this study were also dominated with hypertension (80%) and grade 1 obesity (47%). The mean of FBG is 137.16 ± 49.34. Meanwhile the mean of lipid profiles (TG, HDL, and LDL) are 145.96 ± 75.16, 46.93 ± 13.04, and 112.53 ± 37.45, respectively. Of the 55 subjects enrolled, 78% exhibited echocardiographic findings consistent with normal diastolic function. However, those with diastolic dysfunction had significantly lower cardiometabolic index values. As illustrated in Figure 1, the TG/FBG ratio (Panel A), TG/HDL-C ratio (Panel B), and LDL-C/HDL-C ratio (Panel C) were all significantly reduced in subjects with diastolic dysfunction compared to those with normal diastolic function (p=0.015, 0.003, and 0.044, respectively). These findings suggest a potential association between lower cardiometabolic index values and impaired ventricular relaxation. Other baseline characteristics of the subjects are presented in Table I.

Association between Cardiometabolic Indexes (TG/FBG, TG/HDL, and LDL/HDL) and Diastolic Dysfunction

The comparison of cardiometabolic index values between subjects with normal diastolic function and those with diastolic dysfunction is illustrated in Figure 1. In Panel A, subjects with diastolic dysfunction had significantly lower TG/FBG ratios compared to those with normal diastolic function (median [IQR]: 0.62 [0.38–0.86] vs. 1.00 [0.75–1.41]; p = 0.015). In Panel B, TG/HDL-C was also markedly lower in the diastolic dysfunction group (1.54 [1.16–1.88] vs. 2.82 [2.03–4.11]; p=0.003). Similarly, Panel C shows that LDL-C/HDL-C was significantly reduced in patients with diastolic dysfunction (1.87 [1.18–2.27] vs. 2.40 [1.89–3.23]; p=0.044). These findings demonstrate a consistent pattern: patients with diastolic dysfunction had significantly lower cardiometabolic index values across all three ratios, suggesting a potential link between lower cardiometabolic burden and the presence of subclinical cardiac dysfunction in T2DM patients with HFpEF. The complete result of bivariate analysis is presented in Table II.

DISCUSSION

This cross-sectional study demonstrated a large proportion (78%) of patients in this T2DM-HFpEF cohort were categorized as having normal diastolic function based on echocardiographic parameters. This apparent paradox may reflect the limitations of current diastolic dysfunction grading criteria, particularly in distinguishing early or subclinical forms of dysfunction in patients with T2DM. It is also possible that diastolic function was assessed during a compensated phase, where structural and functional abnormalities may not be overt. Furthermore, the diagnosis of HFpEF relies not only on echocardiographic parameters but also on clinical presentation, natriuretic peptide levels, and comorbidities, as highlighted in the HFA-PEFF diagnostic algorithm. Therefore, echocardiographic classification alone may underestimate diastolic dysfunction in some clinically diagnosed HFpEF cases.

Table I: Baseline characteristics of the participants

Parameters	Overall (n = 55)
Demographics	
Age (years), mean ± SD	62.7 ± 8.3
> 60 years, n (%)	35 (63%)
Female, n (%)	34 (62%)
Diabetes Mellitus Duration, n (%)	
< 5 years	7 (13%)
5–10 years	34 (62%)
> 10 years	14 (25%)
Hypertension, n (%)	44 (80%)
Anthropometric Data	
Body weight (kg), mean ± SD	64.9 ± 13.1
Height (cm), mean ± SD	157.6 ± 6.9
Body Mass Index (kg/m²), mean ± SD	26.0 ± 4.4
< 18.5 (underweight), n (%)	3 (5%)
18.5–22.9 (normal weight), n (%)	8 (15%)
23.0–24.9 (overweight), n (%)	10 (18%)
25.0–29.9 (grade 1 obesity), n (%)	26 (47%)
≥ 30 (grade 2 obesity), n (%)	8 (15%)
Blood Pressure	
Systolic blood pressure (mmHg), mean ± SD	138.1 ± 23.2

Table II: Association between TG/FBG, TG/HDL-C, and LDL-C/HDL-C and diastolic dysfunction

Index	Normal median [IQR]	Diastolic dysfunction median [IQR]	p-value	95% CI
TG/FBG	1.00 [0.75–1.41]	0.62 [0.38–0.86]	0.015	–0.89 to –0.07
TG/HDL-C	2.82 [2.03–4.11]	1.54 [1.16–1.88]	0.003	–2.63 to –0.48
LDL-C/HDL-C	2.40 [1.89–3.23]	1.87 [1.18–2.27]	0.044	–1.47 to –0.01

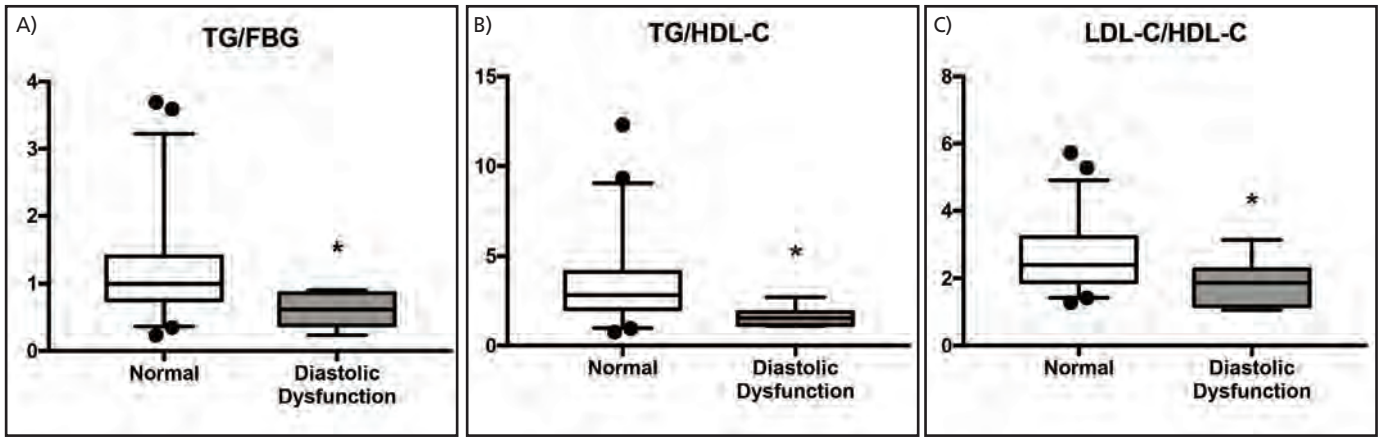

Fig. 1: Distribution of cardiometabolic indexes according to diastolic function status. (A) Triglyceride-to-fasting blood glucose (TG/FBG) ratio; (B) Triglyceride-to-high-density lipoprotein cholesterol (TG/HDL-C) ratio; (C) Low-density lipoprotein-to-high-density lipoprotein cholesterol (LDL-C/HDL-C) ratio. In each plot, the median, interquartile range (IQR), and outliers are shown. Comparisons were made between normal and abnormal diastolic function groups using the Mann–Whitney U test.

Our studies found that there is association between TG/FBG and diastolic dysfunction with p-value 0.015, CI 95% -0.89 to -0.07. TG/FBG was a reliable predictor for metabolic syndrome, including BMI, HbA1c, blood pressure, insulin resistance etc. Recent study also showed that TG/FBG index was strongly associated with greater risk of developing HFpEF. TG/FBG play a role as reliable biomarker to identify asymptomatic HFpEF patients.[17-18] On the other hand, recent studies proved that TG/FBG also significantly associated with other CVD, such as CAD, stroke, and lower limb vascular stenosis.[19-20]

The other parameter which uses TG and FBG as its component is Triglyceride-glucose index (TyG). TyG suggested as a reliable and valuable predictor for risk stratification and prognostic indicator in CHF patients. Increasing TyG value correlated significantly with increasing mortality incidence in CHF patients. The predictive implication of TyG index mainly found in HFmrEF and HFpEF patients.[21] TyG index also can be an independent and causal risk factor for HF incident. Based on some recent studies the TyG index were well correlated with insulin resistance. Insulin resistance contribute to abnormal circulating free

fatty acids and triglycerides, increasing proinflammatory molecules, and maladaptive activation of renin-angiotensin-aldosterone system which cause cardiac dysfunction.[22]

TG/HDL-C index represents the cardiovascular events and part of the cardiometabolic index which is a novel indicator for abdominal fat and strongly correlated with hypertension, hyperuricemia, diabetes, arterial stiffness, and CVD assessment. TG/HDL-C can detect LVH, metabolic disorders, and myocardial infarction.[23-24] TG/HDL-C known as atherogenic index of plasma which is associated with coronary artery disease risk and outcomes.[25] TG/HDL-C index has positive association with metabolic syndrome and insulin resistance which is also the positive risk factor of diastolic dysfunction.[26-27] This study found that there is significant association between the TG/HDL-C index with diastolic dysfunction (p-value 0.0003, CI 95% -2.63 to -0.48). Similar result was also found in a study done by Khedr et al in a T1DM patient. There is positive association between TC/HDL and TG/HDL index with diastolic complication in T1DM patients with p-value 0.016 and 0.028, respectively. HDL, TC/HDL, and TG/HDL can predict diastolic dysfunction only in female patients.[14]

Mechanism of association between TG/HDL-C and diastolic dysfunction remains unclear. There is some speculation of its mechanism. First, TG/HDL-C represent insulin resistance (IR). Higher IR associated with left ventricular diastolic dysfunction. The changes of myocardial diastolic function are already present in subclinical T2DM.[27–29] Secondly, TG/HDL-C also represent metabolic syndrome. Metabolic syndrome associated with high pro-inflammatory cytokines level.[30] Thirdly, decreasing HDL-C and increasing TG can elevate myocellular lipid accumulation and trigger lipoapoptosis which can cause diastolic dysfunction. Low HDL-C concentration also causes arterial stiffness and increasing myocardiocytes hypertrophy which can induce diastolic dysfunction.[26]

In this study the LDL/HDL was significantly associated with diastolic dysfunction The similar result was consistent with previous study (p-value 0.044, CI 95% -1.47 to -0.01). High LDL-C/HDL-C associated with increased HbA1c, decreased eGFR, CHD, and left ventricular hypertrophy in elderly.[31] One of the most important things in left ventricular hypertrophy pathophysiology is myocardial fibrosis which is clinically manifested by diastolic dysfunction.[32] Increasing LDL and decreasing HDL are two components of dyslipidemia which significantly correlated with diastolic dysfunction. The severity of diastolic dysfunction was also significantly correlated with LDL level. Oxidized LDL in the blood inhibit the function of HDL and also cause endothelial dysfunction and cardiomyocytes apoptosis. On the other hands, dyslipidemia induce alterations in myocardial lipid metabolism, increase inflammation, and oxidative stress which ultimately lead to cardiac lipotoxicity and diastolic dysfunction.[32-33]

About 30-60% T2DM patients, either with normal or abnormal glycemic control, have dyslipidemia, specifically high TG, high LDL, and low HDL.[34] In T2DM patients, dyslipidemia tends to be the main factor in the CVD. CVD risk also associated with HbA1c levels, every 1% increase in absolute HbA1C levels increasing the CVD risk about 18%. Poor glycemic index can increase the FBG, TC, TG, and TC/HDL-C.[35] T2DM patients can experienced diabetic myocardial dysfunction with main features impaired in left ventricular diastolic function. The glycemix control level (HbA1C) and duration of T2DM also strongly correlated with diastolic dysfunction.[31,36] Persistent hyperglycemias induce abnormal lipid metabolism, systemic inflammation, oxidative stress, activation of the renin-angiotensin-aldosterone system (RAAS), and myocardial microvasculopathy. Those complex mechanism led to diastolic dysfunction which can cause heart failure.[31]

All of cardiometabolic indexes analyzed in this study, either TG/FBG, TG/HDL, or LDL/HDL are easily calculated, cheap, and commonly available parameters in most laboratories.In this study all of those indexes are significantly associated with diastolic dysfunction in T2DM patient with HFpEF. This result indicates that there is probability of cardiometabolic indexes as diastolic dysfunction predictor in T2DM patients with HFpeF.

This study has several limitations that should be acknowledged. First, the cross-sectional design prevents any inference of causality between cardiometabolic indexes and diastolic dysfunction. Second, the relatively small sample size from a single center may limit the generalizability of our findings to broader populations with T2DM and HFpEF. Third, the reliance on echocardiographic parameters alone to define diastolic dysfunction may underestimate subclinical abnormalities, particularly in patients with preserved ejection fraction. Additionally, the potential influence of medications, glycemic control variability, and unmeasured confounding factors (e.g., natriuretic peptides or left atrial strain) could not be fully accounted for in the analysis. Future prospective studies with larger and more diverse cohorts are warranted to validate these findings.

CONCLUSIONS

This study showed that lower cardiometabolic indexes characterized by TG/FBG, TG/HDL-C, and LDL-C/HDL-C were significantly associated with diastolic dysfunction in T2DM patients with HFpEF. These findings suggest that these easily calculated lipid ratios could serve as potential biomarkers for identifying T2DM patients at risk for diastolic dysfunction. Further prospective research is warranted to validate these associations and clarify their underlying mechanisms and clinical utility.

CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Cytomegalovirus infection impact on cholangitis in patients with biliary atresia following the Kasai procedure

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ABSTRACT

Introduction: Biliary atresia (BA) is a congenital anomaly often found in neonates, with an incidence reaching 1:5500 per birth. BA is frequently associated with cytomegalovirus (CMV) infection in the patient, which causes a clinical appearance different from other types of BA. BA is usually treated by Kasai procedure, with cholangitis being the most common complication of this procedure. CMV infection is found to affect post-operative survival and bilirubin levels. However, it remains unclear whether the infection may affect the incidence of cholangitis in BA patients post-Kasai procedure.

Materials and Methods: This retrospective study used the medical records of 33 BA patients who underwent the Kasai procedure in Dr. Sardjito Hospital between 2017 and 2021.

Results: Among 33 patients, 17 (51.5%) were infected with CMV, and 12 (36.4%) developed cholangitis. The frequency of cholangitis following the Kasai procedure is not significantly influenced by the CMV infection (p=0.615). Interestingly, the incidence of cholangitis is significantly associated with the pre-operative gamma-glutamyl transferase (GGT) levels (p=0.026). Furthermore, pre-operative ALP appears to have a protective effect against cholangitis, with these associations nearly reaching a significant level (p=0.093).

Conclusion: CMV infection is unlikely to impact the incidence of cholangitis after the Kasai procedure in BA patients. Notably, the pre-operative GGT level might affect the incidence of cholangitis following the Kasai procedure, thereby increasing their risk.

KEYWORDS:
Cytomegalovirus, biliary atresia, Kasai procedure, cholangitis, pre-operative gamma-glutamyl transferase

INTRODUCTION

Biliary atresia (BA) is a disorder in which the obliteration of the biliary duct obstructs bile flow.[1] This condition is the most common cause of neonatal jaundice in the world.[2] In Indonesia, the incidence of BA reached up to 1:7000 births.[3] Symptoms of BA include hyperbilirubinemia, pale stools, dark urine, progressive renal failure, and even death if not promptly treated.[2] The most common treatment for this disorder is the Kasai procedure.[3]

The etiopathogenesis of BA is multifactorial and remains elusive. The two most widely accepted etiologies are embryological malformation of biliary ducts during fetal development and inflammation due to perinatal viral infection that leads to fibrosis and obliteration of the duct.[4-5] Cytomegalovirus (CMV) infection is among the most widely studied. It is known to cause clinically distinct BA symptoms with a greater inflammation and higher mortality, reaching 25% in CMV-associated BA compared to 6.5% in non- CMV-associated BA.[6-7]

There remain conflicting and debatable results on the impact of CMV infections on BA patients post-Kasai procedure. Cholangitis might be found in patients with BA that develop complications after a Kasai procedure, and it serves as a significant predictor for survival and successful outcome of the Kasai procedure. Therefore, this research aimed to compare the incidence of cholangitis between BA patients with and without accompanying CMV infection.

MATERIALS AND METHODS

A descriptive retrospective study was conducted to analyze the association between CMV infection and cholangitis incidence in BA patients post-Kasai procedure. This study was conducted using medical records of BA patients who underwent the Kasai surgery in Dr. Sardjito Hospital in Yogyakarta, Indonesia, between 2017 and 2021. Patients without a complete medical record were excluded.

The diagnosis of BA and cholangitis was based on the patient's clinical medical record. At the same time, CMV infection was defined as the presence of anti-CMV antibodies or a positive result on a PCR CMV test.

Prognostic Factors

In total, 15 variables were collected, including demographic, clinical, and laboratory data. These variables are age at the time of surgery, sex, BA subtype, CMV infection status, pre-operative total and direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), INR, albumin, gamma-

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Table I: Baseline characteristics of patients with BA

Characteristics	N (%)	Mean ± SD
Sex		
▪ Male	12 (36.4)	
▪ Female	21 (63.6)	
Age at Kasai surgery (days)		92.18 ± 22.65
BA type		
▪ I	1 (3.0)	
▪ IIA	20 (60.6)	
▪ IIB	2 (6.1)	
▪ III	10 (30.3)	
CMV infection		
▪ Negative	17 (51.5)	
▪ Positive	16 (48.5)	
Pre-operative lab		
▪ Total Bilirubin (mg/dL)		10.24 ± 3.25
▪ Direct Bilirubin (mg/dL)		8.82 ± 2.84
▪ Aspartate Aminotransferase (U/L)		220.76 ± 116.61
▪ Alanine Aminotransferase (U/L)		167.45 ± 129.1
▪ Gamma-glutamyl transferase (U/L)		694.33 ± 506.73
▪ Alkaline Phosphatase (U/L)		472.91 ± 155.8
▪ INR		1.11 ± 0.63
▪ Platelet (103/µL)		361.97 ± 126.25
Post-operative lab		
▪ Total Bilirubin POD7 (mg/dL)		9.79 ± 3.66
▪ Direct Bilirubin POD7 (mg/dL)		8.21 ± 3.25
Cholangitis		
▪ Yes	12 (36.4)	
▪ No	21 (63.6)	

SD, standard deviation; IQR, interquartile range; POD, postoperative day; BA, biliary atresia

Table II: The cut-off points of variables according to the ROC curve

Characteristics	Cut-off	Sn (%)	Sp (%)
Age at surgery (days)	≥99	58.3	76.2
Total Bilirubin (mg/dL)	≥9.215	83.3	47.6
Direct Bilirubin (mg/dL)	<8.825	33.3	47.6
Aspartate Aminotransferase (U/L)	≥152.5	91.7	28.6
Alanine Aminotransferase (U/L)	<220.5	16.7	71.4
Gamma-glutamyl transferase (U/L)	≥979.5	50	90.5
Alkaline phosphatase (U/L)	<368	58.3	9.5
Albumin (g/dL)	≥4.17	41.7	85.7
INR ≥0.975	83.3	52.4	
Platelet (103/µL)	≥315	83.3	47.6
Total bilirubin POD7 (mg/dL)	≥11.78	50	76.2
Direct bilirubin POD7 (mg/dL)	≥9.345	58.3	76.2

Sn: Sensitivity, Specificity; POD, postoperative day

glutamyl transferase (GGT), and alkaline phosphatase (ALP) levels, pre-operative platelet count, and post-operative total and direct bilirubin levels.

Ethics Approval

This study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital (KE/FK/0439/EC/2021). Before participating, the parents or legal guardians of BA patients and controls signed written informed consent forms. The research was performed following the Declaration of Helsinki.

Statistical Analysis

The data were presented as nominal data, and bivariate analysis was performed using Fisher's exact or chi-square tests. Then, further multivariate analysis was performed using binomial logistic regression. For statistical analysis, type I BA was treated as one group alongside type IIA BA, while type IIB BA was categorized with type III BA. All statistical analyses were performed using IBM SPSS Statistics 23.

RESULTS

Forty-six patients were identified, with 13 patients excluded due to incomplete medical records. Of the 33 patients, 17 (51.5%) were infected with CMV, and 12 (36.4%) developed cholangitis (Table I).

Table III: Association between prognostic variables and cholangitis

Characteristic	Cholangitis		p-value	OR (95% CI)
	Yes	No		
Sex				
▪ Male	9	12	0.457	2.25 (0.47-10.78)
▪ Female	3	9		
Age at Kasai surgery (days)				
▪ ≥99	7	5	0.067	4.48 (0.98-20.59)
▪ <99	5	16		
BA type				
▪ IIB/III	5	7	0.716	1.429 (0.33-6.17)
▪ IIA/I	7	14		
Total bilirubin (mg/dL)				
▪ ≥9.22	10	11	0.133	4.55 (0.80-25.98)
▪ <9.22	2	10		
Direct bilirubin (mg/dL)				
▪ ≥8.83	7	12	0.947	1.05 (0.25-4.42)
▪ <8.83	5	9		
Aspartate aminotransferase (U/L)				
▪ ≥152.5	11	15	0.223	4.40 (0.46-41.97)
▪ <152.5	1	6		
Alanine Aminotransferase (U/L)				
▪ ≥220.5	2	6	0.678	0.50 (0.08-2.99)
▪ <220.5	10	15		
Gamma-glutamyl transferase (U/L)				
▪ ≥979.5	6	2	0.015	9.50 (1.5-60.11)*
▪ <979.5	6	19		
Alkaline Phosphatase (U/L)				
▪ ≥368	7	19	0.071	0.15 (0.02-0.94)
▪ <368	5	2		
Albumin (g/dL)				
▪ ≥4.17	5	3	0.106	4.29 (0.8-22.92)
▪ <4.17	7	18		
INR				
≥0.975	10	10	0.067	5.50 (0.96-31.43)
<0.975	2	11		
Platelet (103/µL)				
≥315	10	11	0.133	4.55 (0.80-25.98)
<315	2	10		
Total Bilirubin POD7 (mg/dL)				
≥11.78	6	5	0.149	3.20 (0.71-14.53)
<11.78	6	16		
Direct Bilirubin POD7 (mg/dL)				
≥9.345	7	5	0.067	4.48 (0.98-20.59)
<9.345	5	16		
CMV				
Positive	6	10	0.895	1.10 (0.27-4.55)
Negative	6	11		

POD, post-operative day

Table IV: Multivariate analysis of the association between independent variables and cholangitis

Characteristic	p-value	OR	95% CI
Age at Kasai surgery	0.438	5.11	0.08-313.16
Pre-operative total bilirubin	0.75	1.6	0.09-28.16
Gamma-glutamyl transferase	0.026*	29.37	1.5-576.29
Alkaline phosphatase	0.093	0.04	0.001-1.74
Albumin	0.844	1.45	0.03-65.58
INR	0.44	3.17	0.17-59.09
Post-operative total bilirubin	0.597	2.97	0.05-167.8
Post-operative direct bilirubin	0.987	1.03	0.03-36.04
CMV	0.615	2.94	0.04-197.2

All numerical data were analyzed using the receiver-operating characteristic (ROC) curve to determine their cut-off points (Table II).

Association between prognostic variables and cholangitis
We found no significant association between CMV infection and the incidence of cholangitis (p=0.895). Pre-operative GGT was significantly associated with cholangitis post-surgery, where patients with GGT levels ≥979.5 U/L had a 9.5 times increased risk of developing cholangitis (95% CI=1.50-60.11; p=0.015). Interestingly, we also found that the pre-operative ALP shows a protective effect (OR = 0.15 [95% CI=0.02-0.94]), and these associations almost reached a significant level (p=0.071) (Table III).

Multivariate analysis of the association between independent variables and cholangitis
We found that only pre-operative GGT levels were an independent risk factor for cholangitis incidence post-Kasai surgery (p=0.026) (Table IV).

DISCUSSION

CMV infection is postulated to be involved in the pathogenesis of BA and affects the clinical manifestation and severity of the condition.[7-8] Cholangitis post-Kasai surgery is due to inflammation of the anastomosis site and colonization by intestinal flora, worsened by cholestasis.[9-10] In our study, we were unable to show that the presence of CMV infection has any significant association with the development of cholangitis as a complication of the Kasai procedure in BA patients. This result agrees with previous reports that show no association between CMV infection and cholangitis in BA patients.[8,11] Despite this, other studies have shown an association between cholangitis risk and inflammation in BA patients after a Kasai surgery.[7,12-13] The difference in our results may be due to our small sample size, and further studies with larger sample sizes will help elucidate a better conclusion.

Most of our subjects were female (63.6% vs 36.4%), with a ratio of 7:4 or 1.75:1. These ratios vary among studies.[3,14-19] We did not find any statistically significant association between sex and the incidence of cholangitis. This supports earlier studies that show no significant association between the patient's sex and the success of the Kasai procedure.[3,20]

The association between cytomegalovirus (CMV) infection and biliary atresia appears to vary by geographic region. In Asian populations, several studies have demonstrated regional differences in the prevalence of CMV-positive BA.[21-23] In Asian countries, such as China and Taiwan, CMV DNA has been detected in a significantly higher percentage of BA patients, ranging from 30% to over 50%, compared to Western countries, where reported rates are generally lower, between 10% and 20%.[7,24] The reported incidence of BA varies by geography, with higher incidence found in the Asia and Pacific region. About 1 in 5000 live births in Taiwan, 1 in 10,000 in Japan, 1 in 17,000–19,000 in the UK and France, 1 in 19,000 in the Netherlands, and 1 in 15,000 in the United States have the condition. There are no clear reports regarding the incidence of biliary atresia in Indonesia.[2,25] Extrahepatic biliary atresia, subsequently mentioned as biliary atresia, was reported to occur in 1 in 18,000-20,000 live births, more commonly in Asians (1 in 5.000-8.000).[25-26] Our findings align with this trend, supporting the hypothesis that CMV may play a more prominent role in the pathogenesis of BA in Asian settings.

Our patients' average age at surgery was around 92.8 days, 36.37% of whom were older than 99 days old. It is often reported that earlier age at surgery leads to better outcomes in BA patients, with one study showing that older age at surgery is a risk factor for recurrent cholangitis.[9,20,27] This conclusion, however, has also been often debated, with other reports showing no significant association between earlier surgery and better surgical outcomes.[3,19,28-30] The choice of cut-off point for earlier and later surgery definition might explain the discrepancy, as a report from Song et al. shows no significant difference in survival between >90 days surgery patient and 61-90 days surgery patient but shows a substantial difference between <60 days surgery patient and >90 days surgery patient.[31]

Early surgery for biliary atresia, ideally within 60 to 70 days of life, has been shown to improve outcomes following Kasai portoenterostomy[32-33] significantly. In our cohort, the mean age at surgery was 92 days, which may have negatively influenced postoperative bile flow. This delay was primarily due to late presentation, limited parental awareness, and referral system inefficiencies, common challenges in our healthcare setting. This highlights the urgent need to strengthen early detection programs and streamline referral pathways. A previous study showed that performing the Kasai operation beyond the age of 60 days was not associated with a worse outcome and that a high percentage of patients could still achieve good bile flow with normal bilirubin postoperatively.[34] Thus, it is believed that until the age of 100 days, the age of the patients does not play a significant role in determining the success of the Kasai operation.34 Another study also suggested that when faced with cholestasis, significant clinical attention is typically directed towards diagnosing or excluding BA.[35] Of all cholestatic conditions, BA is the only one that is "time-sensitive" because early diagnosis significantly improves outcomes, and if missed, can have adverse consequences.[35] The benefit of early intervention on survival continues to show a positive correlation with younger age at surgery. Traditionally, the first 60 days of life are critical in establishing bile flow to prevent or ameliorate liver-related morbidity and mortality in BA patients. However, the cutoff of 60 days is somewhat arbitrary because establishing bile flow with Kasai portoenterostomy even beyond that age may still result in favorable outcomes. In the present cohort, almost all cases underwent surgery at or beyond the age of 60 days. Accordingly, the success and survival rates are comparable to previous reports.[35] The optimum treatment for late BA presentation remains controversial, especially considering the difficulty in predicting Kasai procedure prognosis.[35] On the one hand, survival with the native liver for those undergoing the Kasai procedure beyond 60 days appears to be more favorable than previously suggested.

Biliary atresia (BA) is increasingly recognized as a multifactorial disease due to genetic predisposition, immune dysregulation, and environmental or viral triggers. Developmental genes such as Sox17 and Hes1 regulate bile

duct specification, while Lgr4 is essential for gallbladder and cystic duct formation. Polymorphisms in VEGF may contribute to inflammatory angiogenesis, and mutations in CFC1, associated with biliary atresia splenic malformation (BASM) syndrome, further support a genetic role.[36] In Indonesian patients, overexpression of miRNA-21 and downregulation of PTEN correlate with liver fibrosis, while altered expression of collagen genes (COL6A1, COL6A2, COL6A3, COL1A1) suggests a genetic basis for fibrogenesis.[37-38] Cholangiocytes also exhibit innate immune activation via TLR3 in response to viral dsRNA, such as RRV. Stimulation with poly(I:C), a synthetic analogue of viral dsRNA, induces EMT and fibrosis-related pathways, and the failure to develop TLR tolerance sustains biliary injury.[36]

Etiopathogenesis of the perinatal form of BA may be caused by primary viral infection targeting the bile duct epithelium (cholangiotropic infection), which then initiates the destructive cascades.[2,39] It has been proposed that several viruses, not limited to CMV, Reovirus, and Rotavirus, can infect and directly damage bile duct epithelial cells.[39-40] This process may provoke a secondary, damaging immune or autoimmune response, leading to progressive inflammation, fibrosis, and eventual obliteration of the biliary tree, the pathognomonic hallmark of BA.[39] Functional studies further support this hypothesis, as demonstrated by a rotavirus-induced mouse model, which shows that a viral infection in newborns can lead to a phenotype of biliary inflammation and obstruction similar to that seen in human infants.[41] While this evidence suggests a potential mechanism for how CMV initiates inflammation and destruction of the biliary system, its impact on postoperative complications, such as cholangitis, is still unclear. In our study, we were unable to demonstrate a significant association between CMV infection and the development of cholangitis as a complication of the Kasai procedure in BA patients. This result agrees with previous reports of no association between CMV infection and cholangitis in BA patients.[2,8] A 2021 meta-analysis, further supported our inference as although CMV-positive BA patients had significantly lower rates of jaundice clearance after the Kasai procedure, there was no significant difference in the overall incidence of cholangitis.[8] We presume that cholangitis after the Kasai procedure observed in our cases may also be due to inflammation at the anastomosis site and bacterial colonization from the intestine, which is exacerbated by cholestasis.[2,8] The difference in our results compared to studies that do show an association may be due to our small sample size, and further studies with larger cohorts are needed to draw a more definitive conclusion.

Recurrent cholangitis is a well-known complication after Kasai portoenterostomy and is associated with progressive liver damage and poorer prognosis due to progressive liver injury and fibrosis.[42-44] One preventive strategy is ensuring an adequate length of the Roux-en-Y limb. A length of at least 40–50 cm has been recommended to minimize the risk of ascending bacterial infection from the intestine.[42-43,45] A randomized controlled trial comparing standard (30–40 cm) versus shorter (13–20 cm) Roux limbs showed comparable rates of cholangitis and bile flow, indicating shorter loops may be equally effective in selected populations.[42] Furthermore, in cases of recurrent cholangitis without mechanical obstruction, extending the Roux limb, including lengths up to 90 cm, has been reported to resolve symptoms completely by reducing bile reflux and bacterial stasis.[43] These findings emphasize that while limb length is essential, optimal outcomes likely depend on individualized surgical planning and vigilant postoperative management.[42-45]

Gamma-glutamyl transferase is one of the ductal enzymes that serves as an indicator of ductal and canaliculi damage.[46] A higher level of GGT would indicate more significant ductal damage and inflammation. Our study shows that pre-operative GGT level ≥979.5 U/L is associated with up to a 9.5-fold increase in the risk of cholangitis. We also show that pre-operative GGT is an independent predictor for cholangitis in patients that undergo the Kasai procedure. This agrees with another report showing that patients with cholangitis have greater GGT levels.[9] Other studies also support our result by showing the association between higher GGT levels and failure in jaundice clearance and lower native liver survival.[27,47]

Alkaline phosphatase is another ductal enzyme that indicates damage to the canaliculi and bile system, and its elevation is one indicator of cholestasis and cholangitis.[48] However, our result did not show the association between pre-operative ALP level and the incidence of cholangitis. Plenty of previous studies reported similar results, showing no association with other outcomes of Kasai surgery.[14,18,20,27,49]

One of the common indicators of a successful Kasai surgery is jaundice clearance, often defined as a serum level of total bilirubin under 20 µmol/L.[9,35] Another standard definition is serum bilirubin level under 2 mg/dL or 1.2 mg/dL.27-28 Our patients show an average total bilirubin level of 9.79 mg/dL 7 days after Kasai surgery and an average of 8.21 mg/dL for direct bilirubin level 7 days after the surgery. We fail to show any significant association between pre-operative and post-operative total and direct bilirubin levels and cholangitis incidence post-surgery. These findings vary among reports. Another study agrees that earlier jaundice clearance is not associated with cholangitis post-Kasai surgery, and another reported that direct bilirubin level shows no association with a patient's prognosis two weeks after the surgery.[9,50] One study even reported that early jaundice clearance is a risk factor for cholangitis after Kasai surgery.[10] Meanwhile, other studies report a better prognosis in patients with lower bilirubin levels post-surgery.[27,49,51-52] The discrepancies between these results might be explained by the varying definitions of the "post-surgery" period, ranging from one week to several months.

LIMITATIONS

Our study is constrained by its small sample size and the single-center source of our samples. Research involving a larger sample size from multiple centers could further validate our results.

CONCLUSIONS

CMV infections might not be linked to the risk of cholangitis in BA patients who undergo the Kasai procedure. Pre-operative GGT levels might serve as a valuable predictor for the incidence of cholangitis in BA patients after Kasai surgery.

Abbreviations

BA, biliary atresia; IQR, interquartile range

CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

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Comparative outcomes of single incision laparoscopy versus conventional laparoscopy in paediatric population: a meta analysis

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ABSTRACT

Introduction: Single-incision laparoscopic surgery (SILS) and conventional laparoscopic surgery (CLS) are minimally invasive surgical techniques that are widely used as the treatment of appendicitis. While both techniques have demonstrated several advantages over laparotomy, such as reduced postoperative pain and faster recovery, SILS is hypothesized to further enhance clinical outcomes due to its less invasive approach. Hence, systematic comparisons of SILS and CLS are necessary to delineate the most efficient surgery for pediatric surgeons. This study aimed to compare postoperative outcomes between SILS and CLS in pediatric appendicitis, specifically focusing on complication rates, length of hospital stay (LOS), and duration of surgery.

Materials and Methods: A comprehensive search of four databases (PubMed, ProQuest, Scopus, and ScienceDirect) was conducted in October 2024 to identify the eligible studies. Meta-analysis was performed using Comprehensive Meta-Analysis software, applying a random-effects model to calculate pooled effect sizes. Heterogeneity was evaluated using the I^2 and Q statistics to assess consistency across studies.

Results: Six studies, encompassing a total of 838 children who underwent laparoscopic appendectomy, were included. The pooled analysis showed no statistically significant difference in complication rates between SILS and CLS (OR: 1.421, 95% Confidence Interval: 0.609–3.314, p=0.416), nor in length of hospital stay (SMD: -0.003, 95% Confidence Interval: -0.252–0.247, p=0.983).

Conclusion: Both SILS and CLS demonstrate favorable outcomes for pediatric appendicitis surgery, with minimal differences in complication rates and recovery times. These findings suggest that both techniques were feasible choices, allowing for flexibility in choosing the surgical approach based on patient-specific factors and surgical expertise.

KEYWORDS:
Conventional laparoscopic, meta-analysis, single incision laparoscopic

INTRODUCTION

Acute appendicitis remains one of the most common surgical emergencies worldwide. While its exact aetiology is not fully understood, luminal obstruction caused by factors such as fecaliths, hyperplastic lymphoid tissue, foreign bodies, parasitic infections, or tumors is thought to play a significant role.[1] Appendectomy has long been recognized as the standard treatment for this condition, with its first recorded performance by Amyand in 1735 during the repair of an inguinal hernia containing an inflamed appendix. The introduction of the right iliac fossa incision by McBurney in 1894 revolutionized appendectomy, providing the foundation for modern surgical approaches.[2]

The advent of minimally invasive techniques marked a significant milestone in appendectomy. In 1983, the first laparoscopic appendectomy was performed, ushering in a new era of surgical innovation. Laparoscopic appendectomy (LA) has since become the preferred choice for treating suspected appendicitis due to its numerous advantages, including reduced postoperative pain, faster recovery times, fewer complications, and improved cosmetic outcomes.

Furthermore, Single-incision laparoscopic surgery (SILS) represents a further evolution of minimally invasive surgery, aiming to enhance patient recovery and satisfaction by minimizing abdominal wall trauma. SILS reduces the number of incisions to a single-entry point, potentially offering even better cosmetic outcomes and reduced postoperative pain compared to conventional laparoscopic surgery (CLS). Despite these theoretical advantages, SILS has yet to achieve widespread adoption, largely due to technical challenges such as limited instrument maneuverability, ergonomic difficulties, and increased surgeon fatigue.[3]

The current literature on SILS versus CLS is characterized by heterogeneity, with studies reporting varying results and utilizing diverse methodologies. While some studies suggest potential benefits of SILS, others have found no significant differences in outcomes compared to CLS. The absence of consensus highlighted the need for a comprehensive synthesis of the available evidence.

This study aims to address this gap by conducting a systematic review and meta-analysis to compare SILS and

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CLS in the treatment of appendicitis, especially in children. The primary outcomes evaluated include complication rates and hospital stay. Through this analysis, we seek to provide a clearer understanding of the relative benefits or limitations from these two surgical approaches.

MATERIALS AND METHODS

Search strategy

The protocol for the meta-analysis was developed in alignment with the Cochrane Collaboration guidelines.[4] This study was conducted following the updated of 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses as reporting guidelines.[5]

An extensive search across four databases (PubMed, ProQuest, Scopus, and ScienceDirect) was conducted in October 2024 to identify relevant articles, without restrictions on language or publication year. The search terms were conducted using a combination of Boolean operators and Medical Subject Headings keywords: (laparoscopy OR peritoneoscopy OR surgery, laparoscopic OR laparoscopic surgical procedures) AND (appendicitis) AND (SILS OR CLS OR single OR conventional). The specific search terms and syntaxes used across four databases are outlined in Supplementary 1.

Eligibility criteria

The inclusion criteria for this meta-analysis were studies that reported the effectiveness of SILS versus CLS in treating children with appendicitis, adhering to the diagnostic criteria outlined in the International Classification of Diseases. To maintain consistency throughout the study, we included studies that delineated the effectiveness of SILS vs CLS based on the availability of 2x2 contingency table, using either raw or modified data. Our initial search included all comparative studies, including randomized controlled trials and quasi-experimental studies. While the exclusion criteria for this meta-analysis were applied to studies that failed to meet the following conditions: (1) relevance to the designated topic, (2) adherence to an appropriate study design, (3) classified as non-meta analysis or systematic review, and (4) sufficient raw data.

Data extraction

Articles were screened by two independent reviewers. Initially, duplicated articles were identified and removed, followed by an assessment based on titles and abstracts. Subsequently, a thorough review of the full-text articles was performed on the basis of predetermined inclusion and exclusion criteria. Any discrepancies between the reviewers were addressed through discussion for resolution. To ensure accuracy and relevance, a post hoc exploration of the reference lists from relevant previous systematic reviews and meta-analyses was also been carried out. Finally, after completing the screening and reviewing process, the following data were extracted from each included study: (1) author names, country, and study design; (2) sample size; (3) demographic characteristics (e.g., age, gender distribution, weight, height, and comorbid if data were available); and (4) effectiveness of SILS versus CLS.

Data synthesis and analysis

The pooled effect size of SILS versus CLS effectiveness was evaluated using a random effects model in CMA version 3. I2 and Cochran's Q tests were assessed to determine the heterogeneity within the included studies, with an I2 value exceeding 30%, a small Q value, and a p value of <0.1 indicating a significant heterogeneity. Study outcomes were presented as odds ratio (OR) and standardized mean difference (SMD) with corresponding 95% confidence intervals (95% CIs). In the presence of heterogeneity, meta-regression analyses were conducted to identify potential moderators such as age, weight, height, and child's gender. A p-value of <0.05 was considered as significant moderator variable.

Sensitivity analysis based on study weight was not carried out because of the small number in our included studies. However, to maintain the consistency and study robustness, we conducted the identification of publication bias using visual inspection of a funnel plot and analyzes using Peters' regression test.[6] Alteration in the funnel plot and p-value of Peters' regression test ≤ 0.10 suggested the potential presence of publication bias. In cases where publication bias was identified, a trim-and-fill procedure will be implemented to correct the bias.

RESULTS

Overview of included studies

A comprehensive search across the four databases yielded a total of 732 relevant studies. After excluding 122 duplicates, the remaining 610 studies underwent initial screening based on titles and abstracts. Subsequently, 593 studies were excluded due to irrelevant topic, design, or a non-research nature. This process led to the identification of 17 eligible studies for full-text examination. Out of these, 11 studies were excluded for not meeting our inclusion criteria, leaving 6 studies that fulfilled the specified criteria and were included in the analysis (Figure 1).

Study characteristics

All studies included in this meta-analysis were published from 2011 to 2019 and were conducted in diverse countries, including United States (n=3), Poland (n=1), China (n=1), and Japan (n=1). All of the studies were designed using randomized controlled trial and comprised of 838 children diagnosed with appendicitis. The majority of participants in the included studies were male (52.9%) with the mean age at 9.9 years. Average weight of the participant was 36.21 kg. In the SILS group, observed complications included wound seroma (1 case), abdominal collection (1 case), postoperative wound infection (10 cases), and intra-abdominal abscess (1 case). In comparison, the CLS group reported postoperative wound infection (7 cases) and intra-abdominal abscess (2 cases). Notably, no severe complications such as postoperative ileus requiring reoperation via laparotomy were reported in either group (Table I).

Result of the meta-analysis

Our meta-analysis showed no significant difference between the measured variables when comparing SILS to CLS (Figure 2,3 and 4). The effect size for complication rates between SILS

Table I: Characteristics of the included studies

No	Author(s) (year)	Study design, country	Sample size	Demographic characteristic, n (%) or mean±SD	n-event (%) or mean±SD
1	Peter et al, (2011)[10]	Randomized controlled trial, United States	360	Type of appendicitis: Acute and chronic Age: 11.10±3.50 Gender, male 191 (53.05) female 169 (46.94)	Complication rate SILS: 6/180 (3.33) CLS: 4/180 (2.22) LOS duration SILS: 0.94±0.26 CLS: 0.92±0.28
2	Knott et al, (2012)1[1]	Randomized controlled trial, United States	274	Weight: 42.70±18.50 Type of appendicitis: Acute Age: 11.00±3.50 Gender, male 143 (52.19) female 131 (47.81) Weight: 38.30±14.50	Surgery duration SILS: 35.2±14.5 CLS: 29.8±11.6 Complication rate SILS: 2/135 (1.48) CLS: 3/139 (2.16) LOS duration SILS: 0.92±0.24 CLS: 0.94±0.30 Surgery duration SILS: 34.0±13.6 CLS: 29.6±13.6
3	Perez et al, (2012)[12]	Randomized controlled trial, United States	50	Type of appendicitis: Acute Age: 8.70±0.60 Gender, male: 25 (50) female: 25 (50) Weight: 36.25±NA	Complication rate SILS: 1/25 (4.00) CLS: 0/25 (0.00) LOS duration SILS: 1.70±NA CLS: 1.50±NA Surgery duration SILS: 46.8±3.7 CLS: 34.8±2.5
4	Wu et al, (2014)[13]	Randomized controlled trial, China	60	Type of appendicitis: Acute Age: 8.90±1.80 Gender, male 37 (61.67) female 23 (38.33) Weight: 27.6±3.8	Complication rate SILS: 1/30 (3.33) CLS: 1/30 (3.33) LOS duration SILS: 4.00±0.84 CLS: 4.50±1.17 Surgery duration SILS: 64.3±3.1 CLS: 53.0±2.8
5	Moriguchi et al, (2018)[14]	Randomized controlled trial, Japan	44	Type of appendicitis: NA Age: 8.70±2.40 Gender, male 26 (59.09) female 18 (40.90)	Complication rate SILS: 1/20 (3.33) CLS: 0/24 (2.22) LOS duration SILS: 9.20±5.90 CLS: 10.00±7.90 Surgery duration SILS: 85.1±36.2 CLS: 78.5±30.3
6	Golebiewski et al, (2019)[15]	Randomized controlled trial, Poland	50	Type of appendicitis: Acute Age: 11±3 Gender, male 21 (42) female 29 (58)	Complication rate SILS: 2/25 (8.00) CLS: 1/25 (4.00) LOS duration SILS: 6.00±4.00 CLS: 4.00±2.00 Surgery duration SILS: 68.0±15.0 CLS: 58.0±15.0

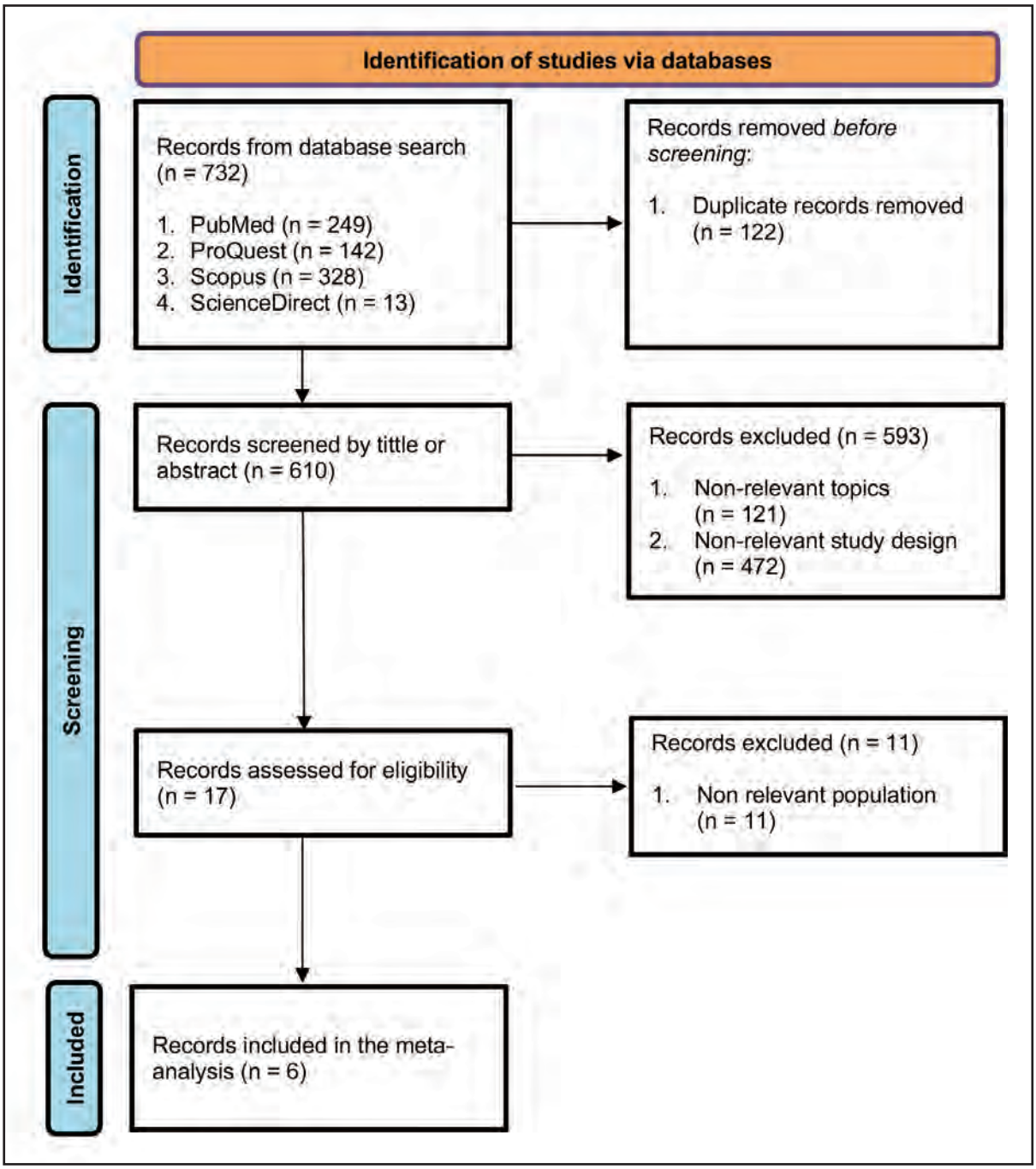

Fig. 1: Prisma flow diagram

and CLS was measured in odds ratio and valued as 1.421 (95% CI: 0.609–3.314, Q=1.370, p=0.416, and I2=0%). Similarly, in terms of hospital stay comparison that was measured in SMD, yielded the value of -0.003 (95% CI: -0.252–0.247, Q=9.271, p = 0.983, and I2 = 56.8%) (Figure 2,3 and 4). Visual examination of publication bias using the funnel plot displayed no apparent asymmetry. This observation was further substantiated by Egger's regression test that yielded p-value of 0.402 and 0.963 (p>0.05) for complication rate and hospital stay respectively, both indicating the absence of publication bias in our meta-analysis (Supplementary 2).

DISCUSSION

The results of this meta-analysis indicate that there was no significant difference in complication rates or hospital stay between SILS and CLS when treating children with appendicitis. Our findings align with those studies conducted by Aly et al (2016) and Deng et al (2017), who compared complication rates between SILS and CLS for adult appendicitis, both studies also reported no significant difference in complication rates following appendicitis surgery. However, our findings differ from study that conducted by Ding et al (2013) in term of hospital stay, who reported a better outcome in SILS surgeries. This discrepancy might be attributed to the differences in the characteristics of

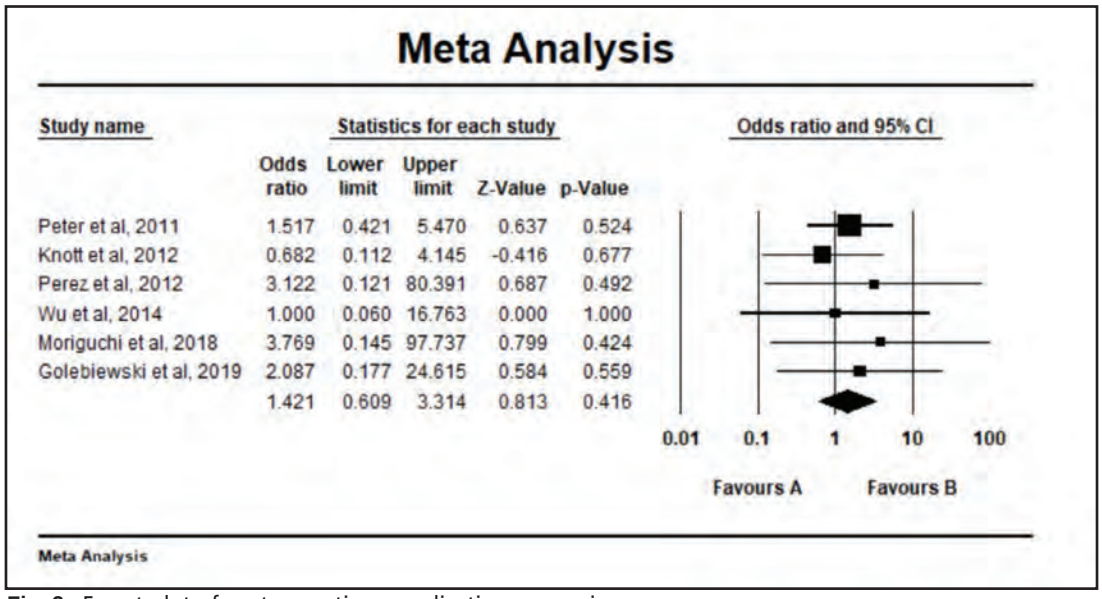

Fig. 2: Forest plot of post-operative complication comparison

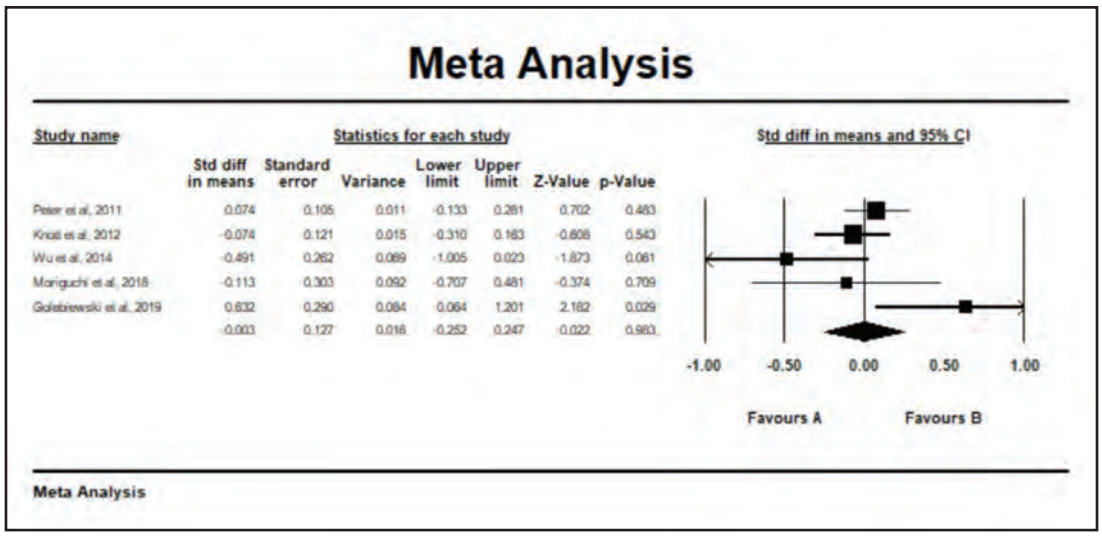

Fig. 3: Forest plot of length of stay comparison

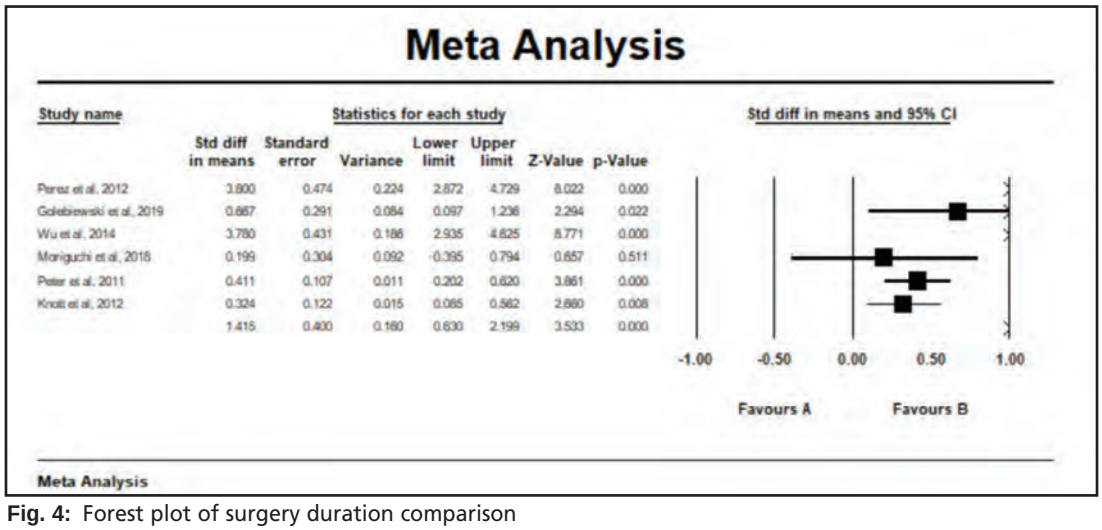

Fig. 4: Forest plot of surgery duration comparison

the study populations. While it has been demonstrated that SILS results in lesser wounds, which could hypothetically accelerate recovery, the actual healing process in children might be influenced by the immaturity of their immune system. This factor could potentially negate any differences in hospital stay between the two surgical techniques.[7,8,9]

Notably, this study holds several strengths. Firstly, to the best of our knowledge, this study was the first meta-analysis that comprehensively evaluated the global comparison between SILS and CLS in the treatment of child appendicitis. Secondly, we conducted an extensive search across four databases without implementing any limitations such as publication date, region, or language restrictions. Finally, we followed a clear and detailed methodological procedure for data extraction using the Cochrane guidelines.

However, this study is not without its limitations. Firstly, we identified a moderate level of heterogeneity in one of our analyzed variables, which could alter the generalizability of our findings. Secondly, we were unable to perform further analysis regarding these findings due to the lack of measured other variables within the included studies, such as anthropometric factors, which might pose as potential moderators. Therefore, future research incorporating larger data pool and additional analyses, such as meta-regression, is essential to address these limitations.

CONCLUSION

Both SILS and CLS demonstrate favorable outcomes for pediatric appendicitis surgery, with minimal differences in complication rates and recovery times. These findings suggest that both techniques are feasible choices, allowing for flexibility in choosing the surgical approach based on patient-specific factors and surgical expertise.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare.

ACKNOWLEDGEMENTS

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Supplementary 1
Syntaxes Used in the PubMed, ProQuest, Cochrane, Scopus, and Sage

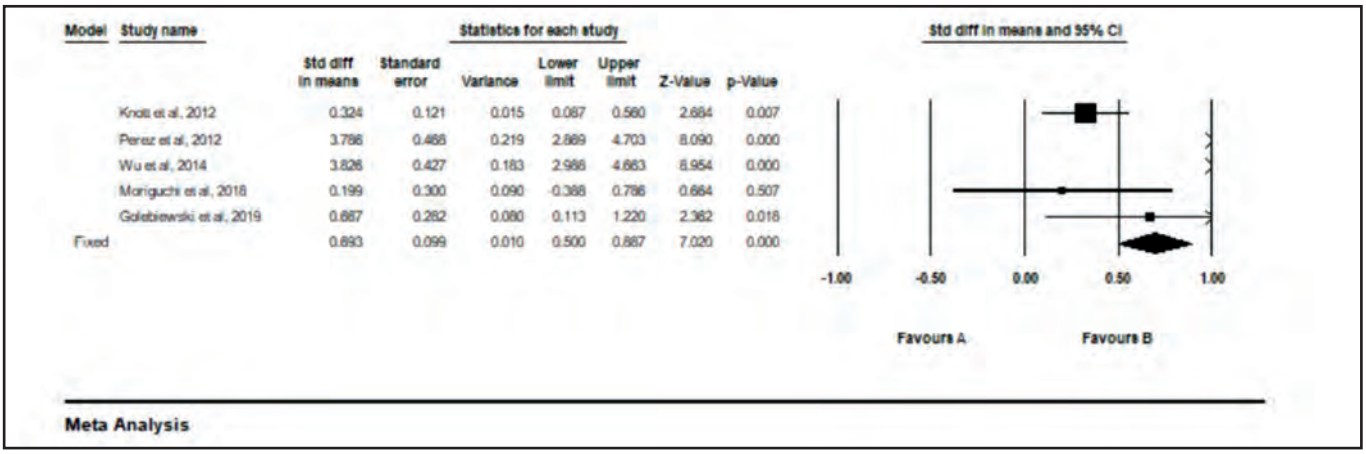
PubMed	
"Laparoscopy"[Mesh] OR "Peritoneoscopy"[tw] OR "Surgery, Laparoscopic"[tw] OR "Laparoscopic Surgical Procedures"[tw]	123,959
"Appendicitis"[Mesh]	21.799
"SIL"[Title] OR "CL"[Title] OR "single"[Title] OR "conventional"[Title]	391,486
(("Laparoscopy"[Mesh] OR "Peritoneoscopy"[tw] OR "Surgery, Laparoscopic"[tw] OR "Laparoscopic Surgical Procedures"[tw]) AND ("Appendicitis"[Mesh])) AND ("SIL"[Title] OR "CL"[Title] OR "single"[Title] OR "conventional"[Title])	249
ProQuest	
"Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures"	75,270
"Appendicitis"	25,051
title ("SIL" OR "CL" OR "single" OR "conventional")	4,124,754
("Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures") AND "Appendicitis" AND title("SIL" OR "CL" OR "single" OR "conventional")	142
Scopus	
ALL("Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures")	162,562
TITLE-ABS("Appendicitis")	27,805
TITLE ("SIL" OR "CL" OR "single" OR "conventional")	911,885
(ALL("Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures") AND TITLE-ABS("Appendicitis") AND TITLE("SIL" OR "CL" OR "single" OR "conventional"))	328
ScienceDirect	
("Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures")	83,810
("Appendicitis")	39,884
("SIL" OR "CL" OR "single" OR "conventional")	+1,000,000,000
((("Laparoscopy" OR "Peritoneoscopy" OR "Surgery, Laparoscopic" OR "Laparoscopic Surgical Procedures") AND ("Appendicitis"))) AND ("SIL" OR "CL" OR "single" OR "conventional")	13

2. Edger regression for length of stay (LOS)'s funnel plot analysis

Egger's regression intercept

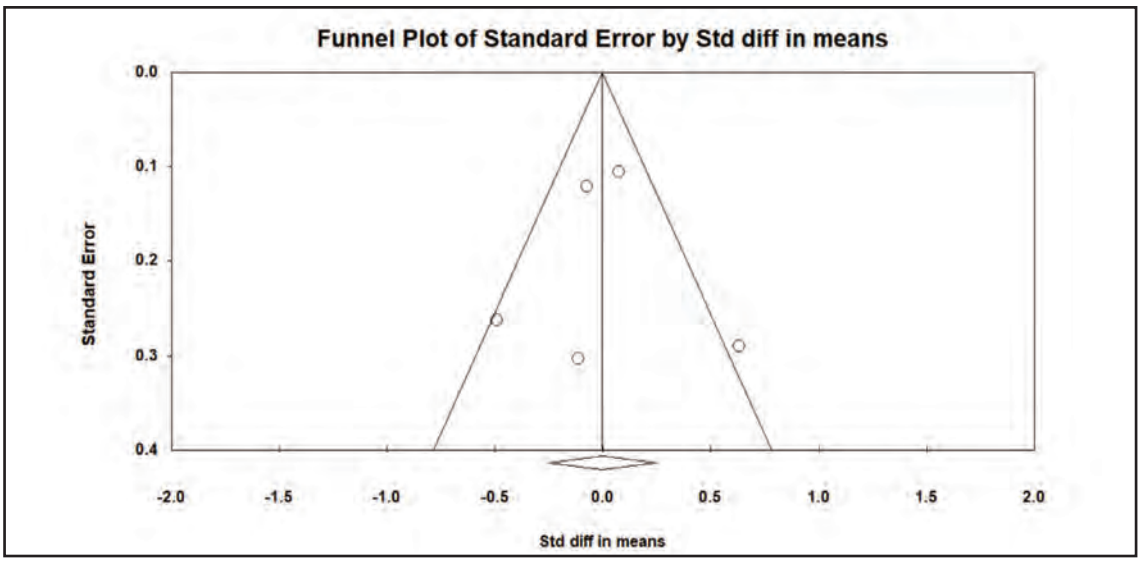
Intercept	-0.09205
Standard error	1.85050
95% lower limit (2-tailed)	-5.98118
95% upper limit (2-tailed)	5.79708
t-value	0.04974
df	3.00000
P-value (1-tailed)	0.48173
P-value (2-tailed)	0.96345

3. Forest plot of surgery duration SILS vs CL comparison



Model	Effect size and 95% confidence interval						Test of null (2-Tail)		Heterogeneity				Tau-squared			
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value	I-squared	Tau Squared	Standard Error	Variance	Tau
Fixed	5	0.004	0.071	0.005	-0.136	0.144	0.055	0.956	9.271	4	0.055	56.853	0.041	0.057	0.003	0.203
Random	5	-0.003	0.127	0.016	-0.252	0.247	-0.022	0.983								

Funnel plot of surgery duration SILS vs CL comparison



Egger's regression intercept

Intercept	7.40100
Standard error	3.36864
95% lower limit (2-tailed)	-3.31951
95% upper limit (2-tailed)	18.12152
t-value	2.19703
df	3.00000
P-value (1-tailed)	0.05775
P-value (2-tailed)	0.11549

Duodenojejunostomy versus Duodenoduodenostomy in Congenital Duodenal Obstruction: A retrospective study

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ABSTRACT

Introduction: Congenital duodenal obstruction (CDO) is a common cause of neonatal intestinal obstruction, most requiring surgery to restore bowel continuity. Duodenoduodenostomy (DD) is the preferred procedure, but duodenojejunostomy (DJ) may be performed when DD is not feasible due to anatomy, particularly in small or premature infants. In our centre, choice of procedure was based on intraoperative findings, tension-free anastomosis feasibility, and surgeon preference. This study compared short-term outcomes of DJ and DD in CDO.

Materials and Methods: This is a retrospective study with a cross-sectional about outcome of congenital duodenal obstruction at Hasan Sadikin Bandung General Hospital in 2019-2024. Data were collected through medical records, including gestational age, age at surgery, surgical technique, operation time, time to first feed, time to full feed, length of stay, and mortality.

Results: There were a total of 36 congenital duodenal obstruction patients. There were 4 patients (11%) who died before surgery, and 32 patients (89%) underwent surgery. All procedures were performed open surgery. Duodenojejunostomy was performed in 10 patients (31%), duodenoduodenostomy in 22 patients (69%). Time to first feed (mean) is post operative day 8 vs 9 (P = 0.3), time to full feed (mean) post operative day 27.1 vs 25.4 (p=0.8) and length of stay (mean) 27.6 days vs 34.6 days (p=0.9). Three patient of each group died post operative due to sepsis.

Conclusion: Duodenojejunostomy and duodenoduodenostomy have similar outcomes in neonates with congenital duodenal obstruction. These findings are relevant for surgeons who repair congenital duodenal obstruction with duodenojejunostomy or duodenoduodenostomy in open surgery.

KEYWORDS:
Congenital duodenal obstruction, duodenojejunostomy, duodenoduodenostomy, outcome

INTRODUCTION

Congenital duodenal obstruction (CDO) is a common cause of intestinal obstruction in the newborn period, occurring in approximately 1 per 5,000–10,000 live births and affecting males more frequently than females.[1] Associated anomalies have been reported in 45–65% of cases. Most commonly, trisomy 21 is found in almost half the cases, cardiac malformations in 25–65% of cases, and malrotation in 30% of cases.[2]

Congenital duodenal obstruction can occur due to an intrinsic or extrinsic lesion.[2] Intrinsic CDO may be caused by duodenal atresia, stenosis, diaphragm, a perforated diaphragm, or a "wind-sock" web. Extrinsic CDO may be caused by annular pancreas, malrotation, or preduodenal portal vein.1 The most common cause of duodenal obstruction is atresia. Duodenal atresias have been traditionally classified by into three types: type 1—obstructing perforate or imperforate web within continuous bowel; type 2—fibrous cord connecting the two blind ends of the duodenum with an intact mesentery; and type 3—complete bowel discontinuity and a V-shaped mesenteric defect.[3] Errors of duodenal re-canalization during the eighth to the tenth week of embryological development leads to duodenal atresia. Approximately 50% of all cases of CDO are detected antenatally on routine foetal ultrasounds when the characteristic 'double bubble' sign is noted in the upper abdomen due to dilated fluid-filled stomach and proximal duodenum.[4]

In almost all situations, a duodenoduodenostomy, joining the bowel just proximal and distal to the obstruction, is the best corrective option. It is the most direct, physiologic repair and, of the available options, has the least potential for later complications. When this procedure is difficult because of patient anatomy, particularly in some small, premature infants, duodenojejunostomy is the next best choice. A loop of proximal jejunum is chosen that will comfortably reach the proximal duodenal segment and is brought through the mesentery of the right transverse colon in a retrocolic position. Duodenojejunostomy provides postoperative results that are generally equivalent to those obtained with duodenoduodenostomy. Gastrojejunostomy, the third bypass option, suffers from the frequent late complications of marginal ulceration and blind loop syndrome, and therefore should be avoided. The study aims to evaluate the post operative outcome in patient with congenital duodenal obstruction treated with duodeno-jejunostomy compared to duodeno-duodenostomy repair.[2,5]

MATERIALS AND METHODS

This was a retrospective, cross-sectional study conducted at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from January 2019 to July 2024. All neonates diagnosed with

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CDO based on clinical presentation and radiological findings were included. Patients with incomplete medical records were excluded.

The decision to perform duodenoduodenostomy (DD) or duodenojejunostomy (DJ) was made intraoperatively, based on anatomical feasibility of a tension-free anastomosis. DJ was selected when DD was not technically feasible despite maximal mobilisation, such as in cases with short distal duodenum, marked calibre discrepancy, or abnormal bowel rotation. All surgeries were performed via open laparotomy by multiple paediatric surgeons.

We divided our patients into two groups: Group 1 consisted of 10 neonates treated with DJ, and Group 2 consisted of 22 neonates treated with DD. Outcome measures compared between the two groups included demographics (sex, gestational age, birth weight, reason for surgical delay, age at surgery, comorbidities, and operative time), time to first feed, time to full feed, length of stay (LOS), postoperative complications (wound infection, wound dehiscence, anastomotic leak), and mortality. Postoperative follow-up was conducted through outpatient clinic visits and review of medical records. Short-term outcomes were defined a priori as events occurring during the index admission or within 90 days after surgery. Longer follow-up beyond 90 days was recorded descriptively when available.

The DJ and DD groups were compared using non-parametric statistical tests for continuous variables and the Chi-square or Fisher's exact test for categorical variables. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess associations between potential risk factors and outcomes. Data are presented as numbers with percentages or as medians with ranges, as appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

There were 36 congenital duodenal obstruction patients. There were 4 patients (11%) died before surgery and 32 patients (89%) underwent surgery. From 32 patients, there were 16 boys and 17 girls (Table I). Most patients were full-term baby (53 %). We found comoborbid such as congenital heart anomaly (VSD, PDA), trisomy 21 and pneumonia . Age at surgery in DJ range from 4 – 31 days (mean: 15,1 days), in DD range from 5 – 32 days (mean: 17,7 days). Operation time in DJ range from 90 – 160 minutes (mean: 129 minutes), and in DD range from 100 – 190 minutes (mean: 150 minutes).

All procedures were performed open surgery. Duodenojejunostomy was performed in 10 patients (31%) and duodenoduodenostomy in 22 patients (69%). In DJ feeding start range from POD 7–12 (mean: 8) and in DD range from POD 6-13 (mean: 9) (Table II). In DJ group time to full feed reached in range from POD 23-32 (mean: 27.1), in DD group range from 22– 37 (mean: 25.4). Length of stay (LOS) of DJ group range from 28 – 47 days (mean:38,8) and in DD group range from 25–56 days (mean: 34,63).

We found no differences between two groups for time to first feed (p=0.3), time to full feed (p=0.8) and length of stay (p=0.9) (Table II). Of 32 patients who underwent surgery, 6 patients died with 3 patients per group.

DISCUSSION

Comorbidities in this study found there were Down Syndrome, congenital heart anomaly and pneumonia. Down Syndrome found more frequent especially in DJ group. This result had similar result with study by Zani et al, they recorded the most common comorbid was Down Syndrome.[6] In our cohort, four patients died before surgery due to severe comorbidities, and six patients died postoperatively—three in each group. All postoperative deaths were attributable to sepsis, often secondary to pneumonia or wound infection.[6]

The standard bypass procedure for CDO has long been a retrocolic DJ. DD is reported to be the most physiologic operative procedure, with a lower complication rate and higher survival. Another technique described is a diamond-shaped DD. This method was compared with conventional DD and was found superior to side-toside DJ or end-to-end DD. The advantage of this method lay in earlier feeding and discharge of the patients from the hospital. DJ is considered an easy method perform with DD, on the other hand, is considered a more physiologic bypass procedure for intrinsic duodenal obstruction. However, it is reported to require considerably more extensive and difficult disection, with reflection of the right colon to mobilize a greater length of duodenum by Kocher's maneuver. The experienced surgeon performing a DD does not consider that type of anastomosis technically more difficult, as it needs only minimal dissection.[7-9] Study by Weber et al, showed similar result where DD's outcome was better and allows significantly earlier oral feeding and shorter hospital stay when compared to DJ. Safety and efficacy of DD also reported by previous series.[10-12] In contrast, our study found no statistically significant differences between DJ and DD in terms of time to first feed, time to full feed, and length of stay, although DD showed numerically shorter feeding times and hospital stay. DJ, however, had a shorter mean operating time than DD.

Potential long-term complications of DJ, such as blind loop syndrome or bacterial overgrowth, may not be evident in the short term. In our study, the median follow-up was only 3 months (range 1–12 months), which is insufficient to reliably detect these late events. Ideally, a minimum of 12 months' follow-up is required to assess these risks. No anastomotic leaks or recurrent obstructions were observed during the follow-up period.

Duodenojejunostomy remains the next best choice when DD is not feasible due to anatomical constraints—such as short distal duodenum, marked calibre discrepancy, or unusual rotation—despite maximal mobilisation. A loop of proximal jejunum is chosen that will comfortably reach the proximal duodenal segment and brought through the mesentery of the right transverse colon in a retrocolic position. Study by Zani et al, that compared DD and DJ reported had similar rates of postoperative outcome. This study also reported the two groups that there were no differences for time to first feed, time to full feed and length of stay.[6]

Table I: Demographics of neonates treated with duodeno-jejunostomy (DJ) or duodeno-duodenostomy (DD) for congenital duodenal obstruction

Demographic variables	DJ	DD
Age (days, mean)	15,2	16,22
Sex (n)		
Boy	5	13
Girl	5	9
Gestational age		
Preterm	6	9
Full term	4	13
Comorbid (n)		
Congenital Heart Anomaly	4	4
Pneumonia	5	4
Trisomy 21	5	1
Age at surgery (days, mean)	15,1	17,1
Operation Time (minute, mean)	129	150

Table II: Outcome of neonates treated with duodeno-jejunostomy (DJ) or duodeno-duodenostomy (DD) for congenital duodenal obstruction

Variables	DJ	DD	p-value
Time to First feed (days)	8 (7-12)	9 (6-13)	0,300
Time to Full feed (days)	27,1 (23-32)	25,4 (22-37)	0,800
Length of stay (days)	38.8 (28-47)	34,63 (25-56)	0,900
Mortality (%)	30%	13,6%	

This study has several limitations. It is retrospective in nature with non-randomised allocation of surgical technique. The procedures were performed by multiple pediatric surgeons rather than a single operator, introducing potential operator-dependent variability. The sample size was relatively small, and follow-up duration was short and heterogeneous, limiting the ability to detect rare or late complications, particularly after DJ. Future prospective studies with longer follow-up and standardised operative technique are warranted to clarify any long-term differences between DD and DJ.

CONCLUSIONS

This study has shown that duodeno-jejunostomy repair for neonates with congenital duodenal obstruction provides postoperative results that are equivalent with duodeno-duodenostomy. These findings are relevant for surgeons who perform duodeno-jejunostomy and this technique has equal clinical outcomes and in some cases could be easier to perform.

CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

ACKNOWLEDGMENT

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ORIGINAL ARTICLE

Alterations in intestinal microbiota composition in children with hirschsprung disease: A comparative study with healthy controls

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ABSTRACT

Introduction: Hirschsprung disease (HSCR) is a congenital condition characterized by the absence of ganglion cells in the distal colon, resulting in bowel obstruction and motility disorders. Recent studies have highlighted the role of intestinal microbiota in intestinal health and disease, yet its specific alterations in HSCR patients remain unclear. This study aimed to investigate the intestinal microbiota profile of children with HSCR and compare it with healthy controls to identify potential microbial signatures associated with the disease.

Materials and Methods: This comparative cross-sectional study analyzed fecal samples from 7 preoperative HSCR patients and 3 healthy controls using 16S rRNA gene sequencing. Inclusion criteria required no antibiotic use within the previous two weeks. Clinical data, including nutritional status, medication history, bowel management methods, and history of HAEC, were recorded. Microbiota composition was compared at the phylum and family levels.

Results: HSCR patients exhibited significantly higher relative abundance of Enterobacteriaceae (mean 0.2582) compared to healthy controls (mean 0.0236), representing an approximately eleven-fold increase. HSCR patients also showed decreased proportions of Firmicutes, Actinobacteria, Bacilli, and Clostridia, while Bacteroidales were increased. Classification of taxa revealed a reduction in beneficial bacteria (e.g., *Lactobacillus, Bifidobacterium*) and enrichment of potentially pathogenic taxa (e.g., Escherichia-Shigella).

Conclusion: HSCR patients demonstrate a distinct dysbiotic microbiota profile, with reduced beneficial taxa and elevated Enterobacteriaceae levels. These findings highlight potential microbiota-targeted strategies for clinical management of HSCR.

KEYWORDS:
Hirschsprung disease, intestinal microbiota, dysbiosis, 16S rRNA sequencing

INTRODUCTION

Several studies have reported various alterations in the intestinal microbiota in both animal and clinical studies of Hirschsprung's disease (HSCR). Research indicates significant alterations in the intestinal microbiota associated with HSCR and its complications, particularly Hirschsprung-associated enterocolitis (HAEC). These changes are observed in both clinical and animal studies, highlighting the microbiota's role in disease pathogenesis and potential therapeutic avenues.[1-2]

In HAEC patients, a notable decrease in beneficial bacteria such as Bifidobacterium and Lactobacillus was observed, alongside an increase in Enterococcus. Studies in HSCR mice showed increased alpha diversity over time but a decrease post-surgery, indicating surgical impact on microbiota.[1]

The presence of Proteobacteria, particularly Escherichia, was linked to HAEC occurrences, suggesting a pathogenic role.[3] Functional analyses revealed that virulence factors associated with these bacteria may contribute to HAEC development.3 Fecal microbiota transplantation (FMT) has shown promise in enhancing cell therapy outcomes in related conditions, indicating potential for HSCR treatment.[3-4] While these findings underscore the microbiota's critical role in HSCR and HAEC, the variability in microbiota responses post-surgery suggests a complex interplay that warrants further investigation.

The lack of preliminary data on intestinal microbiota in HSCR patients compared to healthy children in Makassar, Indonesia, highlights a significant gap in understanding the disease's pathogenesis.

MATERIALS AND METHODS

The authors collected fresh fecal samples from 7 HSCR patients and 3 healthy children. This study utilized purposive sampling to select samples that met specific inclusion criteria. The subjects were pediatric surgical patients diagnosed with either HSCR or other non-HSCR conditions who were hospitalized at our center. The subjects were divided into two

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groups: Group 1 for HSCR and Group 2 for non-HSCR or healthy children, with participants selected randomly within these groups.

The authors extracted bacterial DNA from the fecal samples and then amplified it using target-specific primers (16S V3-V4). After amplification, we prepared a library using the PCR products and sequenced the final library on the Illumina platform to generate paired-end raw reads at the Molecular Biology and Immunology Laboratory, Microbiology Section, Faculty of Medicine, Hasanuddin University.

Data collection took place from January to August 2024. The study excluded patients with HSCR who had received antibiotic therapy within the last week, non-HSCR patients with accompanying gastrointestinal disorders, and those unwilling to participate.

The study processed various variables, including age, gender, source of infection, type of organism, bacterial classification, and outcome. HSCR are those whose clinical and histopathological tests revealing the presence of aganglionic tissue, as seen in HSCR, through either a rectal biopsy or a biopsy during laparotomy. Non-HSCR patients have diagnoses other than HSCR and show no signs of gastrointestinal symptoms. The ethical recommendations have been approved by the Hasanuddin University of Faculty of Medicine Ethics Commission (No:605/UN4.6.4.5.31/PP36/2024)

RESULTS

The demographic data for the patients in this study show that the youngest patient was 7 months and the oldest was 8 years. Regarding gender distribution, 6 out of the 10 patients (60%) were male, while 2 patients (40%) were female (Table I).

Based on histopathological examination, the data revealed that 7 samples were from biopsies that showed aganglionic tissue, confirming a diagnosis of HSCR. The remaining data pertained to healthy children.

Figure 1a illustrates the intestinal microbiota profile for all samples based on Phylum-level taxonomy, highlighting the five most abundant phyla: *Firmicutes, Proteobacteria, Bacteroidota, Verrucomicrobiota,* and *Actinobacteriota*. Our findings demonstrated that HSCR patients showed lower relative abundance of Firmicutes (mean 37.6% vs 55.4%) and Actinobacteria (mean 4.1% vs 9.8%), with higher Bacteroidetes (mean 28.9% vs 21.3%) and Proteobacteria (mean 29.4% vs 11.2%).

Based on the taxonomic class division shown in Figure 1b, the intestinal microbiota profiles of all samples were mainly characterized by the classes *Bacteroidia, Clostridia, Gammaprotobacteria, Bacilli,* and *Verrucomicrobiae*. In HSCR patients, there was a decrease in the presence of Bacilli and Actinobacteria at the class level.

The analysis based on order taxonomy reveals that the top five intestinal microbiota orders across all samples are Bacteroidales, Lactobacillales, Enterobacterales, Verrucomicrobiales, and Oscillospirales, in sequential order. Among HSCR patients, the highest-ranking order is Enterobacterales, followed by Bacteroidales and Verrucomicrobiales. Conversely, in non-HSCR individuals, the predominant order is Bacteroidales, followed by Bifidobacteriales and Lachnospirales, respectively, as illustrated in Figure 1c.

Following, a family-level ordered evaluation uncovered that the intestinal microbiota from the Enterococcaceae, Enterobacteriaceae, Bacteroidaceae, Akkermansiaceae, and Pseudomonadaceae families were the five most copious among all tests (Figure 1d). At this level, there's more inconstancy among the tests. In HSCR persistent tests, three families were reliably found: Enterobacteriaceae, Bacteroidaceae, and Akkermansiaceae. In any case, two tests appeared a dominance of the Enterococcaceae family. In non-HSCR quiet tests, the foremost predominant families were Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae.

The analysis of the genus taxonomic (Figure 1e) revealed that the most common intestinal microbiota genera across all samples, in order, were Enterococcus, Escherichia-Shigella, Bacteroides, Akkermansia, and Pseudomonas. In the HSCR disease group, the identified genus showed considerable variety, with the most consistent sequences in the samples being Escherichia-Shigella, Akkermansia, and Bacteroides. On the other hand, the non-HSCR group displayed more uniformity, with Bacteroides, Bifidobacterium, and a relatively even distribution between Faecalibacterium and Akkermansia. There were increased relative abundances of Escherichia-Shigella and decreased abundances of Faecalibacterium in HSCR patients compared to healthy children.

An analysis of diversity was conducted to identify the presence of intestinal microbiota dysbiosis. Beta diversity, as shown in Figure 2, was used to visualize the distribution and profile of individual sample microbiota. The PCoA plots offer a clear visualization of the differences in intestinal microbial communities between samples. It is important to note that there is no distinct group clustering immediately apparent from this visualization, suggesting some overlap in microbial diversity between samples.

DISCUSSION

Whereas investigating microbiota from all parts of the human body is interesting, the intestinal microbiota has gathered specific intrigued among analysts from differing areas. Information on typical intestinal microbiota are significant for understanding a extend of human gut-related infections. A version of the diary Nature Microbiology in 2015 dove into the setting of microbiome investigate, characterizing the microbiome as a multi-species community of microorganisms in different situations, counting the have, environment, or biological system. One of the conclusions drawn from these considers is the require for large-scale data, including information from different ages, sorts of illnesses, assorted populaces, and broad dispersal of this information

Table I: Characteristics of the samples

Participant ID	Group	Age (months)	Sex status	Nutritional (WHO z-score)	Laxative use	Bowel management method	Stoma present	Antibiotic use (past 2 weeks)
HSCR-01	HSCR	12	M	-1.2 (normal)	No	Rectal washout	No	No
HSCR-02	HSCR	7	F	-0.8 (normal)	Yes	Rectal washout	No	No
HSCR-03	HSCR	15	M	-2.1 (moderate underweight)	Yes	Stoma	Yes	No
HSCR-04	HSCR	10	M	-1.5 (normal)	No	Stoma	Yes	No
HSCR-05	HSCR	14	M	-0.7 (normal)	No	Stoma	Yes	No
HSCR-06	HSCR	9	F	-1.0 (normal)	No	Stoma	Yes	No
HSCR-07	HSCR	11	M	-1.8 (mild underweight)	Yes	Rectal washout	Yes	No
CTRL-01	Healthy control	96	F	0.2 (normal)	No	N/A	No	No
CTRL-02	Healthy control	72	M	0.5 (normal)	No	N/A	No	No
CTRL-03	Healthy control	12	F	-0.1 (normal)	No	N/A	No	No

Notes: Nutritional status classified according to WHO z-scores: normal (> -2), mild underweight (-2 to -3), moderate underweight (< -3). HAEC = Hirschsprung-associated enterocolitis. N/A = Not applicable.

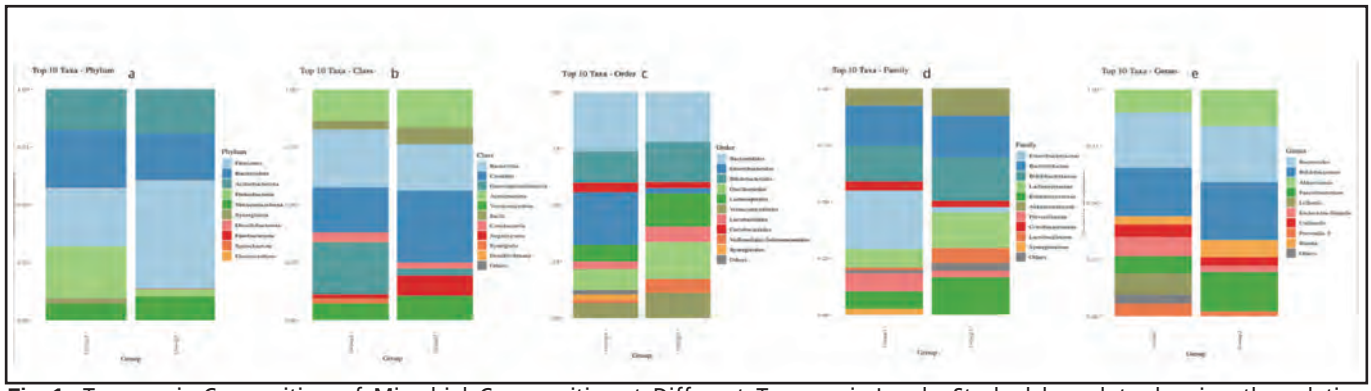

Fig. 1: Taxonomic Composition of Microbial Communities at Different Taxonomic Levels. Stacked bar plots showing the relative abundance of the top 10 microbial taxa in two different groups at five taxonomic levels: (a) Phylum, (b) Class, (c) Order, (d) Family, and (e) Genus. The y-axis represents the relative abundance of taxa, while the x-axis represents different groups under comparison

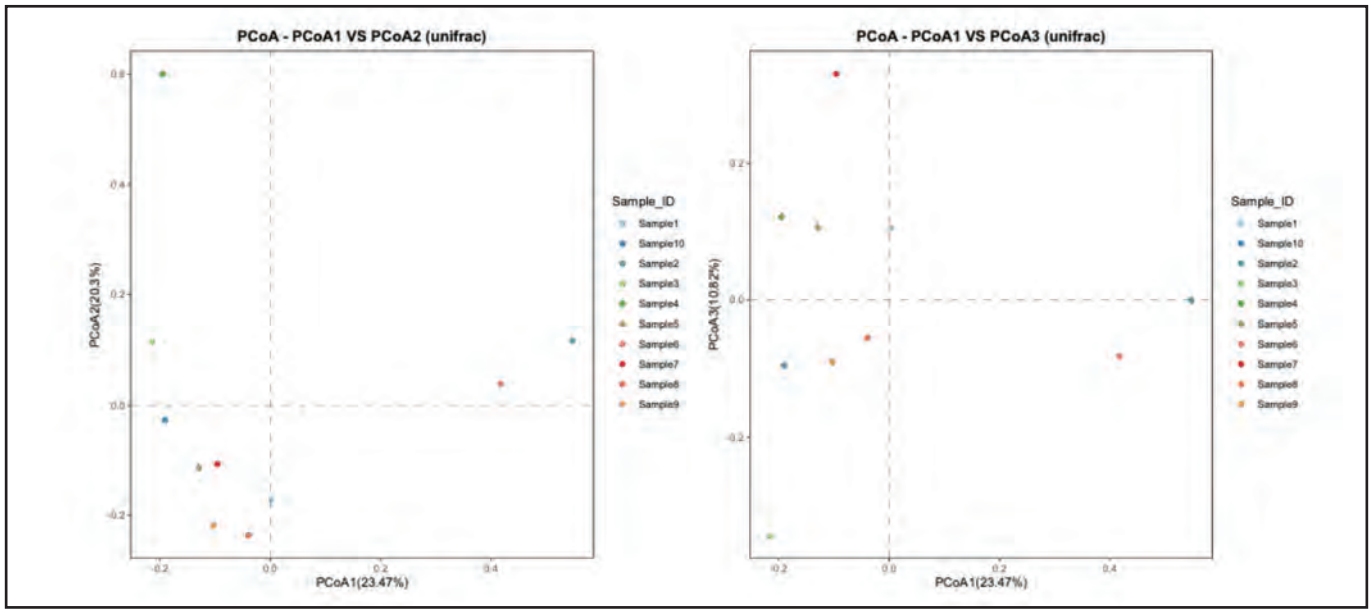

Fig. 2: PCoA on Weighted UniFrac Distance

for meta-analysis studies.6 Hence, in this think about we spearheaded a gene-based ponder in following intestinal microbiota profiles, particularly for sufferers of HSCR in our center.

Metagenomic study currently entails extracting nucleotide sequences from a collection of reference databases including microorganisms or genes, followed by computer analysis employing gene sequence reading algorithms to identify

microbe kinds and measure their abundance.[6] The genetic sequences of all detected organisms are shown separately, allowing for comparisons between HSCR and non-HSCR groups. To investigate the particular intestinal microbiota profile in HSCR, we collected samples from two groups: HSCR and non- HSCR, and compared their intestinal microbiota profiles at the phylum, class, order, family, and genus levels. We then determined the top five groups in each category.[7-8]

In this investigation, we employed 16S Amplicon Sequencing, a DNA sequencing approach that targets particular portions of the 16S or 18S ribosomal RNA (rRNA), known as amplicons, using universal primers. Next-generation sequencing of the 16S rRNA gene is commonly used in clinical microbiota research to explore the diversity of bacteria and archaea, whereas the 18S rRNA gene aids in identifying various species in eukaryotic samples. The 16S rRNA gene has nine hypervariable regions (V1-V9) that allow for taxonomic distinction. Regions V3–V4, which encompass locations 341–805 of the E. coli 16S rRNA gene, can differentiate distinct taxonomies to varying degrees.[8-9]

We amplified genomic DNA (gDNA) from our samples using primers that targeted the 16SV3-V4 region. The PCR findings were utilized to create genomic data, which was subsequently sequenced using Illumina technology to identify matches in a reference database. The R Studio tool (R version 4.2.3) and PCoA were used to analyze and visualize the data. This technique allowed us to show bacterial profiles in samples from HSCR and non-HSCR patients, as well as categorize the microbiota into key taxonomic categories such as phylum, class, order, family, and genus.

This study concludes that mapping intestinal microbiota profiles for various clinical needs, such as diagnostics, therapy planning, and treatment method selection, as well as developing databases on a disease's genomic and microbiotic profiles, is possible using gene sequencing methods, as we demonstrated. If performed on a bigger scale and with a more representative number of samples, the results will be extremely beneficial for future microbiota analysis-based research and therapeutic guidelines.

Two animal studies looked at intestinal microbiome alterations in genetically engineered mice with HSCR.[9-10] Both investigations found that mutant mice had more intestinal microbiota diversity than wild-type mice. Another research by Hegde discovered the variety of intestinal bacteria in mice with partial colon blockage.[11] These findings are congruent with those reported in functional gastrointestinal diseases such as constipation. This study implies that higher variability in intestinal obstruction may be due to slower transit rates in the blocked gut.

The bulk of research on the intestinal microbiota in HSCR has found a considerable rise in Proteobacteria and Bacteroidetes, whereas Firmicutes have decreased at the phylum level. The same finding with our research, with Bacteroidetes being the second most common phylum among non-HSCR cases.[12-13]

When identifiable microbes in HSCR are examined at more specific taxonomic levels, most studies report an increase in the presence of Escherichia, particularly Escherichia coli, from the Proteobacteria phylum, as well as higher levels of Bacteroides and Tannerella from Bacteroidetes, while Lactobacillus and Staphylococcus from the Firmicutes phylum are noted to be reduced. Further research on intestinal microbiota in children with HSCR discovered higher amounts of Escherichia and Pseudomonas from the Proteobacteria phylum, as well as more Prevotella and Actinomyces from the Bacteroidetes and Actinobacteria phyla, respectively. These patterns are consistent with our findings, which showed that the most prevalent genera in HSCR illness were Escherichia-Shigella, Akkermansia, and Bacteroides [10-11,13]

Previous research has suggested a relationship between intestinal microbial dysbiosis and HSCR, albeit the specific etiology is uncertain. In this study, we used taxonomic tracing techniques to examine the intestinal microbiota makeup of HSCR patients. We then compared this intestinal microbiota profile to that of people without the disease. The findings provided important information on the nature of intestinal microbiota dysbiosis in gastrointestinal obstructive illnesses, specifically HSCR.[14]

Intestinal microbiota dysbiosis in HSCR may be caused by delayed bowel transit due to intestinal blockage or inadequate motility. Some studies imply that dysbiosis can remain even after surgery to remove the blockage. In individuals with HSCR, an aberrant mucosal barrier and decreased immunological activity in the intestines may lead to gut dysbiosis. Dysbiosis in HSCR impairs mucosal defense and gut immunity via many mechanisms, increasing the risk of colonization and invasion by pathogenic microorganisms. This increases the risk of individuals developing enterocolitis and other HSCR complications.[12-13]

This study demonstrated distinct gut microbiota alterations in HSCR patients compared to healthy controls, characterized by reduced beneficial taxa and increased potentially pathogenic bacteria. Several factors may contribute to these alterations: Dietary patterns: HSCR patients often have modified diets with lower fiber intake due to gastrointestinal symptoms, potentially reducing beneficial taxa. Bowel management: The presence of stomas or frequent rectal washouts can alter gut environment and microbiota composition. Chronic constipation: Prolonged intestinal transit time in HSCR creates a milieu favorable for Enterobacteriaceae proliferation. Comorbidities: Nutritional deficits and recurrent infections may further impact microbial diversity.

LIMITATIONS

The small sample size limits generalizability. The cross-sectional design precludes possible causal inference. Hospital-based recruitment and lack of dietary records may introduce potential biases.

CONCLUSION

HSCR patients exhibit a distinct dysbiotic microbiota profile, with significantly higher relative abundance of

Enterobacteriaceae and reduced beneficial taxa compared to healthy controls. These alterations may have clinical relevance for monitoring and preventing complications such as HAEC.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare.

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Patient's outcome after definitive surgery using krickenbeck continence score in anorectal malformation

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ABSTRACT

Introduction: Anorectal malformations (ARM) are a common type of congenital anomaly seen in pediatric surgery, with an approximate incidence ranging from 1 in 2,000 to 5,000 live births. The main aim of neonatal care in patients with ARM is to ensure that patients achieve optimal functionality after definitive surgery. The Krickenbeck continence score is used to assess the outcomes of ARM patients. So, this study aimed to evaluate patient outcomes after definitive surgery in Arifin Achmad General Hospital.

Materials and Methods: This was a cross-sectional retrospective study utilizing electronic medical records of patients with ARM who had undergone a definitive surgery procedure at Arifin Achmad General Hospital, Indonesia, from 2019 to 2022.

Results: In this study, twenty-six patients with ARM were identified, including 12 males and 14 females. Approximately 76.9% of the patients had normal birth weight. The study found that 61.5% of the patients had ARM without fistula, 15.4% had ARM with rectourethral and perineal fistula, 3.8% had ARM with vestibular fistula, and 3.8% had ARM with cloaca. According to the Krickenbeck continence score, 61.5% of the patients achieved Voluntary Bowel Movement (VBM), while 11.5% experienced soiling and 7.7% experienced constipation. It was observed that normal birth weight patients had finer VBM compared to those with low birth weight (P <0.001), and male patients had finer VBM than females (P = 0.005).

Conclusion: The results of ARM patients for functional outcomes at our hospital are generally positive. More than half of the children exhibit voluntary bowel movements (VBM), and only a little experience soiling and constipation. The number of VBM may be linked to the child's birth weight and gender.

KEYWORDS:
Anorectal malformation, Krickenbeck classification, outcome

INTRODUCTION

Anorectal malformations (ARM) are the most frequent of the gastrointestinal tract congenital anomalies encountered in pediatric surgery practice, consisting of structural anomalies of the rectum, anal sphincter complex, and genitourinary apparatus.[1–3] The reported rate is between 1 in 2,000 and 5,000 live births with no apparent sex predilection.[4,5] Although no defined etiology of ARM has been established, genetic predispositions as well as environmental factors have been thought to be causative.[1] These malformations occur during embryogenesis,[6] characteristically because of urorectal septum arrested caudal migration to the cloacal membrane,[2] or else because of pathologic recanalization processes occurring in the ninth week of gestation, resulting in ectopia of the anal canal in the cloacal structure.[7]

The diagnosis of ARM is primarily established by the observation of meconium or feces passing through ectopic sites. In males, this would be from the perineum or urethra, and in females, this may be observed to pass through the vestibule or perineum. When there is no passage of stool, radiographic evaluation with a prone cross-table lateral view can be indicated to aid in anatomical localization.[8] The Krickenbeck classification system categorizes ARM into three broad domains: diagnostic, surgical management, and long-term functional outcomes following definitive surgery. [9] The main diagnostic subtypes are perineal fistula, rectourethral fistula (with bulbar and prostatic subtypes), rectovesical fistula, rectovestibular fistula, cloacal malformation, absence of fistula, and anal stenosis.7[-10] Rectourethral fistulas are most commonly found in males, whereas rectovestibular fistulas are more commonly found in females.[1,9]

The management of ARMs is based on anatomical classification and the degree of the defect.11 The initiated technique described by Peña and DeVries, the posterior sagittal anorectoplasty (PSARP), has been a very successful procedure. But management is more complex in patients with a poorer prognosis for continence.[8,12–14] Fecal incontinence, in addition to being a physiological problem, is also a significant psychosocial issue, impacting a child's sense of autonomy and subjective quality of life.[6,15] Current surgical goals have evolved beyond ensuring the simple survival of infancy, with a greater emphasis being placed on providing acceptable long-term bowel function to enhance the child's daily living and social integration.[4,6,16,17] Functional outcomes are evaluated by three parameters, as distinguished by Krickenbeck: voluntary bowel movements (VBM), soiling, and constipation.[2,5] This study will determine ARM patient functional outcomes through the Krickenbeck continence scoring system.

MATERIALS AND METHODS

This retrospective study aimed to evaluate the functional outcomes of patients with ARM treated with posterior sagittal

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Table I: Association between the characteristics of ARM patients and functional outcomes

Characteristic	N (%)	VBM		p-value	Soiling		p-value	Constipation		p-value
		Yes	No		Yes	No		Yes	No	
Gender										
▪ Male	12 (46.2%)	11	1	0.005	1	11	1.00	0	12	0.483
▪ Female	14 (52.8%)	5	9		2	12		2	12	
Birth Weight										
▪ Normal	20 (76.9 %)	16	4	<0.001	2	18	1.00	2	18	1.00
▪ Low	6 (23.1 %)	0	6		1	5		0	6	
ARM Type										
▪ No Fistula	16 (61.5%)	9	7	0.741	2	14	0.065	1	15	0.012
▪ Rectoperineal Fistula	4 (15.4%)	2	2		0	4		0	4	
▪ Rectourethra Fistula	4 (15.4%)	3	1		0	4		0	4	
▪ Rectovestibular Fistula	1 (3.8%)	1	0		0	1		0	1	
▪ Cloaca	1 (3.8%)	1	0		1	0		1	0	

anorectoplasty (PSARP) or minimal anoplasty in the Pediatric Surgery Department of Arifin Achmad General Hospital from December 2019 to December 2022. The inclusion criteria were patients aged ≥3 years who had completed all surgical treatment stages at least six months before follow-up, allowing for valid continuity assessment. Participants who had comorbid conditions or who declined to take part in the evaluation were excluded. All the eligible patients received adequate postoperative follow-up to measure the outcome.

This study investigated correlations of gender, birth weight, type of anorectal anomaly, and postoperative functional outcomes. Anomalies were categorized according to the Krickenbeck classification system. Functional outcomes assessed after definitive surgical intervention were voluntary bowel movements (VBM), soiling, and constipation. Voluntary bowel movements were defined as perception of the urge to defecate, communication of need, and control over defecation. Soiling was graded into three levels: grade 1, intermittent soiling (1–2 episodes a week); grade 2, daily soiling with no effect on social interaction; and grade 3, chronic soiling with social handicap. Constipation was graded as well: grade 1, manageable with diet adjustment; grade 2, necessitating pharmacologic therapy with laxatives; and grade 3, not responsive to either diet change or laxatives.[18]

Categorical variables were presented as frequencies and accompanying percentages. Association between the categorical variables was ascertained through Fisher's exact test. A p-value of less than 0.05 was considered statistically significant. IBM SPSS Statistics version 27.0 was used for statistical analysis.

RESULTS

A total of 26 patients with ARM were admitted to Arifin Achmad General Hospital. Among them, 12 patients (46.2%) were male and 14 (53.8%) were female. Most of the patients (76.9%) had a normal birth weight. According to Krickenbeck's classification, the most common anomaly was ARM without fistula, occurring in 61.5% of cases. It was followed by rectourethral and rectoperineal fistulas (15.4% each), vestibular fistula (3.8%), and cloacal malformation (3.8%).

Voluntary bowel movements (VBM) with adequate sphincter control were achieved in 61.5% of patients. Some patients, however, experienced soiling, which happened in 11. 5% of cases, and others had constipation, which was seen in 7. 7% of patients. Normal birth weight patients had significantly greater rates of VBM compared to low-birth-weight patients (p<0.001). Male patients also had significantly improved functional outcomes compared to female patients (p=0.005). On the other hand, the type of anorectal malformation did not make a statistically significant difference in whether or not a patient could achieve voluntary bowel movements (p=0.741).

Additionally, between gender, birth weight, or ARM type, there was no correlation with the occurrence of soiling and constipation, with p-values of 1.0, 1.0, 0.065, and 0.483, 1.0, 0.012, respectively (Table I).

DISCUSSION

Anorectal malformations are a range of congenital anomalies that can vary from mild anal misplacement with a good functional outcome to serious malformations that present significant surgical and management challenges.[9,19] Cassina et al. hold that ARM occurs in about 1 per 5,000 live births, making them comparatively common among congenital abnormalities. Unlike the 2:1 male predominance reported by Houben et al., in the present study, the gender distribution was equal.[1] This result aligns with findings from Mfinanga et al. and Narad et al., who also reported female predominance among ARM patients.[5,15]

Pediatric surgeons taking care of patients with ARM not only for the surgical treatment but also for the follow-up care to achieve bowel continence. All but a few patients with ARM experience some form of defecation dysfunction, and it can significantly impact their quality of life (QOL). Children with ARM have much poorer bowel function than children of similar ages. The extent of functional impairment directly correlates with ARM severity and long-term effects of constipation and/or fecal incontinence, frequently extending into adulthood.[7,13,16]

Krickenbeck classification was developed to standardize the measurement of functional outcomes across different subgroups of surgery so that comparisons between groups are

more meaningful. Bowel function is the most important predictor of long-term functional outcome in children with ARM because fecal incontinence and chronic constipation are serious postoperative complications. These may seriously undermine the social and psychological health of the affected.[6,9] In measuring functional outcomes during assessment, certain critical parameters must be determined: continence, the voluntary control of defecation without episodes of soiling; bowel regularity, more typically described as the frequency of absence of irregular or hard stools; and quality of life in general.[9]

Fecal continence is maintained by a blend of voluntary sphincter control, normal anorectal sensation, and orderly bowel motility. Certain anatomical and clinical characteristics have been shown to function as predictors for excellent functional outcomes in ARM patients. These include normal sacral and spinal anatomy, developed gluteal cleft, presence of anal dimple, ARM types, and the lack of presacral or sacral masses. Despite the presence of all these favorable features, the majority of patients ultimately develop defecatory dysfunction, such as constipation and/or incontinence, as permanent sequelae.[2,7] "True incontinence" has frequently been linked with sacral agenesis and rudimentary pelvic neuromuscular development.[20] Sacral agenesis has an estimated prevalence of between 1 in 25,000 to 100,000 live births, with a rate of 1 in 350 live births occurring among mothers with diabetes mellitus. This condition disrupts neural control, resulting in muscle weakness and sphincter dysfunction, which can lead to issues such as fecal incontinence and constipation.[21]

In some cases, fecal incontinence is a result of surgical complications, e.g., neorectum misplacement. In the majority of children, fecal incontinence is a result of an intrinsic anatomic defect. In men, the greatest incidence of incontinence is present in cases with rectal fistulas with communication to the bladder neck, while in women, high confluence cloacal malformation is most commonly associated with poor continence outcomes.[4] In congruence with Pelizzo et al.'s study, patients who underwent definitive surgical management within the first three months of life had higher rates of fecal continence.[6] Hakalmaz et al., however, mentioned inconsistent long-term rates of incontinence between 16% and 76% in ARM patients.[20] Postoperative continence was achieved in 70.8% of the patients in the present study.[15]

In a study by Hakalmaz et al., 75% of the patients' ARM were able to have voluntary bowel movements (VBM), and almost half became continent without adjunctive treatment.[20] The six low-birth-weight patients in this study did not have success with VBM. Malnutrition, a phenomenon common in low birth weight, is recognized to be a possible factor for compromised neuromuscular function. Voluntary bowel movements rely upon the integrity and coordinated function of pelvic floor musculature, rectum, and anal sphincter, all of which depend on sufficient innervation. Malnutrition may compromise the latter and functional maturation and potentially aggravate the severity of defective dysfunction in low-birth-weight infants with ARM compared to their normal-birth-weight peers. Previous research has also established a positive correlation between increased birth weight and improved survival rates among ARM patients. Defective voluntary control over the bowel may also occur secondary to anatomical defects or as a complication of reconstructive surgical procedures that affect the pelvic floor, rectum, or sphincteric mechanisms.[2]

The frequency of constipation following definitive repair of ARM has ranged extensively in the literature from less than 22% to as high as 86%.[20] The frequency of constipation following surgery was relatively low in the present study, occurring in only 7.6% of patients. The pathophysiology of constipation in ARM patients is not entirely clear. One of the proposed mechanisms is interference with rectal sensory innervation from the extensive anorectal mobilization in surgery, which could cause reduced rectal sensation. Hypomotility of the rectosigmoid area has also been implicated. Constipation also seems to be more common in operations that involve preservation of the internal anal sphincter, perhaps because of changed anorectal reflexes and motility.[4]

Chronic dilation of the rectal pouch may disrupt effective defecation by impairing peristaltic function, thus causing constipation.[2,7,9] It is one of the most prevalent and aggravating postoperative complications in patients with ARM who undergo definitive surgical repair.[1,8,14] Although constipation would be theoretically more prevalent in low-type anomalies, due to preservation of the internal anal sphincter and increased rectal reservoir function, it is often seen in both low and high ARM subtypes. In the study by Narad et al., a higher prevalence of constipation was reported in recto-bulbar and recto-prostatic fistulas, and to a lesser extent in recto-vesical fistulas. Effective management of constipation is necessary since it can otherwise lead to overflow fecal incontinence and gradual rectal or sigmoid dilation (megarectum or megasigmoid), significantly complicating subsequent treatment.[15]

In our research, approximately 61.5% of the patients achieved voluntary bowel movements with satisfactory sphincter control; however, a subgroup of patients continued to experience soiling episodes. Soiling developed in 11.5% of patients, less than in some past series.[1] Soiling is usually attributed to overflow incontinence, possibly due to sphincteric dysfunction or chronic constipation.[9] The height of the rectal blind pouch or fistula is usually inversely correlated with functional outcomes, with higher lesions correlated with poorer continence.[17] Lower-type anorectal malformations are usually preceded by a more favorable functional outcome than higher lesions.[2,22] It needs to be remembered that four patients (15.4%) of this series underwent minimal anoplasty, whose introduction of potential bias must be taken into account when assessing postoperative continence outcomes.

Despite advances in the surgical techniques for anorectal malformations, a subset of patients continues to experience severe postoperative complications, including overflow incontinence secondary to constipation and bowel motility abnormalities.[8] It should be kept in mind that the present study was not able to consider a variety of other possible factors that could influence functional outcomes following definitive repair. These include the presence of associated

anomalies, sacral and spinal morphology, value of sacral ratio, variability in surgical methods, and intra- or postoperative complications. Therefore, QOL evaluation must be included in the comprehensive follow-up of ARM patients, as reported in some of the earlier studies. Anatomic considerations, such as the nature of malformation and repair, are merely one group of factors, however, that influence fecal continence and QOL; sensory function and motility are also significant.[6]

CONCLUSIONS

Anorectal malformations (ARM) in our society are frequent. According to the Krickenbeck continence score used at our hospital, the functional outcomes for patients with ARM are generally positive. More than fifty percent of the children achieve bowel management (VBM), and relatively a few experience issues with soiling or constipation. Furthermore, the frequency of achieving VBM may be linked to factors such as gender and birth weight, but not to the ARM types. In contrast, constipation and soiling don't seem to be linked to gender, birth weight, or the type of malformation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Anti-inflammatory effect of UC-MSC secretome on diabetic ulcer model rats: Study of Ccl22 and Cxcl12 mRNA expression

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder which is characterized by chronic hyperglycemia condition and this condition can cause some complications such as diabetic ulcers. Secretome from UC-MSC is an alternative treatment that being developed for accelerating wound healing of diabetic ulcers. The aim of this research is to study the effect of UC-MSC secretome for wound healing of diabetic ulcers in Nicotinamide-Streptozotocin (NA-STZ) induced Wistar rats and the mechanism through mRNA CCL22 and CXCL12 expression study.

Materials and Methods: Fifteen male Wistar rats were divided into the diabetes group and the normal group. The diabetes group consists of the diabetes control group given by 0.02 ml MEM-α, the secretome group 0.004 ml, the secretome group 0.02 ml, and the secretome group 0.4 ml, and the normal group. Wounds are created on the back of the hyperglycemia rats and given the treatment intradermally. Wounded skin was collected for RNA extraction. RNA total yield used for cDNA synthesis. cDNA used as a template for Polymerase Chain Reaction (PCR) and using Agarose electrophoresis to view mRNA CCL22 and CXCL12 expression. Gene expression was normalized using mRNA GAPDH. Online software Targetscan and miRTarbase are used for selection of miRNA which have potentially targeted CCL22 and CXCL12 mRNA.

Results: NA-STZ affect increasing blood glucose. Injection of 0.02 ml UC-MSC secretome showed a better impact on wound healing than the diabetes control group. Lower expression of mRNA CCL22 and CXCL12 showed in male Wistar rats induced NA-STZ with UC-MSC secretome treatments.

Conclusion: UC-MSC secretomes have potential therapy as anti-ulcer on hyperglycemia male Wistar rats induced NA-STZ with probability via hsa-23a-3p in nuclear factor-kappa β (NF-κβ) pathway via inflammation mechanism.

KEYWORDS:
Diabetic ulcer, UC-MSC secretome, Nicotinamide-Streptozotocin (NA-STZ), mRNA CCL22, mRNA CXCL12

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by insufficient production of insulin or insulin resistance at the insulin receptors on the cell membrane. Based on the International Diabetes Federation (IDF), there are 536.6 million cases of diabetes worldwide. The prevalence of diabetes in persons between the ages of 20 and 79 was estimated to be 10.5 % in 2021 and is expected to rise to 12.5 % in 2045. Type 2 diabetes (DMT-2) is a subtype of diabetes mellitus characterized by hyperglycemia, which is can be caused by either impaired or insulin secretion failure in the body. About 80-90 % of diabetes mellitus patients have DMT-2.[1] Hyperglycemia is a dysregulation of blood glucose causes microvascular and macrovascular disease such as peripheral neuropathy and peripheral arterial disease which is involved in pathogenesis of diabetic foot ulcers (DFU).[2] The wound dressing treatment for diabetic ulcers can promotes wound healing through the angiogenesis process. However, if wound dressing not routinely monitored and replaced by an expert it will cause an infection. Thus, adequate inhibition of peripheral neuropathy and angiopathy is crucial for the diabetic ulcers wound healing.

Mesenchymal stem cells (MSC) are multipotent non-hematopoietic cells with spindle shape, highly replicative in

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vitro, that derive from the mesoderm germ layer from different tissue. Based on their origin, MSC can be classified into neonatal tissues, such as placenta, fetal blood, umbilical cord tissue, and adult tissues consists bone marrow, thymus, brain, liver, and adipose tissue. The different sources of MSC lead to diverge of proliferation and growth rates, differentiation and immunomodulatory potentials and regenerative properties.[3]

Thus, MSC have great potential for tissue repair and recently used for medical treatment. Nevertheless, there are controversial issues such as autoimmune effect and ethical clearance on using of MSC.

Secretome is the MSC secrete that consist of many crucial biological molecules such as cytokine, chemokines, enzymes, growth factors and extracellular vesicle that can modified by conditioned media.[4] The CM represents the complete culture medium together with the secretome and biological factors secreted into the extracellular space by the cells.[5] The cytokine responsible for immunomodulation such as interleukin 1 receptor antagonist (IL-Ra), competitive inhibitor of interleukin such as IL-13, IL-10, IL-17, and monocyte chemoattractant protein such as CXC motif ligand 12 (CXCL12) and C-C motif chemokine ligand 22 (CCL22). The presence of multiple growth factors consists vascular endothelial growth factor (VEGF), hepatocyte growth factor 1 (HGF-1), and tumor growth factor B (TGF-B). Extracellular vesicle (EV) derived from MSC considered as "cellular waste", recently get recognized for important players in intercellular communication. EV have ability to transport key molecules including functional RNA (mRNA and miRNA).[6] Therefore, secretome have potential as therapeutic agent. In vivo study showed that hWMSC-EC can repair ischemic tissue by promoting neovascularization and re-endothelialization through the secretion of pro-angiogenic factors.[7] Many studies shows that wound repair involves many growth factors such as PDGF and VEGF from BM-MSC for enhancing the wound healing in mice via recruitment, survival, and proliferation of keratinocyte, fibroblast, and endothelial cell to the wound and subsequent neovascularization.[8]

Umbilical Cord Mesenchymal Stem Cells (UC-MSC) have been shown in dozens of studies to be a prospective and optimal therapeutic approach in all kinds of MSC, owing to their advantageous characteristics, including easy extraction, low cost, noninvasive collection procedure, plentiful content, and low immunogenicity.[9-10] UC-MSC can promote tissue regeneration via the secretion of bioactive factors known as UC-MSC secretome. Compared to cell-based therapy with stem cell transplantation, which have a risk such as infection and pro-tumorigenicity, cell-free therapy with secretome injection is less immunogenic, safer, less expensive, and more practical application.[11] Due to the bunch of benefits, this study using In vivo model for evaluate the secretome efficiency in wound healing.

In vivo model are chosen for this wound healing pre-clinical study due to highly medical relevant if the wound is generated to mimic the wound seen in clinical practice. Compared with In vitro studies which is have limitation cannot reproduce biological conitions such as enzymes, cells, and tissue interactions.[12] Wistar rats are frequently used in pre-clinical studies to evaluate the novelty of medicine. A popular DMT-2 mimic model is the NA-STZ (Nicotinamide-Streptozotocin) rat model.

NA have a function on protecting β cells pancreas from the β-cytotoxic effects of STZ. This compound combination produces a model of insulin-deficient, but not insulin-resistant. It is characterized by moderate hyperglycemia associated with an approximately 60 % loss of β-cell function.[13] The mechanism NA-STZ in DMT-2 rat model starts with STZ entering cells by the glucose-2 transporter (GLUT-2) because it have structural similarity to glucose.[14] STZ in the cell, damaged the DNA and activate poly-ADP-ribose polymerase-1 (PARP-1), a polymerase that uses NAD^+ as a substrate to repair damaged DNA. As a result, NAD+ potentially depleted by the activated PARP-1, thereby leading to cell death. Therefore, the NA injection before STZ injection in mouse can decrease the effects of STZ in two ways. One is that NA is a direct inhibitor of PARP-1, the other way NA is precursor of NAD^+.[15]

Hyperglycemia in DMT-2 causes wounds to exhibit excessive oxidative stress which is break the balance between oxidative and antioxidative activities. The escalation of oxidative activity in the cells inhibit the cell function normally and delayed wound healing. In this condition, important regulators nuclear factor kappa B (NF-κβ) pathways play the key role oxidative stress in diabetic wounds.16 The vascular endothelial cell membrane contains the AGE receptor (RAGE), which can activate the transcription factor nuclear factor-kappa β (NF-κβ) and resulting oxidative stress, inflammation, and complications related to diabetes.[17] This activation modify the responses of switch control signals to pro-inflammatory gene transcription.[18]

C-C Motif Chemokine Ligand (CCL22) and C-X-C Motif Chemokine Ligand (CXCL12) are pro-inflammatory that involve in control the immune system. Innate immune cells such as macrophages, dendritic cells, and endothelial cells release CCL22 or macrophage-derived chemokine (MDC) and has CC chemokine receptor 4 (CCR4). CCL22 were able to induce keratinocyte migration and/or proliferation in vitro. CCL22 also has a role to Treg cell recruitment which can cause dysregulation of wound healing.[19]

The other pro-inflammatory, CXCL12 can promote the function of CD57+ CD8+ T cells via Erk 1/2 signaling which is activate the pro-inflammatory cytokine such as IL-15.[20] Higher expression of CXCR4/CXCL12 can recruit M1 macrophages, produce inflammatory cytokines, and cause insulin resistance.[21] CXCR4/CXCL12 is widely expressed in various tissues and organs. It is involved in physiology pathological processes such as growth and development, immune-inflammation, tumor invasion, wound healing, fibrosis through various pathogenic mechanism. Hyperglycemic condition in DMT-2 can lead into CXCR4/CXCL12 dysregulation which is significantly hampers the wound healing process.[22]

The aim of this study is to observe the effect of UC-MSC secretome injection on wound healing of diabetic ulcers and CCL22 and CXCL12 mRNA expression in a wistar rat induced NA-STZ model. In this study, secretome are collected from PT

Tristem Medika Indonesia who collaborated with the Faculty of Medicine UGM team for UC-MSC secretome production for diabetic ulcer therapy. Secretome products consist miRNA which have an advantage as cell-free therapy and targeted gene therapy has been profiled and normalized using β-actin and obtain top 10 highest miRNA expression (hsa-miR-23a-3p, hsa-miR-4445 + miR-7975, hsa-miR-1915-3p, hsa-miR-4488, has-miR-125b-5p, hsa-miR-21-5p, hsa-miR-130a-3p, hsa-miR-320e, hsa-miR-494-3p, and hsa-miR-4516).[23] The highest miRNA expression is miR-23a-3p which also potentially targets mRNA CCL22 and CXCL12.

MATERIALS AND METHODS

Animals Handling

The fifteen male Wistar rats, weighing between 180-230 g were purchased from the Pharmacology and Therapy Department, Gadjah Mada University. The rats are used in this study because it is sensitive to Streptozotocin, unlike rabbit which is resistant even with higher dose.24 Rats were kept in broad cable-based polypropylene cages and the rats were given a week to adjust the room temperature (24 – 25 oC), humidity (ranging from 55 – 60 %), and light/dark (12/12 hours). Animals had unrestricted access to rodent laboratory pellets and water. The handling of experimental animals was conducted according to BPOM No.10/2022 guidelines for the care and use of laboratory animals. This study protocol was approved by the Medical and Health Research Ethics Committee Faculty of Medicine, Public Health and Nursing University Gadjah Mada (Reference KE/FK/1116/EC/2023).

NA-STZ Wistar Rats Model Induction

A minimum sample size of animals involved was calculated according to the following equation: E = total number of animals – total number of groups. The "E" value should be between 10 and 20 to be considered as adequate. Higher animals should be added if the E value is less than 10, and the sample size should be reduced if it is higher than 20.[25] The final number of subjects in this study is 15. Rats were chosen randomly and divided into two groups: diabetes group and normal group. The diabetes group consist rats injected by Nicotinamide which is dissolved 0.9 % sodium chloride solution and obtained 230 mg/kg in concentration. NA administration using 1 ml syringe and inject it intraperitoneally into the rat. Fill a 1.5 ml microcentrifuge tube with 32.5 mg of STZ, cover it with aluminum foil, and prepare for the citrate buffer. Dissolve STZ to a concentration of 65 mg/kg in citrate buffer (pH 4,5). STZ injection intraperitoneally is conducted after a 15-minute NA injection.[26]

Diabetic Ulcer Model Induction

Fasting blood glucose of the diabetic rat group in 14 days after NA-STZ is measured using a glucose meter. Rats that had a fasting blood glucose level of more than 150 mg/dl were confirmed as hyperglycemia conditions. The normal group and the diabetes group injected 0.2 ml of ketamine intramuscularly, then returned the rats to their cage, and waited until the rats no longer reacted to pain. The wound is conducted on rat backs after removing the hair on the back clearly. The excision wound model size is 64 cm^2 square-shaped and made with sterile surgical scissors. Povidone-iodine was applied to the wound, and then covered with clear tape and sterile gauze. The rats return to their cage and are placed in a sterile area.

Administration of Secretome and Wound Closure Measurement

The diabetes group was separated into four subgroups which are given the following treatments: The normal group (A) which is not giving any treatments, diabetes control (B) which is injected by MEM-α 0.02 ml, secretome 0.4 ml (C), secretome 0.02 ml (D), and secretome 0.004 ml (E). At each point of the wound one injection in five days. A mobile phone camera is used to take a photo and a millimeter block is used to measure the closure of the wound. Next, the wound measured with ImageJ (Ver 1.54) and the data analyzed using One Way ANNOVA. Analysis of wound closure percentage using the following formula:

Symbol:

A_0 = wound area in 0 day (mm^2)

A_n = wound area in n-day (mm^2)

$$\textit{Wound healing rate}\ (\%) = \frac{A_0 - A_n}{A_0} \times 100\ \%$$

Skin Sample Collection

The skin with scar after wound conduction is collected on day twenty-two. Rats are anesthetized with 0.2 ml ketamine hair on the back was removed clearly before skin collection. The scar and ± 5 cm from the scar area are collected and preserved in 0.2 ml RNAlater and stored at -20 °C.

RNA Extraction and cDNA Synthesis

The Quick-RNA Miniprep Plus Kit (Zymo) protocol is used for RNA extraction. 150 grams of collected skin were weighed. RNA concentration was determined with Nanodrop spectrophotometry, yielding results with good purity and concentration. The concentration of RNA determines the RNA dilution in NFW. RNA is used as a template in cDNA synthesis using The Excel RTTM Reverse Transcriptase (SMOBIO) protocol. A total of 10 µl volume in the PCR tube contains 1 µl OligoDT and 9 µl RNA template diluted in NFW. Then, incubate it for 5 minutes at 70o C and left in an ice flake for 1 minute. Add 4 µl 5x RT buffer, 5 µl DEPC-Treated H2O, and 1 µl RTase mix into the mixture and get the 20 µl in volume total. Homogenize the mixture, and incubate for 5 minutes, then incubate in a thermocycler on first strand synthesis condition with temperature setting: 25 °C for 10 minutes; 42 °C for 50 minutes, 85 °C for 5 minutes, 4 °C for 10 minutes. cDNA then stores at -20 °C.

CCL22 and CXCL2 mRNA Expression

CCL22 and CXCL2 mRNA were amplified with specific primers using the Polymerase Chain Reaction (PCR) method. Add 1 µl cDNA from RNA template with 5 µl Powerpol 2X PCR with dye (ABclonal), 0.4 µl specific primer of CCL22 (F: 5'ACTTCAGACCTCCGATGCAG 3' ; R : 5 'GGGTGACGGATGTAGTCCTG 3') , CXCL12 (F: 5' GCCTTAAACAAGAGGCTCAAG 3'; R:5' CTTCAGACCTAGGCTCCTTCT 3') , GAPDH (F : 5' TGAGAAGCTGGTCATCAAC 3' ; R:5' GCATCACCCCATTTGATGTT 3') , and 3.2 µl NFW and mix it.

The mixture conducted for PCR method using a thermocycler with temperature setting: Pre-Denaturation: 98 oC for 45 seconds; Denaturation: 98 °C for 10 seconds; Annealing with gradation temperature 52 °C (CCL22), 58 °C (CXCL12, GAPDH) for 30 seconds; Extension: 72 °C for 20 seconds: post-Extension: 72 °C for 5 minutes; Hold: 4 °C for 10 minutes.

In this study, PCR method followed by electrophoresis are used to ensure the PCR amplicon primer binds to the target gene and to validate the mRNA expression. 0.5 grams of Agarose are weighed, then add it to 50 ml of 1X TBE buffer and heat it in the microwave for 1 minute. Add 1 µl loading dye and mix it with pipetting. Run the electrophoresis chamber at 50 volts for 20 minutes. Agarose was then visualized under UV light and the result was taken by camera. The density of the specific band result was then measured using ImageJ software.

miRNA Targeting CCL22 and CXCL12 mRNA Selection
Selection of miRNA which have potentially targeted CCL22 and CXCL12 are using online software TargetScan (https://www.targetscan.org) and miRTarbase (https://mirtarbase.cuhk.edu.cn). The acquired mRNA gene target position on conserved miRNA was compared to miRNA profiled from the UC-MSC secretome by the PT Tristem Indonesia team and the Faculty of Medicine, Public Health, and Nursing UGM.

Data Analysis
Body weight, fasting blood glucose, wound closure, and densitometry of electrophoresis band data were analyzed using computerized statistical program, GraphPad Software. The statistical analysis that used on this study is the ANOVA with normality, homogeneity, and independence assumption are checked. Each group have same sample size (n=5 rat) and rat are placed in the different cage, so this study meets the ANOVA assumption requirements. If the ANOVA test result is p-value <0.05, the Tukey post-hoc test is conducted and using p-value <0,001 for significant threshold.

The Tukey post-hoc test is used due to there are equal numbers of subjects contained in each group for which comparisons of the data are being made. It is used to determine whether any group or set of treatment conditions significantly differs from one or more others.

RESULTS

Body Weight and Fasting Blood Sugar
Based on the ANOVA test followed by Tukey Post Hoc test (Figure 1), the body weight of diabetes group is decreased in week 1 (Figure 1B) and week 2 (Figure 1C) after the NA-STZ injection and the body weight on week 3 (Figure 1D) shows the body weight is increasing.

Macroscopic wound observation on day 7 (Figure 2), shows that all groups are on inflammation phase which is characterized by heat, read color, swelling, and pain. The purposes of inflammation phase to remove necrosis tissue and prevent microbe infection. The diabetes control group (B) compared with the diabetes group that received 0.02 ml secretome (D) showed a significant result ($p<0.01$) (Figure 3A) which indicate diabetes group that received 0.02 ml secretome (D) had better effect on wound healing than normal group.

Macroscopic wound observation (Figure 2) and One Way ANOVA followed Tukey Post Hoc Test on day 14 (Figure 3B) showed that the diabetes group received 0.02 ml secretome (D) perfectly closed (100%). Normal group (A), diabetes control group (B), and the diabetes group received 0.004 ml (E) still not closed perfectly (± 90%). Significant results ($p<0.01$) in the normal group and diabetes control group compared with the diabetes group received 0.4 ml secretome (C) (Figure 3B).

CCL22 and CXCL12 mRNA Expression
The PCR method was used to observe the CCL22 and CXCL12 mRNA expression in the diabetes group and normal group. ImageJ software was used to measure the densitometry of the CCL22 and CXCL12 mRNA. Gene target amplification showed that all three genes-CCL22 (78 bp), CXCL12 (96 bp), and GAPDH (78 bp) were amplified on a specific target. Target gene amplification with various densities was shown by agarose electrophoresis. A thin band visualized the CCL22 gene on the diabetes group received 0.4 mL secretome (C) and diabetes group received 0.02 mL secretome (D). The CXCL12 gene band in the diabetes group received 0.4 mL secretome (C) also looks thinner than in another group. This result may secretome have a paracrine effect in decreasing CCL22 and CXCL12 mRNA expression.

One Way ANOVA followed Tukey Post Hoc Test also showed a lower expression of CCL22 and CXCL12 mRNA on day 21 (Figure 4A and Figure 4B) in the the diabetes group received 0.4 mL secretome (C), diabetes group received 0.02 mL secretome (D), and diabetes group received 0.004 mL secretome (E).

Gene target prediction analysis using Targetscan (https://www.targetscan.org) and miRTarbase (https://mirtarbase.cuhk.edu.cn), showed hsa-miR-23a-3p targeting CCL22 3' UTR gene on 842-849 bp position with strong binding affinity on duplex structure (MFE value) is -17,7 (kcal/mol), -15,80 (kcal/mol), -10,50 (kcal/mol) (Figure 5) and targeting CXCL12 3' UTR on 1452-1458 bp position with strong binding affinity on duplex structure (MFE value) is 13,10 (kcal/mol), -10,00 (kcal/mol), -12,00 (kcal/mol) (Figure 5).

DISCUSSION

Body Weight and Fasting Blood Sugar
Body weight is one of the developed DMT-2 indicators. Polyuria, polydipsia, polyphagia, and body weight loss are signs of diabetes mellitus (DM). Body weight loss may be caused by decreasing insulin production or insulin resistance. The inability of glucose to enter the cells was due to insulin resistance. Lipolysis and the breakdown of fat into energy are conducted to restore the energy. Subsequently, a decrease in body weight is caused by the breakdown of structural muscle proteins due to insufficient energy.

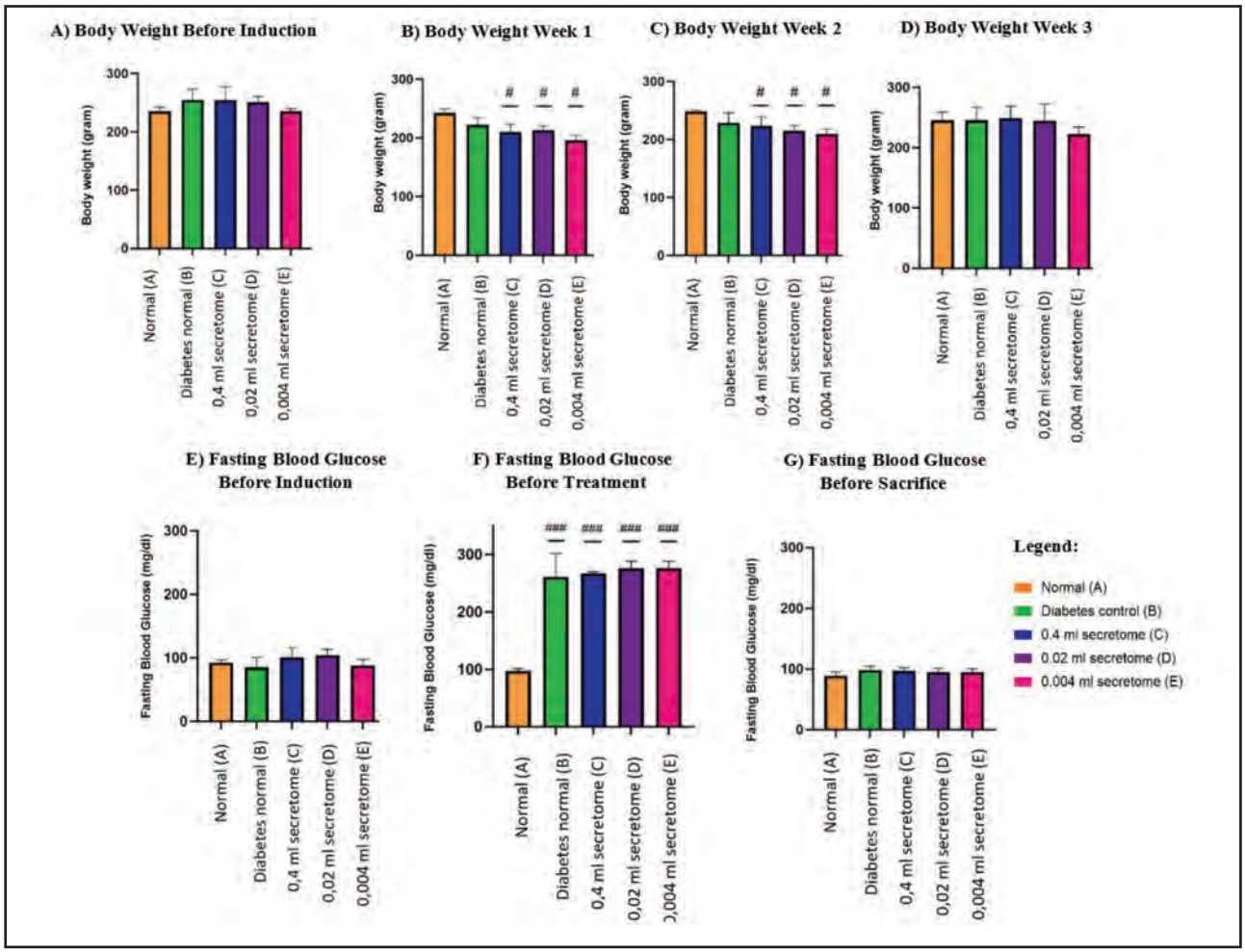


Fig. 1: Body weight and fasting blood glucose of 5 sample NA-STZ induced male Wistar. ANOVA followed by Tukey Post Hoc Test to evaluate the significance. The p-value ($p < 0.05$ vs normal) was statistically significant

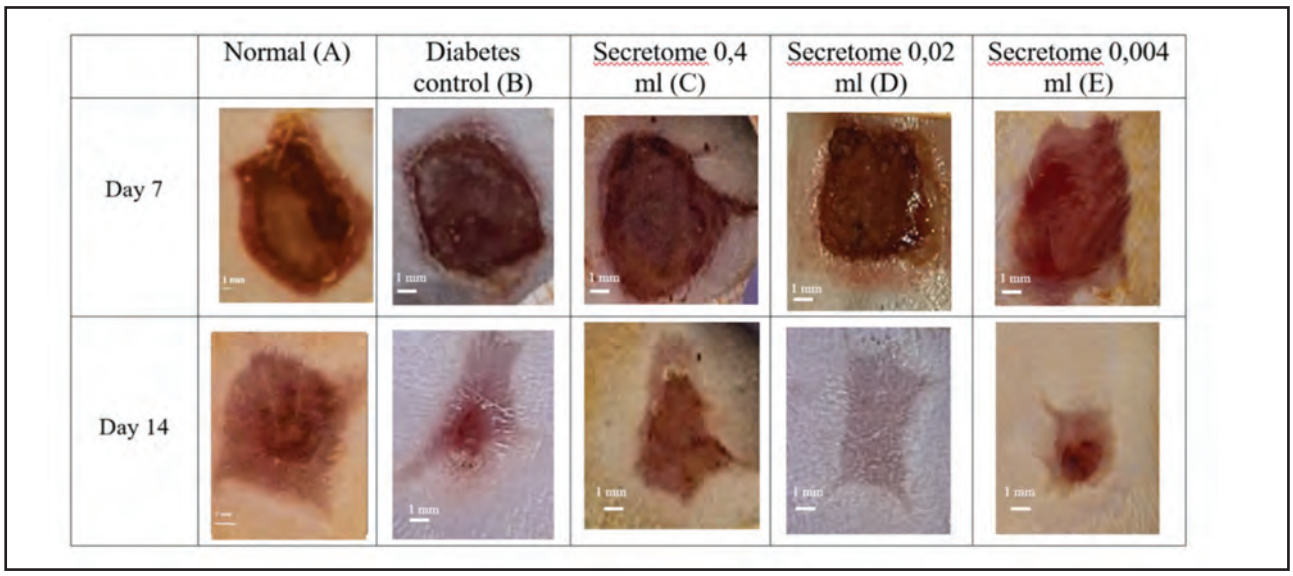


Fig. 2: Macroscopic wound healing observation on day 7 and day 14. Image scale = 1 mm/px

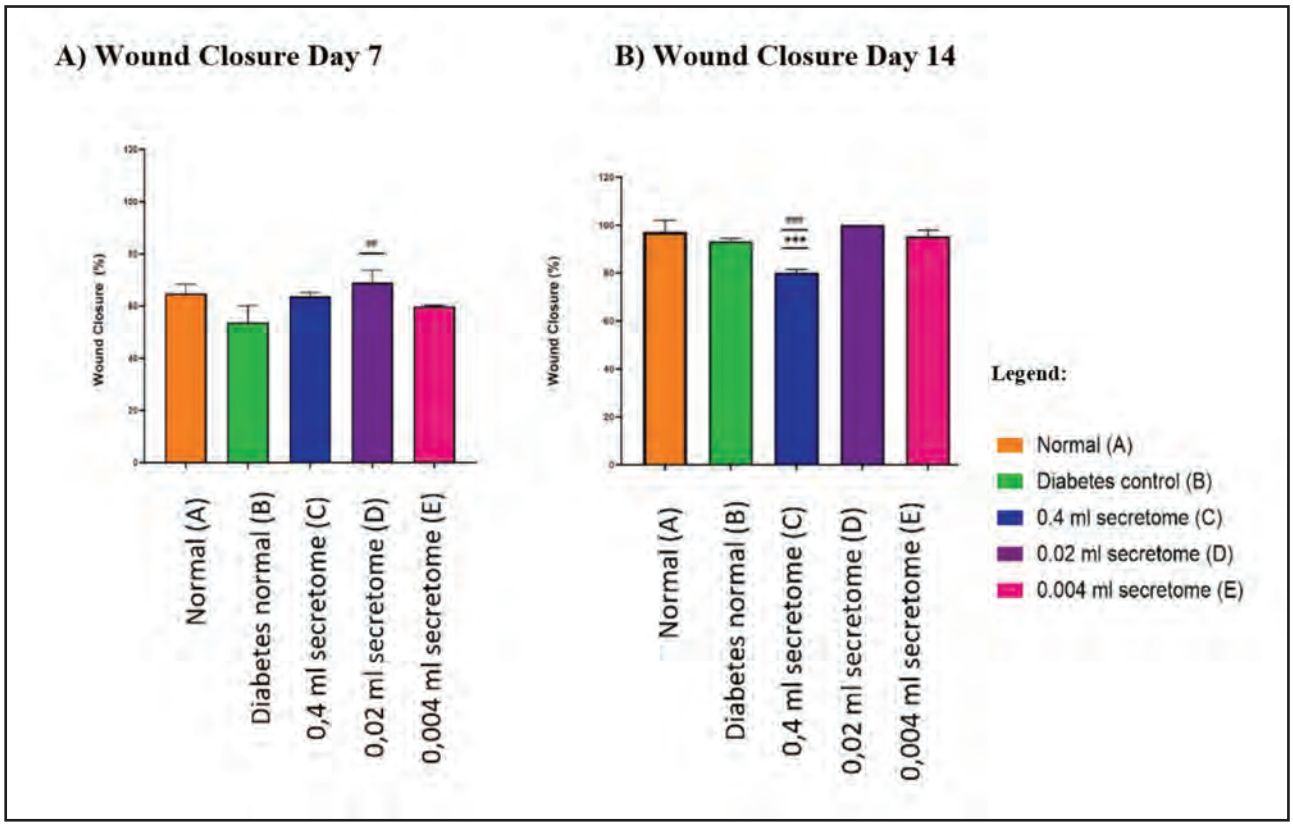


Fig. 3: Wound closure of 5 sample NA-STZ induced male Wistar. ANOVA followed by Tukey Post Hoc Test to evaluate the significance. The p-value (##p < 0,01, ###p<0,001 vs normal; ***p < 0,001 vs diabetes control) was statistically significant

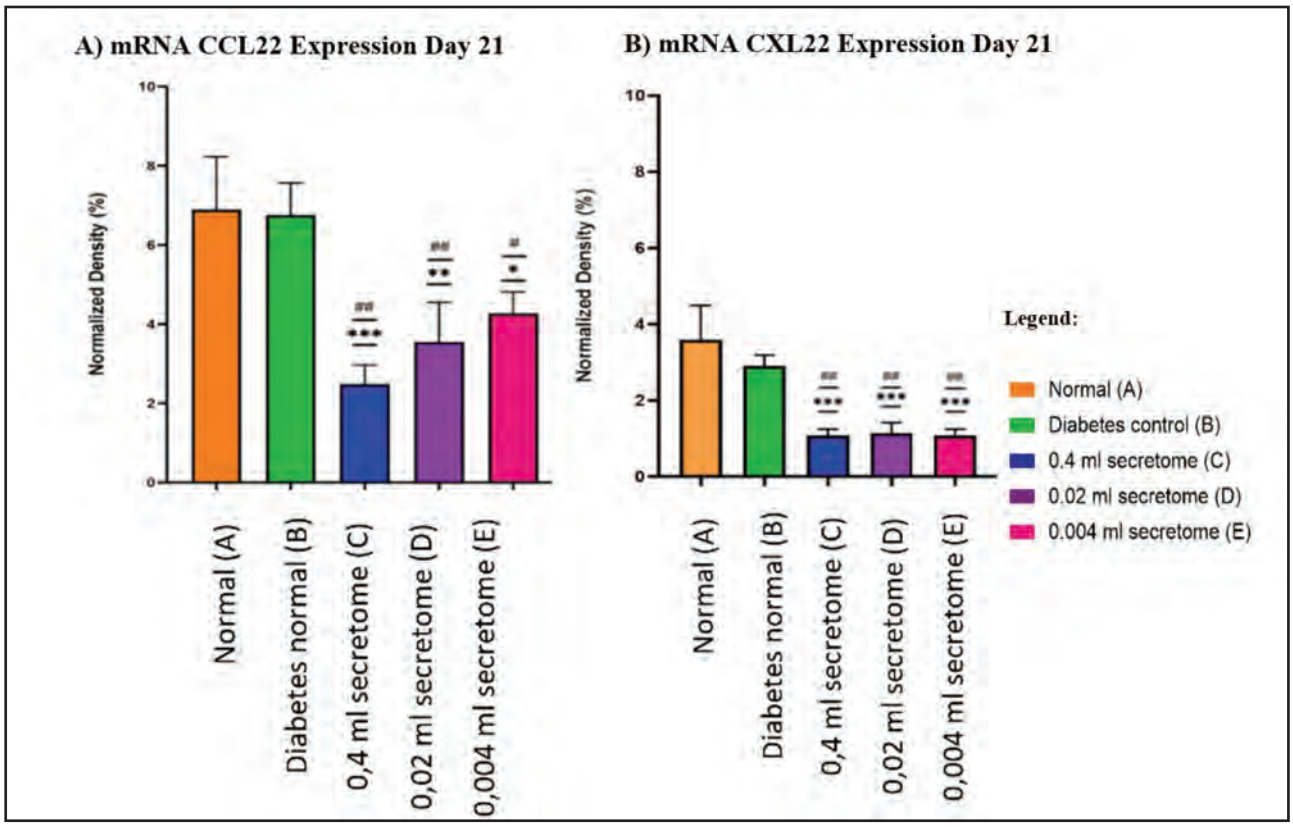
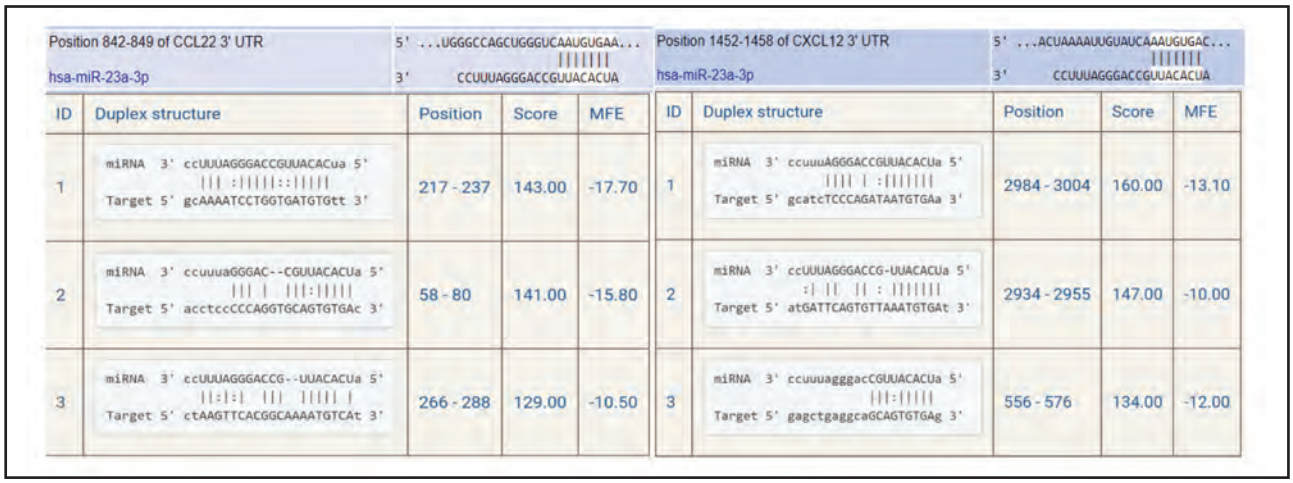


Fig. 4: mRNA CCL22 and mRNA CXCL12 expression of 5 sample NA-STZ induced male Wistar. ANOVA followed by Tukey Post Hoc Test to evaluate the significance. The p-value (*p < 0,05, **p< 0,01, ***p< 0,001 vs kontrol diabetes; #p< 0,05, ##p < 0,01 vs normal) was statistically significant



ID	Duplex structure	Position	Score	MFE
1	miRNA 3' ccUUUAGGGACCGUUACACua 5' Target 5' gcAAAATCCTGGTGATGTGtt 3'	217 - 237	143.00	-17.70
2	miRNA 3' ccuuuaGGGAC--CGUUACACUa 5' Target 5' acctcccCCAGGTGCAGTGTGAc 3'	58 - 80	141.00	-15.80
3	miRNA 3' ccUUUAGGGACCG--UUACACUa 5' Target 5' ctAAGTTCACGGCAAAATGTCAt 3'	266 - 288	129.00	-10.50

ID	Duplex structure	Position	Score	MFE
1	miRNA 3' ccuuuAGGGACCGUUACACUa 5' Target 5' gcatcTCCCAGATAATGTGAa 3'	2984 - 3004	160.00	-13.10
2	miRNA 3' ccUUUAGGGACCG-UUACACUa 5' Target 5' atGATTCAGTGTTAAATGTGAt 3'	2934 - 2955	147.00	-10.00
3	miRNA 3' ccuuuagggacCGUUACACUa 5' Target 5' gagctgaggcaGCAGTGTGAg 3'	556 - 576	134.00	-12.00

Fig. 4: Position and duplex structure has-miR-23a-3p targeting mRNA CCL22 and CXCL12 sequence

The injection of secretome can improve the repairment and regeneration of β-cells. Consequently, normal glucose regulation including weight growth is caused by the regeneration of β-cells. Based on the other researcher, in a rat model of mild hyperglycemia, STZ can trigger the proliferation which can lead to β-cells regeneration. Fasting blood glucose is checked before NA-STZ injection to ensure normal glucose level. NA-STZ injection was conducted after the adaptation period, and then fasting blood glucose was checked 14 days post-injection and before sacrifice. Fasting blood glucose before treatment of NA-STZ analyzed by ANOVA and followed by Tukey Post Hoc Test, showed significant results between the diabetes group and normal groups (p<0,001) (Figure 1.F). This result caused by the toxicity of STZ on the β-cells pancreas which is can effect on increase of blood glucose or hyperglycemia. Fasting blood glucose measurement before sacrifice (Figure 1.G) showed blood glucose returned to a normal condition which is caused by β-cells regeneration after injection of NA-STZ on a mild dose.[27]

Wound Healing after UC-MSC Secretome Treatment

In the diabetes group received 0.02 mL of secretome (D) shows better performance on wound healing than the normal group (A) and other diabetes group (B, C, E). This result caused by secretome injection which is contains soluble factors (growth factors, cytokines, chemokines, and enzymes) and extracellular vesicles that have a paracrine effect on tissue regeneration and reduce the pro-inflammatory production such as TNF-α, IL-6, and IL-10.[28] The use of secretome as cell-free therapy is to overcome the limitation of the stem cell therapy which have insufficient integration of the cells at the site of the implantation which can significantly reduce the efficacy of the treatment. The cost of cell therapy, adequately qualified staff and suitable facility core are other limitations on its everyday clinical use.

Based on wound healing image (Figure 2) and ANOVA Test followed Tukey Post Hoc test (Figure 3) can be concluded that wound healing (A) and diabetes group received 0.02 mL of secretome (D) has a better performance than the diabetes group received 0.4 ml secretome (C). This effect known as by paradoxical drug reaction, which is constitute an outcome that is opposite from the outcome that would be expected from the drug's known actions. Apparent clinical paradoxical or bidirectional effects and reactions ensue when conflicts arise at different levels in self-regulating biological systems, as complexity increases from subcellular components, such as receptors, to cells, tissues, organs, and the whole individual.[29] This effect case might related to the aggregation of the high dose secretome components in wound area which is also increases the immunogenic response.[30] Exosome-derived bone marrow-mesenchymal stem cell (BM-MSC) lower doses also found to be neuroprotective (anti-apoptotic, anti-necrotic, and anti-oxidant), but higher doses harm the neuron.[31]

CCL22 and CXCL12 mRNA Expression

The result on CCL22 and CXCL12 mRNA gene expression based on Figure.3 shows lower expression in diabetes group received secretome (C, D, E). This effect might cause by secretome which is contains molecular bioactive including soluble factors such as cytokines, chemokines, enzymes and extracellular vesicles. Extracellular vesicles contain exosomes which have an important role in cellular communication involving miRNA and protein delivery.[32] miRNA is a short nucleotide with 19-28 nucleotides including a short noncoding single-stranded RNA class that regulate cellular processes like cell death, and differentiation miRNA regulates gene target expression via incomplete pairing on the 3'-untranslated region (3'-UTR) from the mRNA target.[33]

Secretome UC-MSC which has been profiled showed the 10 highest miRNA expression and hsa-miR-23a-3p is the top highest miRNA expression which was normalized using β-actin.23 Gene target prediction analysis using Targetscan (https://www.targetscan.org) and miRTarbase (https://mirtarbase.cuhk.edu.cn), showed hsa-miR-23a-3p targeting CCL22 3' UTR gene on 842-849 bp position with strong binding affinity on duplex structure (MFE value) is -17,7 (kcal/mol), -15,80 (kcal/mol), -10,50 (kcal/mol) and targeting CXCL12 3' UTR on 1452-1458 bp position with strong binding affinity on duplex structure (MFE value) is 13,10 (kcal/mol), -10,00 (kcal/mol), -12,00 (kcal/mol). MFE

value showed the lowest energy from the secondary structure for mRNA binding whereas more negative MFE value, the binding of the target gene was stronger.[34]

Thus, indicating hsa-miR-23a-3p affected on lowering CCL22 and CXCL12 mRNA expression in the nuclear factor-kappa β (NF-κβ) pathway via inflammation mechanism. The availability of hsa-miR-23a-3p in UC-MSC secretomes may have a potential targeting therapy on diabetic ulcer therapy. The potential can be used for the development of the secretomes by standardizing the composition of biomaterials, especially miRNA in sccretome that shows quality, stability, security, and efficacy as important factors for clinical trials. The limitation in our study is no validation of miRNA expression on gene target. Future studies should investigate whether miR-23a-3p actually target the CCL22 and CXCL12 gene.

CONCLUSIONS

UC-MSC secretome injection at 0.02 ml gives wound healing effect on NA-STZ- induced male Wistar rats. Giving UC-MSC secretome to NA-STZ-induced Wistar rats showed lower CCL22 and CXCL12 compared to NA-STZ induced Wistar rats that were not given UC-MSC secretome.

CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

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ORIGINAL ARTICLE

Wanagama honey modulate macrophage phagocytic activity in mice

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ABSTRACT

Introduction: Honey is one of the natural ingredients that have long been used in the community to cure certain diseases and to maintain health. Several studies have shown that honey contains many active ingredients that have pharmacological effects, including immunomodulatory effects. The active compound of honey varies depending on the bee that produces honey and also the food source of the bees. Wanagama honey is honey produced in the forest in Gunung Kidul, Yogyakarta, Indonesia. Despite the use of Wanagama honey to improve immune system, there is no study regarding its immunomodulatory effect. This study aimed to study the immunomodulatory effects of Wanagama forest honey.

Materials and Methods: Twenty Balb c mice divided into 4 different groups received different treatment which were water (CONTROL), honey 10 mg/kg BW (DOSE 1), honey 25 mg/kg BW (DOSE 2), and honey 50 mg/kg BW (DOSE 3). The immunomodulatory effect evaluation was using the carbon clearance assay. Complete blood count and delta body weight were also measured.

Results: Mice in DOSE 3 group showed lowest delta body weight. the complete blood count parameters were not different significantly between groups of treatment. however, the phagocytic index was higher significantly in the groups receiving honey compare to those without honey treatment.

Conclusion: This study showed the potential of Wanagama honey as immunomodulator.

KEYWORDS:
Wanagama honey; immunomodulator; carbon clearance assay; phagocytic index; weight gain

INTRODUCTION

The immune system is the system in the body which responsible to maintain integrity of the body in the face of dangers that can be caused by the environment. Macrophages are the effectors in the immune system that act as pathogens or germs that will damage systems in the body[1], either directly through intracellular phagocytosis or indirectly by releasing Nitric Oxide (NO), Intermediate Reactive Oxygen (ROI) and cytokines.[2]

Immune system can be enhanced with immunomodulators. Immunomodulators are pharmacological agents that can partially modulate the immune response induced by the immune response and on the other hand it inhibits several other immune systems.[3] One of the immunomodulators that have long been used is honey. Honey is a natural product, rich in nutrients, which also has economic and ecological benefits, and has been used in traditional medicine since prehistoric times.[4] A small clinical trial showed that honey administration for 2 weeks resulted in increase of antioxidant agents, increase serum iron, decrease plasma ferritin, increase percentage of blood parameter, and reduce immunoglobulin E.[5] The pharmacological effects and biochemical constituents of various types of honey have been extensively investigated.

The pharmacological activity and biochemical composition of honey is highly dependent on its content, which is largely influenced by bee species, nectar source, geographic origin, and post-harvest processing.[6] Indonesia is a tropical country that has abundant types of honey and honey bees. Apis cerata is one of the native bee species that is widely used for traditional honey production, including Wanagama forest honey. Wanagama forest honey, harvested from Wanagama Forest in Gunungkidul, Yogyakarta. This honey is widely marketed and traditionally consumed by local communities as health supplement to enhance immunity.

Wanagama forest, unlike natural forest, is a rehabilitated, man-made ecosystem established by Universitas Gadjah Mada as an ex-situ conservation and land restoration project. The vegetation is composed of seven distinct main stands-pine (Pinus merkusii), mahogany (Switenia mahagoni), kesambi (Schleichera oleosa), teak (Tectona grandis), cajuput (Melaleuca leucadendron), glicidia (Gliricida sepium), and mixed stands which differ significantly in floral composition, canopy structure, and microclimate. These variations leading to a highly heterogenous floral ecosystem that differs markedly from natural tropical forest.[7]

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The mosaic of introduced and local flowering plants in Wanagama forest from which the Apis cerana forage makes the nectar and pollen composition of Wanagama forest honey is expected to be unique, potentially yielding distinct phytochemical profiles and immunomodulatory effects compared with other honey. However, despite its popularity, no scientific investigation has yet been conducted to evaluate the immunomodulatory activity of Wanagama forest honey. Therefore, this study aims to explore the potential immunomodulatory effect of Wanagama forest honey.

MATERIALS AND METHODS

All the procedures regarding animal study have received approval by ethical committee of Faculty of Medicine, Public health and Nursing Universitas Gadjah Mada with ethical approval number KE/FK/0251/EC/2021

WanagamaHoney

Wanagama honey is honey obtained from Wanagama Forest located in Banaran Village, Playen District, Gunung Kidul Regency It is produced by Apis cerana bees. The honey was harvested by taking honeycomb and then extracted by squeezing.

Chromatography test

Chromatography test were conducted using Gas Chromatography and Mass Spectroscopy (GCMS). For gas chromatography (GC) analysis, the honey sample was diluted with distilled water, followed by liquid–liquid extraction using ethyl acetate to separate volatile compounds. The organic layer was then filtered through anhydrous sodium sulphate to remove residual water, and the filtrate was concentrated under reduced pressure before injection into the GC system.

Carbon suspension preparation

A total of 1.6 g of dried Chinese ink, suspended with 25 mL of tween 80 (1%) w/v in 0.9% physiological NaCl solution, until a solution concentration of 64 mg/mL was obtained.

Carbon clearance assays

The study involved 20 Swiss albino mice age 3 – 4 weeks old. They received different treatment based on their group which are group which receive water or negative control group (CONTROL), group which received honey 10 mg/kg BW (DOSE 1), group which received honey 25 mg/kg BW (DOSE 2), and group which received honey 50 mg/kg BW (DOSE 3). They received the treatment for 14 days. The honey sample was diluted in distilled water and administered perorally to mice at a volume of 0.1 mL per 10 g body weight using an oral gavage needle to ensure uniform delivery. In the end of the treatment, the mice received intravenous injection of 0.1 ml carbon suspension via tail vein. Blood collection through orbital vein was conducted before and 15 minutes after carbon suspension injection. The carbon concentration was measured using spectrophotometer UV-Vis 650 nm. Optical density (OD) measured was used to calculate the phagocytic index using the following formula:

$$(K) = \frac{\text{Log A (n)} - \text{Log A (n-1)}}{\text{t (n-1)} - \text{t (n)}}$$

K = Phagocytic constant
A = optical density
t = time
n = time after carbon injection (15 minutes)
n -1 = time before carbon injection (0 minutes)

$$PI = \frac{\text{K mice X}}{\text{Mean K}}$$

PI = Phagocytic Index
K = Phagocytic constant

Hematological examination

The blood examination was conducted using Automatic Veterinary Hematology Analyzer.

Statistical analysis

The data presented are mean ± Standard Deviation. The mean of carbon clearance assay and blood parameter value were analyzed using ONE-WAY ANOVA and continued with Tukey's multiple comparisons test.

RESULTS

Chromatography test

Table I and Figure 1 presents the results of the GC-MS test on Wanagama honey. GC-MS chromatogram (Figure 1) shows the presences of twenty phytochemical constituents. The content of fatty acids and hydrocarbons is 76.45%, the value of furan compounds and other components is 20.42% and 3.15%, respectively.

Body weight examination

The body weight measurement shows the increase of the mice body weight in all groups except in DOSE 3 group. However, the difference of the body weight before and after treatment between groups was not significantly difference. The change of body weight before and after treatment is presented in Figure 2.

Blood examination

Complete blood count examination also shows that the blood parameters were similar among the group. Table II presents the complete blood count result.

Carbon clearance assay

Phagocytic index calculation shows that all group treated with honey have higher phagocytic index compared to those on negative control group. Figure 3 shows the phagocytic index measured using carbon clearance assay.

DISCUSSION

The GC-MS results show the volatile compounds of honey. The compounds are grouped into furans, hydrocarbons and fatty acids, sugars, and other components. Fatty acids and hydrocarbons dominated in Wanagama honey, followed by furan group compounds. Honey mainly consists of carbohydrates which contribute to more than 90% of its dry weight. Other main compounds of honey include proteins, vitamins, amino acids, minerals, and organic acids. Honey also contains flavonoids, polyphenols, alkaloids, glycosides, and volatile compounds. [8–10]

Table 1. The results of GC-MS test of Wanagama Honey

Ret.Time	Area %	Compound	SI
10.63	3.15	Pyranone	92
13.25	20.42	HMF (hydroxymethylfurfural)	93
24.64	3.07	Myristic acid	95
26.38	0.46	Pentadecanoic acid isomer	95
27.89	14.24	cis-9-Hexadecenoic acid	96
28.24	27.07	cis-11-Eicosenoic acid	93
30.99	0.76	9-Hexadecenoic acid	88
31.06	0.89	cis-Vaccenic acid	88
31.32	0.52	Aqua Cera	88
36.01	1.83	Eicosane	95
36.93	0.35	Triphenylphosphanoxid	84
37.32	0.26	Tetracosane	87
38.59	13.22	Heptacosane	96
39.79	0.45	Octacosane	89
40.97	6.86	Tetratetracontane	96
41.34	0.41	13-Methylheptacosane	79
46.44	0.64	Palmitaldehyde	81
47.09	4.24	9-Eicosyne	88
47.36	0.65	2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	76
47.48	0.53	Tetratriacontane	76

Table 2. Complete Blood Count Result after 7 days treatment. There was no statistical different among CBC parameter measured between groups.

Parameter	Control	Dose 1	Dose 2	Dose 3
RBC	9.52	9.09	9.18	9.27
Hb	14.85	14.48	14.52	14.62
Ht	49.23	47.68	49.05	48.66
MCV	51.77	52.53	53.53	54.98
MCH	15.60	15.92	15.95	16.40
MCHC	30.17	30.37	29.85	29.95
RDW	16.77	16.42	16.70	16.63
PLT	1210.67	1143.00	1162.00	1171.89
MPV	4.55	4.43	4.72	4.55
PDW	15.70	15.75	15.90	15.87
PCT	0.51	0.51	0.55	0.57
WBC	2.12	2.10	1.87	2.03
Granulocyte	26.30	40.34	16.30	20.08
Limphocyte (%)	63.60	47.46	73.78	69.45
Monocyte (%)	10.10	12.20	9.92	10.47
Granulocyte (0.29-1.42)	0.72	1.20	0.30	0.32
Limphocyte (0.49-3.92)	1.18	0.94	1.37	1.13
Monocyte (0.0-0.08)	0.22	0.30	0.17	0.15

RBC (Red Blood Cell), Hb (Haemoglobin), Ht (Haematocrite), MCV (Mean Corpuscular Volume), MCH (mean Corpuscular Hemoglobin), MCHC (mean Corpuscular Hemoglobin Concentration), RDW (Red Cell Distribution Width), PLT (Platelet), MPV (mean Platelet Volume), PCT (Platelecrit), WBC (white Blood cell)

Although, volatile compounds are generally low in concentration, approximately 600 volatile compounds have been reported to exhibit pharmacological effect.[11] The volatile compound found in the highest percentage in Wanagama honey, cis-11-Eicosenoic acid, has been known to act as an immune system stimulator.[12] Another major volatile compound, cis-9-Hexadecenoic acid, possessess antiinflammtory properties.[13] Based on these findings, the chemical profile of Wanagama honey supports its potential immunomodulatory activity.

This study examined the potential of Wanagama honey as immunomodulator using carbon clearance assay in mice, where the phagocytic index was determined. The phagocytic index evaluates the capacity of reticular endothelial systems (RES), a systemic network of phagocytic cells, to clear exogen materials from systemic circulation. The injected carbon suspensionis cleared cleared by macrophage, and a higher phagocytic index reflects better RES function.[14] In this study, Wanagama honey modulated macrophage phagocytic activity in vivo at all tested doses (10 mg/kg BW, 25 mg/kg BW, and 50 mg/kg BW).

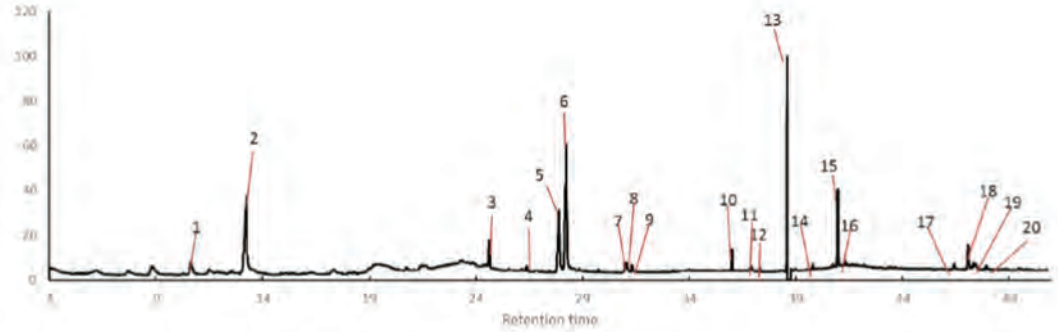

Figure 1. GC-MS result of Wanagama honey

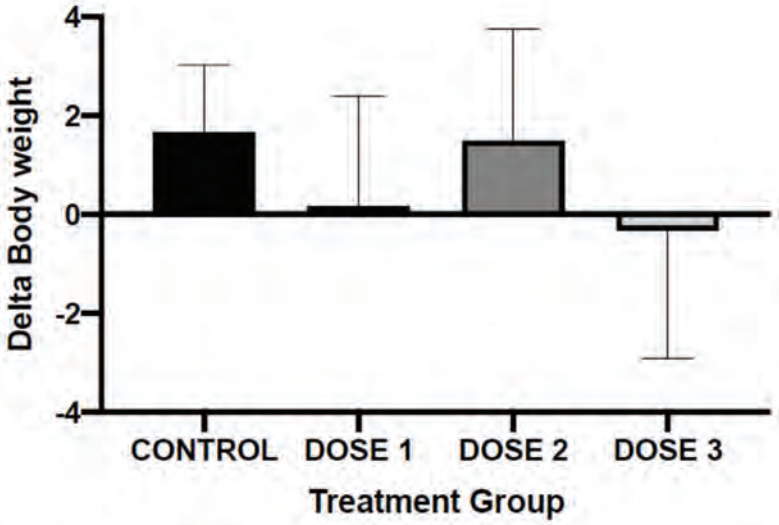

Figure 2. Difference in body weight before and after treatment with honey in different concentration. There was no statistical different of weight change between groups.

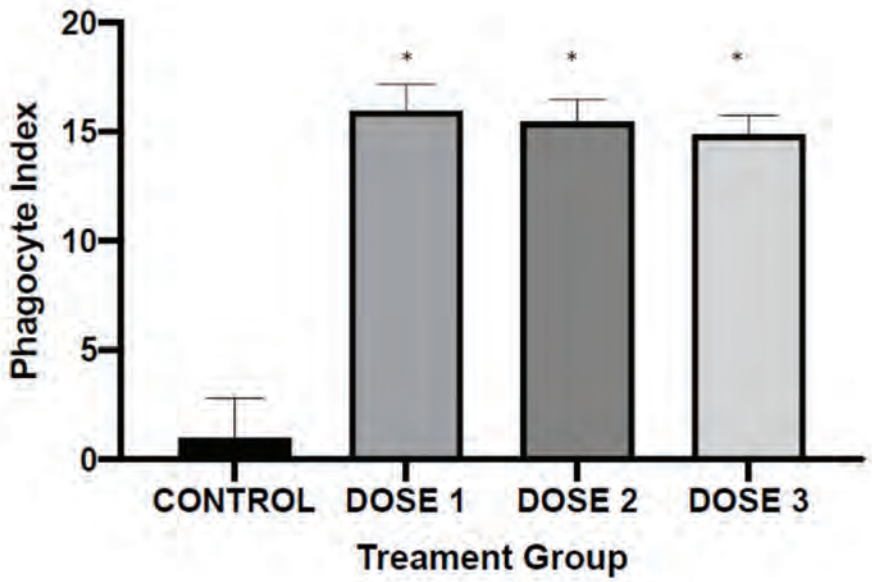

Figure 3. Phagocyte Index measured by carbon clearance assay. * P < 0.05 vs control

A previous study in rat showed that 14 days administration of honey increased several cytokines including cytokines IL-4and IL-10.[15] Ripened honey has high concentration of glucose and fructose and low water content, which prevents the spoilage.[16] Stimulation of cytokines release by honey is partly atributed to H_2O_2 production from glucose oxidation catalyzed by honeybee glucose oxidase (Gox). Low concentration of H_2O_2 is known to stimulate immune responses, while honey also serves as energy substrate for macrophages, enhancing their phagocytic performance.[16-17] furthermore, the sugar content of honey can be metabolized into short chain fatty acids (SCFA), which also possess immunomodulatory properties.[18]

Honey also contains non sugar components with antioxidant effects. The immune system uses reactive oxygen species (ROS) to eliminate pathogens, however, excessive ROS can lead to oxidative stress, impairing immune function and causing tissue damage. Antioxidant compounds in honey may therefore support immune balance by mitigating oxidative stress.[19-20]

In this study, the effect of wanagama forest honey administration on carbon clearance was not showing clear dose response relationship. According to lawa of mass action all pharmacological effect is inherently concentration dependent; however, in practice, some responses may appear non–dose-related when the dose–response curve reaches a plateau at relatively low doses.[21] In our study, the lower and medium doses of Wanagama honey might have been sufficient to achieve near-maximal macrophage activation, such that higher doses produced no additional effect. This saturation or plateau effect is common in immunomodulatory studies where receptor occupancy or cytokine signaling reaches maximal efficiency at submaximal concentrations.

Honey administration in several different doses also potentiated the several hematological parameters such as Hb, RBC, PCV, lymphocytes, and eosinophils in rat model of breast cancer. In our study, all of the blood parameters measured were not significantly different with those on CONTROL group. We suggest that it is due to the different animal model, dose and length of honey treatment. In study by Ahmed et al., 2018[17], the honey administration is higher, 0.2-2g/kg BW, and longer, >120 days. In our study, the honey dose was 10-50 mg/kg BW and the duration of treatment was 14 days.

The absence of significant differences in leukocyte, lymphocyte, or monocyte counts after 7 days of honey administration also suggests that the immunomodulatory effect occurs at the functional level rather than through hematopoietic stimulation. We suggest that Wanagama forest honey modulate immune cell function—such as phagocytosis and cytokine release—without necessarily changing circulating cell numbers.

Avoiding consumption of high-sugar foods often discourages honey use despite its health benefits. However, in this study, mean body weight did not differ significantly between groups. Interestingly, mice in the highest dose group (50 mg/kg BW) showed the lowest or even negative weight gain. This finding aligns with Nemoseck et al. (2011), who reported that after 33 days of honey treatment, rats exhibited reduced weight gain and adiposity, possibly due to decreased food intake compared with sucrose-fed controls.[22]

CONCLUSIONS

This study demonstrated that Wanagama forest honey, produced by Apis cerana in a unique rehabilitated forest ecosystem, contains bioactive volatile compounds with potential immunomodulatory properties. In vivo findings showed enhanced macrophage phagocytic activity at all tested doses, supporting its role as a natural immunomodulator. Further studies with longer duration and positive controls are recommended to confirm its mechanism and therapeutic potential.

CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

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ORIGINAL ARTICLE

The effect of serum eosinophilia and lymphocytosis on functional outcomes of Hirschsprung disease patients after Duhamel procedure

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ABSTRACT

Introduction: Hirschsprung disease (HSCR) is due to the failure of enteric nervous system precursors to colonize the distal intestine in embryonic development, with surgical treatment like the Duhamel procedure as a definitive intervention. However, complications leading to functional disorders may occur, especially with increased eosinophil levels. No studies have assessed the effect of peripheral blood eosinophil and lymphocyte counts on functional outcomes in HSCR patients post-Duhamel procedure.

Materials and Methods: This observational retrospective study involved 54 patients undergoing Duhamel procedures from January 2014 to June 2020 at Dr. Sardjito Hospital. Patient records were analyzed using the Mann-Whitney U test for continuous variables and the Chi-Square test for categorical variables.

Results: The majority of patients had normal eosinophil levels (78.8%) and normal lymphocyte counts (96.2%). Functional outcomes revealed that 49 patients (94.2%) had no voluntary bowel movements (VBM), 45 patients (86.5%) did not experience constipation, and 48 subjects (92.3%) had no soiling issues. There was no significant association was found between eosinophilia and functional outcomes in Hirschsprung disease patients after Duhamel procedure ($p > 0.05$). Similarly, there was no significant association between lymphocytosis and functional outcomes in these patients following Duhamel procedure ($p > 0.05$)

Conclusion: The functional outcomes of patients with Hirschsprung disease post-Duhamel procedure at our institution are favorable with most having normal eosinophil and lymphocyte counts. Eosinophilia and lymphocytosis might not significantly affect the outcomes of Hirschsprung disease patients after undergoing the Duhamel procedure at Dr. Sardjito Hospital.

KEYWORDS:
Hirschsprung; Duhamel; Eosinophilia; Lymphocytosis; Functional Outcomes (Krickenbeck)

INTRODUCTION

Hirschsprung's Disease (HSCR) is a congenital disorder of the enteric nervous system marked by the absence of ganglion cells in the distal colon, leading to chronic bowel obstruction as affected segments cannot perform normal peristalsis.[1] Originating from disrupted migration of neural crest cells during fetal development, this condition affects around 1 in 5,000 live births worldwide, with higher incidence rates, such as 1 in 3,250, reported in Yogyakarta, Indonesia.[2] Newborns with HSCR commonly show signs of bowel obstruction, including abdominal distention, feeding intolerance, and failure to pass meconium within 48 hours of birth. Diagnosis combines clinical evaluation and imaging, with definitive confirmation through rectal suction biopsy showing absent ganglion cells, leading to the necessity for surgical intervention to remove non-functional bowel segments and restore bowel continuity.[3] The Duhamel procedure is a common surgical approach in HSCR, where the ganglionated segment of the colon is pulled through to connect with the rectum, preserving anal sphincter function and minimizing rectal resection.[4] Although effective, postoperative complications like constipation, soiling, and Hirschsprung-associated enterocolitis (HAEC) may impact recovery.[5] Emerging research highlights that preoperative blood markers, such as elevated eosinophil and lymphocyte counts, may correlate with higher risks of gastrointestinal complications.[6] This study explores the role of these hematological markers in predicting functional outcomes in HSCR patients post-Duhamel surgery, aiming to refine preoperative evaluations and improve postoperative care strategies.

MATERIALS AND METHODS

This retrospective observational study aims to evaluate the association between eosinophilia and lymphocytosis on functional outcomes in Hirschsprung's disease patients following Duhamel surgery. The study was carried out in the Pediatric Surgery Department at Dr. Sardjito General Hospital, gathering data from the medical records of patients who underwent the Duhamel procedure. The target population includes Hirschsprung's disease patients in

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Table I: Clinical Characteristics, Hematological Findings, and Postoperative Functional Outcomes of Study Subjects

Parameter	Subjects (n = 52)	Percentage
Characteristics of Study Subjects		
Gender		
- Male	38	73.1%
- Female	14	26.9%
Eosinophil and Lymphocyte Count in Peripheral Blood		
Eosinophil		
- Normal	41	78.8%
- Eosinophilia	11	21.2%
Lymphocyte		
- Normal	50	96.2%
- Lymphocytosis	2	3.8%
Functional Outcomes After Surgery		
Voluntary Bowel Movement		
- Present	49	94.2%
- Absent	3	5.8%
Soiling		
- Present	4	7.7%
- Absent	48	92.3%
Constipation		
- Present	7	13.5%
- Absent	45	86.5%

Table II: The correlation between eosinophil and lymphocyte counts in the peripheral blood and Voluntary Bowel Movement (VBM)

Variable		Presence of VBM		Absence of VBM		p-value	Odds Ratio (95% CI)
		Subjects	%	Subjects	%		
Eosinophils	Normal	38	92.7	3	7.3	1	0.78
	Eosinophilia	11	100	0	0		(0.67-0.9)
Lymphocytes	Normal	47	94	3	6	1	0.96
	Lymphocytosis	2	100	0	0		(0.9-1.02)

Table III: The correlation between eosinophil and lymphocyte counts in the peripheral blood and Soiling

Variable		Presence		Absence		p-value	Odds Ratio (95% CI)
		Subjects	%	Subjects	%		
Eosinophils	Normal	4	9.8	37	90.2	0.57	1.3
	Eosinophilia	0	0	11	100		(1.11-1.51)
Lymphocytes	Normal	4	8	46	92	1	1.04
	Lymphocytosis	0	0	2	100		(0.98-1.11)

Table IV: The correlation between eosinophil and lymphocyte counts in the peripheral blood and Constipation

Variable		Presence		Absence		p-value	Odds Ratio (95% CI)
		Subjects	%	Subjects	%		
Eosinophils	Normal	6	14.6	35	85.4	1	1.71
	Eosinophilia	1	9.1	10	90.9		(0.184-15.95)
Lymphocytes	Normal	7	14	43	86	1	1.05
	Lymphocytosis	0	0	2	100		(0.98-1.12)

Yogyakarta who had Duhamel surgery at Dr. Sardjito General Hospital between January 2014 and June 2020, under the age of 18, and with complete medical records. Patient records were analyzed using the Mann-Whitney U test for continuous variables and the Chi-Square test for categorical variables.[7]

All patients underwent a standardized Duhamel procedure in which the posterior rectal wall and the anterior wall of ganglionated bowel were aligned and stapled using a gastrointestinal stapler. This approach minimizes the formation of a long rectal spur and preserves rectal sensation, as approximately half of the pulled-through segment remains ganglionated. These technical considerations are essential to achieving satisfactory postoperative continence and preventing residual fecal stasis.

Ethics Approval

This study received approval from the Medical and Health Research Ethics Committee (MHREC) at the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (KE/FK/0124/EC/2020).

RESULTS

This study included 52 patients with Hirschsprung's disease who underwent the Duhamel procedure. The majority of patients were male, with 38 males (73.1%) and 14 females (26.9%) (Table I).

Eosinophilia, defined as eosinophil counts >5%, >500 cells/mm³, or >0.5 x 10^9/L, was identified in 11 patients (21.2%), while the remaining 42 patients (78.8%) had normal eosinophil levels. Lymphocytosis, defined as lymphocyte counts >9,000/mm³ or >9.0 x 10^9/L, was present in 2 patients (3.8%), whereas 50 patients (96.2%) had normal lymphocyte levels. (Table I)

Postoperative outcomes were classified into three categories based on the Krickenbeck classification: voluntary bowel movement (VBM), soiling, and constipation. Among the patients, 3 (5.8%) experienced VBM disturbances, while 49 (94.2%) had normal VBM. Regarding soiling, 4 patients (7.7%) reported soiling complaints, whereas 48 patients (92.3%) had no complaints. Constipation was observed in 7 patients (13.5%), while 45 patients (86.5%) did not report constipation issues. (Table I)

Based on eosinophil counts, 38 out of 41 patients with normal eosinophil levels (92.68%) and all 11 patients with eosinophilia (100%) had normal VBM. Three patients with normal eosinophil levels reported VBM disturbances. This difference was not statistically significant (p=1.000).

Regarding lymphocyte counts, 47 out of 50 patients with normal lymphocyte levels and both patients with lymphocytosis (100%) had normal VBM. Three patients with normal lymphocyte levels reported VBM disturbances. This finding was also not statistically significant (p=1.000). (Table II)

For soiling outcomes, 4 out of 41 patients with normal eosinophil levels reported soiling complaints, while no patients with eosinophilia or 37 patients with normal eosinophil counts reported soiling. This result was not statistically significant (p=0.57). In terms of lymphocyte counts, 4 out of 50 patients with normal lymphocyte levels reported soiling complaints, while none of the patients with lymphocytosis experienced soiling. This finding was not statistically significant (p=1.000). (Table III)

Regarding constipation, 6 out of 41 patients with normal eosinophil levels and 1 out of 11 patients with eosinophilia, experienced constipation. This result was not statistically significant (p=1.000). Similarly, 7 out of 50 patients with normal lymphocyte levels experienced constipation, while none of the patients with lymphocytosis reported constipation. This finding was also not statistically significant (p=1.000). (Table IV)

DISCUSSION

The characteristics of the subjects evaluated include gender, type of aganglionosis, age at the time of Duhamel surgery, nutritional status, and the presence or absence of complications during and after surgery. Previous research has found that there is a higher proportion of male subjects compared to females, as Hirschsprung disease is more commonly seen in males than in females.[8-10] It has also been suggested that female patients may be more prone to constipation, possibly due to hormonal influences.[11-12] A previous study demonstrated that male patients were more likely to experience abnormal defecation frequency compared to females, despite having comparable overall bowel function scores between the two groups.[13] Another study reported that male patients generally exhibited less favorable postoperative outcomes.[14] Notably, several other studies have supported the notion that sex may influence outcomes in pediatric patients with various conditions.[14-16] These variations may be attributed to anatomical distinctions between males and females, particularly in pelvic structure and pelvic floor configuration.[13,17]

Regarding the length of the aganglionic segment, more subjects had short-segment aganglionosis compared to long-segment types. This finding aligns with Nelson's study, which found that 60-85% of HSCR cases have short-segment aganglionosis.[8] Furthermore, postoperative functional disturbances can partly result from the absence of coordinated motility in the aganglionic colon. A longer aganglionic segment corresponds to a more proximal obstruction site, which raises intraluminal pressure and worsens dysmotility. Evidence regarding the association between long-segment Hirschsprung disease (HSCR) and poorer functional outcomes remains inconclusive. Some studies have reported higher rates of soiling and incontinence among long-segment HSCR patients, whereas others found no significant correlation.[18-19]

At the time of surgery, age was categorized into one year or less and over one year. More subjects were older than one year compared to those one year and younger. In another study, neonates comprised the majority of HSCR patients undergoing definitive therapy (84%), followed by infants (5%), toddlers (4%), children (3%), and adolescents (1%).[5] Older patients with HSCR tend to present with more severe intestinal obstruction.[20] A recent study reported that while postoperative functional impairments are frequent, intestinal function tends to improve as patients grow older.[21] Conversely, other studies have found that bowel dysfunction remains unchanged with advancing age.[22-23] Motivation and training to help control and distinguish stool types, along with social awareness with age, may reduce soiling and help achieve normal VBM.[6,12]

Nutritional status in children with Hirschsprung disease was generally good. This contrasts with common signs and symptoms of Hirschsprung disease, such as abdominal distention and bowel obstruction, which can lead to difficulty eating and green-colored vomiting.[4]

Postoperative complications are often related to obstructive issues such as anastomotic stricture, anemia, and surgical wound infections, which can impact functional outcomes such as constipation and soiling.[4,24] Newborns with eosinophilia on rectal biopsy but without HSCR showed a higher percentage of gastrointestinal symptoms compared to

those without eosinophilia. However, in studies involving HSCR patients with mucosal eosinophilia, this finding did not increase postoperative complications or affect feeding management.[25-26]

Subjects with eosinophilia were fewer in number compared to normal patients. Eosinophil levels in HSCR patients did not impact outcomes or affect postoperative complications. Although some studies excluding HSCR patients found that those with mucosal eosinophilia reported a high percentage of constipation complaints.[12]

More subjects had normal lymphocyte counts compared to those with lymphocytosis. However, no studies have specifically investigated lymphocytosis in HSCR patients. Voluntary Bowel Movement (VBM), soiling, and constipation are functional outcomes assessed using the Krickenbeck classification. These issues are commonly observed and significant following definitive surgery in HSCR patients.[10]

Postoperatively, HSCR patients often struggle to maintain continence and lack the reflex and sensation to control bowel movements and sphincters, making it challenging to differentiate rectal contents as solid, liquid, or gas.[6,12] Our previous study indicated that the type of definitive surgical procedure could influence long-term bowel function outcomes. Patients who underwent transabdominal Duhamel procedures tended to experience less favorable functional bowel results.[27]

Constipation is a functional bowel disorder that remains a concern in postoperative HSCR patients. In the Duhamel procedure, the incidence of postoperative constipation is 17.2%. Reducing the risk of constipation involves leaving a short native rectum; however, constipation may improve over time.[28] Additionally, increasing fiber intake or using loperamide may be effective.[4]

In this study, 92.68% of patients with normal eosinophil levels and 100% of patients with eosinophilia had normal VBM. The calculated OR value was 0.78, indicating that HSCR patients with normal eosinophil levels are more likely to have normal VBM than those with eosinophilia. However, this study had a p-value >0.05, indicating that the eosinophil level did not significantly impact VBM outcomes. Meanwhile, patients with normal lymphocyte levels were more likely to have normal VBM compared to those with lymphocytosis, with an OR value of 0.96. This data also lacked statistical significance due to a p-value >0.05.

For soiling, the OR was 1.3 for eosinophilia and 1.04 for lymphocytosis, suggesting that patients with eosinophilia are more likely to experience soiling compared to those with normal eosinophil levels, while patients with lymphocytosis are also more likely to experience soiling than those with normal lymphocyte levels. However, both findings lacked statistical significance due to p-values >0.05, indicating no effect on soiling outcomes.

In constipation tests, eosinophil levels had an OR of 1.714, while lymphocyte levels had an OR of 1.05. This indicates that patients with eosinophilia are more likely to experience constipation than those with normal eosinophil levels. Likewise, patients with lymphocytosis are also more likely to experience constipation than those with normal levels. However, neither result was statistically significant due to p-values >0.05, indicating no effect on constipation outcomes. Studies have suggested that while there may be concerns that HSCR patients with mucosal eosinophilia could have worse outcomes than HSCR patients without eosinophilia, especially concerning feeding and defecation, findings indicate similar outcomes between patients with or without mucosal eosinophilia.[25]

CONCLUSIONS

The functional outcomes of patients with Hirschsprung disease post-Duhamel procedure at our institution are favorable with most having normal eosinophil and lymphocyte counts. Eosinophilia and lymphocytosis might not significantly affect the outcomes of Hirschsprung disease patients after undergoing the Duhamel procedure at Dr. Sardjito Hospital.

This study has several limitations. First, as a retrospective single-center review, the data were limited to available records (small sample size) and may be subject to selection bias. Second, we did not include certain potentially confounding factors in the multivariate analysis, which may have affected the statistical associations observed. Third, anal manometry, histopathological confirmation of ganglion cells, and long-term quality-of-life assessments were not routinely performed and could provide more objective evaluation of functional outcomes. Future multicenter, prospective studies incorporating these variables are warranted to better delineate the complex determinants of postoperative function in Hirschsprung disease.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Motor response in monogenic LRRK2 parkinson's disease after deep brain stimulation: A systematic review and meta-analysis

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ABSTRACT

Introduction: Genetic factors are increasingly recognized as crucial contributors to both familial and sporadic forms of Parkinson's disease (PD), including mutations in LRRK2 (Leucine-rich Repeat Kinase 2). Previous studies have indicated that the G2019S variant results in more favorable motor outcomes post-deep brain stimulation (DBS) compared to the R1441G variant. This study was aimed at investigating whether different LRRK2 variants in Parkinson's disease patients with LRRK2 mutations (LRRK2 PD) produce distinct motor responses following DBS.

Materials and Methods: A literature search was conducted across three databases using keywords related to Parkinson's disease, deep brain stimulation, and LRRK2. The inclusion criteria involved studies focusing on LRRK2 PD with DBS intervention, specifically comparing LRRK2 variants, and measuring motor responses pre- and post-DBS using the UPDRS III. A meta-analysis was performed to compare motor responses using a random effects model.

Results: Out of 325 search results, eleven articles were included in the review. Three LRRK2 PD variants—G2019S, R1441G, and G2385R—were associated with DBS intervention. The overall effect of DBS in LRRK2 PD compared to idiopathic PD was not statistically significant, with a mean difference (MD) of -3.00 (-8.52; 2.52). High overall heterogeneity was observed (I^2 = 63.1%; P < 0.05). Subgroup analysis revealed significant differences (P < 0.05), suggesting that different LRRK2 variants may result in varying motor outcomes post-DBS.

Conclusion: LRRK2 PD exhibited diverse motor outcomes depending on the specific mutation variant when subjected to DBS. Patients with LRRK2 variants G2019S and G2385R demonstrated clinically significant improvements in motor responses, while those with the R1441G variant showed inadequate motor response.

KEYWORDS:
Monogenic Parkinson's Disease, LRRK2, motor response, deep brain stimulation

INTRODUCTION

The majority of Parkinson's Disease (PD) cases are idiopathic, although 5–10% of cases are familial, involving mutations in various genes.[1] Among the genes most studied in monogenic PD are SNCA (Synuclein alpha), LRRK2 (Leucine-rich Repeat Kinase 2), GBA (Glucocerebrosidase), VPS13C (Vacuolar Protein Sorting 13 Homologue C), PRKN (Parkin RBR Ubiquitin Protein Ligase), and PINK1 (PTEN Induced Kinase 1).[2-4] Although monogenic PD is relatively rare epidemiologically, studies recruiting individuals with LRRK2 gene mutations, regardless of whether they exhibit PD symptoms, have shown that these mutations are relatively common in certain populations.[2]

The G2019S variant is one of the most commonly identified LRRK2 mutations, contributing to 6–40% of familial Parkinson's Disease cases depending on the ethnic group and accounting for 2% of sporadic cases.[5-8] This mutation has been widely reported in European populations and is particularly common in specific populations, such as Ashkenazi Jews in Israel and Berbers in North Africa.[9-11] However, the G2019S variant is rarely found in Asia. Other notable mutations of LRRK2 PD include the R1441C/G/H, N1437H, Y1699C, I2020T, R1628P, and G2385R variants.[12-13] Among these, the R1441G variant is predominantly found in the Basque population of northern Spain.[14]

Current treatments for PD focus on restoring dopaminergic activity in the striatum, aiming to alleviate motor symptoms effectively.[15] Non-pharmacological approaches, such as basal ganglia surgery, have been utilized as therapeutic modalities for tremors in PD, even before the discovery of levodopa therapy.[16] Deep brain stimulation (DBS) has emerged as a surgical treatment for Parkinson's Disease, initially used exclusively for managing chronic tremors and advanced stages of the disease. However, its application has expanded to include early-stage Parkinson's Disease.[16] This procedure involves implanting electrodes in specific brain regions, primarily targeting the subthalamic nucleus (STN) and the globus pallidus internus (GPi), both of which are recognized as the recommended targets for DBS in Parkinson's Disease.[17-18]

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The effectiveness of deep brain stimulation (DBS) therapy for Parkinson's Disease largely relies on the careful selection of appropriate candidates. Only patients diagnosed with primary Parkinson's Disease are eligible for DBS, while individuals with secondary Parkinson's Disease or atypical parkinsonian syndromes are excluded from this treatment.1[6] Monogenic PD, particularly LRRK2 PD, represents one of the most prevalent genetic abnormalities in this form of the disease, with varying effects across different populations. Considering the diversity of LRRK2 mutation variants, further research is essential to understand their impact on monogenic Parkinson's Disease patients undergoing deep brain stimulation (DBS). In this review, we aim to investigate whether different variants of the LRRK2 mutation in Parkinson's Disease patients (LRRK2 PD) result in varying motor responses following deep brain stimulation (DBS).

MATERIALS AND METHODS

Literature Search

This systematic review was performed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, with a literature search covering publications up to December 30, 2024. The search was conducted using online databases, including MEDLINE (via PubMed), Google Scholar, and Scopus. Keywords were selected to align with the review's objective of comparing the response to deep brain stimulation (DBS) in patients with LRRK2-mutated monogenic Parkinson's Disease (LRRK2 PD) and those with idiopathic Parkinson's Disease. The search terms used as search query were 'Parkinson', 'deep brain stimulation,' and 'LRRK2.' Before commencing the review, the protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews) on April 28, 2022, under registration number CRD42022320770.

Eligibility Criteria

The inclusion criteria for this systematic review were as follows: human studies comparing the effects of deep brain stimulation (DBS) in Parkinson's Disease (PD) patients with LRRK2 mutations versus idiopathic PD. Only articles written in English were included, and studies without full access to the complete text were excluded. The main exposure was the specific LRRK2 variant identified, which was also analyzed separately. The primary outcome was the improvement in motor function, assessed using the Unified Parkinson's Disease Rating Scale Part III (UPDRS-III). Pre-DBS motor status (medication-off) was compared with post-DBS motor status in both medication-off and on-stimulation conditions. Exclusion criteria included studies that failed to confirm the presence of LRRK2 genetic mutations, those with incomplete follow-up data, genetic analyses that did not specifically reference LRRK2, studies that did not utilize DBS as the primary therapy, or those that did not employ UPDRS-III for motor response assessment.

Data Extraction and Analyses

Data were manually extracted using a standardized data extraction form, including: source of the article (first author and year of publication), Number of patients with LRRK2 mutations, Number of control patients (non-monogenic Parkinson's disease), Country of origin, LRRK2 mutation variant, DBS target, Age at onset and at DBS therapy, Pre-DBS UPDRS-III score (medication-off), and Post-DBS UPDRS-III score (medication-off and on-stimulation).

The data synthesis strategy for this review involves both quantitative and narrative approaches. For the quantitative analysis, we will calculate the mean difference of motor response, assessed using UPDRS-III, following DBS. Studies with sufficient comparable data will be included in the meta-analysis, where results will be pooled. The meta-analysis will be conducted using a random-effects model, accounting for the expected heterogeneity between studies. Heterogeneity will be assessed using the I^2 statistic, which quantifies the degree of variation across studies due to heterogeneity rather than chance. Studies reporting LRRK2 mutations with different motor outcomes will be compared to each other and to idiopathic PD cases. For studies with data that cannot be quantitatively synthesized, a narrative report will be provided, summarizing the key findings in descriptive terms. Subgroup analysis will be performed for each specific LRRK2 mutation (e.g., G2019S, R1441G, G2385R), with comparisons made between mutations to explore their effects on motor response post-DBS. This will allow us to examine how different genetic variants of LRRK2 affect treatment outcomes and to identify potential patterns in mutation-specific responses to DBS therapy.

Quality Assessment

The quality of the included studies was assessed using the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist, which consists of 22 items analyzing abstracts, titles, methodology, results, and potential funding influences.[19]

RESULTS

Included studies

The search process across three databases yielded 325 articles in total: 41 from MEDLINE, 242 from Google Scholar (top 20 pages sorted by relevance), and 42 from Scopus. After removing duplicates, 250 unique articles remained. These were then screened based on titles and abstracts using the inclusion and exclusion criteria, resulting in the elimination of 239 articles. The remaining 11 articles underwent a detailed evaluation against the criteria for systematic review eligibility. Ultimately, 7 articles were included in the systematic review and meta-analysis. The article selection process is summarized in the flowchart presented in Figure 1.

LRRK2 variants in PD Patients with DBS

Table I provides an overview of the seven studies included in this systematic review, consisting of longitudinal follow-up, cohort, and case-control studies. Three LRRK2 mutation variants were identified across the studies: G2019S20-24, R1441G25, and G2385R26.

Five studies focusing on the G2019S variant were conducted in diverse regions, including France, North Africa, Israel, and the United Kingdom.[20-24] The study examining the R1441G variant included participants from the Basque region of Spain, where this mutation is believed to have originated.[25] Lastly, the study on the G2385R variant involved individuals of Han Chinese descent.[26]

Table I: Characteristics of the included studies showing LRRK2 mutation variant, origin, DBS target, follow up, and overall outcome

Study	Number of LRRK2 mutation subjects	Number of control subjects	Country/Population origin	LRRK2 mutation variant	DBS target nucleus	Follow-up duration (months)	Outcome
Schüpbach et al., 2007	9	60	France/North Africa (Caucasian)	G2019S (n=7), G2019S + heterozygous PRKN mutation (n=1), T2031S (n=1)	Subthalamic nucleus	6 to 12	LRRK2 PD patients undergoing DBS demonstrated outcomes that were comparable to or better than those of other groups, particularly in terms of motor symptom improvement, daily living activities, and L-DOPA-related complications.
Gómez-Esteban et al., 2008	4	41	Basque Area, Spain	R1441G	Subthalamic nucleus	6	Patients with LRRK2 PD carrying the R1441G mutation variant in the Basque region of Spain experienced poorer outcomes in motor improvement, daily living activities, and quality of life following DBS.
Angeli et al., 2013	5	67	United Kingdom (Caucasian)	G2019S	STN (LRRK2 (+)=5, LRRK2 (-)=65); GPi: (LRRK2 (+)=0; LRRK2 (-)=2)	12	Patients with LRRK2 PD undergoing DBS exhibited superior motor outcomes compared to those with idiopathic Parkinson's disease.
Greenbaum et al., 2013	13	26	Israel (Jewish)	G2019S	Subthalamic nucleus	6 to 12	Patients with LRRK2 PD undergoing DBS demonstrated favorable outcomes, with motor symptom improvements comparable to those seen in idiopathic Parkinson's disease patients.
Sayad et al., 2016	15	12	Algeria, North Africa	G2019S	Subthalamic nucleus	12	Patients with LRRK2 PD exhibited greater motor improvement following DBS compared to those with idiopathic Parkinson's disease.
Chen et al., 2019	8	49	China / Han Chinese	G2385R	Subthalamic nucleus STN (LRRK2 (+) = 18, LRRK2 (-) = 55); STN+GPi (LRRK2 (+) =1;	12	Patients with LRRK2 PD carrying the G2385R mutation variant showed no significant differences in motor function improvement, daily living activities, or L-DOPA dose reduction compared to patients with non-LRRK2 Parkinson's disease.
Anis et al., 2024	19	64	Israel	G2019S	LRRK2 (-) =1); GPi (LRRK2 (-) = 6); VIM (LRRK2(-) = 2)	12	The LRRK2 variant did not influence the motor outcomes of deep brain stimulation (DBS) in Parkinson's disease patients, nor did it increase the risk of psychosis or cognitive decline.

LRRK2: leucine-rich repeat kinase 2; DBS: Deep Brain Stimulation (DBS); STN: Subthalamic Nucleus; GPi: Globus Pallidus Internus; VIM: Ventral intermediate nucleus of thalamus; PRKN: Parkin RBR Ubiquitin Protein Ligase; PD: Parkinson's Disease

The target nuclei for DBS are detailed in Table I. All studies focused on the subthalamic nucleus, although some also targeted the internal Globus Pallidus and the ventral intermediate nucleus of the thalamus. Notably, Sayad et al. (2016)[23] employed a case-control study design with matching between cases and control groups. In contrast, the other studies exhibited class imbalance, with the number of participants in the control group (non-LRRK2/idiopathic PD) exceeding those in the case group (LRRK2 PD). The overall outcomes, encompassing both motoric and non-motoric aspects, are summarized in Table I.

Five of the seven studies included in this systematic review were previously included in the systematic reviews by Kuusimäki et al. (2020) and Artusi et al. (2019).[3,27] These earlier reviews were conducted before the publication of the studies by Anis et al. (2024) and Chen et al. (2019).[24,26] Kuusimäki et al. (2020) and Artusi et al. (2019) focused on all monogenic forms of PD compared to idiopathic PD, with all participants undergoing DBS.[3,27] In their analyses, LRRK2 PD, regardless of mutation variant, was grouped into a single population while comparing various monogenic Parkinson's genes. Additionally, their inclusion criteria encompassed case reports and case series. The current systematic review incorporates the newer studies by Anis et al. (2024) and Chen

Table II: Characteristics of the motoric response of the included studies in LRRK2 PD vs idiopathic PD

Study	LRRK2 Variant	PD Patients with LRRK2 Mutation (LRRK2 PD)				PD Patients without LRRK2 Mutation (Idiopathic PD)			
		Age at DBS	Age at DBS	UPDRS III Pre-DBS (medication-off)	UPDRS III Post-DBS (medication-off, on-stimulation)	Age at onset	Age at DBS	UPDRS III Pre-DBS (medication-off)	UPDRS III Post-DBS (medication-off, on-stimulation)
Schüpbach et al., 2007	G2019S (n=7), G2019S + heterozygous PRKN mutation (n=1), T2031S (n=1)	41.1 (6.1)	54.5 (8.8)	41.4 (12.4)	17.8 (9.6)	43.1 (7.8)	56.1 (9.3)	43.4 (17)	15.7 (9.9)
Gómez-Esteban et al., 2008	R1441G	43.2 (10.8)	56 (10.5)	48.5 (18.5)	39.7 (17.7)	58.03 (1.16)	Duration: 14.19 (6.9)	42.5 (10.6)	26.1 (8.4)
Angeli et al., 2013	G2019S	43 (8.7)	Duration: 12.1 (1.8)	65.4 (14.9)	30.6 (16.1)	40.8 (7.2)	Duration: 15.1 (5.5)	GPi = 40.5 (13.4), STN = 47.8 (14.8)	GPi = 51 (7.1), STN = 24.6 (11.3)
Greenbaum et al., 2013	G2019S	49.5 (6.8)	61.1 (6.6)	42.5 (11.8)	28.5 (13.3)	49.15 (6.6)	62.4 (4.5)	43.4 (12.3)	27.2 (14.1)
Sayad et al., 2016	G2019S	40.1 (9.4)	NA	55.8 (16.4)	27.3 (20.6)	40.3 (8.2)	NA	51.7 (14.4)	38.5 (16.6)
Chen et al., 2019	G2385R	52.13 (7.55)	62.38 (9.84)	43.38 (11.51)	20.5 (7.89)	51.8 (8.51)	61.29 (8.13)	49 (11.62)	29.69 (9.95)
Anis et al., 2024	G2019S	49.6 (7.3)	62.9 (6.8)	49 (40.5 – 50)	19 (14-22)	51.2 (9.8)	61.8 (8.9)	44 (37.2 – 53.7)	22 (16-34)

LRRK2: leucine-rich repeat kinase 2; DBS: Deep Brain Stimulation (DBS); STN: Subthalamic Nucleus; GPi: Globus Pallidus Internus; VIM: Ventral intermediate nucleus of thalamus; PRKN: Parkin RBR Ubiquitin Protein Ligase; PD: Parkinson's Disease

Table III: Quality assessment of the included studies using STROBE

Item STROBE	Schupbäch et al., 2007	Gómez-Esteban et al., 2008	Angeli et al., 2013	Greenbaum et al., 2013	Sayad et al., 2016	Chen et al., 2019	Anis et al., 2024
1	Y	Y	Y	Y	Y	Y	Y
2	Y	Y	Y	Y	Y	Y	Y
3	U	U	U	Y	Y	Y	Y
4	Y	Y	Y	Y	Y	Y	Y
5	Y	U	Y	Y	Y	Y	Y
6	Y	Y	Y	Y	Y	Y	Y
7	U	U	U	U	Y	Y	Y
8	Y	Y	Y	Y	Y	Y	Y
9	U	U	U	U	U	U	U
10	U	U	U	U	U	U	U
11	Y	Y	Y	Y	Y	Y	Y
12	Y	Y	Y	Y	Y	Y	Y
13	U	Y	Y	Y	U	Y	Y
14	Y	Y	Y	Y	Y	Y	Y
15	Y	Y	Y	Y	Y	Y	Y
16	Y	Y	Y	Y	Y	Y	Y
17	U	U	U	U	U	U	U
18	Y	Y	Y	Y	Y	Y	Y
19	U	U	U	U	Y	Y	Y
20	U	U	U	U	U	Y	Y
21	U	U	U	U	U	Y	Y
22	Y	N	N	Y	Y	Y	Y

STROBE: Strengthening the reporting of observational studies in epidemiology; Y: Yes; U: Undetermined; N: No.

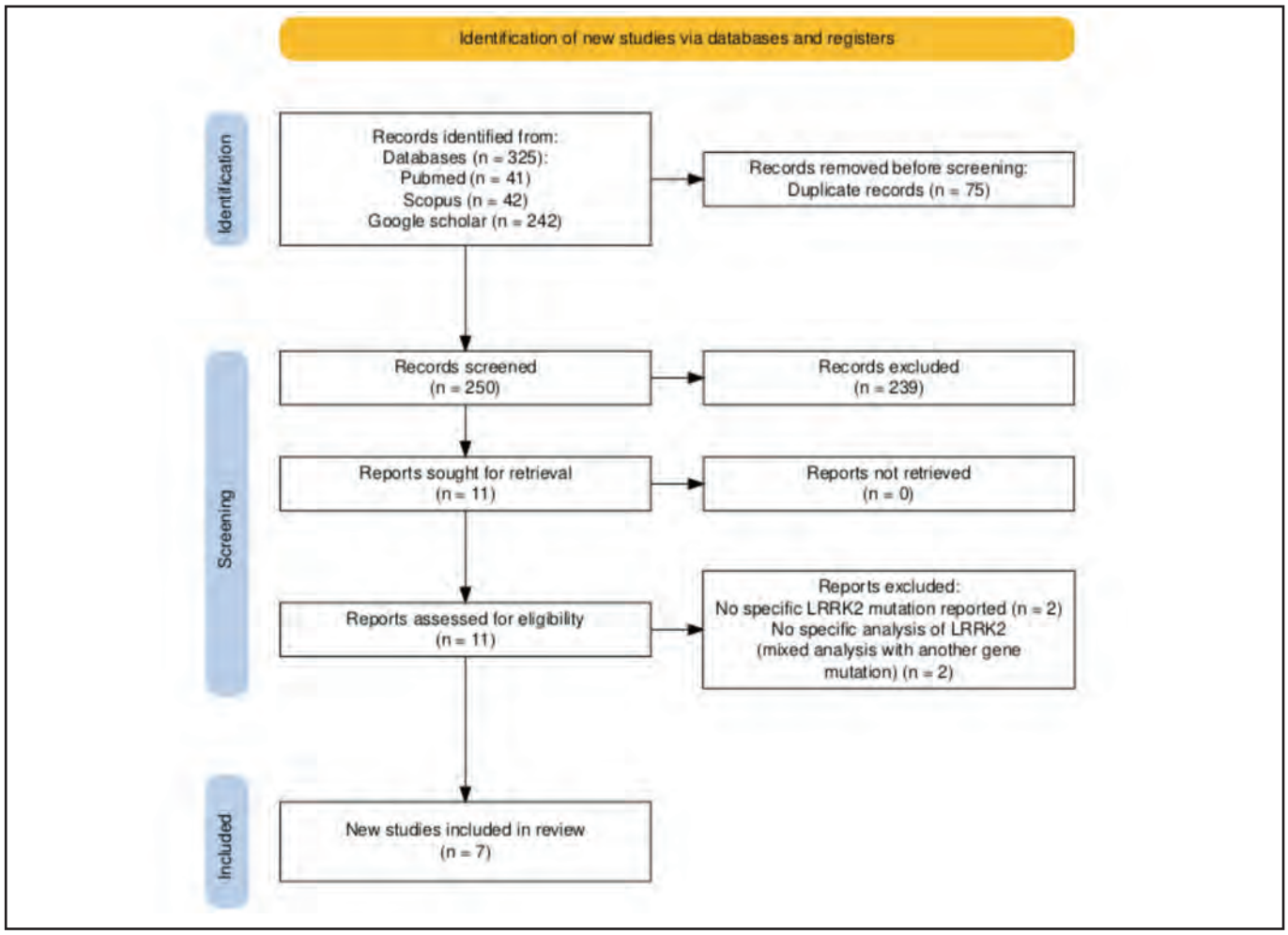

Fig. 1: PRISMA flowchart diagram of the included studies

et al. (2019).[24,26] Furthermore, it distinguishes each LRRK2 mutation variant to specifically evaluate motor responses to DBS treatment, as assessed by UPDRS III scores.

The motor response of LRRK2 PD to DBS

All Parkinson's disease (PD) patients included in this review underwent deep brain stimulation (DBS) after a minimum of 10 years of PD progression (Table II). The mean age of onset and the mean age at which DBS was performed were comparable between the LRRK2 PD and idiopathic PD groups across studies. The motor responses, assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) III, are presented in Table II, which includes baseline motor responses before DBS and post-DBS responses (on stimulation).

The meta-analysis revealed no statistically significant difference in the overall effect of DBS between LRRK2 PD and idiopathic PD, with a mean difference (MD) of -3.00 (-8.52; 2.52) (Figure 2). However, the overall heterogeneity was high (I^2 = 63.1%; $P < 0.05$), suggesting variability across studies. When subdividing the LRRK2 variants, substantial heterogeneity remained, even within the G2019S subgroup (I^2 = 56.9%; $p<0.05$). The other variants, R1441G and G2385R, were each represented by a single study. The results showed that the G2385R variant was associated with a significant improvement in UPDRS III (MD = -9.19; -15.33; -3.05), the G2019S variant showed a statistically insignificant improvement (MD = -2.92; -8.51; 2.68), and the R1441G variant demonstrated no significant improvement (MD = 13.6; -3.94; 31.14). The test for subgroup differences indicated a significant effect ($p<0.05$), suggesting that different LRRK2 variants may lead to distinct motor outcomes following DBS.

Quality assessment

The quality assessment of the studies indicated that the core components of the introduction, methods, results, and discussion were adequately addressed in all cases. However, certain studies lacked detailed information on diagnostic criteria, potential confounders, sample size, the workflow of subject selection, subgroup analysis, limitations, and funding sources (Table III). Given the nature of monogenic Parkinson's disease as a rare subset with a limited number of subjects, the absence of these elements may be considered acceptable.

DISCUSSION

This systematic review identifies the following LRRK2 mutation variants in relation to motor response compared to idiopathic Parkinson's disease: G2019S, G2385R, and R1441G, ranked from most to least effective. The G2019S

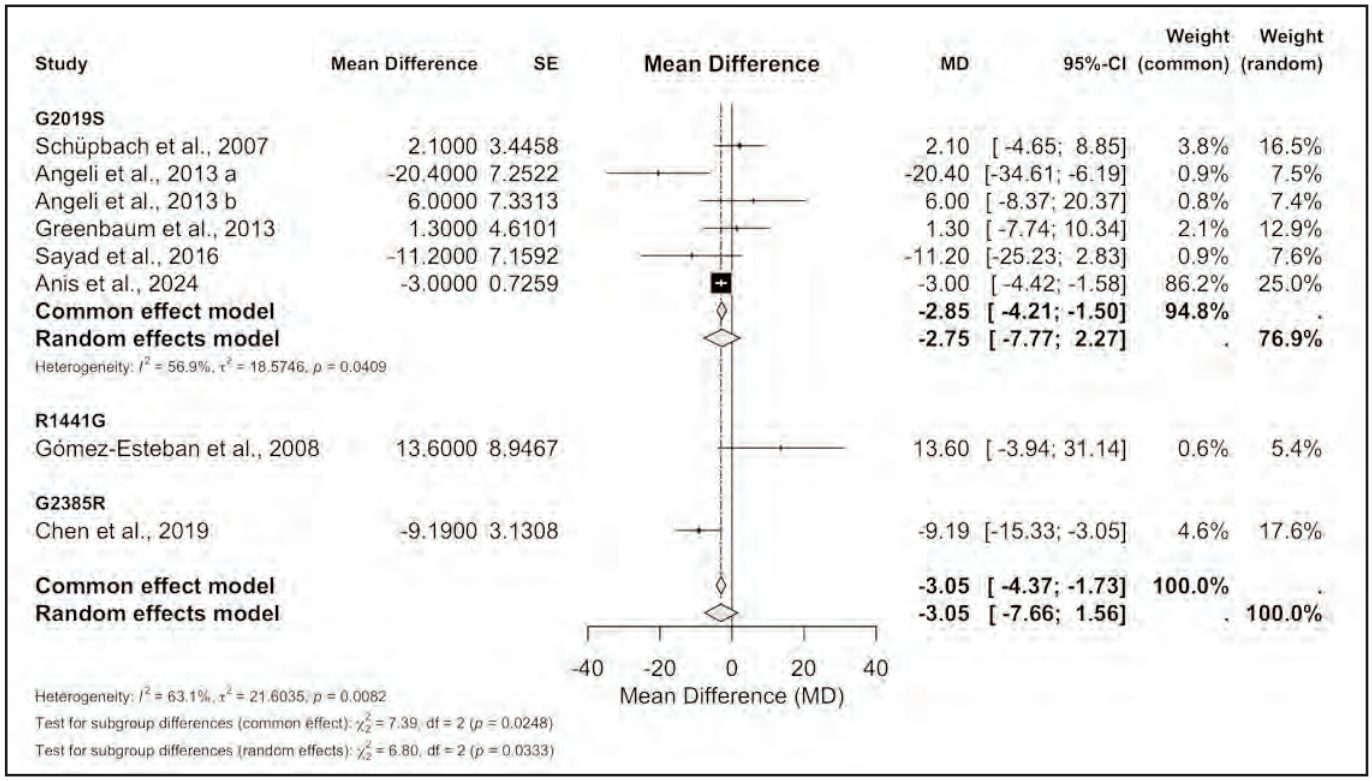

Fig. 2: Forest plot illustrating motor response as assessed by UPDRS III, with subgroup analysis based on specific LRRK2 variants

variant demonstrated a significant motor response, as evidenced by improved UPDRS III scores and reduced levodopa equivalent daily dose (LEDD) after DBS surgery. This mutation, the most common in monogenic PD, was studied in well-established research settings, particularly in regions with a higher prevalence of familial Parkinson's disease. For example, Greenbaum et al. (2013) conducted their study within the Ashkenazi Jewish population in Israel.[22] The G2019S variant showed a superior motor response to DBS when compared to the R1441G variant. In contrast, studies on Parkinson's patients with the LRRK2 R1441G variant indicated clinically insignificant motor improvements, suggesting a limited DBS response.[25] Patients with LRRK2 R1441G Parkinson's disease did, however, show a sustained positive pharmacological response to L-DOPA after DBS surgery.

This finding is supported by evidence suggesting that PD associated with the LRRK2 G2019S variant progresses more slowly in terms of drug-resistant symptoms and experiences milder disease deterioration compared to idiopathic Parkinson's disease.[27] This review includes studies that utilized DBS targeting the subthalamic nucleus in patients with LRRK2 PD. Therefore, the systematic review focuses specifically on this DBS target and its association with motor response, as measured by UPDRS III scores.

A significant limitation identified in nearly all studies included in this systematic review is the class imbalance in the number of subjects between the two groups20-22,24-26, with the exception of the study by Sayad et al. (2013) which reported that 55% of participants had the LRRK2 G2019S variant in comparison to the control group, owing to the case-control study design.[23] The significance of this imbalance lies in its potential to affect statistical analysis, reducing its power due to the unequal distribution of subjects. Class imbalance in genetic research presents notable challenges, mainly due to the rarity of specific genetic mutations. Most studies assessed motor response through UPDRS III scores, which, while practical, may not capture the full spectrum of clinical outcomes, including non-motor symptoms or long-term functional status. The most recent study by Anis et al. (2024) also compared LRRK2 PD with GBA1 PD and idiopathic PD.[24] One key finding was that LRRK2 PD and idiopathic PD exhibited comparable motor, behavioural, and cognitive outcomes after DBS, in contrast to GBA1 PD, which showed an increased risk for psychosis and cognitive decline. Additionally, the studies primarily focused on a single DBS target, the subthalamic nucleus, with less comprehensive exploration of other potential targets, such as the globus pallidus internus. Although detailed reporting of DBS programming parameters and lead placement was limited in the included studies, almost all patients received STN-targeted DBS. The differences in electrode placement or target selection were minimal, thereby reducing the potential impact of such variability on outcomes. This limitation affects the generalizability of the findings to other DBS techniques. Consistent with this limitation, our meta-analysis demonstrated a high degree of heterogeneity (I^2 = 63.1%), likely reflecting the variability introduced by class imbalance and small sample sizes inherent in studies of rare mutations. This high heterogeneity necessitates cautious interpretation of the pooled effect estimates.

The G2019S, G2385R, and R1441G variants are among the reported pathogenic mutations causing LRRK2 PD.[28] All

pathogenic mutations in the LRRK2 gene are located within the ROC (Ras of complex), COR (C-terminal of Roc), and kinase domains.[4] The G2019S variant is situated within the kinase activation loop and leads to increased kinase activity. The R1441G/C/H variants are located in the ROC GTPase domain, resulting in the suppression of GTP hydrolysis and an increased affinity of the LRRK2 protein for GTP, which in turn leads to a three to four fold increase in kinase activity towards the Rab substrate compared to normal. Meanwhile, the N1437H variant of LRRK2 allows the LRRK2 protein to remain bound to GTP by stabilizing the LRRK2 protein dimer, even though the protein's affinity for GTP or its GTPase activity is reduced. The G2385R variant is located in the WD40 domain and disrupts WD40 domain dimerization, thereby enhancing LRRK2 protein activity within the cell.[29] Overall, these pathogenic mutations function by either decreasing GTPase activity encoded by the tandem ROC-COR bidomain or by increasing kinase domain activity.[29-30]

All known pathogenic LRRK2 mutations cluster within a tightly conserved region of the gene that encodes its critical functional domains (ROC, COR, and kinase domains, with risk variants also found in the WD40 domain). According to the NCBI Conserved Domain Database, the most common LRRK2 mutation (G2019S) lies in the kinase domain's activation loop and causes elevated kinase activity, whereas the R1441G mutation in the ROC GTPase domain impairs GTP hydrolysis and similarly results in kinase hyperactivation.[31] The G2385R variant affects the WD40 domain, disrupting normal LRRK2 dimerization and protein interactions, which also enhances LRRK2 activity.[29] These distinct molecular mechanisms trigger different downstream effects – for example, hyperactive LRRK2 leads to excessive phosphorylation of its Rab GTPase substrates and disruption of autophagic pathways – potentially producing variant-specific patterns of neurodegeneration.[28] This provides a biological rationale for the observed differences in DBS outcomes: variants such as G2019S and G2385R, which increase kinase activity but may preserve more typical network function, showed better motor improvement with STN-DBS, whereas the R1441G variant's more disruptive effect corresponded with a relatively limited DBS response.

Subsequent research examining the effects of DBS in monogenic Parkinson's disease associated with LRRK2 mutations should incorporate additional parameters, including various sections of the UPDRS, LEDD, and non-motor outcomes, to yield a more thorough therapeutic response profile. Studies investigating DBS responses in Parkinson's patients with various gene mutations and a detailed analysis of genetic variants are essential for comprehending the broader implications of genetic diversity on therapeutic outcomes.

CONCLUSIONS

Parkinson's disease associated with LRRK2 mutations exhibits diverse motor responses to deep brain stimulation (DBS), depending on the specific mutation variant. Patients with the LRRK2 G2019S and G2385R variants showed clinically significant improvements in motor responses following DBS, as evidenced by the enhancement in UPDRS III scores. These motor responses were either comparable to or superior to those seen in idiopathic Parkinson's disease patients following DBS targeting the subthalamic nucleus. In contrast, Parkinson's disease associated with the LRRK2 R1441G variant demonstrated an inadequate motor response, with no clinically significant improvement in UPDRS III scores. This systematic review highlights the importance of genetic testing in guiding evidence-based decision-making within precision medicine. By providing insight into the genetic foundations and clinical variability of Parkinson's disease, genetic testing enables clinicians to better understand patient responses to DBS, thereby supporting the customization of therapeutic strategies.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare.

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Prospective outlook on negative pressure wound therapy (NPWT) for gastroschisis and ruptured omphalocele: A scoping review

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ABSTRACT

Introduction: In cases of gastroschisis and ruptured omphalocele where primary closure is not feasible, physicians must employ alternative strategies to gradually reduce the herniated contents and promote epithelialization, either through non-surgical methods or with surgical intervention. Negative pressure wound therapy (NPWT) is a device specifically designed to promote wound healing by controlling sub atmospheric pressure. Despite the benefits of NPWT, its use in paediatric patients, particularly congenital abdominal wall defect, remains unclear. This study aimed to assess the benefits of NPWT as part of the treatment strategy for gastroschisis and ruptured omphalocele.

Materials and Methods: A literature search was conducted through electronic databases including Pubmed, ScienceDirect and JSTOR from inception through January 2025. The outcomes identified included NPWT application details (pressure, timing, duration), outcomes related to length of stay, initial feeding, complications, wound progression, and follow-up results. Quality assessment was not conducted as the review aimed to provide a broad overview of the topic.

Results: Sixteen studies were included in the final analysis. Subjects consisted of 41 patients (32 gastroschisis, and 9 ruptured omphalocele), with a mean gestational age of 36.9 weeks and a mean birth weight of 2,216 g. The most common herniated contents were small bowel and liver, with an average defect size of 6 cm. The initial NPWT pressure ranged from -25 mmHg to -100 mmHg, with -40 mmHg being the most common starting pressure. At 10-12 months follow-up, most subjects demonstrated no fascial defects and intact epithelialized skin. NPWT was found to be an effective alternative, particularly as part of immediate primary closure, facilitating wound healing during staged closure, or managing infection. Although most evidence came from case reports or series, and there is a lack of standardized protocols for NPWT, its benefits over conventional care were evident.

Conclusion: NPWT shows its benefit in adjunct to delayed closure, primary suture less closure, and in the management of gastroschisis and ruptured omphalocele. Future research should further investigate the optimal use of NPWT in a larger larger prospective or randomized controlled trials to refine protocols and better understand its long-term benefits and risks.

KEYWORDS:
Negative pressure wound therapy, gastroschisis, omphalocele

INTRODUCTION

Gastroschisis is one of the most common types of abdominal wall defect, characterised by a defect typically to the right of the umbilical cord.[1] A study conducted at a single centre in Indonesia found that the mortality rate of gastroschisis was 69% among 42 subjects. Early closure within 1 day is associated with a lower mortality rate.[2] In southern Brazil, the incidence of gastroschisis has increased by 85%, with an annual incidence of 2.69 per 10,000 live births.[3] Globally, in 24 countries, gastroschisis occurs in 1 per 3,268 births, or 3.06 per 10,000 live births.[4] Omphalocele is another type of abdominal wall defect, occurring through the umbilicus, and it can present with or without a sac.[5] The prevalence of omphalocele between 2000 and 2012 was 2.6 per 10,000 births, with a mortality rate of 32.1%. Most omphalocele related mortalities occur within the first 24 hours of life, with a one-year mortality rate of 30.7%.[6]

Managing both gastroschisis and omphalocele remains a significant challenge for physician. In cases where primary closure cannot be performed, physicians must find an alternative solutions to slowly reduce the herniated contents and allow for epithelialization to form, with or without surgical management.[7,8] This is where negative pressure wound therapy (NPWT) has been introduced as a potential treatment option. NPWT has been widely used for all various types of wound injuries.[9,10] In a study focused on open abdominal wounds in paediatric patients, NPWT was applied until healthy granulation tissue formed, allowing the wound edges to be safely sutured without tension.[9,11–13] Evidence suggests that NPWT promotes faster wound healing by applying negative pressure to the wound bed through a specialized dressing, sponge, and vacuum system. However, wound healing in neonates presents additional challenge including fragile skin, immature immune system, and impaired thermoregulation, all of which can hinder proper restoration.[13–15] To date, there are no established guideline or

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protocols for using NPWT in neonates or paediatric patients.[15] In this scoping review, we aim to explore the characteristics and the clinical benefits of NPWT, particularly in the context of congenital abdominal wall defects such as gastroschisis and ruptured omphalocele.

MATERIALS AND METHODS

This scoping review was conducted in accordance to Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for scoping reviews (PRISMA-ScR).[16]

Search Strategy and Selection of Studies

A comprehensive computerized search was conducted using databases such as Pubmed, ScienceDirect, and JSTOR. We retrieved relevant study from inception through January 2025. The search terms included the following combinations: ((“negative pressure wound therapy") OR ("NPWT") OR ("vacuum assisted closure") OR ("VAC")) AND (("gastroschisis") OR ("omphalocele") OR ("congenital abdominal wall defect")). Boolean operators’ combination was used to broaden and narrow search result. The search was limited to human subjects, with language restricted to only English.

Eligibility Criteria

All publications that met inclusion criteria were considered. Inclusion criteria were as follows: (i) type of studies included randomized or non-randomized trials, observational studies, pilot studies, case report, or case series; (ii) neonates or children diagnosed with gastroschisis or ruptured omphalocele; (iii) studies where negative pressure wound therapy (NPWT) was used as part of the management; (iv) studies reporting outcomes related to NPWT pressure, timing of NPWT application, duration of NPWT, length of stay, initial feeding, and complications. The exclusion criteria included animal studies, irretrievable full texts, non-English language publications, and studies on irrelevant topics.

Data Extraction and Quality Assessment

After removing duplicates, screening was performed on all studies based on their titles and abstracts. Full-text articles of studies that met the inclusion criteria were then retrieved and reviewed in detail. Disagreements regarding the eligibility of studies and data extraction were resolved by consensus among the authors. In cases of persistent disagreement, a third expert was consulted to reach a final decision. Each included study was carefully reviewed to extract the following details: author(s), year of publication, study design and setting, sample size, NPWT application specifics (including pressure, timing, and duration), and the outcomes related to length of stay, initial feeding, complications, wound progression, and follow-up results. Quality assessment of the studies was not conducted as part of this review, as the goal of the scoping review was to provide a comprehensive overview of the topic.

RESULTS

Results of Literature Search

A total of 308 publications were identified through database searches (Figure 1). After removing duplicates and excluding studies marked by the automation tool, we screened 271 titles and abstracts. Thirty-six studies were further assessed based on the inclusion criteria. Ultimately, 16 studies (5 case series 9 case reports, and 2 retrospective observational studies) were included and analysed.

Study Characteristics

The studies were conducted in Canada[17], the USA[10,18–21], Australia[22,23], South Africa[24,25], Japan[26,27], Romania[28], Vietnam[29], Germany[30], and China.[31] The oldest publication was from 2006 [17], and the most recent was from January 2025.[31] All included studies detailed the use of NPWT as part of the management process for either gastroschisis or ruptured omphalocele. Two studies described the use of NPWT in the treatment of an open abdomen but did not specifically focus on gastroschisis or ruptured omphalocele, so these articles were excluded.[11,12]

Patient and Abdominal Defect Characteristics

A total of 41 participants (9 ruptured omphalocele, 32 gastroschisis) were included (Table I). The reported gender distribution was 21 males and 16 females, with a mean gestational age of 36.9 weeks. The average defect size was 6 cm, and the mean birth weight was 2,216 grams. Herniated contents primarily consisted of small bowel loops and liver. In gastroschisis cases, the bowels were generally eviscerated, matted, and oedematous. Congenital dysmorphic features in ruptured omphalocele patients included low-set ears, frontal head bossing, short neck [25] patent omphalomesenteric duct and cleft palate.[30]

Indications for using negative pressure wound therapy (NPWT) included the need to accelerate wound closure, inadequate conditions for primary closure, and failure of staged abdominal closure. Some subjects were diagnosed prenatally via ultrasonography.[10,18,21,30] After reduction, there are cases where the abdominal wall was non-compliant, and closing the defect would create excessive tension. In the included studies, NPWT was generally used after silo reduction. Two studies achieved complete reduction using a silo, and then NPWT was used to aid in skin closure.[10,18] In the case of ruptured omphalocele, it was treated similarly to gastroschisis by placing a silo or attempting to suture the bloody sac.[25,27,29] One patient with a history of ruptured omphalocele was admitted for NPWT due to delays in developmental milestones such as rolling and crawling. This patient was also fed via nasogastric tube due to feeding intolerance.[23] Another patient with gastroschisis treated with NPWT was also put on a vacuum trial due to abdominal viscera disproportion.[23] One omphalocele case involved the use of NPWT to treat wound dehiscence and infection during staged closure.[27] Detailed characteristics of each case are provided in Table II and Table III.

Technique of implementing NPWT

Several options were used for dressing the herniated abdominal contents, including clear sheets, hydrocolloid, mesh, and biological patches.[21,26-28] These were followed by polyurethane ether[24], white foam[20,26], or granufoam/blackfoam.[20,26] A clear film or transparent tape was then applied over everything, with the apex cut to allow insertion of the suction tube. Hattori et al., (2017) used a

Table I: Characteristics and demographics of included studies (N=41)

No	Author, Year	Region	Study Design	N	Population	Gender (F/M)	GA	Birth Weight	Defect Size (cm)	Herniated Stomach Content	Associated Condition
1	Gabriel, 2006 [17]	Canada	CS	3	GC	M	36	N/A	8 x 10.5	N/A	Noncompliant abdominal wall after reduction using silo
					GC	N/A	35	N/A	5 x 6,5	N/A	Noncompliant abdominal wall after reduction using silo
					GC	N/A	38	N/A	5 x 5,5	N/A	Abdominal distention and noncompliant abdominal wall after reduction using silo
2	Hubbard, 2009 [18]	USA	CS	2	GC	M	37	N/A	4	Small bowel, large bowel, and bladder	N/A
					GC	M	37	N/A	4	Small bowel, large bowel, and bladder	Meconium aspiration and desaturation that required one day of mechanical ventilation
3	Hassan, 2011 [10]	USA	OBV	15	GC	F(7)/M(8)	33-39 (35)[a]	1.6-2.960 (2.371)[a]	4-5 (4.75)[a]	N/A	N/A
4	Choi, 2011 [22]	Australia	CS	4	GC	F	34	3070	5	Eviscerated oedematous and matted bowel	N/A
					GC	M	32	1730	1	Eviscerated bowel	N/A
					GC	M	35	1760	5	Stomach, small and large intestine thickened at birth.	N/A
					GC	F	36	2300	5	Features of both gastroschisis and omphalocele because the isolated loop of terminal ileum, caecum and appendix was contained in a pre-existing cavity within the umbilical cord.	Type IV intestinal atresia with a blind ending ileum isolated loop of terminal ileum, caecum and appendix; as well as a blind-starting colon that began 20 cm from the rectum.
5	Morris, 2013 [19]	USA	CR	1	GC	M	36	N/A	N/A	Small bowel, colon, stomach, 30% of liver	N/A
6	Mcbride, 2014 [23]	Australia	CS	3	RO	F	37	3000	N/A	N/A	N/A
					GC	M	35	2460	N/A	Bowel	N/A
					RO	M	37	2620	N/A	Gut and Liver	Congenital renal disease
7	Hattori, 2016 [24]	South Africa	CR	2	GC	N/A	35	1620[b]	N/A	N/A	N/A
					GC	N/A	35	1700[b]	N/A	N/A	N/A
8	Butler, 2018 [20]	USA	CR	1	GC	M	35	2120	4	Stomach, small intestines, colon	N/A
9	Horiike, 2020 [26]	Japan	CR	1	RO	F	38	3047	6	All abdominal organs except duodenum, rectum, kidneys, uterus, and ovaries	No other associated abnormalities in echocardiography or g-band analysis.
10	Morulana, 2020 [25]	South Africa	CR	1	RO	F	34	2090	12	Visible bowel and liver through the sac	Low set ears, frontal head bossing and short neck.
11	David, 2021 [28]	Romania	CR	1	RO	M	37	2600	8	Small bowel loops and liver	Small ventricular septal defect with patent ductus arteriosus
12	Tri, 2021 [29]	Vietnam	CS	3	RO	F	36	3000	12	N/A	N/A
					GC	F	36,5	2500	N/A	N/A	N/A
					GC	M	35	2600	N/A	N/A	N/A
13	Chen, 2021 [21]	USA	CR	1	GC	F	37	2300	N/A	Indurated bowel, bladder	Bladder fused to the inferior aspect of the defect wall.
14	Nakagawa, 2022 [27]	Japan	CR	1	RO	M	38	1896	10 x 10	Total Intestine and liver	N/A
15	Nissen, 2022 [30]	Germany	OBV	1	RO	F	37	3000	N/A	Bowel and liver	Patent omphalomesenteric duct Cleft Palate
16	Hou, 2025 [31]	China	CR	1	RO	M	N/A	2930	8	Bowel and liver	N/A

a = quantitative data are presented as median or mean; b = weight measured when NPWT was first used; CR = case report; CS = case series, OBV = retrospective observational; GC = gastroschisis; RO = ruptured omphalocele.
GA = gestational age; N/A = not available;

Table II: Characteristics negative pressure wound therapy application in gastroschisis cases (N=32)

No	Author, Year	N	Initial Treatment	Surgical Intervention	Technique of NPWT	NPWT pressure (mmHg)	NPWT initiation	NPWT duration (days)	Additional Treatment
1	Gabriel, 2006 [17]	3	Case 1: 7,5 cm, Case 2 and 3: 5 cm Silo spring loaded	Not applied.	Visceral is covered by surgisis ES and SIS was secured to the fascial edge and sutured to the peritoneal surface of the abdominal wall	-75	15 days; 9 days; 13 days	54 28 58	Dressing changes every 2-3 days with additional SIS coverage
2	Hubbard, 2009 [18]	2	Application of spring-loaded silo (bedside procedure)	Not applied.	Approximate edges with steri strips. Small perforation in the center to create an exit for fluid. Tegaderm to protect surrounding skin from sponge	-50 to -75	4 days after silo usage. 5 days after silo usage.	4 17	Application of dressing after NPWT machine removal. Dressing changes on day 12 and 19 during the use of NPWT.
3	Hassan, 2011 [10]	15	Application of Silo performed at bedside under sedation.	Not applied.	Approximate the defect edges. Several steri strips over the edges. Cut a small hole in the center and vacuum sponge is placed on top.	-100 to -125	4-5 days after silo usage.	14	N/A
4	Choi, 2011 [22]	4	Application of spring-loaded silo	After 9 days of silo, the isolated loop of terminal ileum, caecum, and appendix was removed. An ileocolic anastomosis was performed with formation of tube jejunostomy (Case 4)	Acticoat as interface between bowel and RENSYS as gauze dressing. NPWT applied on top of it.	-80	17 days;8 days;6 days;9 days after silo usage	26 21 14 21	Dressings were changed weekly for Case 1 and 2
5	Morris, 2013 [19]	1	Silo placement with a 5 cm per-formed appliance.	Partial fascial closure at 7 DOL	NPWT applied in the operating room.	-75	5 days after silo usage.	7	N/A
6	Mcbride, 2014 [23]	1	Partial reduction using silo, subsequent NPWT and split thickness skin graft.	Surgical reduction by removing excess skin and adhesions. Bowel was returned to abdominal cavity and full fascial and skin closure was done.	A ring of hydrocolloid is applied to the surrounding the abdominal hernia. Open weave gauze is wrapped around the mass in a fashion similar to a head dressing. An adhesive plastic dressing is applied to attain a seal. A port is then sited at the apex of the mass and NPWT device is sealed.	-80	Initial at birth and 9 months old	3 weeks after birth; 9 days during 1st admission	N/A
7	Hattori, 2016 [24]	2	Attempt to close abdomen	Attempt to close abdomen	Sterile polyvinyl chloride sheet cut from an intravenous fluid bag as an interface between bowel and a polyurethane ether foam. Foam trimmed to wound size. Transparent and adhesive film dressing to seal the wound. A small hole in the center of the film over the foam and the vacuum device is placed over the hole	-40	7 DOL 11 DOL	4 2	The wound was inspected for either closure or reapplication of the dressing in the operating room routinely at 2-day intervals

Table II: Characteristics negative pressure wound therapy application in gastroschisis cases (N=32)

No	Author, Year	N	Initial Treatment	Surgical Intervention	Technique of NPWT	NPWT pressure (mmHg)	NPWT initiation	NPWT duration (days)	Additional Treatment
8	Butler, 2018 [20]	1	Spring loaded 5 cm silo bag placed at birth, upsized to 7.5 cm	N/A	Whitefoam and GranuFoam dressings were cut to half their thickness, fashioned in the shape of a cup by sewing strips together, placed over the eviscerated bowel. Strips of adhesive drapes were used to secure the dressing, circumferentially wrapping the infant. After puncturing the drape, a SensaT.R.A.C. Pad was placed over the dressing, connected to a VAC unit	-25 to -75	22 DOL	28 19	Biweekly dressing change until the viscera were consolidated. After the viscera consolidated mepitel was placed over the viscera prior to whitefoam and granufoam dressings.
9	Tri, 2021 [29]	2	Suture of bloody sac Use bloody sac, silo sac, abdominal wall closure with gore tex band	Suture of bloody sac	The VAC system includes a suction machine, a sponge, transparent tape, and a suction tube.	-30	15 DOL 47 DOL	14	VAC was change 3 times over 19 days and daily wound care. Wound cleaning and daily bandages changes for 7 days
10	Chen, 2021 [21]	1	Blunt and cautery dissection the separate the bladder from the defect and ligation of persistent urachal remnant followed by insertion of 5 cm spring loaded silo	Failed primary abdominal closure followed by biologic patch to cover the defect and sutured to the fascia circumferentially	StratticeTM biologic patch was cut to the size of the defect and sutured to the fascia circumferentially in an interrupted manner using 3–0 PDS suture without any changes to ventilator pressures or tidal volume. The patch was then dressed with a negative pressure wound vacuum device,	N/A	Immediately after surgery, removed after 3 days to petroleum gauze dressing	3	Biological path under the NPWT device

DOL = day of life; N/A = not available; SIS = small intestinal submucosa

Table III: Characteristics of negative pressure wound therapy application in ruptured omphalocele cases (N=9).

No	Author, Year	N	Initial Treatment	Surgical Intervention	Technique of NPWT	NPWT pressure (mmHg)	NPWT initiation	NPWT duration (days)	Additional Treatment
1	Mcbride, 2014 [23]	2	Applying a silo, NPWT, split thickness skin graft. Primary Closure which is then released put in silo	Staged Surgical Closure: bowel was separated from previous skin graft then mesh was applied (no skin closure) 4:1 meshed split skin graft was placed, minimal debridement.	A ring of hydrocolloid is applied to the surrounding the abdominal hernia. Open weave gauze is wrapped around the mass in a fashion similar to a head dressing. An adhesive plastic dressing is applied to attain a seal. A port is then sited at the apex of the mass and NPWT device is sealed.	-40 to -80 N/A	8 months old first admission; followed by 9 months old second admission after staged surgical closure Use multiple times before and after Surgery. Also while waiting for renal transplant.	9 days for 1st admission; 14 days for 2nd admission Was used multiple times. Specifically 7 days until pressure garment was available.	NPWT dressing is changed once a week
2	Horiike, 2020 [26]	1	Applying a silo	Silo using small size wound retractor and biomaterial silo	Non-adherent wound dressings, white foam, granufoam were applied in this order, and then a clear film was applied to the entire omphalocele. The apex was cut into 1 cm squares and connected to the negative pressure system.	-25	65 DOL	6	A continuous infusion of dialysate was started, in the hope that it would remove fluid and electrolytes in its passage through the abdomen from the catheter tip up through the gauze and out via the NPWT suction tubing. Collagen based artificial dermis
3	Morulana, 2020 [25]	1	Ruptured membrane were sutured with absorbable monofilament suture.	Prolene mesh was sutured to the edge of the abdominal defect over the repaired membranes.	Dressings applied over mesh	N/A	After initial treatment	14	Sac painted with gentian violet dressings changed every 72 days.
4	David, 2021 [28]	1	Immediate Staged Closure	Staged closure (1st: partial resection and attachment of the membrane, 2nd: deatched, partial resection, and re attached of the membrane. 3rd: reduced hernia to abdominal cavity, but defect could not be closed.	Defect closed with gore tex mesh, NPWT, and anti-microbial silver dressing.	-40	50 DOL	28	Dressing changed every 4 days

Table III: Characteristics of negative pressure wound therapy application in ruptured omphalocele cases (N=9).

No	Author, Year	N	Initial Treatment	Surgical Intervention	Technique of NPWT	NPWT pressure (mmHg)	NPWT initiation	NPWT duration (days)	Additional Treatment
5	Tri, 2021 [29]	1	Suture of bloody sac		The VAC system includes a suction machine, a sponge, transparent tape, and a suction tube.	-30	24 DOL	14	N/A
6	Nakagawa, 2022 [27]	1	Silo placement using with artificial mesh	Not applied.	Artificial dermis then the NPWT device on top	-50	33 DOL	27	Artificial mesh, collagen based artificial dermis, dualmesh Dressing changed every 3-4 days
7	Nissen, 2022 [30]	1	The use of NPWT immediately	Continuous sutures of omphalocele membrane	White foam attached to OC surface; cutaneous sagittal and horizontal tight four point fixation; no sting barrier film; transparent film adhesive drape; soft port connector	-80	1 DOL	10	Dressing changed every 3 days
8	Hou, 2025 [31]	1	Silo placement	Silo sutured at the base	Vaseline gauze, CMC-Ag dressing patched on the wound then NPWT machine on top	-60 continuous → -70 for 5 min -40 for 2 min variable therapy mode	13 DOL	19	CMC-Ag dressing; wound dressing changed every 5-7 days; Rh-bFGF after NPWT device was removed

DOL = day of life; CMC-Ag = Carboxymethylcellulose; Rh-bFGF = recombinant human basic fibroblast growth factor; N/A = not available

Table IV: Outcomes of negative pressure wound therapy

No	Author, Year	N	Population	LOS (days)	Time to first/full feed (days)	Wound Healing	Follow Up
1	Gabriel, 2006 [17]	3	GC GC GC	270 66 90	N/A N/A N/A	Wound epithelialized after 54 days Epithelialized wound Epithelialized wound	N/A 6 months follow up → small umbilical hernia that has subsequently closed Small reducible umbilical hernia that was managed conservatively
2	Hubbard, 2009 [18]	2	GC GC	25 N/A	Bolus tube feeding on day 11. Full feed on day 18. TPN discontinued on day 20. Bolus tube feeding on day 15. TPN discontinued on day 21	8 mm wide defect after NPWT removal followed by healed umbilical scar after hospital discharge Defect changes to 1-2 mm on 19 DOL	2 weeks of follow up showed appropriate growth curve 2 weeks of follow up showed scarless healed wound
3	Hassan, 2011 [10]	15	GC	18-131 (24)[a]	Time to initiate feed 6-61 (13) days[a] time to full feed 12-91 (20)	N/A	N/A
4	Choi, 2011 [22]	4	GC GC GC GC	45 56 22 74	N/A N/A N/A Difficulties in establish feeding.	Wound contracted significantly with epithelisation around wound edges → residual defect had epithelialized sufficiently Wound epithelized leaving behind small area of granulation tissue Wound contracted significantly with near complete epithelisation Wound contracted significantly with near complete epithelisation	6 months of age → large non problematic umbilical hernia 6 months of age small umbilical hernia Patient discharged with outpatient dressing changes. Follow up regularly as an outpatient.
5	Morris, 2013 [19]	1	GC	80	Initiated at 18 DOL, but goal feed was not achieved thus gastrotomy tube was placed	Fascia defect completely closed.	Follow up of 6 months of age without readmission.
6	Mcbride, 2014 [23]	3	RO GC RO	9 days for 1st admission ; 37 days for 2nd admission 9 days for 1st admission; 2 days for 2nd admission N/A	Oral intake improved and only required top-up nasogastric feeds. N/A N/A	Full and skin closure Suture line was healed with no further dressing required after 6 days Developed layer of granulation tissue over liver and bowel, facilitating graft take.	10 months post closure, patient was able to walk unaided. Patient has been followed up annually and there have been no further surgical requirements 3 years post-closure At 19 months old patient received adult cadaveric renal transplant, kidney functioning well at 10 months posttransplant.
7	Hattori, 2016 [24]	2	GC GC	N/A N/A	N/A N/A	N/A N/A	N/A N/A
8	Butler, 2018 [20]	1	GC	115	Feed started on DOL 57 and increased to goal by DOL 86.	Skin completely epithelialized	By age of 13 months weight was at 50th percentile for his age.
9	Horiike, 2020 [26]	1	RO	328	N/A N/A	N/A	1 year follow up without postoperative complications
10	Morulana, 2020 [25]	1	RO	N/A	N/A	Wound was epithelizing from edges and covered with dried scab under mush	By 3rd month follow up, mesh had fallen off
11	David, 2021 [28]	1	RO	60		After first two dressings, granulation was formed at edge of tegument and wall defect became progressively smaller from 7 cm to 3cm.	

Table IV: Outcomes of negative pressure wound therapy

No	Author, Year	N	Population	LOS (days)	Time to first/full feed (days)	Wound Healing	Follow Up
12	Tri, 2021 [29]	3	RO GC GC	78 69 67	N/A N/A N/A	Skin of abdominal completely recovered. Abdominal wall and fascia appeared intact. Granulation of skin epithelized	14 months old → intact abdominal wall and skin that was able to protect the viscera. Procedure for abdominal fascia will be planned 24 months old → abdominal wall and fascia appeared intact 24 months old → no fascia defects, and intact abdominal wall skin
13	Chen, 2021 21	1	GC	N/A	4 months post op → open gastrotomy	Healthy granulation tissue underneath	5,6,10 months old → epithelialized tissue leaving 2x1 cm ventral hernia (resolved at age 2.5 y.o); At 3 y.o → very small asymptomatic fascia defect and tolerate regular diet
14	Nakagawa, 2022 [27]	1	RO	N/A	N/A	Complete epithelization at 5 months of age after managed with prostandin ointment and debridement	Compression bandage wrapped around abdomen since 5 m.o → reduced to the abdominal cavity at 8 m.o
15	Nissen, 2022 [30]	1	RO	19	Full oral feed achieved after 18 DOL	Tension free closure of abdominal fascia and skin is possible	Abdominoplasty is planned 9 months of follow up found delayed umbilical cord rest drop off and omphalitis
16	Hou, 2025 [31]	1	RO	60	N/A	Epithelization of the wound and size was reduced to 1x1 cm	N/A

[a] = quantitative data are presented as median or mean ; DOL = day of life; GC = gastroschisis; RO = ruptured omphalocele; N/A = not available

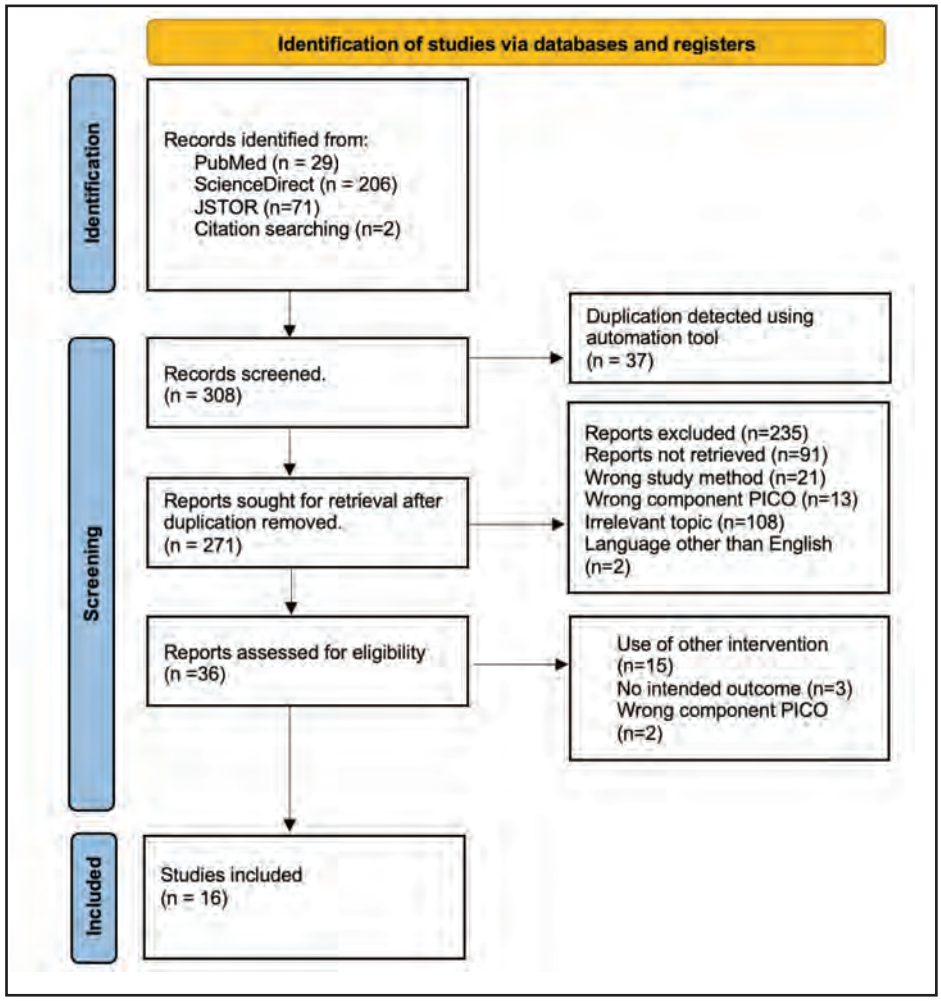

Fig. 1: PRISMA diagram with search strategy

sterile polyvinyl chloride sheet, cut from an intravenous fluid bag, as an interface between the bowel and foam.[24] The lowest starting pressure is -25 mmHg for both gastroschisis 20 and ruptured omphalocele [26], with -40 mmHg being the most common starting pressure across studies.[23-24,28] The highest starting pressure in gastroschisis is -100mmHg 10 and -80 mmHg for ruptured omphalocele.[30] One study employed variable pressure therapy, such as -70 mmHg for 5 minutes and -40 mmHg for 2 minutes.[31]

Outcome of NPWT

The length of stay in NICU varied among subjects, with the shortest being 19 days [30], and the longest being 328 days.[26] Full oral feeding was achieved on 18 day of life (DOL) [18,30], and total parenteral nutrition (TPN) was generally discontinued around day 20-21.[10,18] Two studies reported the need for gastrostomy due to unsuccessful feeding goal.[19,21] In the case described by Horiike et al., (2020), a conventional silo placement was attempted, but adhesion between the portal vein and fascia occurred.[26] Then the new biomaterial patch became infected, and NPWT was used, but a jejunal perforation developed. The perforation was repaired, and the giant omphalocele was subsequently treated using a collagen-based artificial dermis for epithelialization. Another complication associated with NPWT is the formation of enterocutaneous fistula.[24] One case of mortality reported by Hassan et al.[10] due to necrotizing enterocolitis. By 10 to 12 months of follow-up the subjects present no fascial defects with intact epithelialized skin. In the study by Tri et al., (2021) an additional suture of the abdominal wall was performed after the NPWT was removed.[29] Detailed outcomes are presented in Table IV.

DISCUSSION

This scoping review of sixteen studies displayed that NPWT is an effective tool for managing gastroschisis and ruptured omphalocele management in various clinical settings. Three strategies for managing congenital abdominal wall defects were identified: (1) immediate closure of the abdominal wall, (2) delayed closure, and (3) non-surgical treatment through spontaneous skin epithelialization.[32] A primary suture-less closure using NPWT can only be performed once the abdominal contents are reduced, and the wall defects are closed, which is a critical prerequisite for successful NPWT application.[10,18] Gastroschisis with exposed organs is particularly vulnerable to fluid loss and infection, making it essential to wait for the consolidation of the herniated abdominal contents before closure.[20,29] Suture-less closure is a gentle technique and minimizes concerns about skin tension.

In cases requiring long-term stability for gradual reduction of the herniated content, NPWT offers less frequent dressing changes (every 2–3 days) compared with traditional gauze dressings, which need to be removed daily. [10,18,33] However, the frequency of dressing changes should be based on the patient's condition, especially if there is more fluid production than usual.[29] Furthermore, the sponge is covered by a polyurethane drape that is impermeable to proteins and microorganisms, helping to prevent bacterial colonization. The semi-occlusive membrane also has limited permeability to gases and water vapor, which helps maintain moisture and warmth in the wound by reducing heat transfer due to water evaporation.[33,34]

Previous studies on gastroschisis have shown that earlier enteral feeding correlates with significant beneficial outcomes, such as quicker regaining of birth weight, reduced duration of parenteral nutrition, and shorter NICU stays.[35] If feasible, portable NPWT is an option for reducing length of hospital stay.[23] NPWT is generally considered a safe approach and rarely leads to complications. However, some complications of varying severity have been reported, most of which are attributable to poor technique or inadequate patient selection. In other words, most adverse events are preventable. Complications reported after NPWT placement in our review include intestinal perforation 26 and fistula formation.[24] Fistula formation was the least common adverse event, while infection-related conditions were the most common, including surgical site infection (2.5%), cellulitis or abscess (2%), and bleeding (0.6%).[14] To prevent fistula formation, steri-strips can be applied after complete approximation of the wound edges before placing the wound vacuum[10] or absorbable biologic or synthetic mesh can be attached to the organ as an interface organ as interface.[24] The most effective way to prevent infection is to ensure that the wound is clean before applying the device and to change the dressings at regular intervals. The incidence of enteric infections is significantly higher when the dressing is applied directly over exposed organs.[33,34] Depending on the wound's size and location, a significant amount of fluid may be suctioned during the initial days of NPWT therapy, which can sometimes result in hemodynamic instability. It's crucial to monitor patients closely and provide fluid and electrolyte support when necessary. If the foam dressing is left in place for an extended period, or if granulation tissue develops rapidly, it can adhere to the foam. When the sponge is removed, the capillary buds in the tissue may be disrupted, leading to potential bleeding. Most bleeding episodes can be controlled with manual pressure, but in cases of substantial bleeding, electrocoagulation or surgical intervention may be required. Infection risks can be minimized by properly debriding nonviable tissue before applying NPWT and ensuring sterile technique during sponge changes.[33,34]

Standardized protocols for NPWT must be developed, tailored to the clinical needs of gastroschisis and ruptured omphalocele patients. Given the diverse patient population, ranging from preterm neonates to full-term infants, protocols should remain adaptable, accounting for factors such as defect size, status of wound infection, and patient stability. Key components of the protocol include: (i) patient selection criteria: criteria for NPWT inclusion should be viable tissue, and the absence of severe infection or necrosis. Other considerations should include overall clinical state, and the presence of comorbidities; (ii) wound management: the technique should be standardized, ensuring sterile application, and thorough necrotic tissue debridement prior to application; (iii) monitoring and adjustments: a protocol for daily wound assessment, and fluid balance should be implemented. Clinical staff should oversee dressing changes and evaluate patient stability to identify and address complications such as bleeding, or infection; (iv) complication prevention and management. If infection is suspected, thorough debridement and the use of antibiotics are warranted.

The current literature on NPWT in gastroschisis and ruptured omphalocele is limited by small study sizes, lack of control groups, and variability in protocols. Furthermore, there was variability in the reported outcomes and the duration of follow-up. Complications were reported, but these were limited to only two studies.[24,26] Research should also aim to identify the ideal frequency of dressing changes, duration of therapy, and the best approach for managing complications. A more standardized approach to NPWT application will help ensure that this promising technique can be utilized safely and effectively across different clinical settings.

CONCLUSIONS

Based on the findings of this scoping review, negative pressure wound therapy (NPWT) offers a major improvement of wound healing in addition to primary closure or delayed closure. Given the severity of abdominal compartment syndrome, early intervention and management is crucial to optimize therapeutic approach. Future research should further investigate the optimal use of NPWT in these challenging cases to refine protocols and better understand its long-term benefits and risks.

CONFLICT OF INTEREST

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Anesthetic management for enhanced recovery after major surgery in children

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ABSTRACT

Introduction: Perioperative Enhanced Recovery After Surgery (ERAS) protocols, though originally created in adult surgery, are increasingly gaining applications in pediatric practice. This application addresses the unique challenge in children, who possess more complex surgical stress responses. The main goal of ERAS is to maximize postoperative recovery and improve clinical outcomes through a standardized multimodal perioperative care approach.

Materials and Methods: This review article integrates current literature and expert consensus regarding the use of ERAS principles in pediatric anesthetic care in the preoperative, intraoperative, and postoperative phases in children undergoing major surgery, also explores the implementation, benefits, and challenges of ERAS in pediatric patients, highlighting recent advancements and the impact of these protocols on recovery.

Results: The key pillars of pediatric ERAS include preoperative education and dietary optimization, multimodal opioid-sparing analgesia (e.g., regional anesthesia, non-opioid systemic analgesics), minimally invasive surgery, and goal-directed fluid resuscitation. Postoperative care includes early enteral nutrition, early mobilization, and planned discharge. Existing evidence, although further emerging, indicates that adherence to these protocols is associated with significant benefits, including shorter hospital length of stay, reduced opioid consumption, reduced intraoperative fluid administration, and faster return to normal diet.

Conclusion: ERAS protocols implemented in pediatric anesthesia are successful in optimizing recovery by reducing complications, improving pain control, and reducing length of stay. Implementation is case-specific anesthetic planning and multidisciplinary collaboration, finally maximizing outcomes and improving satisfaction for parents and children.

KEYWORDS:
Pediatric ERAS, Multimodal Perioperative Care, hospital stay reduction

INTRODUCTION

Children requiring major surgery have unique and special needs because they have more complex surgical stress reactions compared to adults, are frequently more severely affected by the external environmental disruptions and physical stress response brought on by traditional perioperative care. Consequently, it is even more crucial to optimize perioperative care for pediatrics populations.[1]

In 2018, Short et al. reported a study on the development of a pediatric-specific ERAS protocol, which adopted 19 out of the 21 ERAS elements used for adult colorectal patients. This protocol has increased awareness within the field of pediatric care by highlighting the importance of a multimodal approach to improve postoperative outcomes. ERAS protocols in pediatric surgery focus on multimodal analgesia, opioid minimization, early feeding and mobilization, and maintaining hemodynamic stability, all of which contribute to enhanced recovery and reduced hospital stays.[2-4]

This narrative review explores the implementation, benefits, and challenges of ERAS in pediatric patients, highlighting recent advancements and the impact of these protocols on recovery.

Key Components of ERAS Protocols in Pediatric Patients

The ERAS concept aims at reducing postoperative inflammatory, neurohormonal, and pain response, thereby allowing faster return to baseline function, decreasing in-patient Length of Stay (LOS), and decreasing postoperative complications.

Enhanced Recovery After Surgery (ERAS) protocols in pediatric patients encompass several key components that contribute to improved perioperative outcomes and long-term benefits. ERAS protocols involve a multimodal approach to perioperative care, focusing on reducing the stress response and associated physiological changes that accompany surgery.[1-2]

Using the modified Delphi process, Short et al. ultimately produced a concise list of suggested ERAS elements in paediatrics as follows:[2]

- Preoperative: Preoperative ERAS education, optimize medical comorbidities, avoid prolonged preoperative fasting and administer non-opioid analgesia
- Intra-operative: Venous thromboembolism prophylaxis, pre-incision antibiotic prophylaxis, standard anesthetic protocol, minimally invasive technique, prevention of nausea/vomiting, no nasogastric tubes and standardized hypothermia prevention

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- Postoperative: No intraperitoneal perianastomotic drains, goal-directed/near-zero fluid therapy, early removal of urinary catheters, prevention of postoperative ileus, opioid-sparing pain regimen, early mobilization, audit protocol compliance and outcomes and perioperative nutritional screening.

The ERAS elements are discussed in detail as follows:

Preoperative Management

In preoperative stage all patients get preoperative education and nutritional evaluation prior to surgery, and nutritional support is initiated individually before and/or after surgery. The preoperative fasting time is reduced from stopping clear fluids for at least two hours and solid foods for six hours prior to anesthesia.[2]

Preoperative education for patients and families helps alleviate anxiety, which can significantly impact recovery. Preoperative education and counselling include the goals and procedures of the ERAS protocol, pain treatment strategies, parental expectations of surgery, discharge criteria, and follow-up plans. Furthermore, ERAS protocols enhance patient and family satisfaction. This educational aspect ensures that families are well-informed about the surgical process and recovery expectations, leading to a more positive experience overall.[4]

Adequate preoperative nutrition screening and support are vital yet underrecognized aspects of ensuring optimal surgical outcomes, often due to an inconsistent use of available screening tools in the inpatient pediatric setting, and a lack of validated screening tools in the outpatient setting. Screening in pediatric patients undergoing surgery to identify individuals with malnutrition or those at risk of malnutrition is important. Malnutrition is more prevalent in pediatric patients with underlying chronic medical conditions but can be present in the general pediatric population as well. Screening may identify malnourished patients who are either underweight or overweight. Nutritional optimization is critical, as a pre-operative malnourished state, can hinder recovery.[5]

Patients need to be encouraged to drink clear water up to a maximum capacity of 3 ml/kg up to one hour before to elective general anesthesia.[2] However, the American Society of Anesthesiologists (ASA) continues to advise a two-hour clear fluid fast despite the evidence that within 30 minutes, water leaves the stomach, and within an hour, other clear fluids are nearly gone. Clear fluid fasting in children does not result in any significant change in their gastric volume or pH. Long-term fasting triggers immunological and metabolic reactions that raise insulin resistance, cause a catabolic state, and perhaps lower intravascular volume.[6] Therefore, prolonged preoperative fasting in children should be avoided to minimize metabolic stress and maintain hydration without increasing aspiration risk.

ERAS protocols emphasize the use of multimodal analgesia that combines various analgesic modalities, including neuraxial anesthesia (e.g. epidural or intrathecal morphine), regional blocks (e.g. TAP block), and non-opioid systemic analgesics (e.g. paracetamol, NSAIDs), achieve optimal pain control while minimizing opioid use and its side effects. This approach is crucial in pediatric care, where the risks associated with opioid use can be particularly concerning. The use of adjuncts like dexmedetomidine has been shown to effectively manage pain while also reducing anxiety and emergence agitation in pediatric patients.[7-9]

Intraoperative Management

The choice of anesthetic technique plays a pivotal role in the success of ERAS protocols. General anesthesia remains a common choice for many pediatric surgeries. However, the incorporation of multimodal analgesia strategies is essential to enhance recovery. The anesthetic team must carefully consider the pharmacokinetics and pharmacodynamics of anesthetic agents in children, as their responses can differ significantly from adults. For example, children have a higher cardiac output and greater total body water, resulting in a larger volume of distribution for water-soluble drugs and faster redistribution of lipophilic agents, which can alter both onset and duration of anesthetic effects compared to adults. Regional anesthesia, such as nerve blocks, has been shown to provide effective analgesia while reducing the need for opioids, which can have adverse effects on recovery. The use of caudal blocks in children undergoing lower abdominal surgeries has been associated with significantly decreased postoperative pain and opioid consumption, leading to faster recovery times and shorter hospital stays. For instance, a randomized trial evaluating epidural analgesia in major surgery (MASTER study) demonstrated improved pain scores at rest on postoperative day one and on coughing from days one to three compared to systemic opioid analgesia, highlighting the potential of regional techniques to enhance postoperative outcomes.[10]

Intraoperative management is another critical component of anesthetic care in ERAS protocols. Maintaining hemodynamic stability and minimizing fluid overload are essential to reduce the risk of postoperative complications. The use of goal-directed fluid therapy, guided by hemodynamic monitoring such as stroke volume variation, pulse pressure variation, or esophageal doppler measurements, can help optimize fluid management during surgery. Rather than administering fluids at a fixed dose, goal-directed fluid therapy, which tailors fluid administration based on specific indicators of fluid responsiveness, has been shown to improve outcomes in adults, although data in pediatric populations remain limited.[8] ERAS protocols help ensure more precise management of pediatric patients' hemodynamics by advocating for restrictive or goal-directed fluid administration, which aims to avoid excessive fluid loading that has been associated with impaired recovery, including longer hospital stays and delayed return of bowel function. Additionally, minimizing the duration of anesthesia and surgical time is vital, as prolonged procedures can lead to increased stress responses and delayed recovery. This stability contributes to better overall outcomes and fewer complications.[11]

Recommendations from adult surgical literature have identified antibiotic prophylaxis as a protective factor against surgical site infections. Neonates are at particularly high risk with rates of surgical site infections reported as high

as 13.5%. Neonatal intestinal surgery has a high prevalence of surgical site infections (SSIs), which can have serious consequences. Even though the evidence supporting antimicrobial prophylaxis in neonatal surgery is still lacking, the recommendations from the adult surgical literature in the benefits of antibiotics prophylaxis should still be considered given the SSI rates, immunocompromised state, and unpredictable pharmacodynamics in neonates. The morbidity associated with these infections is reflected in a length of stay for these infants that is three times longer than the stay of their uninfected counterparts.[12]

The anesthetic team should also focus on minimizing the use of volatile anesthetics, which can contribute to postoperative nausea and vomiting (PONV). Utilizing total intravenous anesthesia (TIVA) techniques may reduce the incidence of PONV and enhance recovery outcomes. Furthermore, the implementation of antiemetic protocols can further mitigate the risk of PONV, ensuring a smoother postoperative course for pediatric patients.[13]

In adults, hyperosmotic mechanical bowel preparation (MBP) may increase the risk of SSI, bowel wall oedema, bowel leak, and anastomotic dehiscence. A Multicentre review of 272 children underwent colostomy showed that MBP was associated with an increased risk of wound infection, increased LOS and no reduction in any other complications. These findings suggest that omitting MBP in pediatric patients may reduce postoperative morbidity, hospital costs, and patient discomfort, without increasing the risk of other complications.[14]

Postoperative Care

Postoperative care is integral to the success of ERAS protocols. Effective pain management strategies initiated in the operating room should continue into the postoperative period. The use of patient-controlled analgesia (PCA) can empower children to manage their pain effectively, promoting early mobilization and recovery. Additionally, early resumption of oral intake is encouraged, as it has been associated with improved recovery outcomes and reduced length of hospital stay.

Multimodal analgesia strategies aim to control pain effectively while minimizing the use of opioids, thereby reducing the risk of opioid-related side effects. Early mobilization encourages patients to resume normal activities as soon as possible, which is vital for recovery. Studies have demonstrated that ERAS protocols can significantly reduce the need for intraoperative and postoperative opioids, which helps in faster recovery and fewer complications.[14-15] For example, a scoping review of pediatric gastrointestinal surgeries found that ERAS protocols reduced opioid use, with one study reporting a decrease in postoperative morphine equivalent use following colostomy closure.[15] Additionally, cryoanalgesia, an innovative technique involving freezing the nerves to provide pain relief, has been integrated into some ERAS protocols for pediatric thoracic surgeries, showing promising results in reducing opioid use, enhancing physical rehabilitation, and shortening the LOS.[16] These findings support the efficacy of multimodal analgesia strategies, including cryoanalgesia, in enhancing perioperative outcomes in children.[14-16]

Postoperative early enteral nutrition and mobilization are critical components of ERAS. These practices have been associated with shorter hospital stays and quicker recovery of bowel function.[15,17-18]

A systematic review and meta-analysis including 1,416 adult patients undergoing elective colon and rectum surgery found that routine nasogastric (NG) decompression did not accelerate the return of gastrointestinal function and was associated with increased risks of pharyngolaryngitis and respiratory infections. Although this analysis was conducted in adults, its findings have important implications for pediatric patients, as avoiding routine NG tube placement may similarly reduce discomfort and complications without delaying gut recovery, aligning with ERAS principles promoting early feeding and enhanced recovery in children.[19]

Challenges in Implementing ERAS in Pediatric Patients

Despite the clear benefits, the implementation of ERAS protocols in pediatric settings faces several challenges. These include the need for multidisciplinary collaboration among healthcare providers, the necessity of tailored protocols for different age groups and surgical procedures, and the potential resistance to change from traditional practices. Furthermore, the variability in institutional resources and training can impact the consistency of ERAS protocol application.

Future Directions

Looking ahead, further research is needed to refine ERAS protocols specifically for pediatric populations. This includes exploring the long-term outcomes of ERAS implementation, understanding the psychological impacts on children undergoing surgery, and developing standardized protocols that can be adapted across various surgical specialties. The integration of technology, such as telemedicine, may also play a role in enhancing the delivery of ERAS protocols, particularly in the context of postoperative follow-up and patient education.

CONCLUSIONS

In summary, the advantages of ERAS in pediatric patients may include reduced postoperative complications, improved pain management, faster recovery times, shorter hospital stays, and enhanced patient and family satisfaction. By integrating tailored anesthetics strategies and fostering ongoing education and collaboration among healthcare providers involved in pediatric surgical care, ERAS can optimize outcomes and improve the overall experience for young patients and their families.

CONFLICTS OF INTERESTS

The author declares no conflicts of interest.

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Gut Microbiome profile on hirschsprung diseases with hirschsprung associated enterocolitis and non-hirschsprung associated enterocolitis: A systematic review

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ABSTRACT

Introduction: Hirschsprung's disease (HSCR), commonly known as aganglionic megacolon, is a rare congenital intestinal illness. Hirschsprung-associated enterocolitis (HAEC), an HSCR complication, is the major cause of morbidity and mortality in patients. Many research has highlighted specific microbiomes that promote HAEC, although there is still controversy on microbiome management. The aim of this study is to profile the gut microbiome of paediatric patients on HSCR with or without HAEC.

Materials and Methods: We conducted an analytical descriptive systematic review of relevant case reports from inception research articles between January 2014 to October 2024 using 3 databases following PRISMA guidelines. We extracted data of gut microbiomes in humans with HSCR with or without HAEC. Data about microbiome's effects on gut physiology were also extracted.

Results: This study identified 244 citations; 17 articles were included and analyzed. Proteobacteria were the most common bacteria in HSCR patients developing HAEC and Bacteroidetes were the most common bacteria found in HSCR patients without HAEC.

Conclusion: Proteobacteria were associated in HSCR patient developing HAEC. Therefore, gut microbiome dysbiosis may also be the key point to prevent HAEC.

KEYWORDS:
Gut microbiome, Hirschsprung diseases, Aganglionic megacolon, Hirschsprung Associated Enterocolitis

INTRODUCTION

Hirschsprung's disease (HSCR), commonly known as aganglionic megacolon, is a rare congenital intestinal illness caused by the early termination of craniocaudal migration of enteric neuroblasts during development, resulting in distal intestine aganglionosis and bowel obstruction.[1-2,4,8] This condition can extend into the proximal colon but rarely affects the small intestine.[2] Consequently, various lengths of the distal colon are unable to relax, resulting in functional colonic blockage over time.[2-3,6]

It affects around 1 in every 5,000 births.[2] Most individuals with heritable variation are detected through rectal biopsy in the newborn period due to substantial constipation; nevertheless, a subset of infants shows symptoms until early childhood or even adolescence.[2,5]

The most serious complication is HSCR associated enterocolitis (HAEC), which occurs in up to between 17.3% and 35% of cases and can have severe long-term implications, including death, with a mortality rate of 1 to 10%.[2,5,7]

Hirschsprung's associated enterocolitis (HAEC), defined as a clinical syndrome characterized by diarrhea, stomach discomfort, fever, and, eventually, septic shock.[4,6]

The specific pathophysiology of HAEC is mostly unknown, even though various explanations have been hypothesized, including intestinal barrier failure, decreased gastrointestinal mucosal immunity, and dysbiosis of the enteric microbiota may contribute to HAEC.[3,5]

Most critically, HAEC remains the major cause of death among infants and children with HSCR HAEC is distinguished by inflammation of the intestinal crypts, crypt dilatation with mucus retention, abscess formation, mucosal ulceration, and transmural necrosis of the colon near the affected section.[4] Bacterial translocations across the intestinal wall in conjunction with particular changes of the intestinal microbiota have been hypothesized as a contributing mechanism in the development of HAEC.[4]

Recent studies suggest that an aberrant intestinal milieu, particularly gut microbiota, plays a role in the etiology of HSCR.[2]

The microbiome is the complete set of bacteria, genes, and genomes in a given environment.[5] The human body contains approximately 10 times more bacteria than somatic and germ cells.[5] Most live in human gastrointestinal system, which is thought to contain 10 to 100 trillion microbial cells and over 1000 species.[5] The gut microbiota is a diverse collection of bacteria. Most live in the distal ileum and colon, which have the ideal habitat for microbial nutrients (e.g., vital amino acids, vitamins) and indigestible substances (e.g., plant polysaccharides).[5]

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The gut microbiome begins to colonize after birth and changes dramatically.[2] Indeed, abnormal microbiome composition and dynamics have been found in HSCR patients, particularly those with HAEC.[1,2] Intestinal microbiome changes composition and relative microbial abundance during growth from infancy to adulthood.[1-2] Early childhood is characterized by differential temporal development of gut microbiota.[1,2] Certain microorganisms (Proteobacteria and Enterobacteriaceae amongst all) show major abundance after birth and decrease later, towards adulthood.[1]

Although certain microbes, bacteria, or viruses resemble pathogens, the majority of intestinal microorganisms provide numerous benefits, including as strengthening the gut barrier, creating nutrition, boosting pathogen interception, and modifying host immunity.[5] A healthy gut environment is distinguished by a diversified and plentiful microbiota that is dominated by Bacteroidetes, Firmicutes, and Actinobacteria.[5] Other typical characteristics include an intact mucosal barrier and high short chain fatty acid (SCFA) synthesis.[5] These balancing processes can be disrupted by altered microbial composition and function, known as dysbiosis, which is generally characterized by changes in the microbial population and its function, as well as disturbance of mucus and epithelial barriers.[5] Dysbiosis is linked to a variety of GI illnesses, including HSCR.[5,8]

Based on these considerations, our study aims is to profile the gut microbiome of pediatric patients on HSCR with or without HAEC.

MATERIALS AND METHODS

Following the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), we developed the procedures for the current systematic review.

Literature Search

For this review, keywords and Medical Subject Heading phrases were utilized to search PubMed/Medical Literature Analysis and Retrieval System Online (MEDLINE) (2014-October 2024), Science Direct (2014-October 2024), and Springer (2014-October 2024) focusing on four key concepts: studies investigating microbiota in Hirschsprung Diseases (HSRC), HSRC with Hirschsprung Associated Enterocolitis (HAEC), HSRC without HAEC, and the microbiota profile leading to HAEC in HSRC. References found in the identified papers were reviewed to generate additional studies. After retrieving all full texts, 17 articles were included in this review. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) technique was used for search screening and article selection (Figure 1).

The inclusion criteria were English-language items only, journals focused on the gut microbiome of Hirschsprung Diseases with or without HAEC, and publications from the last 10 years with available full texts.

Exclusion criteria included Systematic Review journals, research data before 2014, and studies that did not discuss gut microbiota. Data collection yielded 203 papers after screening titles and abstracts out of 250 initial studies found, with 17 papers qualifying for analysis after full-text reading (Figure 1).

RESULTS

Out of 244 literatures, we include and analyze 17 articles, 16 of them were studies in humans and 1 of them was genome study. The sample used mostly came from fecal and soft tissue, but there were some studies using intestinal content and genome database for the sample.

In Figure 2 and Table I, we could see that most of the study found out that Bacteroidetes was the dominant gut microbiome in HSCR patients and Proteobacteria was the dominant gut microbiome in HAEC patients.

DISCUSSION

Hirschsprung-associated enterocolitis is severe and lethal complication that can happen in HSCR patient, this condition mostly characterized by fever, abdominal distention, diarrhea, and sepsis; this complication is leading cause of morbidity of HSCR patients and can occur pre- and postoperatively.[9]

There are several risk factors known that can develop HAEC; pre-operatively factor such as family history of HSCR, long segment HSCR, trisomy 21, presence of associated congenital anomalies, and delay in diagnosis of HSCR can lead to HAEC; postoperative risk factors such as the presence of the residual aganglionic segment after definitive surgery that can happen if an area of bowel or the transition zone between normal and aganglionic bowel is not entirely resected, bowel torsion proximal to the anastomosis site, formation of an anastomotic stricture, cuff stenosis on the pulled-through, bowel dysmotility or functional obstruction after pulled-through procedure.[9]

Dysbiosis is the novel insight of HAEC risk factor, dysbiosis is condition that happens when there is unbalance of gut microbiome.10 Our study found that microbiome in HSCR patients were dominated by Bacteroidetes, on the other hand HAEC patient dominated by Proteobacteria.

Bacteroidetes

Bacteroidetes frequently inhabit soil ecosystems and are associated with diverse eukaryotic hosts like plants, animals, and humans. Alterations in gut and plant microbiome community structures often align with dysbiosis and changes in host performance. The main bacterial phyla in eukaryotic microbiomes are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria.[11]

Bacteroides species are usually benign commensals in the gut but can turn into opportunistic pathogens when they move to other parts of the body. For instance, Bacteroides fragilis, a gut symbiont, is often found as an opportunistic pathogen and is the most common isolation from intra-abdominal abscesses. In the gut, Bacteroides species perform various roles, such as defending against pathogens and supplying nutrients to other gut microbes.[12]

Table I: Studies related to Gut Microbiome and the Pathomechanism

No	Title	Author	Years	Sample	Subject	Specimen	Gut Microbiome	Additional Treatment
1	16S rRNA Sequencing Reveals Alterations of Gut Bacteria in Hirschsprung-Associated Enterocolitis	HaoShi, YongShe, WuMao, YiXiang, LuXu, SanjunYin QiZhao	2024	30	Human	Intestinal content	Bifidobacterium, Lactobacillus, and Veillonella	The S group, the dominant phylum was Firmicutes, whereas Bifidobacterium, Lactobacillus, and Veillonella were notablyenriched in the H group, whereas Enterococcus showed a striking enrichment in the S group. Bifidobacterium is a commonly used probiotic, and recent studies have demonstrated its potential to attenuate major depressive disorder by regulating gut microbiome and tryptophan metabolism Lactobacillus, another probiotic, has been found to enhance immune checkpoint blockade therapy, highlighting its potential benefits in respiratory diseases
2	Association between gut microbiota and Hirschsprung disease: a bidirectional two-sample Mendelian randomization study	Wei Liu, Hanlei Yan, Wanying Jia, Jingjing Huang, Zihao Fu, Wenyao Xu, Hui Yu, Weili Yang, Weikang Pan, Baijun Zheng, Yong Liu, Xinlin Chen, Ya Gao, Donghao Tian	2024	18.340	Human	Genome-wide genotype	Eggerthella, Peptococcus, Ruminococcus, Clostridiaceae, Mollicutes RF9, Ruminococcaceae, and Paraprevotella	The abnormal intestinal microenvironment contributed crucially to the HSCR pathogenesis, especially gut GM, but without details flora colonizing the intestine
3	Prospective study reveals a microbiome signature that predicts the occurrence of post-operative enterocolitis in Hirschsprung disease (HSCR) patients A Metagenomics	Weibing Tang, Yang Su, Chen Yuan, Yuqing Zhang, Lingling Zhou, Lei Peng, Pin Wang, Guanglin Chen, Yang Li, Hongxing Li, Zhengke Zhi, Hang Chang, Bo Hang, Jian-Hua Mao, Antoine M Snijders, Yankai Xia.	2020	75	Human	Soft tissue	Gram-negative bacteria, especially Enterobacteriaceae family	Gram-negative bacteria, a major cause of enteric infection, can activate mucosal inflammation by binding LPS, a component of the outer membrane, to enteric toll-like receptor HSCR patients who were exclusively breastfed tended to have a lower abundance of Gram-negative bacteria, particularly Enterobacteriaceae HSCR patients without exclusive breast feeding had an enteric microbiome enriched for LPS biosynthesis proteins
4	Study on Hirschsprung's Disease Associated Enterocolitis: Biodiversity and Gut Microbial Homeostasis Depend on Resection Length and Patient's Clinical History	Alessio Pini Prato, Casey, Bartow-McKenney, Kelly Hudspeth, Manuela Mosconi, Valentina Rossi, Stefano Avanzini, Maria G. Faticato, Isabella Ceccherini, Francesca Lantieri, Girolamo Mattioli, Denise Larson, William Pavan, Carlotta De Filippo, Monica Di Paola, Domenico Mavilio, Duccio Cavalieri.	2019	31	Human	Fecal	Bacteroidetes, Alistipes, Enterococcus, Proteobacteria Non-HAEC : dominated by Bacteroidetes HAEC : dominated by Proteobacteria TCSA Total Colonic Aganglionosis showed lower biodiversity and increased Proteobacteria/Bacteroidetes relative abundance ratio.	Total Colonic aganglionosis had absence of Bacteroidetes affects the production of short chain fatty acids (SCFAs) which are fundamental for intestinal homeostasis. dysmotility and absence of bacteroidetes affect overgrowth of potentially harmful microbial species like Proteobacteria that can lead to HAEC colon resections can change the composition of intestinal microbiota and to dramatically reduce microbial diversity. The subsequent reduction of system robustness could expose TCSA patients to environmental microbes that might not be part of the normal microbiota. bacterial overgrowth, a potentially harmful microbial species, can outcompete commensals very rapidly and lead to HAEC as a result of systemic reaction to this dysbiosis

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5	Intestinal Microbiota in Hirschsprung Disease	Malla I. Neuvonen, Katri Korpela, Kristiina Kyrklund, Risto J. Rintala, Mikko P. Pakarinen.	2018	175	Human	Fecal	Abundances of Proteobacteria, Escherichia and Lactobacillus were significantly increased, Abundances of Clostridia, Oscillospira and Holdemania and Prevotella significantly decreased in patients with a history of recurrent HAEC	Patients with HD and HAEC had a significantly altered intestinal microbiome compared to healthy individuals, characterized by a lack of richness and pathologic expansions of taxa, particularly Enterobacteria and Bacilli.
6	Characterization of Bacterial and Fungal Microbiome in Children with Hirschsprung Disease with and without a History of Enterocolitis: A Multicenter Study	Philip K. Frykman, Agneta Nordenskjöld, Akemi Kawaguchi, Thomas T. Hui, Anna L. Granström, Zhi Cheng, Jie Tang, David M. Underhill, Iliyan Iliev, Vince A. Funari, Tomas Wester, HAEC Collaborative Research Group (HCRG).	2015	9	Human	Fecal	Lower proportion of Firmicutes and Verrucomicrobia, and a relatively increased proportion of Bacteroidetes and Proteobacteria in the HAEC group	These findings suggest a dysequilibrium in the gut microbial ecosystem of HAEC patients, such that there may be dominance of bacteria and fungi predisposing patients to development of HAEC. the microbiota differences between HSCR and HAEC groups may be caused by treatment; may be caused by HAEC; and may, or may not, actually contribute to HAEC.
7	Characterization of Intestinal Microbiomes of Hirschsprung's Disease Patients with or without Enterocolitis Using Illumina-MiSeq High-Throughput Sequencing	Yuqing Li, Valeriy Poroyko, Zhilong Yan, Liya Pan, Yi Feng, Peihua Zhao, Zhoulonglong Xie, Li Hong.	2016	13	Human	Fecal	HSCR patients (characterized by the prevalence of Bacteroidetes) and HAEC patients (characterized by the prevalence of Proteobacteria)	microbiota has been implicated in a variety of inflammatory gut disorders bacterial overgrowth or the presence of specific bacterial or viral pathogens

Table I: Studies related to Gut Microbiome and the Pathomechanism

No	Title	Author	Years	Sample	Subject	Specimen	Gut Microbiome	Additional Treatment
							HSCR patients was characterized by high levels of Bacteroidetes (45%), Firmicutes (24%), and Proteobacteria (16%). HAEC patients was Proteobacteria (60%), followed by Firmicutes (30%) the HAEC-R patients was similar to that of the HAEC patients in that it was characterized by the abundance of Proteobacteria (70%) and Firmicutes (18%)	
8	Hirschsprung's Associated Enterocolitis (HAEC) Personalized Treatment with Probiotics Based on Gene Sequencing Analysis of the Fecal Microbiome	Georg Singer , Karl Kashofer, Christoph Castellani , and Holger Till	2018	1	Human	Fecal	Bacteroidetes, Proteobacteria, Firmicutes	disruption of the intestinal mucosal barrier ("leaky gut"), an increase of inflammatory parameters, an abnormal immune response of the intestinal tract, and infection due to specific pathogens dysbiosis, inflammatory bowel disease
9	Pathways and microbiome modifications related to surgery and enterocolitis in Hirschsprung disease	Roberto Biassoni · Eddi Di Marco · Margherita Squillario · Elisabetta Ugolotti · Manuela Mosconi · Maria Grazia Faticato · Girolamo Mattioli · Stefano Avanzini · Alessio Pini Prato	2022	31	Human	Fecal	Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria	HAEC phase we found bacteria displaying Type I pili known to bind tightly to the gut wall, while after the acute HAEC phase, the taxa were characterized by virulence factors belonging to the polyamines dysbiosis or maturation of microbiome in infancy. several genes encoding for Type 1 pilus assembly proteins, biofilm formation, and antibiotic resistance virulence factors might be associated with severe colonic disease and are typical of entero-hemorrhagic E. coli (EHEC) pathogens. Of note, the inferred functional analysis of patients that have been sampled during or close to a HAEC episode showed bacteria strains characterized by type I pili found on enterotoxigenic E. coli and other bacteria which bind to intestinal epithelial cells, that are known to play an essential role in the virulence of bacteria pathogens leading to microbial invasion and colonization of the gut wall Bacteroidetes had a generic protective role in gut inflammation

Table I: Studies related to Gut Microbiome and the Pathomechanism

No	Title	Author	Years	Sample	Subject	Specimen	Gut Microbiome	Additional Treatment
10	A pilot study characterizing longitudinal changes in fecal microbiota of patients with Hirschsprung-associated enterocolitis	Kristopher D Parker , Jessica L Mueller, Maggie Westfal , Allan M Goldstein , Naomi L Ward	2022	5	Human	Fecal	Proteobacteria, Bacteriodes, Firmicutes, B. fragilis	B. fragilis was primarily associated with active HAEC and disappearance in remission samples. Oral antibiotics have also been associated with depletion of beneficial lactobacilli and bifidobacterial
11	Exclusive breastfeeding reduces risk of enterocolitis by modulating the enteric microbiome in patients with Hirschsprung's disease	Weibing Tang,;Yang Su, MSc;Chen Yuan,;Yuqing Zhang,;Lingling Zhou, ;Lei Peng,;Pin Wang, ;Guanglin Chen;Yang Li,;Hongxing Li,;Zhengke Zhi,;Hang Chang, Bo Hang, ;Jian-Hua Mao, Antoine M. Snijders,and Yankai Xia	2019	253	Human	Soft tissue	HSCR patients observed increased Proteobacteria and Bacteroidetes as well as decreased Firmicutes, Increased Proteobacteria and decreased Bacteroidetes in HAEC cases Recurrent HAEC had increased Proteobacteria and Bacteroidete	Exclusive breastfeeding is an effective approach to reduce HAEC occurrence and can reduce the risk by 40%. Moreover, exclusive breastfeeding may decrease the biosynthesis and release of LPS and reduce postoperative HAEC occurrence. Breast milk ingestion may facilitate the enrichment of microbes during acquisition of the enteric microbiome (14). Exclusive breastfeeding contributes to an enteric microbiome characterized by higher a diversity, lower abundance of Gram-negative bacteria (particularly Enterobacteriaceae), and lower LPS concentrations, which subsequently reduced postoperative HAEC occurrence.
12	Fecal Microbial Profiling of Young Hirschsprung Disease Children After Pull-Through Operation	Kanokrat Thaiwatcharamas, Watcharin Loilome, Sinobol Chusilp1, Patchareeporn Tanming, Poramate Klanrit, Jutarop Phetcharaburanin	2022	10	Human	Fecal	The HD group had reduction of Bacteroidetes, Actinobacteria, Fusobacteria and TM7, while Firmicutes and Proteobacteria were increased compared to the healthy group. Firmicutes was the most prevalent in both the HD with and without HAEC groups The HAEC group had a reduction of Bacteroidetes and increased Proteobacteria compared to the HD without HAEC patients Our study found that Bacilli significantly increased in the HD	Most microbes reside in the distal ileum and colon that contain the majority of microbial nutrients including essential amino acids and vitamins, together with indigestible components such as plant polysaccharides that can be microbially fermented into SCFAs

Table I: Studies related to Gut Microbiome and the Pathomechanism

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							group, while Erysipelotrichi and Actinobacteria significantly decreased. Although Bacilli are usually abundant in infants and are among the most common groups of probiotic bacteria, dysbiosis with a higher abundance of Bacilli was reported to be linked to diseases including HD	
13	Effectiveness of Irrigation Frequency Per Rectal on Intestinal Microbiota Colonies in Hirschsprung Associate Enterocolitis Patients	Shalita Dastamuar, Cesario Budi Prayitno1, Phey Liana, Theodorus M	2022	21	Human	Rectal swab	the most common bacteria found were Escherichia coli (52%) and then Klebsiela pneumonia (30%).	E. coli is the predominant facultative anaerobic flora of the human gastrointestinal tract. Most strains of E. coli are harmless in the lumen of the large intestine. However, more pathogenic strains have been identified and are commonly associated with urinary tract infections, enteric infections, and systemic infections, including bloodstream infections, neonatal meningitis, and pneumonia.
14	Evidence for Differentiation of Colon Tissue Microbiota in Patients with and without Postoperative Hirschsprung's Associated Enterocolitis: A Pilot Study	Ricardo A. Arbizu ,1 David Collins ,2 Robert C. Wilson,3 and Alexander V. Alekseyenko	2021	8	Human	Soft tissue	Bacteroidetes, Firmicutes and Cyanobacteria in HAEC patients Fusobacteria, Actinobacteria and Proteobacteria in HSCR patients	inflammatory, intestinal dysbiosis Total fecal SCFA composition of children with a history of HAEC
15	Altered fecal short chain fatty acid composition in children with a history of Hirschsprung-associated enterocolitis	Farokh R. Demehri, Philip K. Frykman, Zhi Cheng, Chunhai Ruan, TomasWester, Agneta Nordenskjöld, Akemi Kawaguchi, Thomas T. Hui, Anna L. Granström, Vince Funari, Daniel H. Teitelbaum	2016	18	Human	Fecal	Anaerobic microbiota (didn't mention the bacteria)	was 4-fold lower than that of HD patients without a history of HAEC. Anaerobic microbiota which produces fecal SCFAs children with HAEC history were found to have markedly reduced fecal SCFAs, and an altered SCFA profile. These findings suggest a complex interplay between altered local environment and changes in intestinal microbiota, which may influence the pathogenesis of HAEC

Table I: Studies related to Gut Microbiome and the Pathomechanism

No	Title	Author	Years	Sample	Subject	Specimen	Gut Microbiome	Additional Treatment
16	Characterization of the intestinal microbiome of Hirschsprung's disease with and without enterocolitis	Zhilong Yan, Valeriy Poroyko, Song Gu, Zheng Zhang, Liya Pan, Jing Wang, Nan Bao, Li Hong	2014	4	Human	Intestinal content	Bacteroidetes occupied the largest portion (46%) of the genomic sequences in HD patients, followed by Proteobacteria (21%); In contrast, Proteobacteria occupied the largest portion (55%) in HAEC patients, followed by Firmicutes (18%).	The bacteria pathomechanism didn't mentioned
17	Reduced expression of the NLRP6 inflammasome in the colon of patients with Hirschsprung's disease	Christian Tomuschat, Caroline Rouget Virbel, Anne Marie O'Donnell, Prem Puri	2019	10	Human	Soft tissue	Bacteroidetes are dominant in HSCR Proteobacteria dominant in HAEC	Our results show significant differences exist in the relative levels of NLRP6 in the colon of patients with HSCR compared to healthy control colon. The pathomechanism of Proteobacteria (abundant in those patients suffering from recurrent HAEC), is penetration of the inner mucus layer by production of virulence factors with mucinase activity; however, if the inner mucus layer in patients with HSCR is defective, the clearance of those pathogens is diminished, rendering patients with HSCR to be prone to colonization with proteobacteria which may lead to HAEC.

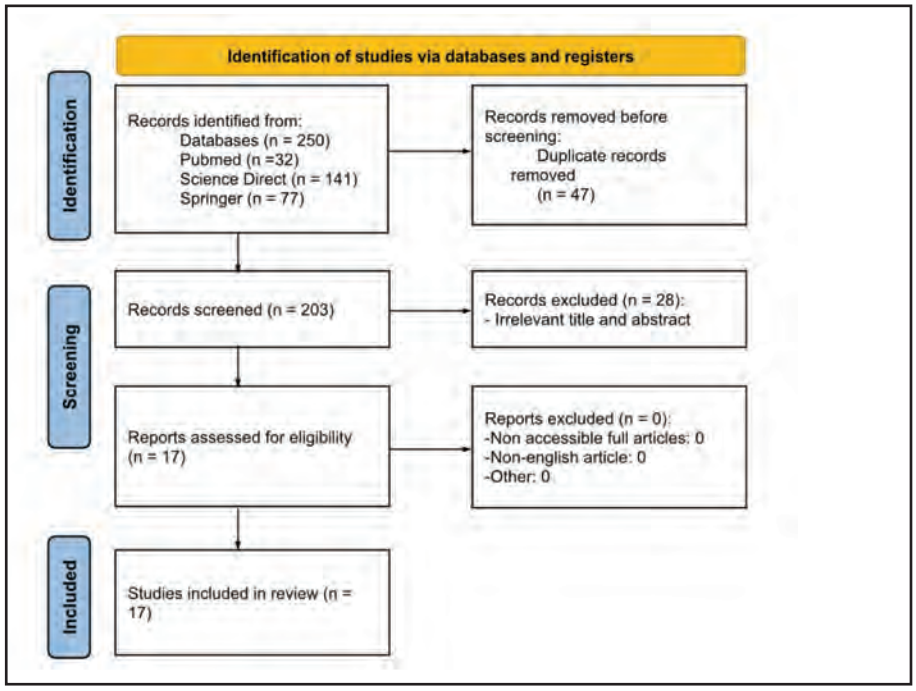

Fig. 1: Distribution of microbiome in HAEC and non-HAEC

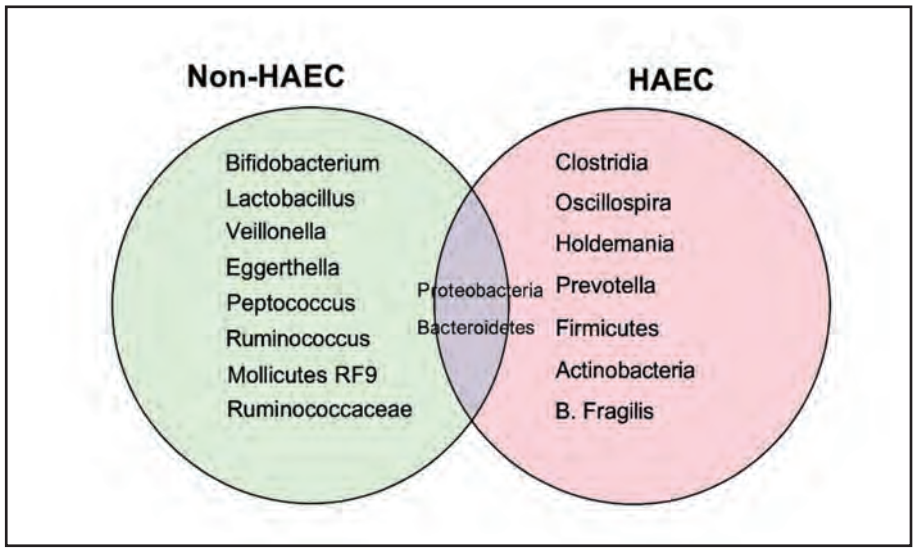

Fig. 2: Distribution of microbiome in HAEC and non-HAEC

Classifying Gram-negative Bacteroidetes has been challenging, but recent whole-genome sequencing data has improved their phylogenetic delineation. With over 32,000 genomes sequenced and assembled, the Bacteroidetes (Bacteroidota) phylum is now divided into six main classes: Bacteroidia, Cytophagia, Flavobacteriia, Chitinophagia, Sphingobacteriia, and Saprospira.[13]

A study by Prato et al. on gut microbiomes found that nearly 70% of RSA patients had gut communities composed of over 33% Bacteroidetes, while all TCSA communities had less than 2% Bacteroidetes, showing a significant difference. Additionally, the TCSA microbiota exhibited a greater presence of Proteobacteria compared to the RSA microbiota. At the genus level, Bacteroides made up more than 25% of the gut microbiota in most RSA patients, whereas all TCSA patients had less than 1% Bacteroides. Furthermore, Prato et al.'s study found no clear correlations between HEAC status, genetic background, phenotype, and gut microbiota both before and after surgery.[13]

Collectively, these findings suggest that Bacteroidetes may play a protective role in active HAEC and may return to baseline levels in HSCR patients after an HAEC episode.[14]

Proteobacteria

Proteobacteria — a diverse phylum that includes many facultative Gram-negative pathogens such as Escherichia and members of the Enterobacteriaceae — are consistently overrepresented in the guts of patients who experience recurrent HAEC.13 The pathomechanism of Proteobacteria, which are prevalent in patients with recurrent HAEC, involves breaching the inner mucus layer by releasing virulence factors that have mucinase activity (mucin-degrading enzymes, adhesins, endotoxin/LPS and secretion systems). However, if patients with HSCR have a compromised inner mucus layer, their ability to clear these pathogens is reduced, making them more susceptible to Proteobacteria colonization, which can potentially lead to HAEC.[15]

In children with HSCR the inner mucus layer and other barrier defenses are commonly altered — studies report reduced mucus integrity, disturbed short-chain fatty acid profiles that weaken mucosal defense and altered local immunity (decreased protective secretory IgA or dysregulated antimicrobial peptide expression).[20] When mucus production or organization is compromised, the normal mechanical and biochemical clearance of invading or overgrowing Proteobacteria is impaired; reduced motility from the aganglionic bowel segments further slows luminal clearance and favours overgrowth.[14]

These factors can create a permissive niche in which mucin-degrading Proteobacteria can expand, contact the epithelium more readily, and trigger an exaggerated mucosal inflammatory response — the clinical syndrome recognized as HAEC. This pathobiological model helps explain why some HSCR patients are repeatedly susceptible to enterocolitis even after surgery: persistent dysbiosis, a weakened mucus barrier, and impaired local immune/motility defenses act together to reduce pathogen clearance and permit Proteobacteria-driven inflammation.[13,20]

Decreasing the risk

Some studies mention that breast feeding can reduce the risk of development of HAEC by 40%, that proved by the decreasing biosynthesis and release of LPS and reduce postoperative HAEC occurrence moreover some studies also mentioned that HSCR patients with exclusive breastfeeding had diversity of gut microbiome.[17,18] Probiotic administrations especially that contain Bifodobacterium can reduce the risk of HAEC in HSCR patients, Bifidobacterium can suppress inflammation process using the SCFA that it produced; that was proved by some studies that found that SCFA in HAEC patients 4 times lower than in HSCR patients, moreover Bifodobacterium also higher in healthy individuals than in HSCR patients with history of HAEC.[1,16]

LIMITATIONS AND RECOMMENDATION

The numbers of patients and samples were limited. Additional research is needed to understand more fully the role and effect of microbiota and complex interactions that cause HAEC. There is a lack of clinical trials available in the field, and thus, the management strategies remain quite limited.With increasing knowledge of the role of gut microbiota dysbiosis in HAEC can provide novel specific interventions to improve dysbiosis and protect against the development of enterocolitis in the future.

CONCLUSIONS

We conclude that Proteobacteria were associated in dysbiosis HSCR patient developing HAEC. and Bacteroidetes were found in non-HAEC. With this information, Bacteriodetes microbiota can be used to control HAEC and Procteobacteria populations. In the future, microbiome nutritional therapy can be created so that microbiota can become dominant and avoid HAEC.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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CASE REPORT

Evaluating single incision laparoscopy-assisted extracorporeal biopsy as an alternative to frozen sections in the management of Hirschsprung disease

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SUMMARY

Hirschsprung disease (HD) is a congenital condition characterized by the absence of ganglion cells in the distal intestine, leading to bowel obstruction. While the use of frozen sections during biopsy is common practice, discrepancies with immunohistochemistry results and the unavailability of frozen section technology in certain pediatric surgical facilities in Indonesia highlight the need for alternative diagnostic approaches. This study evaluates the effectiveness of Single Incision Laparoscopy-Assisted Extracorporeal (SI-ECo) leveling biopsy with immunohistochemistry as a reliable alternative to frozen sections in the preoperative management of Hirschsprung disease, especially in facilities lacking frozen section capabilities. We present three cases of pediatric patients diagnosed with HD confirmed through rectal biopsy. Each patient underwent an SI-ECo leveling biopsy, successfully identifying the ganglionic zone by locating ganglion cells in the distal sigmoid. Based on these findings, subsequent transanal endorectal pull-through (TEPT) procedures were performed. The results demonstrated that SI-ECo leveling biopsy effectively identifies the ganglionic zone, providing a less invasive and precise method for preoperative planning. Leveling biopsy with SILS offers an effective method for identifying the ganglionic zone in Hirschsprung disease. SI-ECo reduces diagnostic discrepancies and provides higher specificity for detecting ganglion cells compared to frozen sections before the pull-through procedure. SI-ECo leveling biopsy with immunohistochemistry offers a practical, accurate, and less invasive alternative for diagnosing and managing Hirschsprung disease. It reduces the risk of discrepancies observed with frozen sections, making it a viable option for facilities without access to frozen section technology.

KEYWORDS:
Hirschsprung disease, Single Incision Laparoscopy, Extracorporeal Biopsy, Frozen Section Alternative, Pediatric Surgery

INTRODUCTION

Hirschsprung disease is a congenital disorder characterized by the absence of enteric ganglion cells in the distal intestine, resulting in functional obstruction.[1] The gold standard for diagnosis remains rectal biopsy with histopathological examination to confirm the absence of ganglion cells.[2] Traditionally, intraoperative frozen section biopsies have been used to determine the level of aganglionosis and guide surgical resection.[1] However, frozen sections have limitations, including potential discrepancies with permanent section results and the need for specialized pathology services that may not be available in all settings.[1]

Single-incision laparoscopic surgery (SILS) has emerged as a minimally invasive technique in pediatric surgery, offering benefits such as reduced postoperative pain and improved cosmesis.[3] The application of SILS for extracorporeal biopsies presents a potential alternative to frozen sections in managing HD. This approach allows for full-thickness biopsies to be obtained and processed for immunohistochemistry, which has shown higher sensitivity and specificity for detecting ganglion cells than frozen sections.

In Indonesia and other resource-limited settings, the unavailability of frozen section technology in many pediatric surgical facilities poses a significant challenge in the optimal management of HD. This highlights the need for alternative diagnostic approaches that are accurate and feasible in diverse healthcare environments.

This study aims to evaluate the effectiveness of Single Incision Laparoscopy-Assisted Extracorporeal (SI-ECo) leveling biopsy with immunohistochemistry as a reliable alternative to frozen sections in the preoperative management of Hirschsprung disease. By presenting a series of cases utilizing this technique, we seek to demonstrate its potential as a less invasive and more precise method for determining the level of aganglionosis before definitive surgery.

CASE PRESENTATION

We present three cases of pediatric patients diagnosed with HD who underwent Single Incision Laparoscopy-Assisted Extracorporeal (SI-ECo) leveling biopsy as an alternative to frozen sections. All patients were initially diagnosed with HD through rectal suction biopsy, which remains the gold standard for diagnosis.[4]

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Case 1
A 3-month-old male infant presented with a history of delayed passage of meconium, abdominal distension, and constipation since birth. The initial contrast enema showed a transition zone at the rectosigmoid junction, suggesting short-segment HD. The patient underwent an SI-ECo leveling biopsy under general anesthesia. A 10 mm incision was made at the umbilicus for camera port insertion with Single incision laparoscopic surgery (SILS).

Full-thickness biopsies were taken at 5 cm intervals, starting from the rectosigmoid junction and moving proximally. The biopsies were sent for immunohistochemistry (IHC) staining using calretinin, Bcl2, and S100 which has shown high sensitivity and specificity for detecting ganglion cells. The results confirmed the presence of ganglion cells in the distal sigmoid colon, 5 cm above the peritoneal reflection. A transanal endorectal pull-through (TEPT) procedure was performed based on these findings. The patient's postoperative course was uneventful, with regular bowel movements achieved by the 6-month follow-up.

Case 2
A 2-year-old female child presented with chronic constipation and failure to thrive. SI-ECo leveling biopsy was performed as described in Case 1. IHC staining with calretinin, Bcl2, and S100 antibodies, which have shown improved accuracy in detecting ganglion cells compared to conventional H&E staining, revealed the presence of ganglion cells in the mid-sigmoid colon.

A transanal endorectal pull-through (TEPT) procedure was subsequently performed. At the 1-year follow-up, the patient had normal bowel function and showed catch-up growth.

Case 3
A 6-month-old male infant, previously diagnosed with Down syndrome, presented with recurrent episodes of abdominal distension and enterocolitis. Rectal suction biopsy confirmed the diagnosis of HD. Given the higher risk of extended aganglionosis in patients with Down syndrome, SI-ECo leveling biopsy was crucial for the accurate determination of the transition zone.

The procedure was performed as in the previous cases, with biopsies taken at closer intervals (3 cm) due to the potential for skip lesions. IHC staining using a panel of neuronal markers, including calretinin, Bcl2, and S100, identified ganglion cells in the proximal sigmoid colon. At the 1 month follow-up, the patient had satisfactory bowel function with three to four daily bowel movements.

In all three cases, the SI-ECo leveling biopsy technique accurately identified the ganglionic zone, allowing for precise planning of the definitive pull-through procedure. IHC staining on full-thickness biopsies offered superior diagnostic accuracy compared to traditional frozen sections.

Operative times for the SI-ECo leveling biopsy ranged from 45 to 60 minutes, with minimal blood loss (<10 mL) in all cases. No intraoperative complications were observed. The single-incision approach resulted in excellent cosmetic outcomes, with the umbilical scar barely visible at follow-up visits.

The definitive pull-through procedures were performed within 1-2 weeks of the leveling biopsies. Histopathological examination of the resected specimens confirmed the accuracy of the transition zone identification in all cases, with no instances of retained aganglionosis.

This case series demonstrates that SI-ECo leveling biopsy with immunohistochemistry is a safe and effective alternative to frozen sections in the preoperative management of Hirschsprung disease. The technique allows for accurately determining the transition zone, facilitating precise surgical planning, and potentially reducing the risk of postoperative complications related to retained aganglionosis. Moreover, the single-incision approach offers the benefits of minimally invasive surgery, including decreased postoperative pain and improved cosmesis.

DISCUSSION

The management of HD has evolved significantly over the past decade, with a trend towards minimally invasive approaches and single-stage procedures. Our study evaluates the effectiveness of Single Incision Laparoscopy-Assisted Extracorporeal (SI-ECo) leveling biopsy with immunohistochemistry as an alternative to frozen sections in the preoperative management of HD. The results demonstrate that SI-ECo leveling biopsy effectively identifies the ganglionic zone, providing a less invasive and precise method for preoperative planning.

The accuracy of intraoperative diagnosis is crucial for the successful management of HD. Traditionally, frozen sections have been used to determine the level of aganglionosis during surgery. However, several studies have reported discrepancies between frozen section results and permanent section findings.[2] In our case series, SI-ECo leveling biopsy with immunohistochemistry provided consistent and reliable results, aligning with the final histopathological diagnosis in all cases.

The use of immunohistochemistry (IHC) markers has significantly improved the accuracy of HD diagnosis. Calretinin, in particular, has emerged as a highly sensitive and specific marker for the presence of ganglion cells.[5] Our study utilized a panel of IHC markers, including calretinin, CD56, and S-100, showing high sensitivity and specificity in detecting ganglion cells. This approach allows for a more precise identification of the transition zone compared to traditional hematoxylin and eosin staining.

Single-incision laparoscopic surgery (SILS) has gained popularity in pediatric surgery due to its minimally invasive nature and improved cosmetic outcomes. The SI-ECo technique combines the benefits of SILS with the accuracy of extracorporeal biopsy and IHC analysis. This approach offers several advantages over conventional multi-port laparoscopy, including reduced postoperative pain, faster recovery, and better cosmesis.[1]

One of the critical benefits of SI-ECo leveling biopsy is the ability to obtain full-thickness biopsies, which are essential for the accurate diagnosis of HD. This is particularly important in ultrashort- segment HD or when dealing with the transition zone, where partial-thickness biopsies may lead to false-negative results.[1]

Using suspension sutures in our SI-ECo technique, as described by Tran et al., facilitates better exposure and simplifies the procedure. This modification addresses some technical challenges of single-incision laparoscopy in infants and small children, making the procedure more feasible and reproducible.

In resource-limited settings, where frozen section capabilities may not be available, SI-ECo leveling biopsy with IHC offers a practical alternative. This approach allows for accurate preoperative planning without requiring specialized intraoperative pathology services. Moreover, it potentially reduces the risk of retained aganglionosis, which can occur when relying solely on frozen sections.

The long-term outcomes of patients undergoing SI-ECo leveling biopsy followed by transanal endorectal pull-through (TEPT) are encouraging. Our case series demonstrated good functional results, with no anastomotic stricture or enterocolitis during the follow-up period. These findings are consistent with other studies reporting favorable outcomes for laparoscopic-assisted approaches in HD management.

Despite the promising results, our study has limitations. The small sample size and relatively short follow-up period necessitate further investigation with larger cohorts and longer-term follow-up. Additionally, a direct comparison with frozen section outcomes in a randomized controlled trial would provide more robust evidence for the superiority of SI-ECo leveling biopsy.

Future research should focus on standardizing the IHC panel for HD diagnosis and exploring the potential of novel markers to further improve diagnostic accuracy. The role of advanced imaging techniques, such as confocal laser endomicroscopy, in conjunction with SI-ECo biopsy, may also be worth investigating.

CONCLUSIONS

In conclusion, SI-ECo leveling biopsy with immunohistochemistry offers a practical, accurate, and less invasive alternative for diagnosing and managing Hirschsprung disease. It reduces the risk of discrepancies observed with frozen sections, making it a viable option for facilities without access to frozen section technology. As minimally invasive techniques evolve, this approach may become part of the standard of care in the preoperative management of HD, particularly in resource-limited settings.

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CONSENT STATEMENT

All patients involved in this study provided informed written consent. Their identities have been kept confidential throughout the study.

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One-step surgery for cyanotic heart disease with pectus excavatum: Should it be done?

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SUMMARY

Congenital heart disease accompanied with pectus excavatum is very rare, we present our experienced in one step surgery of pectus and cardiac repair and staged procedure at our institution. We retrospectively reviewed medical records for patients who underwent a hybrid repair of both pectus and CHD between 2022 and 2023 in RSUP dr. Sardjito, Indonesia. Of these patients, 2 patients had both pectus and CHD. The first patient was using one-staged produce operation began with ASD repair than the patient proceeded with pectus repair. During pectus repair, the patient experienced worsening hemodynamics, the total operation took time seven hours. During those seven hours, there was 1400 cc of bleeding. The patient's condition was stationary and worsened within 72 hours. The patient died three days after surgery due to sepsis, MODS, and hyperlactatemia. Meanwhile the second patient underwent two-staged produce showed good result. The total duration of the operation is half shorter than the one-step operation experience, bleeding during the operation also appears to be at least 300 cc. After surgery, the patient's condition was stable and the vital sign was satisfactory. The patient currently has no complaints, including no concerns about unstable hemodynamics caused by the pectus condition. The timing and approach to surgical correction of pectus excavatum in patients with congenital heart disease must be individualized. Factors such as age, anatomical development, cardiopulmonary status, and the complexity of the cardiac defect should guide the decision between single-stage and two-stage procedures. While early intervention carries the risk of recurrence due to ongoing growth, delayed or staged surgery may offer better long-term stability and outcomes, particularly in complex or adult cases.

KEYWORDS:
Pectus Excavatum, Congenital Heart Disease, One-stage repair, Two-stage repair, outcome

INTRODUCTION

Pectus excavatum (PE) is the most common congenital chest wall deformity, seen in up to 1 in 300–1,000 newborns, and predominantly affects males.[1] Meanwhile, congenital heart disease (CHD) affects about 9.4 per 1,000 live births, with acyanotic types like ASD, VSD, and PDA comprising the majority (57.9%) and cyanotic types such as TOF and TGA making up 8.2%.[2] Although both conditions are relatively common on their own, the coexistence of PE and CHD is rare. For instance, only 0.5% of CHD patients undergo PE repair, according to Hasegawa et al.[3] and just 0.17% in a major Boston study.[4]

Management of pectus excavatum (PE) includes a spectrum of approaches—ranging from pain control and conservative monitoring to surgical correction. Surgery is especially recommended for patients with severe chest wall deformities (Haller Index > 3.25), cardiopulmonary impairment, significant cosmetic concerns, or other related symptoms.[5-7] Two widely used surgical techniques include: The Ravitch procedure, a more invasive method that involves resecting the anterior costal cartilage and placing a mesh to support the chest wall.[8] The Nuss procedure, a minimally invasive technique in which a curved metal bar is inserted beneath the sternum to elevate the chest, typically left in place for 2 to 3 years.[9]

Historically, PE and CHD were treated through separate surgical procedures due to concerns over heightened risks of bleeding, infection, and anesthesia-related complications. However, recent studies have demonstrated the safety and success of combined surgical repairs, shifting this traditional paradigm.[10]

Despite these advancements, performing simultaneous repairs remains challenging. Procedures such as pectoral muscle flap elevation, costal cartilage resection, and sternal osteotomy—when combined with cardiopulmonary bypass (CPB) during CHD correction—can significantly increase the risk of intraoperative bleeding. Thus, although feasible, concomitant repair of PE and CHD requires careful planning and surgical expertise.[10]

Therefore, as a tertiary referral center in a developing country, we present our experience in the surgical management of patients with coexisting pectus excavatum

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and congenital heart disease. We performed single-stage repair in patients with acyanotic CHD and two-stage repair in those with cyanotic CHD, providing valuable insight into the safe and effective treatment of this rare and complex dual pathology.

CASE PRESENTATION

This case series features two female patients who have been diagnosed with congenital heart disease, and are scheduled for repair procedures to correct their cardiac anomalies. Notably, these patients exhibit the comorbidity of pectus excavatum, making them eligible for pectus correction. The study, conducted with ethical clearance number KE/FK/0256/EC/2024 from the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada, is a retrospective case series that includes a thorough examination of medical records for patients who underwent a hybrid repair of both pectus and CHD between 2022 and 2023.

Case 1

A 24-year-old female presents with a major complaint of shortness of breath, diagnosed with an atrial septal defect since the age of three months. Despite being urged to have heart surgery at the National Cardiovascular Center Harapan Kita Hospital at the time, she has not yet agreed to the procedure. Over the past year, she has had regular shortness of breath, fainting during intensive activities, and impaired growth and development. She was referred to our tertiary hospital after experiencing severe symptoms and is now receiving propranolol 3x10mg and furosemide 1x40mg. She denies a history of chest pain, cold sweats, swollen feet, heart attacks, heart failure, hypertension, diabetes, dyslipidemia, or family history. On examination, she is conscious and in good general condition, with normal vital signs. Physical examination reveals cardiomegaly, a wide fixed split S2 sound, and a continuous murmur in the sub valvular area. The laboratory results are normal, and chest X-ray findings confirm cardiomegaly and reveal right diaphragmatic eventration. Transthoracic echocardiography (TTE) reveals bidirectional shunt patent ductus arteriosus (PDA) with a diameter of 0.9-1.6cm, multiple left-to-right atrial septal defects (ASD) with diameters of 8mm and 6mm, severe pulmonary stenosis (PS), mild pulmonary regurgitation (PR), and mild tricuspid regurgitation (TR). Right heart catheterization (RHC) and contrast-enhanced cardiology computed tomography (CT) scan further confirm the echocardiography findings, which include no PDA, multiple left-to-right ASD with high flow (largest size 1.3cm), severe valvular PS, mild infundibular PS, proximal left pulmonary artery stenosis, dilated right atrium, and hypertrophic right ventricle. A thoracic CT scan also reveals pectus excavatum with a Haller index of 3.86. The patient undergoes corrective procedures, including ASD closure with transannular patch placement (cribriform ASD size 1cm x 0.5cm), correction of pulmonary stenosis with commissurotomy on fused cusps, widening of Right Ventricular Outflow Tract (RVOT), infundibulectomy, and reconstruction of pectus excavatum by cutting 1 cm of costal cartilage IX-XI and a modified Ravitch procedure with reconstruction plates and miniplates. Postoperative TTE shows an ejection fraction (EF) of 60%, Tricuspid Annular Plane Systolic Excursion (TAPSE) of 8mm, and a residual shunt of 0.8mm. The operation began with ASD repair, after weaning the first CPB with a duration of 88 minutes and Aox 57 minutes, the patient proceeded with pectus repair. During pectus repair, the patient experienced worsening hemodynamics, necessitating a repeat CPB with a duration of 23 minutes and Aox 21 minutes. The total operation took time seven hours. During those seven hours, there was 1400 cc of bleeding. After surgery, the patient was treated in the ICU during the post-operative period. The patient's condition was worsened within 72 hours. The laboratorium showed the finding of leukocytes up to 20.1, procalcitonin 7.26, and a trend of decreasing pH to 7.195 and increasing lactate from 2.9 up to 10.7 (hyperlactatemia). The patient underwent culture tracing, which did not grow germs in blood, phlegm, and urine. The urine results showing gross hematuria and albuminuria. The chest x-ray showed pneumonia, the patient's condition worsened after the vital sign was not improving with support. The patient died three days after surgery due to sepsis, MODS, and hyperlactatemia. The patient was hospitalized for five days.

Case 2

An 18-year-old female patient presented with a progressive onset of shortness of breath and fatigue, particularly during vigorous activities, over the past year. She reported using two pillows for sleeping daily but denied any interruption throughout the night. She was diagnosed with a congenital heart defect at the age of 7 months and reported cyanosis in her lips and nails, as well as shortness of breath and fatigue during excessive exercise. She had been treated at our tertiary hospital since she was toddler, and was taking propranolol 3x10 mg on a regular basis. As a student, she frequently tired during intensive activities but she denied episodes of coughing up blood, fainting, or chest pain in the past year, and she had not been hospitalized. She denies any history of diabetes, hyperthyroidism, hypertension, dyslipidemia, or a family history of similar conditions.

When the patient arrived at the emergency department complaining of shortness of breath, she was conscious and had normal vital signs except for an oxygen saturation of 80%. Physical examination revealed symmetrical chest movement, vesicular breath sounds, and the presence of pulmonary and mitral systolic murmurs with signs of cardiomegaly; clubbing of the fingers was also observed. Further evaluation through transthoracic echocardiography and right heart catheterization revealed a large bidirectional right-to-left shunt ventricular septal defect (VSD) measuring 1.2cm, along with a muscular VSD measuring 3cm. Hypertrophy of the right ventricle, overriding aorta, severe infundibular pulmonary stenosis, and the presence of PDA were also identified. Additional contrast-enhanced heart CT confirmed Tetralogy of Fallot (TOF) with misaligned VSD and pulmonary stenosis with collaterals. A thorax CT scan revealed the presence of pectus excavatum with a Haller index of 3.55.

The patient was scheduled for corrective surgery to treat the malalignment, which included TOF correction with VSD closure via a pericardial patch and infundibulecomy for

Table I: Surgical Data Comparison of Case 1 and 2

	Case 1	Case 2
Surgical management	Atrial septal defect closure with Ravitch procedure	Total correction without Pectus Excavatum repair
Aortic cross clamp time (min)	57 + 21	99
Cardiopulmonary bypass time (min)	88 + 23	124
Surgery time (min)	435	210
Intraoperative bleeding (cc)	1400cc	300cc

min: minute, cc: cubic centimeter

Patent Foramen Ovale (PFO) closure. Despite the high Haller index, no correction for pectus excavatum was undertaken. Postoperative TTE demonstrated improvement in both left and right ventricular function, with Left Ventricular Outflow Tract Diameter (LVOTd) measuring 15mm, Left Ventricular Outflow Tract Velocity Time Integral (LVOTVTi) measuring 14.6mm, Left Ventricular Internal Diastolic Diameter (LVIDd) measuring 30mm, EF of 70%, and TAPSE of 10mm. Based on experience of doing one step surgery resulting with the long duration of surgery and deceased patient outcome. This time, the patient was decided to undergo two step surgery which TOF repair was carried out first. Apart from the condition of the patient's haler index which was lower than the experience of the first patient, the patient underwent total correction surgery with CPB time 124 minutes and aox time 99 minutes. The total duration of the operation is half shorter than the one-step operation experience, bleeding during the operation also appears to be at least 300 cc. After surgery, the patient's condition was stable and the vital sign was satisfactory; laboratory evaluation revealed a modest rise in leukocytes up to 12.2 with normal pH and lactate 2.9; the patient spent 30 hours in the ICU and 23 hours on a ventilator. Patient was in the hospital for 8 days. Patient follow-up has been ongoing for up to 9 months following surgery. The patient currently has no complaints, including no concerns about unstable hemodynamics caused by the pectus condition. Comparative data from Case 1 and Case 2 are presented in Table I.

DISCUSSION

Correction of pectus excavatum is recommended when patients exhibit high Haller index and correction index values. Additionally, indications for surgery include excessive pain, cardiopulmonary dysfunction, exercise intolerance, and cosmetic concerns.[7] Patients are often encouraged to have at least in the mid-adolescent age range, when they have likely reached their maximum growth, to minimize the risk of recurrence.[7] Pectus surgery can be performed in both adult and pediatric populations, with several factor influencing the patient's clinical improvement benefit.1[1] Both of our patients are late adolescent, who match the repair requirement for their PE condition. However, both patients are females, and PE in females is less prevalent than in males.[1] Even more unusual, both patients have congenital heart disease, one with left-to-right shunt atrial septal defect (ASD) with pulmonary stenosis (PS), and the other with Tetralogy of Fallot (TOF). According to Liu et al., ASD account for 15.3% of all congenital heart disease (CHD) cases, whereas TOF has a prevalence of 4.4%.[2] Managing patients with both CHD and PE is challenging due to the rarity of such cases.

Several studies have reported extensive experience with combined surgical correction of cardiac defects and pectus deformities. In these reports, simultaneous procedures were commonly performed in patients with acyanotic congenital heart disease and in individuals with Marfan syndrome, yielding promising outcomes. However, for more complex cardiac anomalies, including tetralogy of Fallot (TOF), transposition of the great arteries (TGA), double outlet right ventricle (DORV), complete atrioventricular canal (CAVC), and hypoplastic right ventricle—a staged approach was preferred, with intracardiac repair performed prior to pectus correction to minimize surgical risk and optimize patient stability.[3,12-13]

A one-stage procedure is regarded safer since correcting pectus excavatum first might have detrimental effect on wound healing, particularly in patients with severe cyanosis. Additionally, if the pectus is not addressed, the compression might cause serious hemodynamic compromise. In contrast, if the cardiac lesion is repaired but the pectus is not treated, compression from the pectus might disrupt postoperative hemodynamics.[3] The advantages of simultaneous surgery include only one general anesthetic and a single hospital stay.[13] It also requires optimal operative exposure during the cardiac procedure while minimizing bleeding risk, as well as optimal chest-wall stabilization postoperative. Furthermore, concomitant pectus repair may be recommended because it improves cardiovascular function by facilitating heart filling, potentially improving postoperative outcomes.[3] Additionally, significant increases in right ventricular end-diastolic volume index (RVEDVI) and right ventricular stroke volume index (RVESVI) have been observed. However, the disadvantages of a one-stage procedure include longer surgical time, more blood loss, and inadequate exposure to the heart.[3]

Therefore, in the first case, we tried to perform one step concomitant surgery by starting with ASD correction and continuing with pectus correction. Considering that one step procedure currently shows more promising outcomes and are already recommended for patients with acyanotic CHD, we opted for a one-stage surgical approach. This decision was based on the generally favorable prognosis of acyanotic CHD and the fact that corrective open-heart surgery in these patients typically does not require prolonged CPB. Our intraoperative findings supported this approach, as the patient remained hemodynamically stable following atrial septal defect (ASD) repair. Based on our first patient experience, prolonged operative time and significant intraoperative bleeding led to postoperative instability, ultimately resulting in serious complications, including sepsis, multiple organ dysfunction syndrome (MODS), and hyperlactatemia.[14] These adverse outcomes were primarily

attributed to several factor such as excessive blood loss and prolonged CPB duration. Excessive blood loss, particularly during cardiac surgery involving CPB, may result in anemia, hypovolemia, and impaired tissue oxygenation. These conditions weaken immune function and increase the risk of infection and sepsis. Prolonged CPB duration is associated with systemic inflammatory response activation and disruption of immune homeostasis, further predisposing patients to sepsis. CPB also contributes to hemodilution and depletion of clotting factors, increasing bleeding risk and the likelihood of transfusion, both of which heighten infection susceptibility.[15]

Additionally, technical challenges such as limited exposure and difficulty in optimal placement of the sternal retractor are frequently encountered during simultaneous cardiac and chest wall corrective procedures, further complicating the surgical field. Some authors experiences stated that a vertical midline incision provides adequate exposure for intracardiac defects. If more exposure is required, a sternotomy is performed after resecting the deformed cartilage, leaving the intercostal muscle attached to the sternum.[3,13]

Some authors have utilized a one-step surgery approach.[3,12-13,16] This can involve the minimally invasive Nuss procedure or the modified Ravitch procedure, which is still considered as superior by some authors, particularly when combine with open cardiac surgery through a midline sternotomy.[3,13-14,16] We decide to repair pectus in the same way as the Ravitch modification approach. The modifications are intended to improve access due to the existence of PE, which displaces the heart posteriorly and results in inadequate cardiac exposure. These modifications include the parasternal approach,[3,13] sternal turnover,[18] and median sternotomy.[16-17] Early wide cartilage dissection during the incision, before fixing the cardiac defect, improves the stability of the sternal retractor, provides optimal exposure for cardiac surgery, and facilitates the subsequent pectus repair procedure.[13,18] Excessive blood loss after costal cartilage resection and postcardiotomy coagulopathy must be managed carefully since they might delay sternal healing. In our example, the patient's first operation resulted in significant bleeding up to 1400 cc, compared to the more complex TOF operation, which only had 300 cc. Therefore, some surgeons recommend a stepwise approach to PE and congenital heart repair.[19] Some surgeons prefer a two-step procedure due to concerns about excessive bleeding, sternal infections, or lengthy surgical hours.17 However, if the surgery involves a complex cardiac procedure with high morbidity, a two-stage approach with the heart surgery is performed first is recommended.[3,13]

This strategy helps to minimize complications and allows for better management of the patient's condition throughout the surgical process, as we did it for our second patient. The use of temporary retrosternal bars in several studies has shown better outcomes in pectus repair by providing superior sternum stability, reducing pain and pulmonary complications, and yielding favorable long-term results.[3,13,18,20] This sternal fixation typically requires 6-12 months for the costal cartilage to develop in correct position.[19] Hasegawa et al. used Kirschner wires to provide firm fixation of the sternum, which has been shown to facilitate earlier union with superior primary osseous healing.[3] Additionally, preserving both internal mammary arteries can improve sternal viability and healing.[19] In the case of the first patient, we used plates and miniplates to maintain the stability of the sternum after removal of the cartilage. Fixation using convex Nuss Bars has also been reported by Okamura et al.[22] The Nuss procedure has significant downsides when combined with or delayed for cardiac surgery and pectus repair.[17,19] These include interference with cardiopulmonary resuscitation and planning for staged repair, potential sternal dehiscence, heart injury, pericardial effusions, and tissue adhesions. Additionally, it has been shown to reduce blood flow in internal mammary arteries postoperative, limiting their suitability for coronary bypass grafting.[17,19]

The ideal age for pectus surgery remains controversial. In our situation, both patients were teenagers and young adults. At this age, bone growth had already stopped, reducing the complication of earlier surgery at a young age. According to Fonkalsrud et al. the Ravitch procedure can be performed to correct pectus in children, as young as 3-6 years old. This timing is technically easier and takes less time than performing the surgery during adolescence and adulthood.[20] On the contrary, some authors believe that operating before the age of 4 or before growth completion increases the risk of recurrent sternal depression. If surgery is required, limit the amount of cartilage resected and avoid suturing the perichondral sheaths behind the sternum. Hysi et al. recommend doing two-step surgery on young patients, postponing the pectus correction until after the second growth spurt. However, in adults, they prefer concurrent surgery.[23] In summary, the timing and approach to surgical correction of pectus excavatum in patients with congenital heart disease must be individualized. Factors such as age, anatomical development, cardiopulmonary status, and the complexity of the cardiac defect should guide the decision between single-stage and two-stage procedures. While early intervention carries the risk of recurrence due to ongoing growth, delayed or staged surgery may offer better long-term stability and outcomes, particularly in complex or adult cases.

A limitation of this study is the small number of cases of patients with PE and CHD in our center. Therefore, we could only report two patients with both PE and CHD. Our study conducted using retrospective descriptive review. We treated patients according to the standard hospital service and the clinical presentation of the patient, so there was no intervention or control patients in our study.

CONCLUSIONS

The decision to perform a one-stage or two-stage procedure must be made carefully, as even patients with acyanotic CHD may present with complicating factors. For instance, in our first case, although the patient had an acyanotic defect, the individual presented in adulthood, by which time significant anatomical changes of the heart and pulmonary hypertension had already developed. These factors made the surgery more complex despite the acyanotic nature of the disease.

Therefore, we recommend that a two-stage procedure should still be considered for acyanotic patients who present with complicating factors, such as delayed diagnosis, structural cardiac changes, or pulmonary hypertension. In general, patients with complex cardiac conditions, regardless of cyanotic status, are better suited for a two-stage approach to reduce operative risks and improve outcomes.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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CASE REPORT

The migration of atrial septal occluder device, is it fatal?

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SUMMARY

Atrial septal defect (ASD) is a defect between right and left atrium. Nowadays, percutaneous closure using an atrial septal occluder (ASO) device is the preferred treatment for secundum ASDs due to its minimal invasiveness and high success rates. Although rare, device dislodgement can occur and may require surgical retrieval. This retrospective case series from RSUP Dr. Sardjito Hospital (January 2022–July 2024) reports three paediatric cases of failed ASD closure due to ASO dislodgement. The patients were aged 9, 12, and 13 years. Dislodgement occurred three years post-implantation in one patient, two months post-implantation in another, and intraoperatively in the third. Migration sites included the left atrium and right ventricle. Surgical removal and patch closure were performed in all cases. Cross-clamp times ranged from 20–24 minutes and cardiopulmonary bypass times from 34–46 minutes. All patients successfully recovered and were discharged without residual defects or further complications. Possible causes of ASO dislodgement include undersized devices, large defects, poor rim support, and procedural factors. Despite the children's relatively normal body weights, improper anchoring or anatomical limitations may have contributed to the device failure. While percutaneous retrieval is possible, chronic dislodgements are best managed surgically. No major complications, such as rupture or embolism, occurred postoperatively. In conclusion, although ASO dislodgement is rare, timely surgical intervention is essential. Since asymptomatic cases may remain undiagnosed, regular follow-up is crucial to detect delayed dislodgement.

KEYWORDS:
Atrial septal defect, dislodgement, embolism

INTRODUCTION

Continuing previous research by Ambarsari et al., titled 'Atrial Septal Defect Occluder Devices and Embolization – A Case Series,' published in the Bali Medical Journal in 2023. The atrial septal defect (ASD) is one of the most prevalent congenital heart diseases (CHDs).[1] ASD allows for the shunting of oxygenated blood from the left atrium to the right atrium, which can lead to significant consequences, including embolism, pulmonary stenosis, cardiomyopathy and heart failure if not treated properly and promptly. It may be divided into several types: ASD primum (ASD I), ASD Secundum (ASD II), sinus venosus, and coronary sinus ASD.[2] A small defect less than 3 mm will usually close naturally. While ASD with diameter 3 to 8 mm frequently can be closed in childhood, the large defects frequently fail to close spontaneously.[3] Furthermore, because an ASD is typically asymptomatic and has quiet murmurs, these CHD problems frequently fail to result in an early diagnosis or referral.[1] Over the last few decades, the paradigm for treating ASD has shifted toward less invasive interventions. Techniques and equipment for transcatheter treatment have been improved and refined. ASD closure using a percutaneous device has become a popular choice because it reduces the risk of surgery and decrease length of stay in hospital.[4]

The use of transcatheter percutaneous device closure in ASD is now acknowledged as the main choice for majority of patients with secundum ASD, demonstrating great efficacy and lower complication rates than open surgery. A recent study in 2023 showed a 98% success rate in 1,395 patients and a 97.3% complete closure rate at 1 year. For 2.7 years, only 8 patients who complained of problems had significant complications.[5] Dislodgement and embolism frequently occur during the first several hours following an occluder device insertion, with reported rates ranging from 0.5 to 1.1%. Only a few studies have been conducted to determine the late incidence dislodgement of these closure devices. Retrieval of the dislodged device is necessary in order to prevent further complications such as arterial and valvular obstruction or damage and usually requires surgery.[2]

More problematic complications are associated with small children, technique, fluoroscopy time, and large defects. Many devices can only be utilized for defects up to 20 mm in diameter. Larger defects with a diameter of 20 or 25 mm necessitate a stable device position and in certain cases "oversizing" for proper placement, which may necessitate lengthy procedures including an invasive thoracotomy, full or median sternotomy and an open-heart surgical approach. Additionally, the fluoroscopy time is linked with the ASD complexity.[5]

Residual shunts, stenosis, embolism, device-related thrombosis, cardiac erosion and perforation, infective endocarditis, and sudden death are the most prevalent complications linked with ASD closure devices. Adding to these potentially fatal sequelae, as mentioned before, while patients with smaller ASD rarely show any symptoms, several patients with a dislodged Atrial Septal Occluder (ASO) were

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Table I: Comparison of the Patient Characteristics

Name	Patient 1	Patient 2	Patient 3
Age (years)	9	12	13
Body Weight (kg)	33.2	34.7	36.5
Height (cm)	129	147	149
Sex	female	Male	Female
Type of Defect	ASD II	ASD II	ASD II
Onset	3 years post-intervention	2 months post-intervention	During the intervention
Symptoms	Shortness of breath	Asymptomatic	Palpitations
Echocardiography finding	ASD II, L to R, mild PH	ASD II, TR mild	ASD II
Defect area (cm2)	6	2	3
Site of Dislodgment	Left atrium	Right ventricle	Right ventricle
Cross Clamp time (minute)	23	20	24
Cardiopulmonary bypass time (minute)	46	38	34
LOS (days)	7	6	5

ASD: atrial septal defect, cm: centimetres, LOS: length of stay, L to R: left-to-right shunt, kg: kilogram, min: minutes, PH: pulmonary hypertension, TR: tricuspid regurgitation, ASD II: Atrial Septal Defect type secundum.

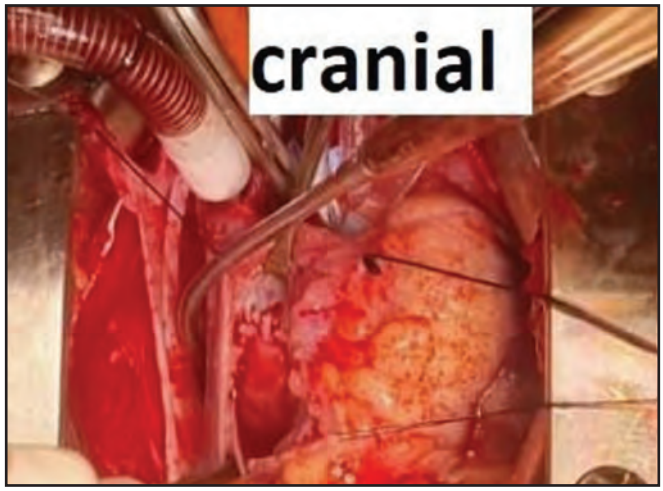

Fig. 1: Atrial septal defect (ASD) closure using an autologous pericardial patch from the first patient

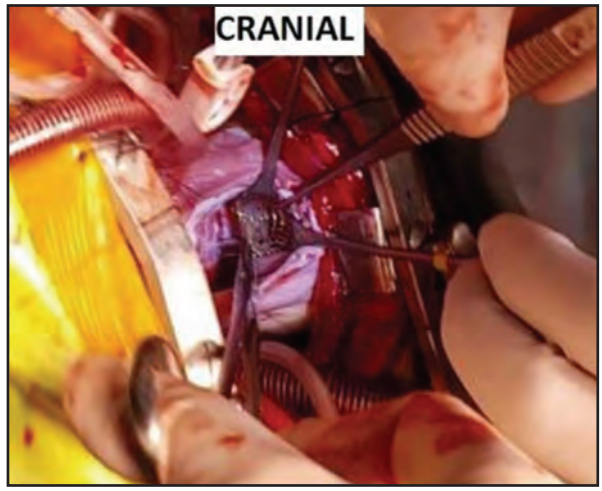

Fig. 2: The dislodgment of the occluder from the second patient

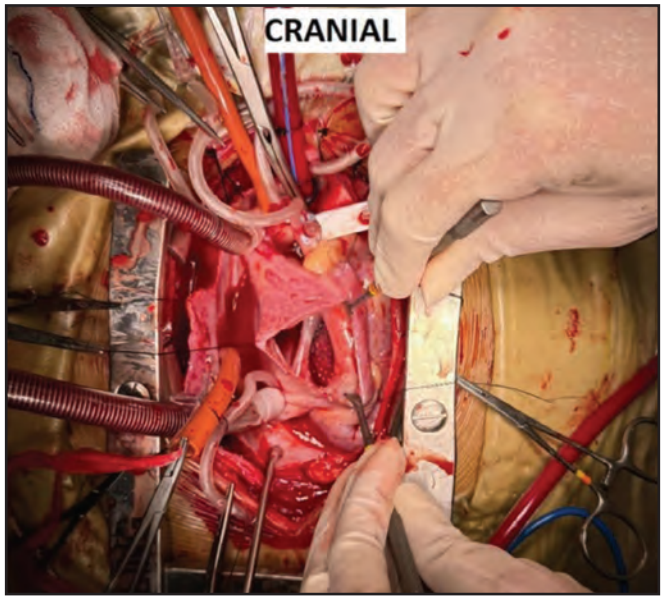

Fig. 3: The dislodgment of the occluder near tricuspid valve from the third patient

also asymptomatic and as a result, they were not recognized to have a potentially life-threatening complication before coming to a hospital with a serious condition.[5] Accordingly, heart failures directly related to complications from an ASD closure using occluder devices have been seldom reported. Hence, in this case series, we report several unsuccessful closure cases using ASO devices which could have been potentially fatal without invasive surgical interventions.

CASE PRESENTATION

This study is a retrospective case series that involved a thorough examination of medical records for patients with ASD who were unsuccessfully closed using an ASO device between January 2022 until July 2024 in RSUP Dr. Sardjito Hospital. This case series reports three patients who were diagnosed with ASD and experienced unsuccessful ASD closure using ASO devices.

Three patients came to our hospital. The first patient complained of difficulty to breath (dyspnea), the second patient had no symptoms (asymptomatic), while the third

patient planned to undergo ASD closure using an ASO device.

Case 1

The first case, female 9-year-old girl had an ASO placement. Three years after the placement, she complained difficulty of breathing. Physical examination revealed that the patient had an increasing respiratory rate and heart beat with normal oxygen saturation. First heart sound and second heart sound were in normal limit with an intermittent split. The radiography examination revealed the migration of an occluder still in atrium. The echocardiography findings are summarized in table I. The operation finding was an ASD II with an area 6 cm2 with a device migration and moving to the right lower Pulmonary Vein (PV). We evacuated the device and followed by ASD closure using an autologous pericardial patch (Figure 1). Follow-up echocardiographic examination revealed no ASD, and the patient had no complaints of dyspnea.

Case 2

The second case, male 12-year-old had an ASO intervention. Two months later, during a routine follow-up visit, transthoracic echocardiography revealed that the occluder device had dislodged and migrated into the right ventricle. The patient remained hemodynamically stable and asymptomatic at the time of discovery. Heart sound demonstrated an intermittent split and pansystolic murmur. The radiology examination revealed the occluder misplaced at the level of Right Ventricle (RV). The echocardiography findings are summarized in table I. Given the risk of further complications, surgical intervention which is ASD closure using autologous patch was planned to retrieve the device and reassess the atrial septal defect for definitive closure. Intraoperative findings revealed an ASD II with an area 2 cm2 and dislodged device in the right ventricle (Figure 2). There was some minimal injury of the Tricuspid Valve (TV) with no rupture was found. Following the operation, the patient remained asymptomatic during the postoperative evaluation with no residual ASD was found in echocardiography evaluation. No health concerns were noted at subsequent follow-up visits.

Case 3

The third case, Female 13-year-old girl complained heart palpitation who was planned to have ASD closure using a percutaneous device. Physical examination revealed that the patient was stable with normal vital sign. We found normal first heart sound, while the second heart sound was accompanied by a systolic ejection murmur. During the procedure, the right and left heart catheterization showed an unsuccessful ASO device replacement, we found an ASD II and the device had escaped into the RV. Thus, the decision was made to perform open surgery on the patient because the operator had failed to replace it correctly. The operation finding was a secundum ASD with an area 3 cm2 and an occluder near the TV of the right ventricle (Figure 3). The evacuated occluder device looked to be undamaged and followed ASD closure using autologous patch. There was no rupture nor heart injury found. Postoperatively, the patient had no complained and echocardiographic evaluation showed no evidence of a residual ASD. Table I presents a comparison of the patient characteristics of the cases.

DISCUSSION

The complications associated with percutaneous ASD closure are diverse and may include vascular trauma, air embolism, device embolization, thromboembolism, venous return blockage, atrial septal damage, aortic perforation, and infective endocarditis. Among these potentially life-threatening outcomes, perforation is considered the most serious and feared complication.[5]

Dislodge of ASO is a rare complication. We only reported three patients with ASO migration in our centre during January 2022 - July 2024. The most common causes for ASO closure complications are undersized devices, small children, large defect sizes, small left atrial, and an inadequate rim.6 In our study we report all the patients were children under 14 years old with the body weight range from 33.2 kg – 36.5 kg. Even though this weight is not defined as small children (BW <15kg)[5], there were several factors such as the operator skill and device patency, which could lead to the unsuccessful ASO placement.

The removal of a dislodged ASO may be achieved via either invasive surgical intervention or percutaneous intervention. Surgical extraction is generally preferred in cases of chronic device dislodgement, particularly when identified several years post-implantation or when associated with right-sided heart failure.[6] In our study we reported two patients with chronic dislodgement but without involving any right heart failure because all of the occluders removed did not create significant blockage of the arterial blood flow. The displacement of ASO may cause mispositioning in several places. The most frequent location is in the RV and PA. As mentioned before, we reported 2 of our 3 patients had mispositioning in the RV near the TV.

Most of these situations need surgical interventions to prevent serious complications.7 Occluder migration into the RV does not always produce clinical symptoms, particularly when pulmonary artery flow remains unobstructed. However, if blood flow to the pulmonary circulation is compromised, the resulting volume overload may precipitate right ventricular failure or trigger arrhythmias.[7-8] In our cases, device migration produced differing outcomes: the first patient experienced dyspnea due to left-sided device displacement, while the second patient remained clinically stable, likely due to the absence of hemodynamic compromise and preserved RV function. Considering the chronicity of the device embolization, we opted against percutaneous retrieval and favoured surgical management. Early intervention in the management of atrial septal defect device dislodgement plays a critical role in ensuring optimal outcomes and preventing complicated sequelae.

CONCLUSIONS

The incidence of ASO migration is considered infrequent because there were only three patients reported between January 2022-July 2024 in our centre. In this study, the three patients did not have any immediate life-threatening conditions such as unstable vital signs due to ASO device displacement. But once found, the patients needed to undergo surgery to remove the device and close the residual defect, to prevent heart deterioration, rupture, or

embolization. Furthermore, surgery to retrieve the devices as soon as possible has a low risk of morbidity and mortality. Complications of ASO device migration in this study did not show any significant results because there was no rupture during open heart surgery due to the device. All the patients were discharged without any health complaints. In our study, we did not follow-up all of the patients with percutaneous placed ASO devices. It is still possible that some patients were having dislodgment of their installed occluder but their condition was underdiagnosed.

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CONSENT STATEMENT

All patients involved in this study provided informed written consent. Their identities have been kept confidential throughout the study.

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12. Dr Mawaddah Rochmah
13. Dr Mohamed Mohamed
14. Dr Mohd Yusof Abdullah
15. Dr Mohd Shahrulsalam Mohd Shah
16. Dr Muhammad Fadli Abdullah
17. Dr Mustofa Mustofa
18. Dr San Thitsa Aung
19. Dr Sandhya Aruljoethy
20. Dr Sanihah Abdul Halim
21. Dr Syukri Ahmad Zubaidi
22. Dr Tan Hui Siu
23. Prof Dr Thameen Ansari
24. Dr Thiyagar Thiyagar
25. Dr Trifosa Stella Chowindra
26. Dr Vasu Pillai A/L Letchumanan
27. Dr Wee Yan Chia
28. Dr Yoon Khee Hon