ORIGINAL ARTICLE

8-Hydroxy-2′-Deoxiguanosine (8-OHdG) as a reliable biomarker for rheumatoid arthritis

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune, destructive inflammatory synovitis that ultimately affects metabolic function, the vascular system, and systemic bone. Many researchers have demonstrated that free radicals induce oxidative stress and are involved in several disease conditions. It was observed that a physiological equilibrium between oxidants and antioxidants is being disrupted by oxidative stress. Free radicals are molecules, ions, or atoms with unpaired electrons. These elements tend to be stabilized. For stabilization, they attack other molecules to get electrons. In the cells, this behavior can cause mutations or even cellular death. This study aimed to evaluate the oxidative stress marker 8-Hydroxy-2-Deoxiguanosine (8-OHdG) as a biomarker for Iraqi RA patients.

Materials and Methods: The estimation of the plasma level of this marker was achieved in human RA patients using ELISA. The current study included 132 adult individuals who were diagnosed as RA patients with joint complications, while the remaining 66 were healthy and served as controls. Blood plasma levels of 8-OHdG were estimated using a commercial ELISA kit. The resultant solution was washed after being incubated, and then a conjugate enzyme was added; finally, the results were read using an ELISA reader at 450nm.

Results: A significant increase in plasma concentration of 8-OHdG was detected in the disease group. The most significant increase in this biomarker (p = 0.001) appeared in RA patients in comparison with the healthy subjects. The significant increase of inflammatory and oxidative biomarkers highlighted in this study might point to a relationship between these biomarkers and the pathogenesis and progression of rheumatoid arthritis in adult subjects.

Conclusion: This study suggests the role of 8-OHdG as an early diagnostic marker of RA. Because 8-OHdG is a strong indicator of oxidative stress-related DNA damage, this result may have significant clinical implications.

KEYWORDS:
8-Hydroxy-2′-Deoxiguanosine (8-OHdG), Rheumatoid Arthritis, Biomarker

INTRODUCTION

Rheumatoid arthritis (RA) is one of the leading causes of disability and labor loss, a chronic autoimmune disease known for its tissue-destructive effects and unresolved synovial inflammation. It impacts between 0.2–1% of the population worldwide.[1-2] In RA, a symptomatic clinical phase after the beginning of systemic immune dysregulation in mucosal surfaces was recognized as the end of the multi-year prodromal phase. Besides causing pain and articular degeneration, the immune reactivity limits itself to the synovium and links to a wider range of comorbidities.[3-4] Persistent inflammatory synovitis, which typically affects peripheral joints in a symmetric distribution, serves as the hallmark of established RA.[5] Oxygen metabolism has an important role in the pathogenesis of many diseases and RA is one of these diseases.[6]

During normal physiological processes, reactive oxygen species (ROS) are molecules that occur when a cell is not under stress. It was detected that 98% of the respired oxygen within the electron transport chain is utilized by mitochondria. This is used to generate adenosine triphosphate (ATP).[7-8] On the other hand, in the course of cellular oxidative phosphorylation, these molecules are produced by activated phagocytic cells during oxidative bursts. When these molecules exceed a physiological buffering capacity, oxidative stress will occur, and ROS will be formed. High levels of production of ROS can damage different cellular elements like proteins, lipids, matrix components and nucleic acids.[9] Furthermore, to amplify a synovial inflammatory–proliferative response, ROS serve as crucial intracellular signaling molecules. Additionally, ROS are linked to many ageing-related disorders and are held responsible for the loss of the structure and function of proteins.[10]

The activation of the two key transcription factors, nuclear factor-κB and hypoxia-inducible factor-1α, is regulated by changes in cytokine stimulation and cellular oxygenation. These two factors are thought to be activated by the recurring cycles of hypoxia and reoxygenation linked to variations in synovial perfusion.[11-12] Furthermore, a variety of ROS molecules, including peroxynitrate, hydrogen peroxide, hydroxyl radicals, superoxide anion radicals and singlet oxygen, can oxidize nucleic acids or their constituent parts to produce a variety of precursors. As a result, the sugar moiety and heterocyclic DNA bases react strongly with the hydroxyl

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radical near or at diffusion-controlled.[13] In recent years, many researchers have confirmed that oxidative stress contributes to many chronic inflammatory conditions.[14] So, understanding the body's oxidative state is quite helpful. Thus, many oxidative stress biomarkers have been measured. Within a living organism, 8-Hydroxyguanine (8-OHGua, the base moiety of 8-OHdG) is a molecule produced as the result of an oxidation of guanine base by ROS.[15] When oxygen starts to damage cellular DNA, free radicals are generated during exposure to environmental oxidants, phagocytosis, cellular respiration, and cell injury.[16] Quanine bases in DNA are the main goal for ROS attack to form 8-hydroxydeoxyguanosine (8-OHdG). Rather than cytosine, this molecule can bind to thymidine. Based on this, the level of 8-OHdG is generally regarded as a biomarker of mutagenesis consequent to oxidative stress.[17]

At the C-8 site, 8-OHdG a product of DNA modification produced by the oxidation of deoxyguanosine, is considered the most sensitive and useful biomarker of oxidative DNA damage.[18] Oxygen derivatives that are commonly linked to RA include lipid peroxides (LOOH), lipid alkoxyl radicals (LOO-), lipid peroxyl radicals (LO-), hydroxyl free radicals (H2O2), and superoxide free radical anions (O2-). In the intense inflammatory environment, these free radicals (LOOH, LOO-, LO-, H2O2 and O2-) are excessively produced by the joints affected in RA patients.[19] One of the most prominent products of oxidative DNA damage is 8-OHdG, which was recently used as a reliable and sensitive marker of oxidative stress.[20] The oxidation potential of the guanine base is the lowest when compared to other bases.[13] This leads to the formation of 8-oxoguanosine (8oxoG) and 8-oxodeoxyguanosine (8oxodG).[21] Thus, the molecule, which is more susceptible to free radical attack, quinine, supports the formation of the 8-OHdG molecule.

Scientific researchers have focused their attention on this molecule, which is a common choice as an oxidative stress biomarker. This molecule indicates DNA damage (8-OHdG residues), which results in transversion mutation by partnering with cytosine or adenine during the replication process (GC to TA).[22] The effect of 8-OHdG is to stimulate the transversion of G-->T, which are among the most frequent somatic mutations.[23] One of the predominant forms of free radical-induced oxidative lesions that occur in nuclear and mitochondrial DNA is 8-OHdG or 8-oxodG. Thus, it has been widely used as a biomarker for oxidative stress and carcinogenesis. To mitigate the harmful effects of oxidative stress-induced DNA lesions, an efficient process removes most of 8-oxoA before the next round of replication. But, the half-life of an 8-oxoG lesion is 4.6 times longer than that of an 8-oxoA lesion induced by H2O2.[24] Thus, the biological significance of alterations in 8-OHdG levels has been investigated in many types of diseases.[25-26]

For more accurate diagnosis and monitoring of RA, many biomarkers, including symptoms, patient-reported measures and laboratory measurements have been used. However, there is no current gold standard measurement because each measure varies in strengths and weaknesses. So, no single 'best' measure of disease activity could be recommended. Protein biomarkers might provide reliable measurements reflecting pathophysiological status. However, erythrocyte sedimentation rate ESR and CRP, incorporated into clinical disease activity measures such as the Disease Activity Score (DAS) and Simplified Disease Activity Index (SDAI), are non-specific indicators because these biomarkers are influenced by age, anemia and the presence of immunoglobulins.[27] Anti-CCP can be used in the diagnosis and prediction of RA especially in recurrence cases. MBDA correlates with and might be a predictor of response to treatment.[28]

Anti-MCV may be used but with anti-CCP and RF negative. Other biomarkers, such as vascular endothelial growth factor-A (VEGF-A) and matrix metalloproteinase 3 (MMP3), are also reported to be correlated with disease activity.[29] Many studies concluded that 8-OHdG is a reliable and promising biomarker of oxidative stress and a valid predictor of functional outcomes in patients.[30-32] Saeed et al. found such a relationship in RA patients.[33] Despite the fact that oxidative stress has been extensively studied in the etiology of RA, it has been established that patients with RA exhibit a much stronger link with their plasma 8-OHdG levels.[34] Some studies have recently employed urinary 8-OHdG as a biomarker for measuring endogenous oxidative DNA damage, as well as for determining the risk of disease development and accelerated aging. Researchers consider it a good biomarker for assessing the risk of various cancers and degenerative diseases.[35]

MATERIALS AND METHODS

This study included 132 individuals (108 females and 24 males) were diagnosed with RA and 66 were healthy subjects (as control). Patients were examined and diagnosed as having definite RA according to the ACR/EULAR 2010 criteria by a specialist physician at Medical City of Baghdad. In addition to the ethical approval of the Baghdad Research Ethics Committee, informed consent was obtained from all participants. The selection of healthy controls was based on the absence of any congenital deformity, malignancy, or infectious diseases such as hepatitis C (HCV) or hepatitis B (HBV), acquired immune deficiency syndrome (AIDS).

In addition, no detection of inflammatory arthritis was made in the blood samples of the control subjects. Whereas, the subjects with a history of antibiotic therapy, corticosteroid drugs and non-steroidal anti-inflammatory drugs in the last three months were not included in this study. Furthermore, the other excluded group was individuals with any history of liver disease, cardiovascular disease, hypertension or diabetes. Smokers were also excluded from this study.

For this investigation, informed consent was obtained from each patient. All Samples were collected during the period between November 2022 and June 2024. The sera were conserved in a freezer at -20 0C until use. 8-OHdG was estimated in the sera of patients using the ELISA kit, Elabscience®, USA. According to the kit protocol, standards were prepared and followed by incubation, washing, conjugate enzyme addition, and the result was read at 450 nm. Furthermore, some blood parameters were detected, which included: white blood cells (WBC), hemoglobin (Hb), and erythrocyte sedimentation rate (ESR).

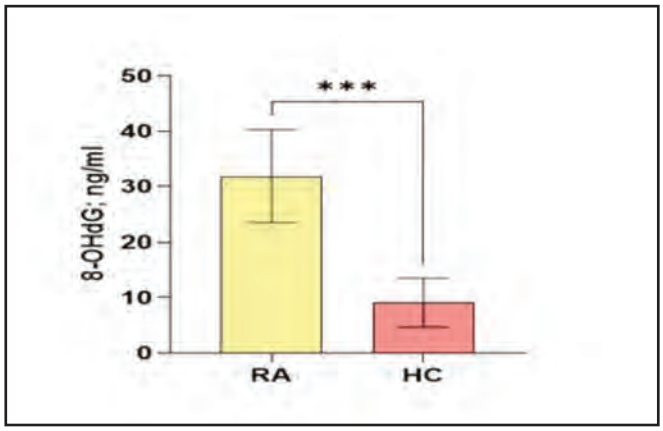

Fig. 1: Level of 8-OHdG in rheumatoid arthritis patient's sera. RA (n=66) and healthy controls HC(n=66). Values are given as mean and standard deviation, 31.9 ± 8.4 vs 9.1 ± 4.4 ng/ml; (*** p < 0.001)

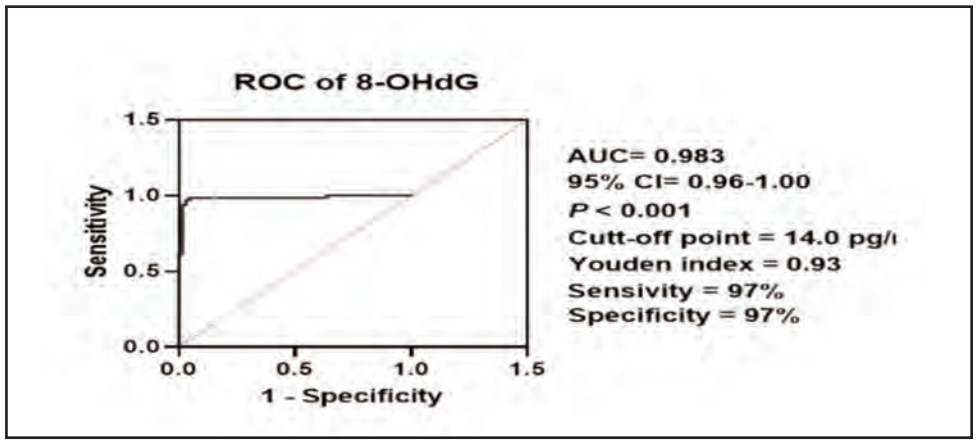

Fig. 2: Receiver operating characteristic (ROC) curve analysis. 8-OHdG in rheumatoid arthritis patients (n=66) versus healthy controls (n-66). The analysis demonstrated a high discriminatory power of 8-OHdG between patients and controls

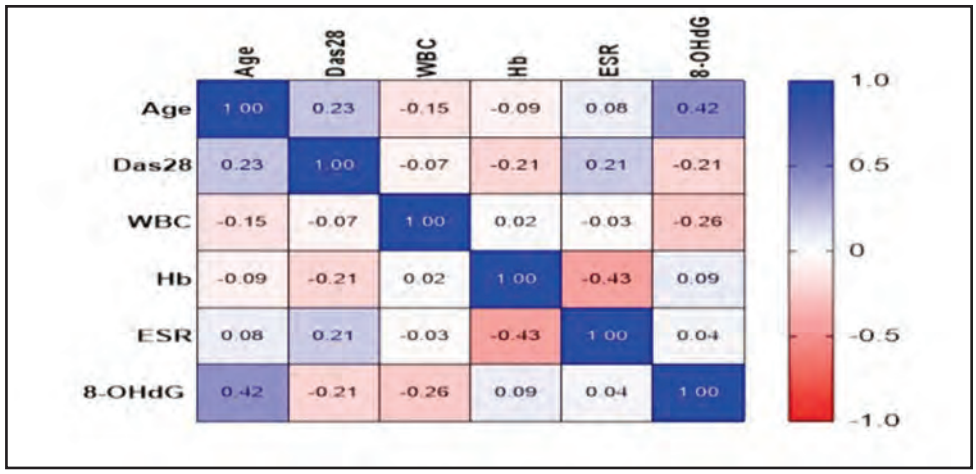

Fig. 3: Heat map matrix of correlation analysis baseline laboratory data in patients with RA. Values inside boxes indicate the correlation coefficient (rSp). The blue color indicates a positive correlation, while the red color indicates a negative correlation

Statistical analysis

The excel sheets had all of the readings, and SPSS v.16 was used to perform the statistics. The results were significant when the mean ± standard deviation ($p < 0.05$). Tests used included the Spearman correlation graph and the independent t-test.

RESULTS

Plasma level of 8-OHdG in RA patients is considered a critical biomarker of oxidative stress. The relationship between 8-OHdG level and RA was confirmed in this study. 8-OHdG measurement was achieved by the colorimetric method (ELISA Kit). The sensitivity range of this test was approximately 0.94-60 ng/ml with an assay time of two hours.

In this study, an analysis of samples aspirated from 66 RA patients revealed a significantly elevated concentration of 8-OHdG ($p = 0.001$). The levels of 8-OHdG were significantly high (31.9 ± 8.4) ng/ml at $p < 0.001$ compared with healthy controls (9.1 ± 4.4) ng/ml at $p < 0.001$ (Figure 1). High discriminatory power of 8-OHdG was shown between patients and controls (Figure 2). Some hematological parameters were studied in both patients and healthy groups in this work to explore the relationship between 8-OHdG and these parameters in RA (Figure 3). The heat map matrix of correlation analysis between baseline laboratory data in patients revealed that there was a correlation between 8-OHdG and the age of patients, but a weak link was shown with the Disease Activity Score 28 (Das28).

DISCUSSION

RA is believed to be one of the main global chronic inflammatory diseases. Its impact is estimated to be 0.5-1.0% of the world's population.[33] Our study demonstrated that a serum level of 8-OHdG is significantly elevated in patients with RA in comparison to healthy controls. Because 8-OHdG is the main oxidized product resulting from DNA damage, the results of this study pointed increase in oxidative DNA deterioration in RA patients, which may take part in the pathogenesis of the disease. The high 8-OHdG levels in patients with RA could be connected to the hyperactivation of immune cells, increased production of pro-inflammatory cytokines, and mitochondrial dysfunction. Also, continual oxidative stress may take part in DNA damage, cellular dysfunction, and apoptosis, triggering synovial inflammation and the destruction of the joint.[35] The ROC (Receiver Operating Characteristic) curve analysis for 8-OHdG in this study estimates its effectiveness in differentiating RA patients from healthy controls. The results suggest that 8-OHdG might be a highly dependable biomarker for the diagnosis of RA, with minimal false positives and false-negative results. The 97% sensitivity and 97% specificity contribute to its high accuracy; as a result, this biomarker could be used for early monitoring of disease progression. The heatmap matrix of correlation analysis between baseline laboratory data in patients with RA indicates correlation coefficients (p-value). 8-OHdG shows a positive correlation with age ($p=0.42$), which suggests that oxidative stress is increased with aging in RA patients. On the other hand, it shows a weak negative correlation with DAS28 ($p=0.21$), suggesting that oxidative stress may not strongly align with clinical disease activity. Finally, it indicates no significant correlation with inflammation markers (ESR, WBC) ($p=0.43$), indicating that 8-OHdG might be more linked to oxidative stress mechanisms than the traditional inflammatory pathway.[36]

CONCLUSION

In this study, selected RA patients' plasma levels revealed elevated levels of 8-OHdG, indicating oxidative DNA damage when compared with the plasma levels of healthy subjects. So, it appears there is a correlation with the pathogenesis and intense inflammatory damage to the joints of patients with RA. Thus, 8-OHdG might be a useful biomarker to monitor RA activity.

CONFLICT OF INTEREST

The authors declare that there is no actual or potential conflict of interest in relation to this article.

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