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Evaluation of 8-Oxoguanine DNA Glycosylase-1(OGG1) Serum Levels in Patients with Type2 Diabetes Mellitus

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ABSTRACT: Chronic hyperglycemia in type 2 diabetes mellitus leads to elevated oxidative stress. As a consequence, the accumulation of reactive oxygen species (ROS)may cause additional damage to various biological macromolecules, including DNA. The current study aims to evaluate the DNA damage in type2 diabetic patients from Wasit Province by estimating the levels of 8-OHdG using ELISA. All samples were collected from the local community of Wasit province, Iraq. Forty-five type 2 diabetes mellitus patients (22 males and 23 females) and 35 healthy controls (17 males and 18 females) were genotyped for *8-oxoguanine DNA glycosylase-1(OGG1)*. Determination of 8-oxoguanine DNA glycosylase-1(OGG1) in sera of patients with T2DM and controls was done by using an Enzyme-linked immunosorbent assay ELISA.The results reveal highly significant differences, the OGG1 levels in the diabetic patients were higher than that of controls (646.96±2.14), controls (326.01±16.66), *P*= 0.0001. The OGG1 levels were elevated significantly among males and female's patients withT2DM compared to the control group, 627.02±66.31 versus 679.68±63.27), *P*= 0.01 (381.85±29.33 versus 355.44±31.45), P = 0.0006, respectively. No significant differences were observed when comparing OGG1 levels between male and female patients with type2 diabetes, as well as in the control group: 627.03±66.31 versus 679.68±63.27, *P*=0.5974;381.85±29.36 vs.355.44±31.45, *P*=0.5478 in patients and controls respectively.679.68267±63.27413 versus627.02830±66.29612 with a non-significant difference *P*=0.5974. In conclusion levels of serum OGG1 are associated with diabetes mellitus

Keywords: 8-oxoguanine DNA glycosylase-1(OGG1), type2 diabetes mellitus

1. INTRODUCTION.

Type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insulin resistance. One of the key factors contributing to the pathogenesis of type 2 diabetes is oxidative stress, which leads to DNA damage. Studies have shown that type 2 diabetic patients exhibit elevated levels of oxidative DNA damage, as evidenced by increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Al-Aubaidy & Jelinek, 2011). This oxidative DNA damage is primarily repaired through the base excision repair pathway, with 8-oxoguanine DNA glycosylase-1 (OGG1) playing a crucial role in initiating the repair process (Pan etal., 2016). OGG1 is an enzyme that specifically recognizes and excises 8-oxoguanine (8-oxoG), one of the most abundant base lesions induced by oxidative stress in DNA (Visnes etal., 2018). The repair of 8-oxoG by OGG1 is essential for maintaining genomic integrity and preventing mutagenesis. Studies have indicated that OGG1 deficiency can exacerbate DNA damage and lead to various pathological conditions, such as cardiac dysfunction (Anene-Nzelu etal., 2022). Furthermore, research has highlighted the association between oxidative DNA damage and diabetic complications, such as nephropathy and retinopathy (Goodarzi etal.,

2010). The extent of DNA damage in diabetic patients has been evaluated using various assays, including the Comet assay, which measures DNA strand breaks as a marker of oxidative stress (Nithya etal., 2017). Additionally, the evaluation of DNA damage in diabetic patients with and without peripheral neuropathy has been studied, emphasizing the importance of understanding the impact of DNA damage on diabetic complications (Prasad etal., 2015). Moreover, the role of OGG1 polymorphisms in cancer susceptibility has been investigated, suggesting that variations in the OGG1 gene may influence an individual's risk of developing cancer (Karahalil etal., 2012). This highlights the significance of understanding the genetic factors that modulate DNA repair mechanisms in the context of disease development. In conclusion, the evaluation of DNA damage in type 2 diabetic patients and its correlation with OGG1 repair gene polymorphisms is crucial for elucidating the mechanisms underlying diabetic complications and potential therapeutic targets. Understanding the interplay between oxidative DNA damage, OGG1 function, and disease pathogenesis can provide valuable insights into the development of personalized treatment strategies for diabetic patients.the current study amis to evaluate the DNA damage in type2 diabetic patients from wasit province by estimating the level of 8-OHdG using ELISA.

2. MATERIAL AND METHODS

This is a case-control study that was carried out on the 10th of October 2023 to the 29th of April 2024, at the College of Education for Pure Sciences / Department biology / University of Wasit. There were eighty people that took part in the study. The people were split up into two groups:
1. The patient group, which included 22 male and 23 female participants from Wasit province, Iraq, with a mean age ± SD of 57.38 ± 7.67 years and a median age of 57 years, included 45 persons with DMT2.
2. The control group, consisting of 35 individuals (17 male and 18 female), had an age range of 40 to 78 years. The average age was 57.38 years with a standard deviation of 7.67 years, and the median age was 57 years. All participants in the control group are apparently healthy. They made their selection from the local Wasit province, Iraqi community.
Five ml of venous blood was collected using a vacuum blood collection tube.The blood were taken;was put in a plain tube and centrifuged for 15 minutes at 3000 revolutions per minute (rpm) in order to extract serum. Sera were dispensed into Eppendrof tubes and preserved at -20C.

Statistical Analysis

The results of the serum level were presented as mean ± standard error and significant differences were analyzed by the genotype and allele.

3.RESULTS AND DISCUSSION

3.1 Assessment of serum levels of 8-oxoguanine DNA glycosylase-1(OGG1)

Determination of 8-oxoguanine DNA glycosylase-1(OGG1) in sera of patients with T2DM and controls was done by using an (ELISA). The results are shown in Tables 1. The results reveal highly significant differences, the OGG1 levels in the diabetic patients were higher than that of controls (646.964023±2.13693), controls (326.00971±16.66097), P= 0.0001.

Table 1: Mean Levels of OGG1 in T2DM patients and control groups

Parameters Groups	ng/ml Mean+SE
Control	326.00971 ±16.66097*
Patients	646.964023±2.13693
***P*-value**	0.0001
Significance level	Sig[1].

ng: nanogram
*Data was shown as Mean ±SE.
Sig[1]. Significant at *P* value ($P \leq 0.01$)

SE: Standard error

3.2 Serum OGG1 levels among males and females

The OGG1 levels were elevated significantly among males and female's patients with T2DM compared to the control group, 627.02830±66.29612 versus679.68267±63.27413), *P*=0.01 (381.850±29.33504 versus 355.44256±31.45145), *P*=0.0006, respectively. No significant differences were observed when comparing OGG1 levels between male and female patients with type2 diabetes, as well as in the control group: 627.02830±66.29612 versus 679.68267±63.27413, *P*=0.5974;381.850±29.33504 vs.355.44256±31.45145, *P*=0.5478 in patients and controls respectively. 679.68267±63.27413 versus627.02830±66.29612 with a non-significant difference *P*=0.5974.

Table 2: Mean comparison of OGG1 in males versus females

Parameters / Groups	ng/ml Mean+SE			
	Male	Female	P-value	Significant
Control	381.850±29.33504	355.44256±31.45145	0.5478	Ns.
Diabetic Patients	627.02830±66.29612	679.68267±63.27413	0.5974	Ns.
P-value	0.0100	0.0006		
Significance level	Sig[1]	Sig[1]		

Ns. Nonsignificant P> 0.05
Sig[1].Significant at *P* value (*P*≤ 0.01)
SE: Standard error

3. DISCUSSION

It is widely accepted that chronic hyperglycemia induces DNA oxidative damage in type 2 diabetes, but little is known about the effect of hyperglycemia on the DNA repair system which plays a critical role in the maintenance of genomic DNA stability in diabetes (Pang *etal*.,2012). Peripheral blood cells are often used for comet assay to detect DNA strand break while urine and serum samples are commonly used for 8-OHdG quantification. The level of 8-oxodG in the serum rather than urinary 8-oxodG was examined in the current study. Although a large body of work has identified urinary 8-oxodG as an informative oxidative stress marker, (Cooke *etal*.,2006; Cooke *etal*.,2008) detection of this biospecimen relies on careful complete collection and storage of 24-hour urine, which may be difficult to obtain. Examination of 8-oxodG in the serum has been widely used to compare 8-oxodG in normal and diseased patients (Shin *etal*.,2001; Kikuchi *etal*.,2002). The accumulation of oxidative damage as a result of chronic hyperglycemia is a causal link to tissue dysfunction in diabetes (Song *etal*.,2007; Golbidi and Laher,2010). DNA oxidative damage can induce transcription and translation errors, which possibly contributed to pancreatic β-cell dysfunction and insulin resistance. Fortunately, the DNA repair system repairs the damaged DNA and maintains normal functions in cells. In previous studies that have investigated the association of polymorphisms of several factors among patients with type2 DM from Wasit province, Yousif and Ghali,2021 revealed that IL-10 is a major contributor to the onset of type 2 diabetes mellitus and there may be a correlation between low levels of interleukin-10 and type two diabetes. Al-Sarray and Ahmed ,2021 found that may be a correlation between high levels of TNF-α and type 2 diabetes mellitus. Shamkhi and Ahmed, 2021 displayed those levels of NAD- dependent deacetylase sirtuin-1 (SIRT1) may be not associated with type2 diabetes mellitus. Furthermore, the cell free mitochondrial DNA increases significantly in patients with type2 diabetes mellitus(Hussein and Ghali,2022). The association analysis of IL-17AG197A gene polymorphism with T2DM displayed that heterozygous AG genotype of IL-

17AG197A showed a risk association among T2DM with OR=1.24 CI95% (0.31 - 5.01) p-value =1.00 and the G allele was associated with an increased risk ofT2DM Khadhum and Ahmed ,2022). Mahmood and Ghali,2022a revealed that there was an association between the polymorphism of Osteoprotegerin (OPG) polymorphism and susceptibility to type2 diabetes mellitus. Mahmood and Ghali,2022 b found also that there may be a correlation between high levels of OPG and T2DM.Thamer etal.,2020 found that IL-4 concentrations had a non-significant difference when compared patients with type-2 diabetes mellitus with the control while patients with T2DM revealed elevated serum levels of IL-6 compared to control group. Ahmed and Ghali,2019 found that different transversion and transition mutations at IL-6-174 (G/C) gene are associated with type-2 diabetes mellitus. Alwan ,2023a revealed that The polymorphism of telomerase reverse transcriptase(TERT) rs 2736100 variant A <C are associated with the susceptibility of type2 diabetes mellitus. The association analysis of (TERT) rs2853669 with susceptibility to type2 diabetes mellitus showed that the individuals carrying the heterozygous AG genotype and homozygous AA genotypes were more likely to have a significantly increased risk of type2 diabetes mellitus (Alwan and,2023 b). The human insulin receptor gene rs1366600 has possible roles in type2 diabetes mellitus susceptibility (Foad and Ahmed ,2023a). A SNPs located within miRNA-binding sites:acyl-CoA synthetase 1 rs2292899 have possible roles in type2 diabetes mellitus susceptibility. (Foad and Ahmed ,2023 b) The homozygous GG genotype of acyl-CoA synthetase 1 rs2292899 is associated with type2 DM. The results of the current study reveal highly significant differences, the OGG1 levels in the diabetic patients were higher than that of controls. Dandona etal.,1996 compared the levels of 8-OHdG in mononuclear cells amongst type 1 diabetic patients (n = 12), type 2 diabetic patients (n = 15) and healthy control subjects (n = 10). They found that both type 1 and type 2 diabetic patients had higher levels of 8-OHdG than the nondiabetic subjects. Production of reactive oxygen species by mononuclear cells was also significantly greater in diabetic patients than the control subjects. Increased serum or urinary levels of 8-OHdG which correlated with poor glycemic control have been confirmed in both type 1 and type 2 diabetes.(Hinokio *etal*.,1999;Goodarzi *etal*.,,2010&Goodarzi *etal*.,2010) In addition to DNA base oxidation, Collins, and co-workers used comet assays on white blood cells and reported higher levels of DNA strand break in people with type 1 diabetes (n = 10) compared to healthy controls (n = 10).Subsequent studies have also confirmed elevated levels of DNA strand break in type 2 diabetes, which, similar to 8-OHdG levels, were correlated with poor glycemic control(Tatsch *etal*.,2012). Pilo *etal*.,2023 found that OGG1 expression was upregulated in Value-Added Tax (VAT) patients with CRC compared to healthy participants ($p < 0.01$). They also found that OGG1 was upregulated in whole blood in patients withCyclic Redundancy Check (CRC) compared to healthy participants ($p < 0.05$). In addition, OGG1 expression was associated with the majority of genes in the Base excision repair (BER) pathways, suggesting a cooperative mechanism (Pilo *etal*.,2023). Previous studies have demonstrated that high glucose treatment decreases OGG1 expression via a redox-dependent activation of Akt in murine proximal tubular epithelial cells, which provides a mechanism of oxidative stress-mediated DNA damage in diabetes (Simone *etal*.,2008). OGG1 is involved in oxidative stress-related pathological progression (Kim *etal*.,2016; Chen *etal*.,2017). OGG1 deficiency results in reduced levels of DNA strand lesions and PARP1 overactivation and promotes hydrogen peroxide-induced cell death induced, suggesting the role of OGG1 in genomic integrity. Gene deletion of OGG1 promotes cell resistance to oxidative stress-induced DNA demethylation. However, the role of OGG1 in endothelial functions in type II diabetes mellitus patients is still unclear (Tao *etal*.,2021)). Damage of DNA by reactive oxygen species can be considered as a direct pathway in diabetes-associated mutation. High serum levels of glucose, Advanced Glycation End Products (AGEs), free fatty acids, and insulin can all promote the production of reactive oxygen species found to be increased in type 2 diabetes compared to nondiabetic subjects. (Orie *etal*.,1999) Besides, people with diabetes had low anti-oxidative capacity such as reduced glutathione synthesis which might contribute to their proneness to oxidative damage.(Seghrouchni *etal*.,2002). Goodarzi *etal*.,2010 report on a significant positive correlation between urinary 8-OHdG, a biomarker of oxidative DNA damage, and fasting blood glucose and HbA1c. Hyperglycemia causes glucose auto-oxidation, glyca - tion of proteins, activation of polyol metabolism and

subsequent formation of ROS. It has also been demonstrated that hyperglycemia is associated with an increased production of free radicals in the mito - chondria and may contribute to a greater DNA damage (Robertson,2004)-). Gao *etal*.,2019 revealed that serum 8-OHdG levels are higher among interventional radiologists than other radiation workers, which reflects a higher degree of oxidative DNA damage in the former's bodies. The three genotypes for type 2 diabetes also showed an increase in the level of OGG1 in comparison to these levels for the same genotypes of the control groups. The current study found that are significantly related to oxidative and genotoxic damage, and mutant type genotype presented higher levels of DNA damage. These results are similar to findings of Chen *et al*., 2003 who showed higher repair activity of *OGG1* Ser/Ser for 8-OHdG than the *OGG1* Cys/Cys. Also, Aka *etal.*,2004 and Pawlowska *etal.*,2009 observed that Cys/Cys and Ser/Cys *OGG1* genotypes had less DNA repair capacity compared to the Ser/Ser *OGG1* genotype.The results show that 8-oxoguanine levels are higher in patients with T2DM than in the control group. At the same time, intra-group analysis by that for all genotypes the 8-oxG level is significantly higher than those of controls. This indicates the probable underlying mechanism of DNA damage. The data about the effect of OGG1 Ser326Cys polymorphism on the repair of the 8-OHdG lesion is limited. We are fully aware of the limitations of our work. First and foremost, the sample size was relatively small. The second limitation is ethnic homogeneity of the studied population; thus, our results cannot be extrapolated to general world population.

4. CONCLUSION

Elevated levels of serum OGG1 are associated with diabetes mellitus.

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