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Correlation of Glycated Hemoglobin with Oxidative Stress in Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) has a heavy disease burden and is one of the leading causes of death worldwide. It is considered to be evolving from a complex and multifactorial metabolic disorder to an inflammatory condition. The strong link between hyperglycemia and oxidative stress has long been established. Oxidative stress leads to the generation of inflammatory mediators and reactive oxygen species, which results in an inflammatory state, which plays a key role in the pathogenesis of diabetic complications. We aimed to correlate the levels of Glycated Hemoglobin with Oxidative Stress.This cross-sectional study included 200 subjects, 100 were type 2 diabetic patients and 100 were healthy non-diabetic individuals. The data were analyzed using a t test. The results showed that as the Glycated Hb increased, the levels of FBS, MDA increased and Serum SOD, Glutathione and Catalase levels decreased. The results showed a positive correlation between HbA1c and fasting blood glucose (r = 0.417, p= 0.000) and MDA (r= 0.340, p=0.000). Whereas negative correlation was observed between HbA1c and other antioxidant parameters, SOD (r= 0.025, p=0.803) Catalase (r= 0.096, p= 0.342), Glutathione (r= -0.164, p= 0.103). It is hereby concluded for the present study that when glycated Hb increases the natural antioxidants that is SOD, catalase and glutathione decrease to combact the increased formation of ROS. Serum MDA, a marker of lipid peroxidation, increased with increased glycated Hb, and shows a positive correlation, indicating that lipid peroxidation increased, when glycation of Hb increased, thus depicting an increased chance of macrovascular complication in type 2 diabetics.

Keywords *Glycated Hemoglobin, type 2 diabetes mellitus, malondialdehyde, superoxide dismutase, glutathione*

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from a defect in insulin secretion, insulin action, or both.[1] Some 382 million people worldwide, or 8.3% of adults, are estimated to have diabetes. About 80% live in low- and middle-income countries. If these trends continue, by 2035, some 592 million people, or one adult in 10, will have diabetes. This equates to approximately three

new cases every 10 seconds or almost 10 million per year. The largest increases will take place in the regions where developing economies are predominant.[2] Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in DM.[3] Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs, which leads to domination of the condition called oxidative stress.[4]

Hemoglobin A1c (HbA1c) is an indication of chronic glycaemia and it can reflect an integrated index of glycaemia over the past 120-day lifespan of the red blood cell. The HbA1c test can be used to diagnose diabetes in which a level of 6.5% is suggested as the cut point for the diagnosis of diabetes.[5] HbA1c has been suggested to be a better indicator for controlling blood glucose levels in patients with diabetes than fasting blood sugar levels.[6]

Malondialdehyde (MDA), a toxic stable aldehyde, is produced by the peroxidation of lipids by the reactive oxygen species.[7] Several protective mechanisms are in place to prevent the histological damage it causes. One is an antioxidant enzyme, superoxide dismutase (SOD), which catalyzes the dismutation of one of the reactive oxygen species, superoxide radical, into oxygen and water.[8] Both MDA and SOD can be used as biological markers to quantify oxidative stress status.[9] Glycated hemoglobin (HbA1c) is the standard test to diagnose and monitor T2DM and can be used to predict the risk of microvascular complications.[10,11]

Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion (-) into hydrogen peroxide and molecular oxygen.[12,13] SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water.[14] Overexpression of SOD or the supplements of antioxidants including SOD mimetics, targeted to overcome oxidative stress, reduce ROS, and increase antioxidant enzymes, has been shown to prevent diabetes mellitus .[15] The elevated level of SOD is shown to reduce oxidative stress; decrease mitochondrial release of cytochrome C and apoptosis in neurons; and, in mice, prevent diabetes-induced glomerular injury, thus suggesting a major role of SOD in the regulation of apoptosis.[16] Decline in the level of SOD in diabetic tissue and blood has been reported in many studies. [17–18]

Glutathione (GSH), a tripeptide, γ-L-glutamyl-L-cysteinylglycine, is present in all mammalian tissues at 1–10 mM concentrations (highest concentration in liver) as the most abundant nonprotein thiol that defends against oxidative stress.[19] GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage.[20] It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level.[21] There are several reports that claim reduced level of GSH in diabetes.[22, 23] Decreased GSH level may be one of the factors in the oxidative DNA damage in type 2 diabetics.[24]

Catalase (CAT) is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases.[25] Catalase acts as main regulator of hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive small molecule formed as natural by-product of energy metabolism. Excessive concentration of hydrogen peroxide may cause significant damages to proteins, DNA, RNA, and lipids.[26] Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. Increased risk of diabetes has been documented in patients with catalase deficiency. The deficiency of this enzyme leads, in the β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS.[27] The aim was to Correlation of Glycated Hemoglobin with Oxidative Stress in Type 2 Diabetes Mellitus.

Material and Methods;

This cross-sectional study was carried out in the Department of Biochemistry, Government Medical College & Guru Nanak Dev Hospital Amritsar, Punjab, from January 2022 to March 2023. Of the 200 subjects, 100 were type 2 diabetic patients and 100 were healthy non-diabetic individuals. Healthy non diabetic individuals serve

as controls for this study and were selected from the general population who visited the hospital's outpatient department. The research was authorized by the Institutional Ethics Committee. All participants provided informed consent. They were exposed to a complete history and examination, as well as biochemical and special tests.

Inclusion criteria

Diabetics: Patients with type II diabetics mellitus confirmed by fasting blood sugar, under medication (hypoglycemic drugs and insulin) in the age group of 26-70 years.

Controls: Normal healthy non diabetic individuals in age group of 26-70 years.

Exclusion criteria

The subjects with liver disease, renal disease, thyroid disease, tuberculosis, hypertension, pancreatitis, Coronary artery disease (CAD, previous history) Stroke, individuals on drugs like gluco-corticoids, Nicotinic acid, Thyroid hormones, β adrenergic antagonists and thiazide diuretics, drug addicts patient with endocrinopathies such as acromegaly, patients with down syndrome were excluded from the present study.

Collection of Blood Sample;

2 mL of fasting venous blood was drawn in an EDTA vial for HbA1c estimate, and 3 mL of venous blood was drawn in a plain vial for serum separation and estimation of Malondialdehyde(MDA), Superoxide dismutase(SOD), Glutathione, and Catalase levels. Fasting blood sugar test required 2 mL of fasting venous blood in a sodium fluoride vial.

Parameters Measured

The following were measured in this study:
1. Fasting blood sugar (FBS) by GOD-POD method[28]
2. Glycated hemoglobin (HbA1c) by Ion Exchange Resin Method[29]
3. Malondialdehyde (MDA) by Kei Satoh[30]
4. Superoxide dismutase (SOD) by Marklund[31]
5. Glutathione (GSH) by ELISA
6. Catalase (CAT) by ELISA

Statistical analysis;

The data thus generated was analyzed Statistically using student ‘t’ test to compare the mean of two groups. ANOVA for comparison of mean in more than two groups. Pearson’s coefficient of correlation was used to calculate the correlation between different parameters. $P < 0.05$ was considered statistically significant.

Results and Discussion

Out of 100 type 2 diabetes participants, 52 were men and 48 were women. Similar to the study group, the control group had 100 non-diabetic participants, 58 men and 42 women. 17 % diabetics and 43 % non-diabetics were sharing the age group <= 40, 57 % diabetics and 41 % non-diabetics were sharing the age group of 41-60, the maximum number of diabetics. On the other hand, age group shared >60, 26 % are diabetics, and 16 % are non-diabetics. Diabetics have a Mean SD and age of 51.33 ± 11.2, while non-diabetics have a Mean SD and age of 44.9 ± 14.4

The HbA1c levels of diabetics were divided into four groups based on their glycated hemoglobin levels in the present study: Group I (=5.4%) had a mean value of 4.29±1.23; Group II (>5.4%-6.4%) had a mean value of 6.06±0.23; Group III (>6.4%-8.0%) had a mean value of 7.29±0.48; and Group IV (>8.0%) had a mean value of 10.62±1.84, which was statistically highly significant (p=0.00).

Table; 1 Segregation of Patients According to levels of glycated (Hb)

S.NO	GROUP	MEAN±SD
		HbA1C %
I	Control	4.11±1.12
II	Group 1 patient<=5.4%	4.29±1.23
III	Group 2 patient >5.4% -6.4%	6.06±0.23**
IV	Group 3 patient >6.4% - 8.0%	7.29±0.48***,*
V	Group 4 patient >8.0	10.62±1.84***,***,***

When the Diabetics were divided according to glycated Hb, which was statistically highly significant. (p=0.00).

Group I was compared to groups II (p= **0.11 not significant), III (p= ***0.00 highly significant), and IV (p= ***0.00 highly significant). Group II was compared to Group III (p=*0.03 significant), IV (p=***0.00 highly significant). When Group III and Group IV were compared, p=***0.00 was found to be highly significant.

Table; 2 Mean Value of FBS in Diabetic Patients According to HbA1C

S.NO	GROUP	MEAN±SD
		FBS (mg%)
I	Control	87.59±7.13
II	Group 1 patient<=5.4%	133.36±31.56
III	Group 2patient >5.4% -6.4%	139.90±31.56**
IV	Group3 patient >6.4% - 8.0%	238.14±247.98**,*
V	Group 4 patient >8.0	252.11±62.26**,***,**

When the Diabetics were divided according to glycated Hb, it was observed that the level of Fasting blood Glucose increased as the glycated Hb levels increased, which was statistically significant. (p <0.05).

Group I was compared to groups II (p=**1.00 not significant), III (p=**0.48 not significant), and IV (p=**0.31 not significant). Group II was compared to Group III (p=*0.04significant), IV (p=***0.00 highly significant). When Group III and Group IV were compared, p=**0.97 was found to be not significant.

Table; 3 Mean Value of Antioxidant status in Diabetic Patients According to HbA1C

S.NO	GROUP	MEAN±SD		
		SOD (ml)	CATALSE (KU/L)	GLUTATHIONE (ng/ml)
I	Control	1.12±0.416	405.2±129.8	25.16±11.27
II	Group 1 patient<=5.4%	0.832±0.331	281.69±75.30	16.98±8.34
III	Group 2 patient >5.4% - 6.4%	0.802±0.279**	258.93±71.71**	15.71±9.14**
IV	Group 3 patient >6.4% - 8.0%	0.752±0.245**,**	255.12±49.15**,**	15.33±6.15**,**
V	Group 4 patient >8.0	0.722±0.196**,**,**	208.91±64.9.90***,*,**	13.75±6.49**,**,**

SOD (p value **0.99, **0.92, **0.84, **0.86, **0.72, **0.96) **Not Significant

Catalase (p value **0.58, **0.90, ***0.00, **1.00, *0.04, **0.63) *Significant, **Not Significant, ***Highly Significant.

Glutathione (p value **0.98, **0.96, **0.89, **0.99, **0.92, **0.94) **NS

When the Diabetics were divided according to glycated Hb, it was observed that levels of SOD, Catalase, and Glutathione decreased as the glycated Hb levels increased. Concerningly, SOD and Glutathione were not statistically significant (p >0.05), but Catalase was significant (p <0.05). When Group I was compared with group II, III and IV. Group II was compared with III and IV. When Group III was compared with group IV.

Table; 4. Mean Value of Lipid Peroxidation in Diabetic Patients According to HbA1C

S.NO	GROUP	MEAN±SD
		MDA (µmol/ml)
I	Control	2.5±1.58
II	Group 1 patient<=5.4%	6.55±1.41
III	Group 2 patient >5.4% -6.4%	11.11±6.21**
IV	Group 3 patient >6.4% - 8.0%	15.39±13.64**,**
V	Group 4 patient >8.0	19.88±16.39**,***,**

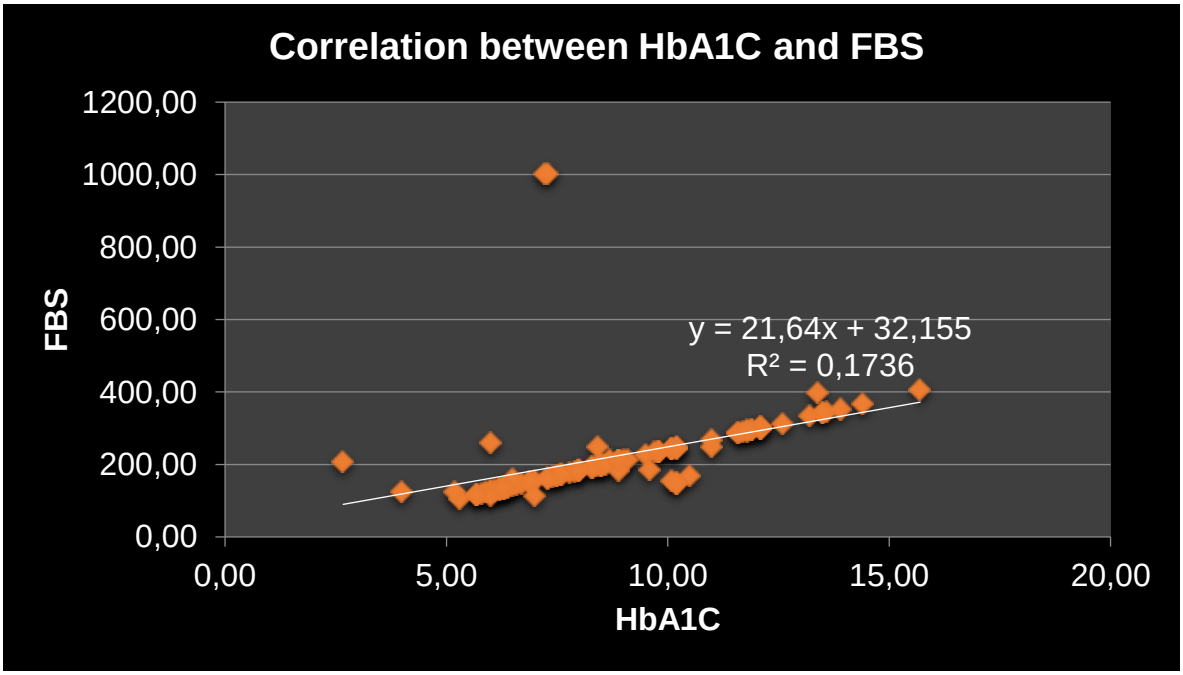
When the Diabetics were divided according to glycated Hb levels, it was discovered that the amount of Lipid Peroxidation increased as the glycated Hb levels increased, which was statistically significant. (p <0.05).

Group I was compared to groups II (p= **0.44 not significant), III (p= **0.54 not significant), and IV (p= **0.11 not significant). Group II was compared to Group III (p=**0.44 not significant), IV (p=***0.00 highly significant). When Group III and Group IV were compared, p=**0.54 was found to be not significant.

Table;5 Correlation of Glycated Hb with Oxidative Stress Parameters

HbA1C (%)	FBS (mg%)	Catalase (KU/L)	SOD (ml))	MDA (µmol/ml)	Glutathione (ng/ml)
	r = 0.417 P= 0.000	r = 0.096 p =0.342	r = 0.025 p=0.803	r =0.340 p = 0.000	r = - 0.164 p= 0.103

Linear regression analysis showed positive correlation between HbA1c and fasting blood Glucose (r =0.417, p= 0.000) (fig.1) and MDA (r= 0.340, p=0.000) with glycated Hb in patients of type 2 diabetes mellitus. Whereas negative correlation was observed between HbA1c and other antioxidant parameters, SOD (r= 0.025, p=0.803) Catalase (r= 0.096, p= 0.342), Glutathione (r= -0.164, p= 0.103).



Figure; 1 Correlation between HbA1c and FBS in diabetic patients

Oxidative stress has focus interest in various clinical research in recent times. There is a growing evidence connecting the action of oxidative stress to the pathogenesis and complications in diabetes mellitus and many other diseases. Oxidative stress plays a role in pathogenesis of insulin resistance and β-cell dysfunction, caused by dysregulation of cell homeostasis and metabolism.[32] Hyperglycaemia is the principal metabolic alteration which is associated with diabetes mellitus, and increased glycaemic levels in body fluids has been implicated to increase oxidants, cause cellular damage, vascular dysfunction and pathogenesis of vascular disease.

Glycated hemoglobin (HbA1c) represents the blood glucose average level within the past 3 months. Therefore, HbA1c is a very important biochemical parameter that provide long term status of blood glucose levels and monitoring tool for measuring glycemic control in Type – 2 diabetic patients.[33]. HbA1c in general, developed when the hemoglobin joined with glucose in the blood and become glycated.[34] According to many studies, HbA1c levels could be used as an independent risk factor for stroke and Cardiovascular disease (CVD) in both healthy and diabetics persons. It has been found that a (0.2%) decrease of HbA1c level can lower the risk of CVD development by 10%.[35] Furthermore, many studies have revealed, newborns moms with high HbA1c levels are more likely suffering from development of CVD in the future.[36] The relationship between these parameters (HbA1c, and oxidative stress parameters) needs to be discussed.

In the present study, HbA1c levels of diabetics were divided into four groups based on their glycated hemoglobin levels in the present study: Group I (=5.4%) had a mean value of 4.29±1.23; Group II (>5.4%-6.4%) had a mean value of 6.06±0.23; Group III (>6.4%-8.0%) had a mean value of 7.29±0.48; and Group IV (>8.0%) had a mean value of 10.62±1.84, which was statistically highly significant (p=0.00). In the Previous study, HbA1c ranged from 6% to 12%, with means of 8.5% (duration <5 years), 8.8% (duration 5–15 years), and 8.5% (duration >15 years). Gradinaru et al reported a much lower value of 7.2%, which might be due to inclusion of subjects with only good and moderate glycemic control(<8.5%).[37] Similarly Aoucheri et al, Zare-Mirzaie et al, and Dhas et al, reported relatively lower values.[38,39,40] Sheth et al reported values of 8.36% ± 1.79%, which were comparable to those of the current study.[41]

Previous study stated that a FBS of patients was 175.5 ± 30.08 (mg/dL) which is significantly higher than control group their mean 74.17 ± 13.76 (mg/dL) (P< 0.01). Concerning to glycated haemoglobin HbA1c, patients with type-2 diabetes had significantly higher mean value 8.852 ± 0.5803 compared to 5.16 ± 0.5049 of control group (P< 0.01). This outcome is consistent with several studies[33,34]. Positive correlation (statically significant) was observed between HbA1c and FBS (r = +0.410, p=0.025). This finding is an agreement with many reported studies[42, 43].

It has been reported that in Diabetic patients had a significant higher HbA1c (p= 0.00) levels and lower SOD (p= 0.00) levels when compared to control subjects. Significant positive correlation (r= 0.60, p= 0.00) and negative correlation (r= - 0.57, p= 0.001) was obtained between duration of diabetes and HbA1c, SOD levels respectively in diabetics. Also a significant negative correlation (r= - 0.75, p= 0.00) was observed between HbA1c and SOD levels in diabetic patients. In diabetics with poor glycemic control, SOD levels were significantly (p= 0.002) lower when compared to patients with good glycemic control.[44]

Several studies on serum and erythrocyte SOD levels have shown increased, decreased as well as unchanged enzyme levels. In this study there was a decrease in SOD activity. This finding is in accordance with Kesavulu et al. We observe a decrease in CAT activity and consequently SOD activity. A possible explanation for the fall in SOD activity could be linked to glycation of the SOD enzyme in serum due to hyperglycaemic condition. Decrease in SOD activity consequently leads to decrease in CAT activity as both the enzymes function in unison to neutralise superoxide ion to water and oxygen molecule.[45]

In spite of low activities of SOD in both groups of diabetes a negative but nonsignificant correlation between the activities of the enzymes and levels of HbA1C was observed. These findings were similar to the findings of Ruiz et al.[46] but not with those of Singhania N etal.[47]

According to another study, production of the oxygen free radicals was directly related to hyperglycaemia and the duration of diabetes. The MDA levels acted as a marker for lipid peroxidation i.e. oxidative stress and it was significantly increased in the cases as compared to the controls (P<0.001). In a state of poor metabolic control, increased serum MDA levels are expected. A positive correlation was found between the mean serum MDA levels and the mean HbA1c levels in the diabetic patients.[48]

Previous study stated that Compared to the controls, T2DM patients had lower erythrocyte GSH concentrations (0.90 ± 0.42 vs. 0.35 ± 0.30 mmol/L; P = 0.001) and absolute synthesis rates (1.03 ± 0.55 vs. 0.50 ± 0.69 mmol/L/day; P = 0.01), but not fractional synthesis rates (114 ± 45 vs. 143 ± 82%/ day; P = 0.07). The magnitudes of changes in patients with complications were greater for both GSH concentrations and absolute synthesis rates (P-values 0.01) compared to controls. There were no differences in GSH concentrations and synthesis rates between T2DM patients with and without complications (P-values > 0.1). Fasting glucose and HbA1c did not correlate with GSH concentration or synthesis rates (P-values > 0.17).[49]

Góth et al. (2016) showed significantly lower CAT activities in patients with T2D when compared with those in controls.[50] Low CAT levels can lead to T2D pathogenesis by decreasing insulin secretion and inducing oxidative damage on pancreatic β-cells.[51] A similar trend was observed by Palekar et al. (2016), who observed a significant decrease in CAT levels in participants with T2D when compared with those in controls.[52] Another study by Lipa et al. revealed that the serum CAT levels were significantly lower in participants with T2D than in those without T2D.[53] Decreased levels of SOD and CAT could result from hyperglycemic conditions that lead to glycation; inactivation by crosslinking enzymes; increased lipid peroxidation; elevated susceptibility of these enzymes to free radicals, resulting in the limited potential to detoxify the radicals; and gene mutations of these enzymes.[54,52,55] Moreover, the decreased SOD and CAT levels might be caused by the activation of protein kinase C and nonenzymatic glycosylation, and the loss of cofactors, including Zn2+ and Cu2+, which are components of these enzymes.[56,57] According to a previous literature review, the downregulation of SOD activity might increase superoxide radicals, leading to CAT inactivation and reduced insulin effectiveness in patients with T2D.[56,50]

Conclusion

It is hereby concluded that when glycated Hb increased the natural antioxidants that is SOD, catalase and glutathione decrease to combact the increased formation of ROS. Serum MDA, a marker of lipid peroxidation, increased with increased glycated Hb, and shows a positive correlation, indicating that lipid peroxidation increased, when glycation of Hb increased, thus depicting an increased chance of macrovascular complication in type 2 diabetics.

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