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CORRELATION BETWEEN GLYCATED HB AND NON-CONVENTIONAL PARAMETER OF OXIDATIVE STRESS

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Abstract

Background; There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia causes oxidative stress, which further exacerbates the development and progression of type 2 diabetes and its complications. Objective; Correlation between Glycated Hb and Non-Conventional Parameter of Oxidative Stress. Material and Methods; This cross-sectional study was carried out in the Department of Biochemistry, Government Medical College & Guru Nanak Dev Hospital Amritsar. 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. Results; Linear regression analysis showed a Positive Correlation between HbA1c and fasting blood glucose (r=0.417, p=0.000) with glycated Hb in patients with type 2 diabetes mellitus. Whereas negative correlation was observed between HbA1c with Non Conventional parameters, Ascorbic acid (r= 0.096, p=0.342), Uric acid (r= 0.025, p= 0.803), protein (r= 0.340, p=0.000), Albumin (r= -0.164, p=0.103), bilirubin (r= 0.069, p=0.494). Conclusions; The study concludes that type 2 diabetes is associated with oxidative stress, evidenced by altered levels of non-conventional oxidative stress markers. Lower levels of antioxidants and proteins in diabetics suggest increased oxidative damage, which may contribute to the progression of diabetic complications..

Key words: Type 2 diabetes mellitus, Oxidative stress, Antioxidants, Non - Conventional parameters, Reactive oxygen species .

INTRODUCTION;

Diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes. It is characterized by the decreased ability of insulin to act on the peripheral tissues (insulin resistance, IR) and an inability of the pancreas to produce sufficient insulin (relative deficiency in early to absolute insulin deficiency in late), either of which may be the predominant feature [1].

Oxidative stress, through the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), has been proposed as the root cause underlying the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and T2DM[2]. Cells can tolerate moderate oxidative loads by increasing gene expression to up-regulate their reductive defense systems and restore the oxidant/antioxidant balance. But when this increased synthesis cannot be achieved due to damage to enzymes, or substrate limitations, or when the oxidative load is overwhelming, an imbalance persists and the result is oxidative stress. Persisting imbalance damages DNA, proteins, lipids, and cell death [3].

Glycated hemoglobin (HbA1c) represents the blood glucose average level within the past 3 months. Therefore, HbA1c is an important biochemical parameter that provides the long-term status of blood glucose levels and a monitoring tool for measuring glycemic control in Type – 2 diabetic patients [4].

Uric acid synthesis and metabolism are complex processes involving numerous parameters that control the hepatic, renal, and gastrointestinal excretion of this compound. Uric acid is the end product of an external purine pool and endogenous purine metabolism. The exogenous pool fluctuates significantly with a meal, and animal proteins significantly contribute to this purine pool. Endogenous uric acid synthesis is mostly from the liver, intestines, and other tissues such as muscles, kidneys, and the vascular endothelium [5]. Diabetes mellitus may cause an increase in uric acid levels in the body, which can lead to gout or other health concerns. This is because diabetes can impair the body's capacity to deal with and reduce uric acid. High uric acid levels can potentially cause kidney stones and other kidney issues over time.

Bilirubin is an orange-yellow pigment of bile that results from the degradation of various heme-containing proteins, especially from hemoglobin catabolism. Heme is broken down into biliverdin, which is converted into unconjugated or indirect bilirubin (UCB). UCB is water-insoluble and enters circulation bound to albumin. In the liver, glucuronic acid is added to UCB (conjugation) to render it water-soluble (direct bilirubin); finally, it is either excreted into bile or recirculated back to the bloodstream, where it is filtrated by the kidneys and excreted through urine [6]. Bilirubin's inhibitory impact on NADPH oxidase activity presumably explains much of the profound antioxidant activity of heme oxygenase, which cleaves heme to yield biliverdin, carbon monoxide, and free iron; biliverdin is then rapidly reduced by the ubiquitously expressed enzyme biliverdin reductase to yield bilirubin [7]. Diabetes mellitus can cause a decrease in bilirubin levels, which can indicate impaired liver function or an increased rate of bilirubin breakdown. High bilirubin levels in the blood can cause jaundice, which can produce look like as skin discoloration. The whites of the eyes turn yellow. Urine that is dark in color.

Albumin has a molecular weight of 66 kDa and 585 amino acids. Human plasma contains this highly soluble protein at typical amounts ranging from 35 to 50 g/l [8]. Albumin serves a variety of physiological and pharmacological activities. Metals, fatty acids, cholesterol, bile pigments, and medicines are all transported via it. It is important in the administration of osmotic pressure and the distribution of fluid between compartments. It has a half-life of about 20 days under normal conditions, and its plasma concentration represents an equilibrium between its synthesis in the liver and catabolism, but also between its transcapillary escape. In general, albumin is the most abundant and controlling antioxidant in plasma, a bodily compartment constantly undergoing oxidative stress. Albumin is an important part of total serum antioxidant properties. Previous research has demonstrated that human serum albumin (HSA) forms more than 70% of the free radical-trapping activity of serum as determined by the free radical-induced hemolysis test [9].

Serum albumin levels can be reduced in diabetes mellitus, indicating a loss of kidney function. Low serum albumin levels can result in a range of complications, including edema, malnutrition, and a greater danger of infection.

Proteins are essential biomolecules in the cell. They play a variety of physiological roles, including cell signaling and transport across cells. Proteins, whose structure and function can be altered by ROS, are another possible target. Protein oxidation has various side-chain targets, including cysteine, methionine, and tyrosine. Carbonyls are protein oxidation products that have been determined as a powerful biomarker of oxidative stress [10]. Protein oxidation can be elevated in diabetes mellitus with high amounts of glucose and free radicals, which can cause oxidative stress and protein degradation. Protein oxidation can have several implications, including protein function loss, protein aggregation, and increased protein fragility.

Ascorbic acid, commonly known as vitamin C plays significant functions in the human body, though its function at the cellular level is not very clear. Vitamin C is needed for collagen synthesis, the protein that serves many connective functions in the body. Among the body's collagen-containing materials and structures are the framework of bone, gums, and binding materials in skin muscle, or scar tissue. Production of certain hormones and neurotransmitters and the metabolism of some amino acids and vitamins require vitamin C. Ascorbic acid is important in the proper function of the immune system. As an antioxidant, it reacts with compounds like histamines and peroxides to reduce inflammatory symptoms. Its antioxidant property is associated with the reduction of cancer incidences [11, 12].

Ascorbic acid levels can be decreased in diabetes mellitus due to increased urinary excretion of the vitamin. This can lead to a higher risk of vitamin C deficiency. Vitamin C deficiency can lead to several health problems, including scurvy, characterized by weakness, fatigue, and bleeding gums. In addition, Vitamin C deficiency has been associated with an increased risk of infection and poor wound healing.

MATERIAL AND METHODS

This cross-sectional study was conducted from January 2022 to March 2023 in the Department of Biochemistry, Government Medical College & Guru Nanak Dev Hospital Amritsar, Punjab. The 200 subjects included 100 type 2

diabetic patients and 100 healthy non-diabetics. This study's controls are healthy, non-diabetic people selected from the general population who visit the hospital's outpatient department. The Institutional Ethics Committee provided their approval to the study. All participants provided their informed consent. They were subjected to a thorough medical history and examination, as well as biochemical and special tests.

Inclusion criteria

Diabetics: Patients with type II diabetes 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 the 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 glucocorticoids, 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.

SAMPLE COLLECTION AND BIOCHEMICAL ANALYSIS;

After an overnight fast of 8-10 hours, 7 mL of peripheral venous blood sample was drawn from the medial cubital vein. One mL of blood was transferred into a test tube containing sodium fluoride and potassium oxalate (1:3 ratio of 20mg/5mL) anticoagulant. 4 mL of the blood was transferred into additive-free tubes. The additive-free tubes were allowed to stand for 30 minutes for clot formation following which they were centrifuged at 3000 rpm for 5 minutes to obtain serum. And 2ml was transferred to the EDTA vial for estimation of HbA1c. The sodium fluoride and potassium oxalate additive tubes were immediately centrifuged at 3000 rpm for 5 minutes to obtain the plasma. Plasma samples were analyzed immediately for blood glucose and ascorbic acid. Serum samples were aliquoted into appropriately labeled vials and stored at -80°C in a deep freezer until analysis of the biochemical parameters. Serum samples were used for the analysis of Uric acid, Bilirubin, Albumin, Protein, and EDTA whole blood samples used for HbA1c.

Parameters Measured

The following were measured in this study:

1. Fasting blood sugar (FBS) by GOD-POD method[13]
2. Glycated hemoglobin (HbA1c) by Ion Exchange Resin method[14]
3. Uric acid by enzymatic method[15]
4. Bilirubin by Jendrassik & Grof method [16]
5. Albumin by BCG method [17]
6. Protein by Biuret method [18]
7. Ascorbic acid by (2,6 dichlorophenolindophenol titration method)[19].

STATISTICAL ANALYSIS;

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

RESULTS AND OBSERVATION

The Present study was conducted in the Department of Biochemistry, Government Medical College & Guru Nanak Dev Hospital Amritsar, Punjab to Study the Correlation between Glycated Hb and Non-Conventional Parameters of Oxidative Stress. 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 % of diabetics and 43 % of non-diabetics were sharing the age group <= 40. 57 % of diabetics and 41 % of non-diabetics were sharing the age group of 41-60, the maximum number of diabetics. On the other hand, the age group shared >60, 26 % were diabetics, and 16 % were non-diabetics. Diabetics have an age of 51.33 ± 11.2, while non-diabetics have a Mean age of 44.9 ± 14.4 years.

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	<=5.4%	4.29±1.23
III	>5.4% - 6.4%	6.06±0.23
IV	>6.4% - 8.0%	7.29±0.48
V	>8.0	10.62±1.84

P value (**0.11, ***0.00, ***0.00, *0.03, ***0.00, ***0.00) *Significant, **Not Significant, ***Highly Significant
When the Diabetics were divided according to glycated Hb, which was statistically highly significant. (p=0.00).
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. 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	<=5.4%	133.36±31.56
III	>5.4% - 6.4%	139.90±31.56
IV	>6.4% - 8.0%	238.14±247.98
V	>8.0	252.11±62.26

P value (**1.00, **0.48, **0.31, *0.04, ***0.00, **O.97) *Significant, **Not Significant, ***Highly Significant
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. ($p<0.05$)
When Group I was compared with Group II, III, and IV. Group II is compared with III and IV when Group III was compared with Group IV.

Table; 3 Mean Value of Ascorbic acid and Uric acid in Diabetics Patients According to HbA1C

S.NO	GROUP	MEAN±SD	
		ASCORBIC ACID (mg/l)	URIC ACID (mg%)
I	Control	6.22±0.66	5.11±1.41
II	<=5.4%	8.87±2.71	5.16±2.00
III	>5.4% - 6.4%	8.10±2.09	5.49±1.84
IV	>6.4% - 8.0%	7.77±1.32	5.73±1.81
V	>8.0	7.68±1.60	5.78±1.48

Ascorbic acid (p value **0.84, **0.65, **0.54, **0.93, **0.77, **0.99) **Not Significant
Uric acid (p value **0.92, **0.97, **0.92, **0.97, **0.99, **1.00) **Not Significant
When Diabetics were separated according to glycated Hb levels, it was observed that levels of Ascorbic acid decreased and Uric acid increased as glycated Hb levels increased. Ascorbic acid levels were statistically significant ($p<0.05$) and uric acid were not statistically 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 Protein & Albumin and Bilirubin in Diabetic Patients According to HbA1C

S.NO	GROUP	MEAN±SD		
		Protein (g/dl)	**Albumin (g/dl)**	**Bilirubin (mg%)**
I	Control	6.15±0.287	3.40±0.322	0.5±0.155
II	<=5.4%	6.88±1.05	3.66±0.511	1.10±3.04
III	>5.4% - 6.4%	6.925±0.386	3.64±0.171	0.635±0.358.
IV	>6.4% - 8.0%	6.927±0.837	3.59±0.596	0.579±0.392
V	>8.0	7.25±0.811	3.55±0.429	0.527±0.177

Protein (p value **1.00, **0.99, **0.47, **1.00, **0.92, **0.69) **Not Significant
Albumin (p value **1.00, **0.95, **0.90, **0.99, **0.98, **0.99) **Not Significant
Bilirubin (p value **0.86, **0.79, **0.96, **1.00, **1.00, **1.00) **Not Significant
When the Diabetics were divided according to glycated Hb, it was observed that levels of Protein increased and Albumin and Bilirubin both decreased as the glycated Hb levels increased. Concerningly, Protein and albumin, were statistically not significant ($p>0.05$) but
Bilirubin was statistically 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 5 Correlations of glycated Hb with Non-Conventional Parameter of Oxidative Stress

HbA1C (%)	FBS (mg%)	Ascorbic acid (mg/l)	Uric acid (mg%))	Protein (g/dl)	Albumin (g/dl)	Bilirubin (mg/dl)
	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	r=0.069 p=0.494

Linear regression analysis showed a Positive Correlation between HbA1c and fasting blood glucose (r=0.417, p=0.000) with glycated Hb in patients of type 2 diabetes mellitus. Whereas negative correlation was observed between HbA1c with Non Conventional parameters, Ascorbic acid (r= 0.096, p=0.342), Uric acid (r= 0.025, p= 0.803), protein (r= 0.340, p=0.000), Albumin (r= -0.164, p=0.103), bilirubin (r= 0.069, p=0.494).

DISCUSSION;
Oxidative stress has focused interest in various clinical research in recent times. There is 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 the pathogenesis of insulin resistance and B-cell dysfunction, caused by dysregulation of cell homeostasis and metabolism [20]. Hyperglycaemia is the principal metabolic alteration that is associated with diabetes mellitus, and increased glycaemic levels in bodily fluids have been implicated in increasing oxidants, causing cellular damage, vascular dysfunction, and pathogenesis of vascular disease.

In our study, according to glycated hemoglobin, diabetics' HbA1c levels were divided into four groups in the present study: Group I (=5.4%) had a mean value of 4.29; Group II (>5.4%–6.4%) had a mean value of 6.06; Group III (>6.4%–8.0%) had a mean value of 7.29; and Group IV (>8.0%) had a mean value of 10.62, which was statistically highly significant (p=0.00). Group I was compared to groups II (p= 0.11 significant), III (p= 0.00 very significant), and IV (p= 0.00 extremely significant). Group II was compared to Group III (p=0.03 significant), and IV (p=0.00 significant). When Group III and Group IV were compared, p=0.00 was found to be highly significant.

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 the

inclusion of subjects with only good and moderate glycemic control(<8.5%).[21] (Similarly Aoucheri et al, Zare-Mirzaie et al, and Dhas et al, reported relatively lower values.[22,23,24] Sheth et al reported values of 8.36% ± 1.79%, which were comparable to those of the current study [25].

According to our study, 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. ($p<0.05$). When the Diabetics were divided according to glycated Hb, which was statistically highly significant. ($p=0.00$). Linear regression analysis showed a positive correlation between fasting blood Glucose and HbA1c ($r = 0.417$, $p= 0.000$) with glycated Hb in patients with type 2 diabetes mellitus.

Our study indicated When Diabetics were separated according to glycated Hb levels, it was discovered that levels of Ascorbic acid decreased as glycated Hb levels increased. Ascorbic acid levels were statistically significant ($p<0.05$). A negative correlation was observed between HbA1c and ascorbic acid ($r=-0.142$, $p= 0.160$). It was not statistically significant ($p>0.05$) when male and female diabetics were separated based on glycated hemoglobin.

Vitamin C is a powerful dietary antioxidant, it donates electrons thus helping in scavenging free radicals. Vitamin C reduces the overall hyperglycaemic state by reducing blood glucose, reducing glycosylation of proteins, and decreasing the production of sorbitol. Several clinical studies have shown that vitamin C levels in diabetics are lowered and that supplementing with vitamin drugs helps to slightly alleviate diabetic complications [26]. In this study, the level of vitamin C is significantly decreased in diabetics. A possible explanation for the low vitamin C levels in diabetics could be linked to increased ascorbic acid oxidation or impaired regeneration from its oxidized state [27].

It was also observed that Diabetics were separated according to glycated Hb levels, and levels of Uric acid increased as glycated Hb levels increased. Which was not statistically significant ($p>0.05$). Linear regression analysis showed a negative correlation between Uric acid and HbA1c ($r= -0.138$, $p= 0.172$) with glycated Hb in patients with type 2 diabetes mellitus. It was not statistically significant ($p>0.05$) when male and female diabetics were separated based on glycated hemoglobin.

A previous study stated that the serum Uric acid for diabetic type-2 patients is significantly higher in comparison with the healthy group 7.95 ± 0.87 and 5.98 ± 0.715 respectively. This finding is similar to earlier reported studies [28] in which hyperuricemia has been correlated with a greater risk for the development of impaired glucose tolerance and type-2 DM [29]. Based on that, the obtained results propose the adverse effect of elevated Serum Uric acid for glycemic control. Other reporters suggest that there is a possible mechanism for the relationship between elevated Serum Uric Acid and HbA1c in DM patients could be linked to the defect of reabsorption of uric acid in the proximal tubal in diabetic individuals with high glucose levels [28].

In diabetic patients, serum uric acid level was found to correlate positively with HbA1c probably reflecting the adverse effect of the elevated serum uric acid in glycemic control, although other researchers assume that the possible mechanism for the association between increasing serum uric acid and uncontrolled hyperglycemia in diabetic patients may be related to the inhibition of uric acid reabsorption in the proximal tubule by high glucose levels in diabetic individuals [30, 31].

When the Diabetics were divided according to glycated Hb, it was observed that levels of Protein increased as the glycated Hb levels increased. Which was statistically not significant ($p > 0.05$). A negative correlation was observed between protein and HbA1c ($r=-0.49$, $p= 0.626$) with glycated HB in patients with type 2 diabetes mellitus. It was not statistically significant ($p>0.05$) when male and female diabetics were separated based on glycated hemoglobin.

According to Another study in the diabetic patients group, Total Protein results were shown significantly elevated (6.15 ± 0.98) in comparison to the control group (4.31 ± 0.73). This result is in agreement with studies by [32,33]. Elevation in the concentration of total protein may be because of the elevation of acute phase proteins (like globulins, fibrinogen, and compounded) by a fractional decrease in the rate of synthesis of albumin due to insulin deficiency or resistance [32]. HbA1c also showed a strong and direct positive correlation with total protein ($r = +0.322$, $p= 0.01$). This hypothesis is consistent with earlier findings [34,35].

When the Diabetics were divided according to glycated Hb, it was observed that levels of Albumin decreased as the glycated Hb levels increased. The Albumin was statistically not significant ($p>0.05$). A negative correlation was observed between Albumin and HbA1c ($r=-0.049$, $p=0.68$) in patients with type 2 diabetes mellitus. It was not statistically significant ($p>0.05$) when male and female diabetics were separated based on glycated hemoglobin.

Previous study in Indian subjects suggests that higher Serum Albumin levels may decrease HbA1c levels and that lower serum albumin levels may raise HbA1c levels as reported previously from Western studies.[36] As we did not measure

glycated albumin levels, we can only cautiously speculate that this could be due to higher albumin levels competing with Hb to get excessively glycated. The results of our study showed a statistically significant negative correlation between HbA1c and serum albumin levels [37].

In the present study, Diabetics were divided according to glycated Hb, it was observed that levels of Bilirubin decreased as the glycated Hb levels increased. Bilirubin was statistically significant ($p < 0.05$). A negative correlation was observed between Bilirubin and HbA1c ($r = 0.069$, $p = 0.494$), with glycated Hb in patients with type 2 diabetes mellitus. When male and female diabetics were separated based on glycated hemoglobin, the male was statistically not significant ($p=0.00$). However, the female was statistically significant.

A study from Choi SW et al. evaluated the relationship between HbA1c and bilirubin in 690 patients with type 2 diabetes mellitus and found that bilirubin concentrations were negatively associated with HbA1c, independent of gender, age, and other confounding factors [38]. Several studies have confirmed that high bilirubin concentrations are inversely associated with insulin resistance [39, 40].

CONCLUSION:

The study concludes that type 2 diabetes is associated with oxidative stress, evidenced by altered levels of non-conventional oxidative stress markers. Lower levels of antioxidants and proteins in diabetics suggest increased oxidative damage, which may contribute to the progression of diabetic complications.

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