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Glycosylated Hemoglobin and Its Relation with Lipid Peroxidation in Type-2 Diabetes Mellitus

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Abstract: Despite massive research, association of glycosylated hemoglobin (HbA1c) with oxidative stress in type 2 diabetes mellitus (T2DM) is poorly understood and still needs further clarification. In addition, free radicals production has been found to be associated with altered glucose levels. The objective of present study was to investigate the relationship between HbA1c, hyperglycemia and lipid peroxidation in T2DM patients and compared it with non-diabetic healthy subjects. Fasting blood glucose levels, HbA1c and malondialdehyde (MDA) levels were estimated by using standard methods in 50 patients of T2DM as Group II and compared it with 50 age matched healthy controls (Group I). The values were expressed as Mean ± SD and data from patients and controls were compared using students't' test. Result: MDA and HbA1c levels along with fasting blood sugar were significantly high ($p<0.001$) in patients group as compared to control. In addition, HbA1c levels were positively correlated with MDA ($r=0.784$) which revealed that oxidative stress had significant association with poor blood glucose management in T2DM. Conclusion: Thus, oxidative stress is associated with hyperglycemia and HbA1c in T2DM patients. Therefore, maintenance of normal glycemic profile, regular monitoring of HbA1c levels along with control on excessive free radical production will hopefully contribute to a more adequate management of T2DM.
Key words: Hyperglycemia, free radicals, oxidative stress, blood glucose.

INTRODUCTION

Diabetes is an endocrine disorder and have found to be associated with serious complications such as cardiac complications, hypothyroidism and renal diseases [1]. Diabetes has evolved into an epidemic in India and it has been projected to rise to a staggering 101.2 million by 2030[2].

Diabetes is characterized by prolonged hyperglycemia along with disturbances in carbohydrate, lipid and protein metabolism [3]. Glycosylated haemoglobin (HbA_1c) is considered as an integrated and reproducible measure of the long term glycemic control. Therefore, attaining the lowest and safest HbA_1c levels remains the 'Standard& Bench Mark' to measure the success of therapy and predict long term complications[4]. Amongst various factors imparting their effect in development of diabetes, free radicals play a significant role in the development of T2DM.

Imbalance between formation and removal of free radicals can lead to a pathological condition called as oxidative stress. Poorly controlled blood glucose levels accelerate hyperglycemia-induced increased free-radical generation and thereby causing oxidative stress. Oxidative damage accumulates during the life cycle, and this has been implicated in aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions. It also causes cellular inflammation thus damaging blood vessels in T2DM [5].

Apart from various other biomolecules, unsaturated fatty acids (lipids) are easily oxidised by free radicals. Membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria, contain high concentration of unsaturated fatty acids. The complete degradation (i.e., peroxidation) of membrane lipids is a hallmark of oxidative damage and can result in the formation of lipid hydroperoxide (R-OOH) which can further breakdown to aldehydes such as malonaldehyde (MDA) [6]. Therefore, the overall aim and objectives of the present study was to investigate the relationship between HbA1c, hyperglycemia and malonaldehyde levels in T2DM patients.

MATERIALS & METHODS

In the present study, 50 healthy subjects, 50 patients with type 2 diabetes mellitus were taken in study group as Group I and Group II respectively. Healthy individuals were selected from the staff of Sharda University and unrelated attendants of patients of either sex. The present study was conducted at the School of Medical Sciences & Research (SMS&R) in the Departments of Biochemistry and Medicine, Sharda Hospital, Greater Noida. This study was approved by the Institutional Ethics Committee. Informed oral consent was obtained from each participant, after explaining the purpose of this study in their own language, before obtaining the blood sample. Complete history was taken from all the participants and patients diagnosed on the basis of history, clinical examination and laboratory investigations.

Inclusion criteria

The inclusion criteria adopted were: age 36 to 60 years, newly diagnosed and untreated cases for T2DM. Diabetes mellitus was diagnosed by following the American Diabetes Association criteria 2015[4].

Exclusion criteria

Patients suffering from cardiovascular disease, hepatic disease, tuberculosis, renal disease and taking drugs like steroid, amiodarone, lithium, antioxidant vitamin supplement or non-steroidal anti-inflammatory drugs, antihypertensive drugs and other medications that alter thyroid functions and lipid levels led to exclusion from the study. Pregnancy and menopause also accounted for exclusion from the study.

Blood samples were collected from the selected participants from midcubital vein after taking aseptic precautions; approx 1ml blood in fluoride vacutainer, 4ml in EDTA vacutainer and 2ml in plain vacutainer after 10-12 hrs of overnight fasting, and the participants were asked to take breakfast and come after 2 hrs for the postprandial blood sample . Vacutainers were centrifuged at 2000 rpm for 5 minutes to obtain serum or plasma.

Fasting blood glucose was estimated by glucose oxidase method. Glucose oxidase converts glucose to gluconic acid. In addition, peroxidase (POD) produces hydrogen peroxide which oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye.[7] Lipid peroxidation was assessed by measuring plasma malondialdehyde (MDA) by Satoh's method.[8]. HbA_1c was estimated in whole blood on Bio-Rad D-10 by NGSP certified method which utilizes principle of ion-exchange high performance liquid chromatography (HPLC).

STATISTICAL ANALYSIS

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2010 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test and distribution of probability (P). Association between different variables was carried out by using Pearson's correlation coefficient.

RESULTS

The clinical and demographic profile of T2DM patients and healthy controls are represented in Table 1. In the present study, study group subjects belonged to age group 30- 60 years i.e. 43.9 ± 6.5 and 47.5 ± 7.2 years in Group I and Group II respectively, as represented in Table 1. Out of the selected 50 subjects of T2DM, 50 patients were male and 50 were female. The recruited diabetic patients have positive family history of T2DM i.e. in 75%. In addition, height and weight measurement followed by BMI calculation revealed that T2DM subjects had significantly high ($p<0.05$) BMI as compared to healthy non diabetic control group subjects. The observation made reveal insignificant increase ($p<0.1$) of waist hip ratio in the patients group subjects as compared to healthy controls.

Table-1: Demographic profile of T2DM patients and healthy controls (Mean ± SD)

S. No.	Particulars	Group I (n=50)	Group II (n=50)	P- value
1)	Age (years)	43.9 ± 6.5	47.5 ± 7.2	p<0.1
2)	M:F ratio	27/23	28/22	p<0.1
5)	BMI (Kg/m^2)	22.1 ± 1.7	23.7 ± 1.8*	p<0.05

Where, * $p < 0.1$: Non-significant; ** $p < 0.05$: Significant

As compared to normal healthy controls, abnormalities in glycemic profile along with markers of oxidative stress were observed in study group subjects with diabetes, as represented in Table 2. In the Group II subjects, fasting blood glucose level was increased significantly (P< 0.001) along with increase in postprandial blood glucose levels (P< 0.001) as compared to Group I subjects or healthy controls.

Table-2: HbA1c, Glycemic profile and lipid peroxidation status in study group subjects (Mean ± S.D.)

S. No.	Particulars	Group I (n=50)	Group II (n=50)	P- value
1.	Fasting Blood Glucose (mg/dl)	90.5 ± 10.2	140.5 ± 4.5	p < 0.001
2.	Post Prandial Blood Glucose (mg/dl)	124.8 ± 13.6	311.9 ± 23.2	p < 0.001
3.	HbA1c (%)	5.7 ± 0.35	9.4± 2.8**	p < 0.001
4.	Malondialdehyde (nmol MDA/ml)	1.90 ± 0.47	6.8 ± 0.48**	p < 0.001

Where, p<0.001: Highly significant;

The mean HbA1c levels in T2DM subjects were significantly high (P<0.001) as compared to controls i.e. 64.9% high. Similarly, malondialdehyde levels were found to be increased significantly (P<0.001) in T2DM patients as compared to healthy controls. In addition, blood HbA1c levels were positively correlated with erythrocyte MDA, fasting and postprandial blood glucose levels as represented in Table 3.

Table-3: Correlation coefficient (r) between HbA1c and malondialdehyde (MDA) along with fasting and post prandial blood glucose levels in T2DM patients

Particulars	MDA	FBS	PPBS
HbA1c	0.784 **	0.610 **	0.559 *

Where, . * p < 0.05 : Significant, ** p < 0.001 : Highly significant

DISCUSSION

It is noticeable that there is a deep underlying relation between diabetes mellitus and oxidative stress [6, 9] previous studies have also found that oxidative stress is much common in diabetic population and its related complications [10]. Recently, tremendous interest has been raised in the alteration of glycosylated hemoglobin (HbA1c) levels and oxidative stress in T2DM patients. When there is reduced insulin production or there is insulin resistance glucose is not transported into the cell from blood circulation, thus glucose levels in blood are not reduced and this leads to hyperglycemia. Further this hyperglycemia leads to increased availability of glycolytic intermediates causing their diversion into other pathways such as polyol pathway, hexosamine pathway, protein kinase C (PKC) pathway and the advanced glycation end (AGE) product, which ultimately is linked to the manifestation of micro and macro-vascular complications [11]. Moreover, with hyperglycemia, there is increased chance of interaction of glucose with hemoglobin, thus leading to increased level of glycosylated hemoglobin. HbA_1c reflects average plasma glucose over the previous 8-12 weeks [12]. Mahajan et al by using HbA_1c for diagnosis of T2DM in India have observed that HbA_1c has advantages over fasting plasma glucose estimation however for diagnosing diabetes mellitus, a number of biochemical, clinical and economical factors limit its use as a single diagnostic test [13].

Persistent hyperglycemia also causes increased production of free radicals, especially reactive oxygen species, major source being mitochondria [14]. However free radicals are disproportionately increased in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [15]. The imbalance between the rate of free radical generation and elimination leads to oxidative stress. There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in T2DM and that the onset of diabetes is closely associated with oxidative stress [16]. Free radicals cause lipid peroxidation. Malondialdehyde (MDA), is highly reactive compound and is one of the final products of polyunsaturated fatty acids peroxidation (lipid peroxidation) in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde is known as a biomarker of oxidative damage to lipids (polyunsaturated fatty acids) thus is a marker of increased free radical production. Our study illustrates the complex interplay between the altered glycemic profile and elevation of oxidative stress in the pathogenesis of T2DM complications. In the present study, marker of lipid peroxidation (MDA) and HbA1c levels were increased significantly (p<0.001) which reflects a possible pathogenic mechanism of excessive free radical production involved in insulin resistance in T2DM patients [17]. In addition, HbA1c levels were positively correlated with MDA levels in T2DM patients.

CONCLUSION

Therefore, the present study authenticates that oxidative stress is associated with Type 2 diabetes.

Hyperglycemias along with elevated HbA1c levels are linked with lipid peroxidation and this deteriorating effect of free radical during Type 2 diabetes seem to have negative consequences on insulin resistance and poor glucose management. These findings could also justify the increased risk for T2DM associated future complications, such as cardiovascular disease and kidney diseases. Therefore, it is very important to control oxidative stress by incorporating antioxidant rich diet and maintain the blood glucose level with proper and timely monitoring of HbA1clevels.

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