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RESEARCH ARTICLE

BIOCHEMISTRY

STUDY ON CORRELATION BETWEEN GLYCATED HEAMOGLOBIN AND OXIDATIVE STRESS IN DIABETICS

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ABSTRACT

Oxidative stress plays an important role in the pathogenesis of diabetic complications. Hyper glycemia causes increased production of free radicals & evidence supports a prominent role for these reactive molecules as mediators of endothelial cell dysfunction in diabetes.

Increased oxidative stress and impaired antioxidant defense have been suggested as contributory factors for initiation and progression of complications in diabetes mellitus.

This study was conducted to evaluate the role of glyclosylation in causing oxidative stress.

Study includes 50 known diabetic subjects. HbA1c, Thiobarbituric acid reactive substances, Total and oxidized ascorbic acid levels were estimated in diabetic subjects & controls. Raised HbA1c levels (p 0.005) in diabetic subjects shows increased glycation of proteins causing increased release of free radicals. Decreased levels of reduced ascorbic acid(p 0.01) are because of its consumption in antioxidant function. Thus the study out-lines the role of HbA1c as an oxidant in DM & points that ascorbic acid is an antioxidant.

KEYWORDS

DM – Diabetes mellitus , MDA - Malandialdehyde ,TBRs – Thiobarbituric acid reactive substances, ,

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder which is associated with the complications resulting in angiopathy & nephropathy[1] .

The complications of diabetes are influenced by the duration of diabetes, free radicals[2,3] & by the average level of chorinc glycemia which is measured most reliably with glycated Hb assay [4].

In normoglycemic subjects a small proportion of Hemoglobin A is attached to carbohydrate moiety; thus it creates a molecule called glycatedhemoglobin (HbA1c) . In diabetic subjects HbA1c levels elevates 2 to 3 folds over the levels found in normal individuals[5].

Hyperglycemia increases the glycation of proteins and produces products called advanced glycation end products (AGEs) which alter the structure & function of proteins. Glycation and oxidative stress are closely linked & both phenomena are referred to as glycoxidation. All steps of glycoxidation generate oxygen free radicals, some of them being common with lipid peroxidation pathways. Glycated proteins activate membrane receptors such as RAGE (Receptor for advanced glycated end products) & induce as intracellular oxidative stress. The knowledge of glycoxidation mechanism may lead to new therapeutic approaches[6].

In addition to being utilized as a diagnostic tool HbA1c is implicated as a culprit molecule to cause injury to vascular endothelium, it can be explained by the release of free iron from HbA1c. H_2O_2 can promote more iron release from HbA1c than that from non glycated Hb. This free iron acting as factor reagent might produce free radicals & degrade cell constituents[7].

Antioxidants play a protective role in the pathophysiology of diabetes. As an electron donor Vit C is a potent water soluble antioxidant in humans.

Increase levels of MDA may be useful marker for oxidative stress. The enhanced lipid peroxidation leads increased free radical activity. This increased free radical activity in diabetes mellitus can lead to activation of stress sensitive pathways which may play an important role in the complications of diabetes[8].

The aim of this study is to evaluate correlation between the increased levels of HbA1c as a marker of hyperglycemia & oxidative stress status by estimation of vit c & lipid peroxide.

MATERIALS AND METHODS

The study was conducted over a period of six months. The study includes fifty diabetic subjects admitted in medicine department in Narayana Hospital, duration of diabetes less than 10 years . They were in the age group of 30 to 60 years. Both the sexes were included.

Blood samples were collected after 12 hours of fasting for estimation of fasting blood glucose and blood is collected in EDTA and heparin tubes for the estimation of HbA_1C and vitamin C, lipid peroxide estimation respectively. PPBS(post prandial blood sugar) sample was collected after two hours of ingestion of food and analyzed.

Glycosylated haemoglobin is estimated in whole blood collected with EDTA.

25 members working in Narayana Hospital having normal fasting blood sugar values, within the age group of 40 years were taken as control subjects. Both the sexes were included.

The same procedure of sample collection and estimation of FBS, PPBS, HbA_1C, vitamin C and lipid peroxide is adopted for control subjects.

Glycated heamoglobin was estimated by cation exchange resin method[9].

Total ascorbic acid was estimated by colorimetric method[10].

Oxidized ascorbic acid was estimated by allowing it to react with acidic 2,4-Dinitrophenyl hydrazine[11].

Reduced ascorbic acid levels obtained by subtracting the oxidized ascorbic acid levels from total ascorbic acid levels

Malandialdehyde was estimated as thiobarbutric acid reacting substances[12].

RESULTS

The results were expressed as Mean (standard deviation). The p value was used to compare the patient mean value with control mean value.

The mean and standard deviation of all the parameters of the study were calculated in patients and control subjects.

Table I

Comparison of parameters in diabetic subjects and controls.

Sl. No	Parameter	PATIENTS		CONTROLS		p VALUE
		Mean	S.D.	Mean	S.D.	
1	HbA_1C	7.38	0.64	6.15	1.1	0.05
2	PPBS	253.9	41.9	105.01	11.7	0.01
3	FBS	203.8	40.8	90.2	9.33	0.01
4	Oxidized Vit. C	0.62	0.1	0.51	0.2	0.05
5	Reduced Vit. C	0.31	0.17	0.54	0.2	0.01
6	Total Vit. C	0.94	0.14	1.06	0.26	0.05
7	Lipoid Peroxidase	8.01	1.04	3.86	0.36	0.01

Table I shows the mean, standard deviation & p value of all parameters in patients & controls. The values of patients and control groups are also graphically represented for comparison. The graphs were plotted using mean values of all the study parameters.

Figure I,II,III,IV shows the mean values of HbA1c, Reduced ascorbic acid , oxidized ascorbic acid , lipid peroxide in diabetic subjects and controls as bar diabgrams.

FIGURE I

Comparison of patients with controls – HbA_1c (Mean)

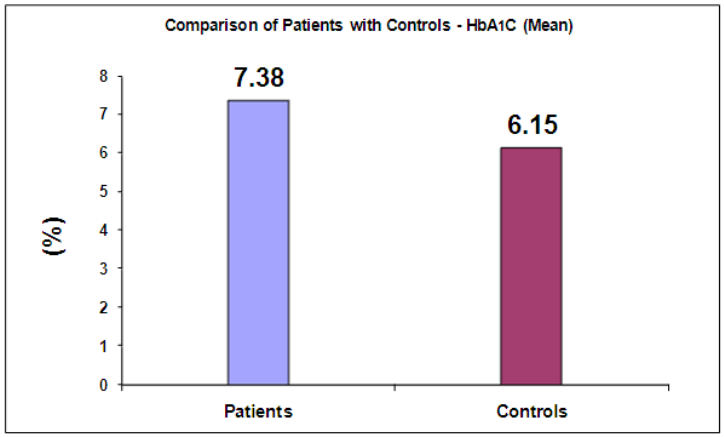
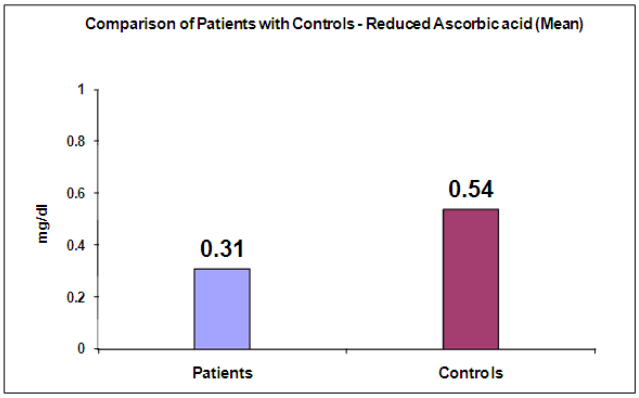


FIGURE II
Comparison of patients with controls – Reduced Ascorbic acid (Mean)



FIGUREIII
Comparison of patients with controls – Oxidized Ascorbic acid (Mean)

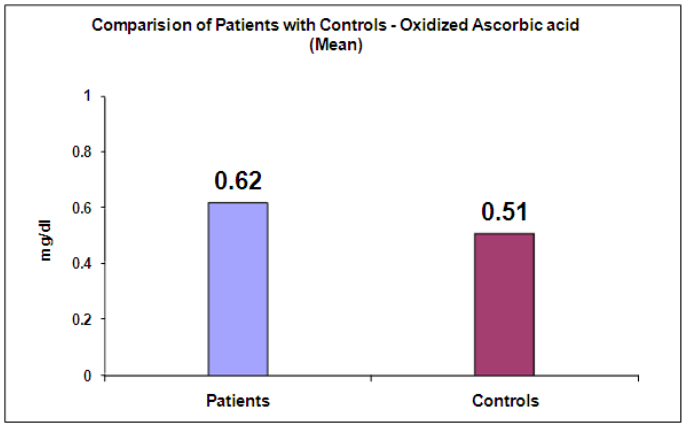
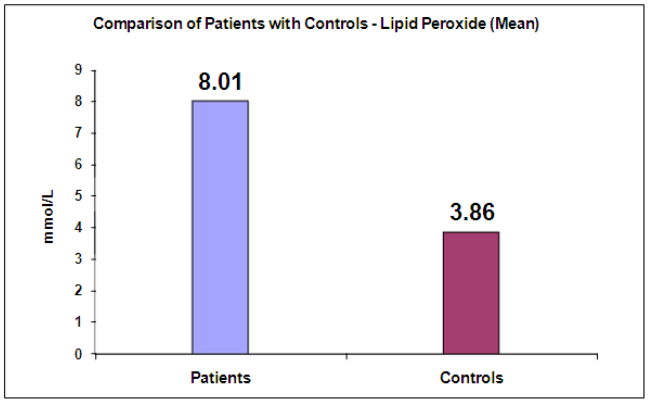


FIGURE IV
Comparison of patients with controls – Lipid peroxide (Mean)

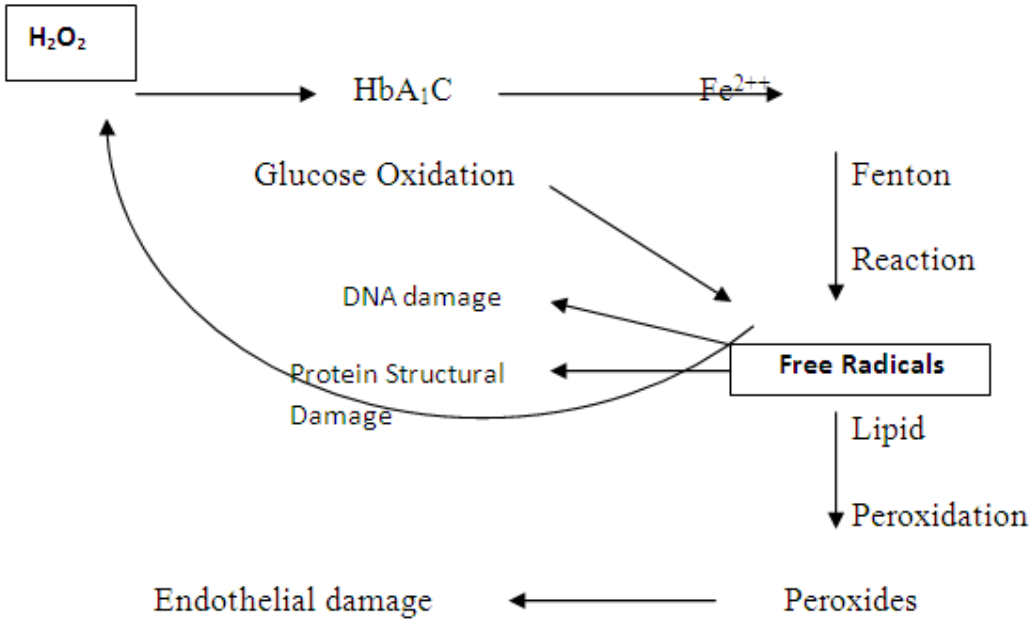


DISCUSSION

Diabetes mellitus is a chronic metabolic disorder primarily due to a defect in glucose utilization, consequently effecting the lipid metabolism. It is characterized by hyperglycemia. Normally fasting blood glucose lies between 60 to 100 mgs/dL and it rises with intake of food and reaches to near the fasting value within two hours. Failure to utilize the glucose within this period leads to hyperglycemia.

Persistent hyperglycemia of higher magnitude leads to a number of disturbances in metabolic status and internal mileau. One of the effects of hyperglycemia is glycosylation of haemoglobin and other proteins. Glycosylation is the interaction of glucose with the N-terminal amino acid of haemoglobin, producing glycolylated haemoglobins. Normally about one third of haemoglobin is glycosylated and it rises to two to three folds in diabetes patients. It persists all through the life span of RBC i.e, 120 days. Hence estimation of glycosylated haemoglobin is an important diagnostic tool to assess the long term control of blood glucose[13].

In addition to utilizing it as a diagnostic tool, it is implicated as a culprit molecule to cause injury to vascular endothelium. It can be explained by the following figure.



Free radicals as ROS and RNS are continuously produced in the body but their effects are counteracted by a number of molecules possessing reducing groups i.e, vitamin A, Ascorbic Acid (reduced), Tocopherols glutathione and enzymes like SOD and glutathione peroxidase and catalase H_2O_2 is produced as ROS and causes the release of iron from HbA_1c more readily than normal haemoglobin (HbA_1). The iron is responsible for generation of free radicals which degrades various cell constituents. They also interact with DNA and proteins and bring out detrimental changes in their structure and properties. HbA_1C is also subjected to auto-oxidation and generate further oxidation stress. The prominent effect of oxidative stress is on vascular endothelium, affecting the vasculature of vital organs like kidney, heart and brain and also cause cataracts of eye[14].

Many scientists worked on this problem and reported on the effects of HbA_1C on complications of diabetes mellitus.

HbA_1C values were elevated in all the diabetic patients along with elevated blood glucose values. (203.8 ± 40.8).

Oxidized and reduced ascorbic acid values were estimated in diabetic patients and control subjects. The reduced ascorbic acid values (0.31 ± 0.17) were significantly reduced and oxidized ascorbic acid values (0.62 ± 0.1) were elevated significantly in the diabetic patients, compared to control subjects (reduced ascorbic acid values 0.54 ± 0.2 and oxidized ascorbic acid values 0.51 ± 0.2, p value of oxidized ascorbic acid is 0.05 and reduced ascorbic acid is 0.01). It indicates that ascorbic acid is subjected to oxidation by oxidant radicals and keeps the reduced ascorbic acid unavailable as anti oxidant[15].

As marker of oxidative stress caused by HbA_1C malandialdehyde values were estimated as TBRs which were found to be significantly elevated (8.01 ± 1.03) compared to control values. It is because of lipid peroxidation[16].

Hence present study supports the view that HbA_1C is not only a parameter for long term control of blood sugar but also it is involved in causing oxidative stress and consequent damage to vascular endothelium. This work also points the need of assaying HbA_1C and parameters of lipid peroxidation and antioxidants like reduced ascorbic acid, right from the earliest stage of diabetes mellitus, so that remedial measures can be instituted to prevent the complicatioins due to microvascular damage by glycosylation and consequent oxidation stress.

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