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A STUDY ON CORRELATIONSHIP OF HBA1C AND PEROXIDASE ACTIVITY OF HB IN DIABETIC CANCER PATIENTS

Biochemistry

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ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycaemia, atherosclerosis, nerve damage, renal failure, infection, retinopathy etc. Oxidative stress can be a causative factor for type 2 diabetes as the increased levels of hydrogen peroxide can damage catalase poor pancreatic beta cells. Objective of the study was to find out any correlation between the diabetic status and antioxidant level of known diabetic cancer patients by measuring HbA1C level and red blood cell peroxidase activity respectively. In this study, known diabetic cancer patients and age and sex matched healthy controls coming to Dept. of Biochemistry, Medical College and Hospital, Kolkata between January 2022 to January 2023 were tested. It was found that the mean value of RBC peroxidase activity of the patients having HbA1C value >6% is 117.55+-11.94KU/L(cases) and of patients having HbA1C value <6% is 233.85+- 36.87 KU/L(controls) (p value < 0.0001). So, the antioxidant status (RBC peroxidase activity) is significantly higher in control population (age and sex matched healthy persons) than test population (known diabetic cancer patients)(p value< 0.001). RBC peroxidase activity correlated negatively with increasing HbA1C value . Tthis conclusion was supported by in vitro experiments done separately. This study can be used in future for better treatment of diabetic cancer patients with antioxidants therapy to lower their long term complications, mortality and morbidity.

KEYWORDS

Cancer, Antioxidants, Diabetes, Hba1C, Oxidative Stress, Red Blood Cell Peroxidase.

I. INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycaemia, atherosclerosis, nerve damage, renal failure, infection, retinopathy etc. Approximately 150 million people are affected by diabetes worldwide currently which will reach 300 million by the year 2025 if recent trends of progression of diabetes is not changed.[1]

According to some studies oxidative stress can be a causative factor for type 2 diabetes as the increased levels of hydrogen peroxide can damage catalase-poor pancreatic beta cells. Increased levels of hydrogen peroxide in muscle cells due to decreased red blood cell peroxidase activity or blood catalase may disrupt insulin signaling via inactivation of the oxidation sensitive tyrosine phosphatases that could not dephosphorylate insulin receptors. [2] So, it can be assumed that there is an imbalance between hydrogen peroxide and antioxidant levels in most of the diabetic cancer patients.

In this study, known diabetic cancer patients and age and sex matched healthy controls coming to the Dept. of Biochemistry , Medical College and Hospital, Kolkata were tested to find out any correlation between their diabetic status and antioxidant levels by measuring HbA1C and red blood cell peroxidase activities respectively.

II. MATERIALAND METHODS

Subject selection and sample collection:

40 cancer patients of Indian origin coming to the dept. of Biochemistry of Medical College and Hospital, Kolkata between January 2022 to January 2023 were used as subjects. 20 of them having HbA1C more than 6% acted as test, 20 of them having HbA1C less than 6% acted as controls. Patients with history of renal disease, cardiac disease, hypo or hyperthyroidism, any other major health problems, history of addiction, patients taking any kind of antioxidants were excluded. Fasting venous blood samples (about 3ml) were obtained from all patients and controls after overnight fasting .2 ml blood is collected in fluoride vials (to obtain plasma) & 1 ml into EDTA vials.

Estimation of red blood cell peroxidase activity:

Red blood cell peroxidase activity was determined by methodology of Goth, 1991 with a little modification which combines the spectrophotometric assay of hydrogen peroxide with an optimized serum catalase determination. In the original method serum was used to obtain serum catalase activity, but in our method we used diluted whole blood which contains only red blood cells, to obtain red blood cell peroxidase/ haemoglobin peroxidase activity.[3]

Optimal conditions for the assay were as follows: 0.02 ml whole blood mixed with 0.18 ml normal saline was incubated in 1.0 ml substrate (65 micromole/lit hydrogen peroxide in 60 mmol/lit sodium potassium phosphate buffer,pH 7.4) at 37 degree centigrade for 1 min. Red blood cell peroxidase activity is linear upto 100 KU/L, if the peroxidase activity exceeded 100 KU/L, the sample was diluted with the phosphate buffer (2 to 10 fold) and the assay was repeated. One unit of red blood cell peroxidasede compose 1 micromol ofH_2O_2 / min under these conditions.

Spectrophotometric assay of hydrogenperoxide :

The enzymatic reaction was stopped with 1 ml of 32.4 mmol/l ammonium molybdate {$(NH_4)_6Mo_7O_{24}$,4 H_2O)} and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against 3 blanks.

$$\text{Red blood cell peroxidase activity(KU)} = \frac{A(\text{Blank1})-A(\text{Blank3})}{A(\text{Blank2})-A(\text{Blank3})} * 271$$

Blank 1 contains 1.0 ml substrate ,1.0 ml molybdate and 0.2 ml sample(i.e. 0.02 ml whole blood & 0.18 ml normal saline). Blank 2 contains 1.0 ml substrate,1.0 ml molybdate and 0.2 ml buffer. Blank 3 contains 1.0 ml buffer,1.0 ml molybdate and 0.2 ml buffer.

Preparation of buffer for red blood cell peroxidase assay:

Na-K-Phosphatebuffer (60mmol/L),pH 7.4

Na_2HPO_4 (0.852gm) and KH_2PO_4 were dissolved in 100ml of distilled water and autoclaved.

Estimation of glycosylated haemoglobin:

Glycosylated haemoglobin was measured by ion exchange resin method with the help of kits from coral clinical systems.[4][5][6][7]

Measurement of plasma glucose:

Plasma fasting glucose was determined by glucose kit (GOD/POD) method supplied by coral diagnostics.

III. Review Of Literature

Glycation of haemoglobin is increased in diabetic cancer patients which is itself an index of long term blood glucose control. The major sites of non-enzymatic glycation in vivo, in order of prevalence ,are

the β-val-1,β-lys- 66,α-lys-61,β-lys-17andα-val-1.The most prevalent species is HbA1c (where glucose binds to β-val-1), the site is also involved with the binding of glycerate-2,3-bisphosphate, CO_2 and hydrogen ions. X-ray diffraction studies have shown that glycerate-2,3-bisphosphate forms ionic bonds with Val-1,His-2 and -143 of both β chains and Lys-82 of either β chain & this blocking of the α –amino position of the β chain gives rise to significant biophysical and biochemical modification of the haemoglobin molecule.[8]

Haemoglobin, due to its iron (III) protoporphyrin IX group, has catalytic features like horse radish peroxidase and cytochrome oxidase. In general,peroxidase enzymes are capable of reducing H_2O_2 to form water, by 2 one electron steps or one 2 electron step.

Manoj Kar et al (2001)[8] showed that HbA1c exhibits less peroxidase activity than $HbA1_0$. These findings on glycosylation induced functional properties of haemoglobin suggest a mechanism of increased formation of free radicals and oxidative stress in diabetes mellitus.

In normal physiological state, iron is tightly bound within protoporphyrin ring of heme pocket, under specific circumstances(e.g. after glycosylation), iron is released from heme & ligated to another moiety(distal histidine in heme pocket), this iron is 'free reactive iron'. This free reactive iron level is increased in diabetic cancer patients as their fasting blood glucose is increased. This free iron may increase the oxidative stress in such patients.

Khoo et al(1994)[9] showed that increased glycation significantly decreases the activity of haemoglobin or RBC peroxidase throughout the pH range.
They also showed that the binding of sugar moiety to haemoglobin molecule significantly lowers the peroxidase activity of haemoglobin mostly in the pH range 4.5 -6.0 and that the presence of 100 mmol/l sorbitol selectively increases the peroxidase activity of glycated haemoglobin.

Y.S.Mahindrakar et al(2007) also found that RBC peroxidase activity was decreased in type2 diabetic patients when compared to control. Further ,significant fall in peroxidase activity was observed in patients with complications as compared to patients without complication.

Increase in lipid peroxidation was observed in that study and glycosylation of the enzyme may result in decreased activity of peroxidase.[10]

A study was done in Russia in 2013 by D.V.Grigorieva et al. They measured haemoglobin peroxidase activities in healthy individuals and patients with type 2 diabetes mellitus and coronary heart disease, high haemoglobin peroxidase activities in both groups of patients indicates disorders in the mechanism of clearance of haemoglobin and its highly reactive derivatives and can serve as specific markers of diseases associated with oxidative stress.[11]

Rationale of this study:
The above background shows that there is considerable role of oxidative stress and antioxidant status in long term diabetic cancer patients with high HbA1C values. So several research with different oxidative stress parameters, in different race, ethnicity, dietary habits should be done to obtain a reliable and authentic finding.

In recent years, there has been a significant rise in diabetic cancer population with significant complication rate, mortality and morbidity.

This kind of studies which are aimed to find the root cause of diabetes in cancer patients and its complications can be used in future for better treatment of diabetic patients with antioxidants and to lower their long term complications.

IV. RESULTS AND ANALYSIS

Statistical analysis were done by Mann-Whitney U test, as the values were not normally distributed (by Kolmogorov- smirnov goodness of fit test).

After statistical analysis, it was found that the mean value of RBC peroxidase activity of cancer patients having HbA1C value >6 % is 117.55 +-11.94 KU/L (cases) and of cancer patients

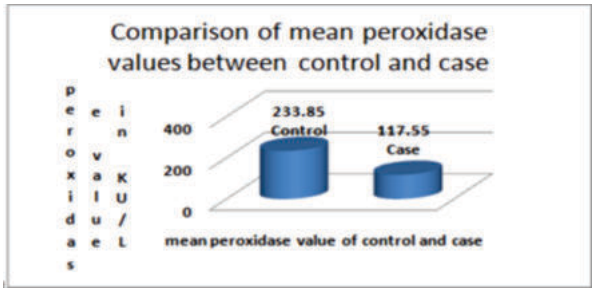

Diagram1
having HbA1C value <6% is 233.85+- 36.87 KU/L(controls).(p value < 0.0001).

Inference-
So, the RBC peroxidase activity is significantly higher in control population than test population (p value< 0.001).

Table–1- Descriptive Statistics Of Numerical Variables - Group Control -Hba1c<6%(sample Number=20)

					95% CONFIDENCE INTERVAL	
	VALID NO.	MEAN	STD. DEV.	MINI MUM	MAXI MUM	LOWER BOUND	UPPER BOUND
Mean peroxidase value	20	233.85	36.87	171	290	160.11	307.59

Table–2- Descriptive Statistics Of Numerical Variables - Group Case -hba1c>6% (sample Number=20)

						95% CONFIDENCE INTERVAL	
	VALID	MEAN	STD.	MINI MUM	MAXI MUM	LOWER	UPPER
	NO.		DEV.			BOUND	BOUND
Mean peroxidase value	20	117.55	11.95	95	136	93.65	141.45

The mean value of RBC peroxidase activity became significantly lower as the HbA1C value increased in both test and control population. The data is as follows -

Table-3 HbA1C Value Mean RBC Peroxidase activity (KU/L)

3-4%	(No.of samples= 6)	268
4-5%	(N=7)	250.14
5-6%	(N=7)	188.29
6-7%	(N=9)	128.11
>7%	(N=11)	108.91

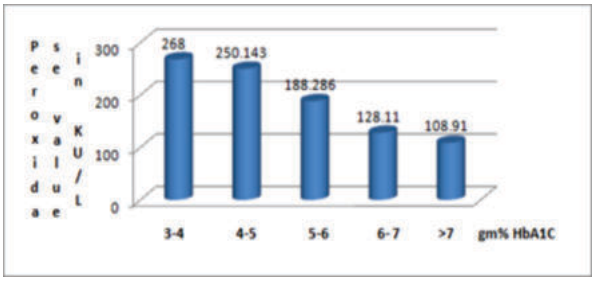

Diagram 2

Inference –
From the data obtained we can assume that RBC peroxidase activity correlated negatively with increasing HbA1C value.(Spearman's correlation coefficient ρ= -0.5412) (vide diagram 2 and 3)

Results of in-vitro study
Fasting whole blood sample was taken from an otherwise healthy control and the red blood cells were washed by phosphate buffered saline three times . Then the washed RBCs were divided into five equal parts (10 microlit each).
1st vial 10 µL washed RBC + 90 µL 500 mg/dl dextrose solution,
2nd vial 10µL washed RBC + 90µL 400 mg/dl dextrose solution,
3rd vial 10µL washed RBC + 90µL 300 mg/dl dextrose solution,
4th vial 10µL washed RBC + 90µL 200 mg/dl dextrose solution,

5th vial 10µL washed RBC + 90µL 100mg/dl dextrose solution.

So, washed RBC and dextrose solution was taken in 1:9 ratio and incubated at 37°C for 12 hours. Then ,from each vial HbA1c and RBC peroxidase activity was measured by modified Goth method separately.

The result is as follows -

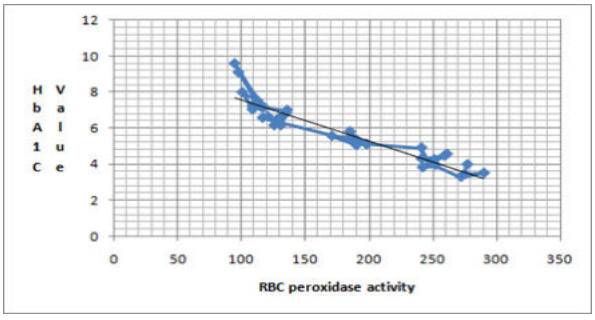

Diagram 3

Table-4

Blood Sugar (mg/dl)	HbA1c	Peroxidase value of RBC (KU/L)
1.)500	8.02%	89
2.)400	6.98%	139
3.)300	5.89%	178
4.)200	4.82%	221
5.)100	4.01%	243

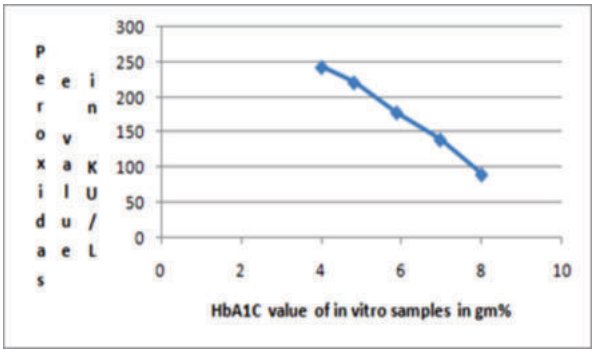

Diagram 4

Inference-

From this data it can be assumed that RBC peroxidase activity progressively decreases or negatively correlated as the HbA1c value increases in in- vitro samples.(vide table 4, diagram 4)
This data supports the data obtained from in vivo study.

V. DISCUSSION

In this study, the mean RBC peroxidase activity was higher in cancer patients having HbA1C value <6% than in cancer patients having HbA1C value >6% and RBC peroxidase activity correlated negatively with increasing HbAIC value. This conclusion was supported by in vitro experiments done separately.

Celik S et al (2009) showed a similar decrease in SOD and catalase activity in diabetic rats and their protective system against oxidative stress were also impaired.[12]

Merzouk et al (2003) stated that they found SOD and other antioxidant activity significantly low in type 2 diabetics compared to control.[13]

Wu et al (1999) found in the experimental study with diabetic rats that, in diabetic rat kidney tissue , SOD , POD(peroxidase) and catalase activities significantly decline at 8th and 16th weeks.[14]

W Chatuphonprasert et al (2014) showed that berberine possesses hypoglycaemic properties and strong potential to improve the oxidant antioxidant balance. In type 2 DM rats, berberine increased the hepatic Cu Zn SOD m RNA expression and the kidney SOD and CAT activities to normal level which was lower before the treatment.[15]

N V Kresyun et al(2014) showed that in 1.5 months after modelling of streptozotocin induced DM, wister rats superoxide dismutase and catalase activities decreased by 40.7% and 32.0% respectively in comparison with healthy wister rats.[16]

Whereas in some other studies they found no alteration in the activity of catalase in plasma or RBC in diabetic patients (Dohietal, 1998;Asayama et al.,1989).[17][18] Others reported decreased catalase activity (Tagami *et al.*, 1992)[19] in aortic endothelial cells of known diabetic patients, while Langenstroer and Piper (1992)[20] reported an increase in these cells. These other investigations used animal models with induced diabetes, the duration of diabetes was not a factor in the other studies.

In diabetic patients auto oxidation of glucose results in the formation of hydrogen peroxide(H_2O_2), which enhances inhibition of superoxide dismutase. Therefore the accumulation of hydrogen peroxide can be the reason behind decreased SOD activity.

VI. CONCLUSION

Our study showed decreased RBC peroxidase activity in diabetic patients where H_2O_2 could have accumulated. The decrease in RBC peroxidase & plasma catalase activity may therefore be a consequence of SOD glaciation or glaciations of catalase. So the decrease in RBC peroxidase or plasma catalase activity which reflects the antioxidant level may be due to exhaustion of the enzymes by H_2O_2. This speculation supports the results obtained in our study.

Our study also showed that there was gradual decrease in RBC peroxidase activity as the HbA1C value in both test and control subjects raised from 3% to > 7%. This finding was supported by another similar study by L Goth (2008) [21]. He found plasma catalase activity in type 2 diabetic subjects were decreased significantly than healthy control population. He suggested that down regulation of catalase synthesis, rather than specific catalase gene mutations, is responsible for decreased catalase activity in diabetics & life-long increased hydrogen peroxide concentrations due to catalase gene mutations may be a risk factor for diabetes , this risk maybe due to peroxide damage of normally catalase-poor pancreatic beta cells. This study was done in a tertiary care hospital of Calcutta and it may not reflect the actual situation so there is always scope to elaborate research with larger sample size, different race and with other oxidative stress markers.

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