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Comparative study of Glycated hemoglobin, Erythrocyte reduced Glutathione, Total protein and Protein Albumin ratio in Maturity onset Diabetes Mellitus.

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ABSTRACT

Purpose of this study is to measure the level of oxidative stress in maturity onset Diabetes Mellitus patients.

Glycated hemoglobin reflects long tern glycemic control and it is more accurate and stable than fasting blood glucose level. It tracts well over time in persons with Diabetes Mellitus and has less measurement error than fasting blood glucose. Erythrocyte reduced glutathione level estimation become important to measure oxidative stress, which may lead to aggravate diabetic complications.

Method of estimating Glycated hemoglobin is "thiobarbituric acid" method , and Erythrocyte reduced Glutathione is estimated by "Dithiobis nitro benzoic acid " (DNBT) method .

In this study correlations between above two parameters have been established, and results found that, Glycated hemoglobin and Erythrocyte reduced glutathione levels are inversely proportional, with hyperglycemia, in most of the uncontrolled Diabetes Mellitus.

Key words: Glycated Hemoglobin, Erythrocyte reduced glutathione, Maturity onset Diabetes Mellitus, Glycemic control,

I. INTRODUCTION

Diabetes Mellitus may be characterized as an insufficiency of insulin, relative to the requirements of the tissue for the hormone. Diabetes is a generalized metabolic disorder manifesting itself, in fully developed form by hyperglycemia, glycosuria, increased protein break down, ketosis and acidosis. If the disease is prolonged, it is usually complicated by degenerative changes of the blood vessels, retina, kidney, and nervous system.

In Diabetes Mellitus there are wide spread biochemical abnormalities, But the fundamental defects to which most of the abnormalities can be traced are: a) A reduced entry of glucose into various peripheral tissues. b) An increased liberation of glucose into circulation from the liver (increased hepatic gluconeogenesis). There is therefore, an extracellular glucose excess and an intracellular glucose deficiency, a situation which has been called, "Starvation in the midst of plenty" and c) A decrease in the entry of amino acids into the peripheral tissues and an increase in lipolysis.

Complications of Diabetes Mellitus:

Acute metabolic complication is diabetic ketoacidosis. Late complications are i) Circulatory abnormalities i.e. arteriosclerosis, intermittent claudication, gangrene. ii) Retinopathy. iii) Diabetic nephropathy. iv) Diabetic neuropathy and v) Diabetic foot ulcer.

What causes the complications of Diabetes mellitus?

The cause of diabetic complications are not known and may be multifactorial. Major emphasis has been placed on the Polyol pathway, which is associated with decrease in myoinositol content in cell, abnormal phosphoinositide metabolism and a decrease in Na^+ , K^+ ATPase activity.

A second mechanism of potential pathognomic importance is glycation of proteins. The effect of such glycation on hemoglobin has been mentioned but multiple proteins in the body are altered in the same way, often with disturbed function. Example include plasma albumin, lens protein. Fibrin, collagen, lipoproteins and the glycoprotein recognition system of the hepatic endothelial cells.

Clinical significance of Glycated protein:

Measurement of Glycated protein is useful in monitoring long term glucose control in individuals of diabetes mellitus. It provides a retrospective index of the integrated plasma glucose values, over a estimated period of time, and is not subjected to the wide fluctuations, when assaying blood glucose level. Glycated protein levels are therefore, are a valuable adjunct of glycemic control. However, these proteins are not reliable for the diagnosis of diabetes mellitus.[1]

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Glycated hemoglobin:

Glycation is the non enzymatic addition of sugar residue to amino groups of proteins, Human adult hemoglobin (Hb) usually consist of HbA (97% of the total) HbA_2 (2.5%) and HbF (0.5%). Chromatographic analysis of HbA identifies a number of minor hemoglobin $HbA_{1a,}$ HbA_{1b} and HbA_{1c}; which are collectively known as HbA_1, fast hemoglobins (because they migrate more rapidly than HbA in the electrical field) or Glycated hemoglobins or glycohemoglobins. HbA consists of four polypeptide chains two alfa chains and two beta chains. Glycated hemoglobin (HbA_{1c}) is formed by condensation of glucose of N-terminal valine of each beta chain of hemoglobin A to an unstable Schiff base (aldimine, $PreA_{1c}$), which can undergo an amadori rearrangement to form a stable ketoamine HbA_{1c}. HbA_{1c} is the major fraction, consisting approximately 80% of HbA_1..

Reduced Glutathione and diabetes mellitus:

It has been reported that, impairment of glutathione metabolism occur in erythrocytes from patient with diabetes mellitus. [2] Erythrocytes contain high concentration of glutathione; normally most of the glutathione in erythrocytes exists in a reduced form (GSH). GSH is synthesized in human erythrocytes with a half life of four days. Glutathione is a important reducing agent in the tissue. Oxidized glutathione (G-S-S-G) is harmful to the tissue, especially to the red blood cells and lens protein and is converted to reduced glutathione(GSH) which is required for integrity of red blood cells membrane and lens protein. By donating H_2 it helps to destroy H_2O_2 and other peroxides in cells. Examination of pattern and magnitude of glutathione abnormalities may clarify whether there is a generalized increase in oxidative stress in diabetes mellitus and whether oxidative stress plays any role in determining why some organs are more susceptible to the development of diabetic complications.[3]

II. METHODS

A**) Estimation of Glycated hemoglobin**[4]: The glucose moiety of Glycated hemoglobin in converted to5-hydroxy methyl furfural by heating with oxalic acid in boiling water both for 1 hour. The supernatant which contains 5-hydrxymethyl furfural is allowed to react with2- thiobarbituric acid, the colour developed is measured photometrically at 443 nm.

C) Estimation of erythrocyte reduced glutathione[5]**:** Virtually all of the nonprotein sulfhydryl groups of RBCs are in the form of GSH. 5-5' Dithiobis (2-nitro benzoic acid) DNBT is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration.

D) Estimation of plasma Glucose: GOD-POD method.

III. RESULTS:

Sixty three subjects suffering from maturity onset diabetes mellitus on oral hypoglycemic agent treatment and thirty healthy controls of both sexes were included in the present study.

The values of glycated hemoglobin, and erythrocyte reduced glutathione along with plasma glucose concentrations (fasting and post prandial) were estimated in normal controls and subjects with maturity onset diabetes mellitus.

Subjects studied were divided into following three groups: Group A: Control, Group B:Subjects with fasting plasma glucose concentration greater than 108 mg% but less than 144mg%, Group C: Subject with plasma glucose concentration equal or greater than 144mg% .

In group A, control subjects aged 20-61 years with 1:2 male female ratio were studied.

Table 1

Age and Sex distribution

Age distribution

Age (Years)	Group A (Control)(n=30)	Group B (n=30)	Group C(n=33)
20-30	05	01	04
31-40	08	nil	06
41-50	09	12	11
51-60	04	11	08
61-70	04	06	04

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Sex distribution

Sex	Group A Controls (n=30)	Group B (n=30)	group C(n=33)
Male	10	16	18
Female	20	14	15

Table 2
Plasma sugar, Glycated hemoglobin, Erythrocyte reduced Glutathione in control subjects.

Sr no.	Parameters group A	Mean value	Standard deviation
1	Plasma glucose level (fasting mg%)	88.2	±12.7
2	Plasma glucose level (Post Prandial mg%)	115.3	±7.19
3	Glycated hemoglobin (percent of total Hb)	7.0	±0.34
4	Erythrocyte reduced glutathione (mg%)	65	±14.4

Table 3
Correlation between degree of hyperglycemia with glycated hemoglobin, and erythrocyte reduced glutathione.

Study group	Mean plasma glucose, Fasting (mg%)	Mean plasma glucose, Post Prandial (mg%)	Mean glycated hemoglobin(% of total hemoglobin)	Mean erythrocyte reduced glutathione (mg%)
Group A	88.2±12.7	115.3±7.19	7.0±0.34	65±14.4
Group B	124.6±9.67	166.1±21.5	8.9±0.87 p<0.01(SS)	55.6±15.0 p<0.05(SS)
Group C	195.1±39.4	247.5±50.5	11.6±1.5 p<0.01(SS)	48.4±21.2 p<0.01(SS)

SS= statistically significant.

IV. DISCUSSION:

In this study fasting venous plasma glucose level were increased 1.4 and 2.2 folds in the disease group i.e. group B and group C.

It is to be noted that, Glycated hemoglobin concentration in this study was significantly elevated in disease group. Normal value of Glycated hemoglobin was found 4-7 % by other authors[3] .In this study the mean value was within this range i.e. 4-7% of total hemoglobin. The result obtained in this study is in good agreement with the studies of other authors [6,7]. Thus we can conclude that, HbA1c is present in increased amount in patients with maturity onset diabetes mellitus as a consequence of increased plasma blood glucose level. In addition to the usefulness of HbA_{1c} levels in the management and care of diabetic patients, it is suggested that, serial measurement of HbA_{1c} levels (every 2 months) may allow an accurate estimate of the degree of hyperglycemia in diabetic patients and therefore, allow long term studies on effects of the adequacy of control of hyperglycemia in the prevention of various diabetic complications.

Erythrocyte reduced glutathione level was found 65±14.4 mg% in control group, whereas in group B and group C (disease group) it was 55.6±15 mg% and 48.4±21.2mg% respectively. In this study the decrease of erythrocyte reduced glutathione level was found only as compared to controls, with a wide range of standard deviation. The decrease as compare to control is statistically significant (in group B $p<0.05$ and in group C $p<0.01$). Results of GSH level in this study is in agreement with earlier studies Matsubara LS et al (1992)[8] and Yoshida K et al (1995)[9].

Yoshida K et al (1995)[9] in similar study found that, the activity of gamma glutamyl cysteine synthetase, the concentration of glutathione and the thiol transport were 77% and 69% respectively in erythrocytes from non insulin dependent diabetic patients compared to normal control subjects . But treatment of patients with antidiabetic agents for 6 months resulted in the restoration of the gamma glutamyl cysteine synthetase activity, the

concentration of glutathione and the thiol transport. This result suggests that inactivation of glutathione synthesis and thiol transport in the diabetic patients increases the sensitivity of the cells to oxidative stress.

The finding of our study was in consensus with the latter two authors; the mean value of erythrocyte reduced glutathione level decreases in diabetic cases by 0.8 fold and 0.7 fold in the group B and group C respectively as compare to controls with wide range of standard deviation because receiving of treatment properly with oral hypoglycemic agents restores the Redox activity of erythrocytes of non insulin dependent diabetes mellitus.

Because of the above mentioned reasons we included serum albumin, globulin and A:G ratio estimation along with estimation of Glycated hemoglobin and reduced glutathione . But in our observations serum albumin, globulin or A:G ratio did not alter significantly in diabetic groups as compared to controls. So their contribution to alter actual serum Glycated hemoglobin and reduced glutathione level was considered insignificant in this study.

V. SUMMARY :

In this present study sixty three subjects suffering from maturity onset diabetes mellitus and thirty normal controls of both sexes were included. All thirty three subjects were on treatment with oral hypoglycemic agents , All the study subjects were grouped as follows :

Group A : Control subjects

Group B : Subject having fasting plasma glucose level >108 mg% < 144mg%.

Group C : Subjects having fasting plasma glucose level >144mg%

The parameters investigated in this study were as follows:

1) Glycated hemoglobin

2) Erythrocyte reduced glutathione level

3) Serum Albumin, globulin and A:G ratio.

We found that, Glycated hemoglobin concentrations were increased by 1.3 and 1.6 folds in disease groups as comparison to controls ($p<0.01$) in this study.

In this study erythrocyte reduced glutathione level were reduced by 0.8 and 0.7 folds in disease groups as compared to controls ($p<0.05$ in group B and $p<0.01$ in group C) with wide range of standard deviation.

Other parameters such as serum albumin , globulin and A: G ratio did not alter significantly.

VI. CONCLUSIONS:

1) Glycated hemoglobin level increases in the maturity onset diabetes mellitus patients and the increase is correlated with plasma glucose level.

2) Erythrocyte reduced glutathione level tends to fall in maturity onset diabetes patients, through treatment by oral hypoglycemic agent try to restore the Redox activity of erythrocytes of diabetic patients.

BIBLIOGRAPHY:

[1]. David B Sacks. Carbohydrates, Text book of Clinical Chemistry, 2nd ed. Carl A Burtis, Edward R Ashwood Ed. W B Saunder Co. Pg 983

[2]. Murakami K, Kondo T, Ohtsuka Y et al: Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus , Metabolism 38(8): 753-58, 1989.

[3]. Mc Lennan SV, Heffernan S, Wright L et al : Changes in hepatic glutathione metabolism in diabetes . Diabetes 40: 344-47, 1991.

[4]. Estimation of Glycated hemoglobin. Text book of Clinical Biochemistry. Principal and practice , 1st ed, 1994 by Praful B Godkar. Pg 115-16

[5]. Bentler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med 61: 882-88, 1963.

[6]. Gonen G, Rubenstein AH, Rockman H et al: Hemoglobin A_1 An indicator of metabolic control of diabetic patients . Lancet 2: 734-37, 1977

[7]. Ditzel J, Kjaergard JJ et al : Hemoglobin A_{1c} concentration after initial insulin treatment for newly discovered diabetes. Brit Med J, 1: 741-42, 1978

[8]. Matsubara LS, Ferrera AL, Tornero MT, Machodo PE. Influence of diabetes mellitus on glutathione redox system of human red blood cells.Braz l Med Biol Res 25(4) ; 331-35, 1992

[9]. Yoshida K, Hirokawa J, Tagami S el al: weakened cellular scavenging activity against oxidative stress in diabetes mellitus: Regulation of glutathione synthesis and efflux. Diabetologia 38(2) : 201-10, 1995.