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Mean Blood Glucose Level and Glycated Hemoglobin Level in Patients of Non-Insulin Dependent Diabeties Mellitus and its Correlation With Serum Ferritin Level

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ABSTRACT

Sub clinical hemochromatosis has been considered as one of the probable cause for diabetes mellitus and this fact gave us an impetus to study the serum ferritin level in type 2 diabetes mellitus and correlate with HbA1c and MBG. This study was conducted in forty seven diabetic patients and compared with forty seven normal age-matched individuals as the control group. Blood samples had been taken from the subject after overnight fasting; the serum ferritin levels, HbA1C and mean blood glucose level were measured. Data were analyzed by student "t" test, chi- square analysis, and correlation coefficient test. Serum ferritin, HbA1c and MBG were significantly increased in type 2 diabetes patients. Out of the forty seven diabetic patients twenty four had hyperferritinemia, and twenty eight cases had poor control of HbA1c and MBG level. From this study it was concluded that 51 per cent of the diabetes patients had significantly higher serum ferritin level when compared to the normal and there is no correlation between serum ferritin, HbA1c and MBG but there is a positive correlation between HbA1c and MBG. Thus elevated serum ferritin concentration was associated with an increased risk of diabetes.

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Diabetes mellitus is a lifelong disease which makes many people worry about the quantity and longevity life after being diagnosed[1]. It is a bipolar in nature with multi-factorial inheritance[2]. People with type-2 diabetes mellitus develop characteristic micro vascular complications such as retinopathy, nephropathy and neuropathy. There is also increased risk of macro vascular complication such as cardiovasculopathy, cerebrova-sculopathy and peripheral vasculopathy[3] although an association between elevated blood glucose level and micro and macro vascular complication has been recognized for a long time[4]. The complications of diabetes mellitus is influenced not only by the duration of the diabetes mellitus but also by the average level of blood glucose along with glycated hemoglobin.

A small proportion of hemoglobin A is attached to a carbohydrate moiety thus creating what is called glycated hemoglobin[5]. It has been established that lowering HbA1C which is a non-enzymatic binding of glucose with free amino group of globulin chain. Thus it is well recognized that near normal glycemic control prevents subsequent diabetic complications[6]

Even smaller accumulation of iron can alter the glucose and insulin homeostasis of the body[7]. This suggestion is based on the observation that increased ferritin level was associated with poor glycemic control[8]. Epidemiological studies also suggest that high iron body stores are associated with insulin resistance and type 2 diabetes mellitus[12]

Serum ferritin is an essential iron stores have been proposed as a component of insulin resistant syndrome. In a study by Salonen and Nyyssnen[10] serum ferritin concentration adds a significant positive correlation with blood glucose and serum triglycerides. Excessive iron accumulation can induce organic damage that leads to diabetes. Saudek and Hemm[11] stated that 50% transfusion treated thalasemia patients have an abnormal glucose, similarly Admas and Kertesz 1991[12] concluded in the study that 65% of hereditary hemochromatosis patients develop diabetes mellitus.

RESEARCH METHODOLOGY

This was a prospective case control study conducted from September 2009 to December

2009 in patients who were attending the general medicine outpatient department of SRM Medical College Hospital and Research Centre, Kattankulathur. The project was approved by institutional ethical committee and done as per Helsinki's ethical guide line of 2000. Each patient gave informed consent to obtain a blood sample. There was no dietary or exercise restriction. The subjects were asked to come in morning with overnight fasting and they were also instructed to give the blood samples for the analysis of parameters like blood glucose, serum ferritin, HbA1c, MBG. After centrifugation sera were stored at 2 - 8^0C until further analysis. We included the patients with diabetes mellitus (Type II), and we excluded the following patients who were diagnosed as patients of type 1diabetes mellitus, bleeding piles, history of acute GI, patient on dialysis, patient having recent trauma and surgery, patient with recent worm infestation and infections, patient of menorrhagia / DUB, and those having micro and macro vascular complications of diabetes mellitus.

This study was conducted on ninety four subjects and they were divided into two groups. We had taken the first group as forty seven healthy controls and second group as forty seven type-2 diabetic patients. Subjects detail history was taken and we also recorded the age, sex, height, weight, basal metabolic rate, body mass index. The subject had been resting in sitting position for at least 5 minutes after that blood samples were collected by venepuncture. The blood was transferred immediately into three plastic tubes. The first tube containing the anticoagulant mixture, potassium oxalate and sodium fluoride in the ratio of 3:1, 2ml of blood was transferred for the analysis of glucose. To the second tube 2ml of blood was transferred and the blood was allowed to clot for serum separation. The serum was analyzed for ferritin only. The third tube containing the anti coagulant EDTA, 5ml of blood was added to that tube for the analysis of HbA1c and MBG.

The HbA1c was estimated by ion exchange resin photometric method[13]. The principle was that a hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. During mixing, HbA0 binds to the iron exchange resin clearing GHb free in the supernatant. After mixing period a filter separator is used to remove the resin from the supernatant. The per cent glycated hemoglobin is determined by measuring absorbance ratio of the glycated hemoglobin and total hemoglobin fraction of the control and the test is used to calculate HbA1c in the sample and calculate the MBG by comparable chart.

The serum ferritin was analyzed by UBI MAGI well enzyme immunoassay[14]. The ELISA's well coated with specific anti-ferritin antibodies were incubated with samples and another anti-ferritin antibody conjugated with horse-raddish peroxidase. The principle basis was that the amount of bound peroxidase is proportional to the concentration of the ferritin present in the sample. Unbound conjugate is washed off with water. Upon addition of the TMB substrate, the intensity of color development is proportional to the amount of ferritin in the samples, and is measure by using 450nm microtitre plate reader. Samples ferritin concentration is obtained by reference to standard.

RESULTS AND DISCUSSION

The diabetes control and complications trial[15] as well as the United Kingdom Prospective Study[4] have used HbA1c as the primary index of glycemic control.Glycated hemoglobin is recommended for both checking blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels then diabetes mellitus.

Glucose levels in blood can vary widely each hour so one or only a few sample from a patient analysed for glucose may be representative of glucose control in the long run. For this reason a blood sample may be analyzed for HbA1c level, which is more representative of glucose control average over a longer time period (determined by the half time of the individual's red blood cells, which is typically 50-55 days). People whose HbA1c runs 7.0% or less show good long- term glucose control. HbA1c values which are more than 8.0% are elevated[16].

The higher the blood glucose sugar, the faster HbA1c will be formed, resulting in higher HbA1c levels. Red blood cells circulate 90-120 days, and the HbA1c is in part affected by blood sugar levels over a 3- month period.

Table 1 : Mean and SD values in patients of diabetes mellitus and health control

Parameters	Experimental sample size	Experimental group	Control sample size	Control group	t- value	P – value
HbA1c	47	8.511± 1.0926	47	5.250 ±.4899	18.670	.000***
MBG	47	236.83 ± 51.520	47	78.49 ± 20.511	19.576	.000***
FERRITIN	47	239.31 ± 77.125	47	100.79 ± 33.780	11.279	.000***

Data are means ± SD *** - Highly significant, HbA1c - Glycated hemoglobin MBG - Mean blood glucose

Table 2 : Correlation coefficients between HbA1c, MBG and serum ferritin

Parameters	HbA1c	MBG	FERRITIN	HB	RBC	PCV
HbA1c	1.000***	.992*	.478	.234	.207	.180
MBG	.992*	1.000***	.491	.223	.214	.166
FERRITIN	.478	0.491	1.000***	-.027	.009	-.091

*** - Highly significance * - Significance

However, it mainly represent level over the past month and is heavily weighted to the past 2 weeks.

Faranak and Sharif[17], reported that the ferritin concentration in glucose-impaired subjects, a high-risk population for type 2diabetes, was significantly higher than that in normal control subjects, implying that hyperferritinaemia occurs. One of these studies was reported from Zanjan, Iran**.** Excessive ferritin concentration can be a marker of iron overload and subclinical haemochromatosis in diabetes patients[18]. There are some reports of a link betweenC282Y and H63D mutations in the HFE gene (haemochromatosisgene) and type 2diabetes[19]. Moreover, first degree relatives of patients with type 2 diabetes mellitus with normal glucose tolerance have higher ferritin concentrations than normal control subjects [20] these observations may suggested genetic predisposition to hyperferritinaemia in type 2 diabetes.

Iron overload decreases insulin sensitivity [21] and can cause earlier complications in diabetes. A small study proved that bloodletting which resulted in a 50 per cent reduction of serum ferritin concentrations, improved glycaemia Therefore, in addition to pancreatic beta cell damage, insulin resistance may be the other explanation for hyperglycaemia following iron overload. Iron is a potent pro-oxidant, and reactive oxygen species have been shown to interfere with insulin signaling at the cellular level[22]. Abnormalities in ferritin metabolism following glycation in a hyperglycaemic state might be a primary cause of hyperferritinaemia in type 2 diabetes.

In the present study based on the chi-square analysis the Table 3 showed that twenty eight diabetic patients had higher HbA1c and the remaining ninteen diabetic patients had good control HbA1c. There are also some physiological factor that affect HbA1c and need consideration when interpreting the result. The most important of these is erythrocyte turnover rate since the longer a given erythrocyte has circulated, the more glycated its hemoglobin becomes. Any condition that shortens erythrocyte life span (such as a hemolytic anemia or hypersplenism) will decrease HbA1c since the average erythrocyte is younger, with less time in circulation to be glycated. Conversly, a sudden cessation of erythropoiesis as in aplastic anemia will result in aged circulating red blood cells and no new reticulocytes entering the pool so HbA1c will progressively rise. The accuracy of HbA1c as an index of glycemic control, therefore, it depends upon the erythrocyte turnover being normal[23]. The reson why the present study we excluded the other organ diseases and hemoglobinopathies.The Table 3 also showed that twenty four diabetic patients had iron over load, and the remaing twenty three patients had the normal iron levels.

Table 3 : Frequency distribution of variables among a case control study of type 2 diabetes and normal subjects and comparison of variables between low and high level of HbA1c, MBG, serum ferritin using chi-square analysis

Parameters	Variations	Experimental group	Control group	Chi-square value	P –value
Serum ferritin level	Normal	23	47	32.229	.000***
	High ferritin level	24	0		
	Total	47	47		
HbA1c	Normal	0	47	94.000	.000***
	Good control	5	0		
	Fair control	14	0		
	Poor control	28	0		
	Total	47	47		
MBG	Normal	0	47	92.000	.000***
	Good control	5	0		
	Fair control	14	0		
	Poor control	28	0		
	Total	47	47		

*** - Highly significant, HbA1c - Glycated hemoglobin MBG - Mean blood glucose

From these results 50 per cent of diabetic patients were affected by iron overload which may be the cause for diabetes mellitus. Although we excluded all subject with acute or chronic infection or inflammatory disorders.

Conclusion:

It was concluded from the study, 50 per cent of the patients with type 2 diabetes mellitus had significantly higher serum ferritin level when compared to the normal and there is no correlation between serum ferritin with HbA1c and MBG but there is a correlation between HbA1c and MBG..

Abbreviations:

HbA1c - Glycated hemoglobin, MBG- Mean Blood Glucose Level, DM- Diabetes Mellitus, UKPDS- United Kingdom Prospective Study, DCCT- Diabetes Control and Complications Trial, HbA0- non glycated hemoglobin

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REFERENCES

1. Stratton, I.M. and Adler, A. I *et al.* (2002). Association of glycemia with macrovascular and micro vascular complications of type 2 diabetes (UKPDS 35), *BMJ,* 321, 405-412.

2. Scheen, A.J. and Lefebvre, P.J. *et al.* (2000). Diabetes in the New Millennium, Wiley and Sons, New York, p. 101–113.

3. Yeolekar, M.E. and Shete, M.M. (2002). Homocysteine and hypertension...Diabetes Complications.

4. United Kingdom Prospective Study (1995). Relative efficacy of randomly allocated diet Sulphonyluria Insulin or metformin in patients with newly diagnosed non- insulin depend diabetes followed for three years", BMJ 310, 83-88.

5. DCCT Research Group (1989). The effect of intensive treatment of diabetes on development and progression of long-term complications in insulin dependent diabetes mellitus, *N. Eng. J. Med.*, **329** : 977–986.

6. Bunn, H.F. and Haney, D.N. (1975). Further identification of the nature and linkage of the carbohydrate in hemoglobin A1C, *Biochem. Biophys. Res. Commun.*, **67** (1) : 103-109.

7. Kim, C.H. and Kim, H.K. (2010) Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. Division of Endocrinology and Metabolism, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon., pp. 420-767.

8. Gallou, G. and Guilhem, I. (1994). Increased serum ferritin in insulin- dependent diabetes mellitus: relation to glycemic control, Clin. Chem., **40** : 947-948.

9. Rajpathak, S. and Ma J., Manson (2006). Iron intake and the risk of type 2 diabetes in women a prospective cohort study, *Diabetes Care*, **29** : 1370-1376.

10. Salonen, J.T. and Nyyssnen, K. (1994). High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men, *Circulation*, **86** : 803-811.

11. Saudek, C.D. and Hemm, R.M. (1977). abnormal glucose tolerance in β- thalassemia. *Metabolism*, 43-25.

12. Adams, P.C. and Kertesz, A.E. (1991). Clinical presentation of hemochromatosis a changing scene, *Am. J. Med.*, **90** : 445-449.

13. Nathan, D.M. and Buse, J.B. (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy a consensus statement of the American Diabetes Association and the European Association for the study of diabetes, *Diabetes Care*, **32** : 193–203.

14. Baur, J.G. (1970). Iron and trace metals. *Gradwohlis clinical laboratory methods and diagnosis*, Chapter 22, **1** : 436-458.

15. DCCT Research Group (1987). The effect of intensive treatment of diabetes on development and progression of long-term complications in insulin dependent diabetes mellitus, *N. Eng. J. Med.*, **329** : 977–986.

16. Pupillo, M. and De Berardis, G. Glycated haemoglobin or mean blood glucose as indicators of metabolic control in Type 2 diabetes? Metabolism and Diabetes Unit, Ospedale Renzetti, Lanciano, Italy, *Diabetes Res. Clin. Pract.*, **80**(3) : 1-3.

17. Frank, L.J. and DSharif, H.M. (2008). Serum ferritin and ceruloplasmin as coronary risk factors in Peripheral Vascular Disease, **337** (7663) : 215-221.

18. Ashourpour, M. and Djalali, M. (2010). Relationship between serum ferritin and inflammatory biomarkers with insulin resistance in a Persian population with type 2 diabetes and healthy people. Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. *Internat. J. Food Sci. Nutr.*, **61**(3) : 316-323.

19. Kankora, K. and Jansen, E.H. *et al.* (2002). Relations among serum ferritin, C282Y and H63D mutations in the HFE gene in type 2 diabetes in the Czech population, *Exp Clin Endocrinol Diabetes*, **110**,223-229.

20. Ren, Y. and Tian, H. (2004). Elevated serum ferritin concentration in a glucose impaired population and in normal glucose tolerance first degree relatives in familial type 2 diabetic pedigrees, *Diabetes Care*, **27** : 622-623.

21. Fernandez Real, J.M. and Ricart-Engel, W. (1998). Serum ferritin as a component of the insulin resistance syndrome, *Diabetes Care,* **21** : 62– 68.

22. Qian, M. and Liu, M. *et al.* (1998). Transition metals bind to glycated proteins forming redox active "glycochelates": implications for the pathogenesis of certain diabetic complications, *Biochem. Biophys. Res. Commun*, **250** : 385-389.

23. Green, B. and Hoyer, J. (2001). Haemoglobin Etobicoke, an incidental finding in an Irish diabetic HbA or HbA1c.

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