Correlation of Fasting Blood Sugar and Glycated Hemoglobin Levels with Fructose 2,6 Bisphosphate Levels in Immune Cells of Diabetic patients

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ABSTRACT

Objective: To study the effect of fasting blood sugar and HbA1c on fructose 2,6 bisphosphate in diabetics and comparing and correlating the same parameter with control subjects.
Material and methods: Two hundred patients with type-II diabetes and fifty control subjects were selected for this study. Fasting blood sugar and glycated hemoglobin were estimated. Fructose 2,6 bisphosphate was estimated and correlated from peripheral blood mononucleocells.
Result: Results showed highly significant fructose 2,6 bisphosphate levels in immune cells in diabetic group. A significantly positive correlation of fructose 2,6 bisphosphate with FBS and HbA1c was seen in diabetic group.
Conclusion: Higher levels of FBS and HbA1c are associated with increased levels of fructose 2,6 bisphosphate of peripheral blood mononuclear cells in diabetes reduces immunity.
Key words: Diabetes mellitus, HbA1c, fructose 2,6 bisphosphate .

INTRODUCTION

Fructose 2, 6 bisphosphate is detected in all mammalian tissue as well as in fungi and plants[12]. Fructose 2,6 bisphosphate potent positive allosteric effector of phosphofructokinase-1 which is rate limiting enzymes for glycolysis15. Fructose 2,6 bisphosphate is formed by phosphofructokinase-2. Phosphofructokinase-2 is a bifunctional protein that has both, the kinase activity that produces fructose 2,6 bisphosphate and phosphatase ectivity that converts fructose 2,6 bisphosphate back to fructose 6 phosphate and Pi[17]. High fructose 2,6-bisphosphate levels mediate enhanced glycolysis and bifunctional enzyme phosphofructokinase-2/fructose 2,6-bisphosphatase catalyses the formation and degradation of fructose 2,6-bisphosphate[1]. Diabetes causes substantial changes in the fructose 2,6-bisphosphate system. In hepatocyte, diabetes mellitus enhances phosphorylation of fructose 2,6-bisphosphate leading to a decrease in the activity of the enzyme causing hyperglycemia. In peripheral blood lymphocytes fructose 2,6-bisphosphate system is slightly different from that of hepatocyte[3]. Hyperglycemia increases intracellular fructose 2,6-bisphosphate in immune cells21. These findings may help to clarify the impaired functions in immune cell, in patients with diabetes[22]. Currently over 230 million patients demonstrate already an epidemic scale of the disease. It is a lifelong progressive disease with a high morbidity and mortality worldwide: every 10 seconds one patient dies on DM-related consequences[6]. As diabetes progress patients are at increased risk of developing infections. Complications due to depressed immunity patient with diabetes mellitus have infections more often than those with diabetes mellitus. The course of the infection is also more complicated in this patient group[9]. Good metabolic control is a major factor in limiting the development and spread of infection and most importantly, the development of diabetes complication which predispose to infection[19], several factors predispose diabetic patient to infection. These factors include genetic susceptibility to infection, altered cellular humoral immune defense mechanism, local factor including poor blood supply and nerve damage and alterations in metabolism associated with diabetes mellitus[10]. Haemoglobin A1c (HbA1c) is widely used to determine levels of long-term blood glucose, judge the adequacy of diabetes management, and adjust therapies[11]. The range for people without diabetes is between 4.0% and 5.95%. In people with poorly controlled diabetes, it is 8.0% or above; in people with good blood glucose control, it is less than 7.0%. Consistently high HbA1c levels increase the risk for long-term disabling and potentially life-threatening complications, including cardiovascular disease, stroke, kidney disease, eye damage and nerve damage[21]. A significant relation ship has been seen in some studies between intracellula fructose 2,6- bisphosphate levels and long term glycemic control as assessed by HbA1c[2].

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In this study FBS and HbA1c are studied to assess the metabolic control over diabetes as reflected by levels of fructose 2,6 bisphosphate. The recognition of impaired immune systems has implication for the diagnosis, treatment and outcome of the infection[14].

MATERIAL METHODS

A total of two hundred and fifty subjects were included in this study. Two hundred subjects were diagnosed type-II diabetics and fifty individuals selected as control were completely a symptomatic for any disease and had normal physical examination. Anemia, liver diseases, renal diseases, other endocrine disease and pregnant were excluded in this study. In all cases age, sex, weight, height and duration of illness were recorded. Diagnosed type-II diabetic patients were taken from diabetic clinics. About 8ml of blood was taken from the patients after an over night fast. Peripheral mononeclaer cells were separated by ficoll gradients from 6ml of venous blood. PBMC were homogenized in 50mM NaOH and incubated 80C for 10mins. After centrifugation the supernatants were used to assay for fructose 2,6 bisphosphate by Van Schaftigen method[18]. The serum glucose was estimated by kit method Cat No. cod1001191 supplied by Spinreact SA Spain. The blank, standard and serum sample tubes were prepared by using 20µl sample serum and 20µl standard. After 30 minutes absorbance was measured against the blank at 505nm in spectronic 20 sphectrophotometer. HbA1c was estimated by fast ion exchange resins separation methods using the kit Cat No. cod10658 supplied by Human Germany. Hemolysate was prepared by mixing 100µl of whole blood with lysing agent. This hemolysate was then used to determine HbA1c and total hemoglobin by measuring the absorbance at 415nm. Statistically analysis was performed with SPSS 15.0.

RESULTS

A total of two hundred and fifty subjects were studied. Fifty healthy normal subjects volunteered for control group-A. two hundred subjects were diagnosed type-II diabetes mellitus were included in group-B. Table-1 shows mean values with standard error (±) of mean (SEM) and comparison of Age, Height, and Weight and body mass index of control with diabetic subjects. Height, weight and body mass index in group-B was significantly higher when compared to group-A. No statistically significant difference was seen when age was compared (Fig.1). Table 2 shows the intergroup comparison of fasting blood glucose, glycated hemoglobin and fructose 2,6 bisphosphate. Here mean fasting blood glucose of group-B significantly higher (P<0.000) when compared to group-A. Same results are seen with HbA1C and fructose 2, 6bisphosphate that is results of diabetic group are significantly higher (P<0.000) when compared to control group. Fig. 2 represents these results in bar diagram. Table-3 depicts the comparison of relationship of fructose 2, 6 bisphosphate and serum fasting blood glucose and Co-efficient of correlation (r) of fructose 2, 6 bisphosphate with HbA1C which shows that in total subjects fructose 2, 6 bisphosphate was significantly positively correlated (P<0.001) with fasting blood glucose. However it also shows no significant relationship was found between diabetic and normal subjects. It also shows that fructose 2, 6 bisphosphate significantly positively correlated with HbA1c (P<0.001). Fig. 1 and 2 shows same results.

Table 1: Comparison of age, height, weight and body mass index of control with the diabetic subjects. The values are expressed as Mean±S.E.

Variables	Control n=50	Diabetic n=200
Age (Years)	44.90±1.36	43.26±0.56
Weight(Kg)	61.23±1.54	63.87**±1.43
Height(m)	1.75±0.002	1.68**±1.33
BMI(kg/m²)	24.12±0.45	27.63**±0.31

*P<0.05,**P<0.01,***P<0.001significant when compared to control.

Fig. 1: Comparison of age, height, weight and body mass index of control with the diabetic subjects

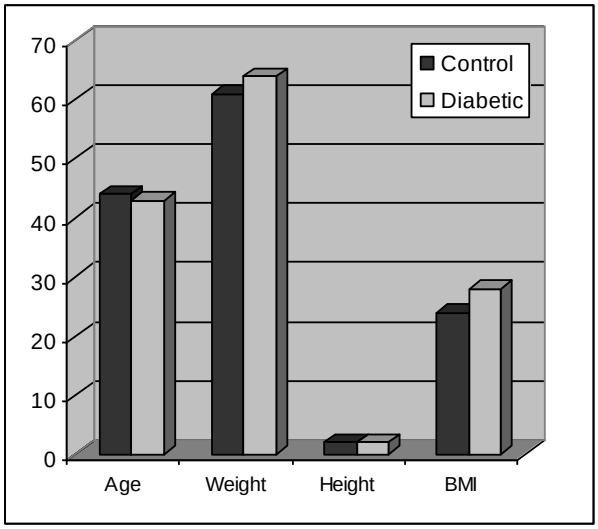
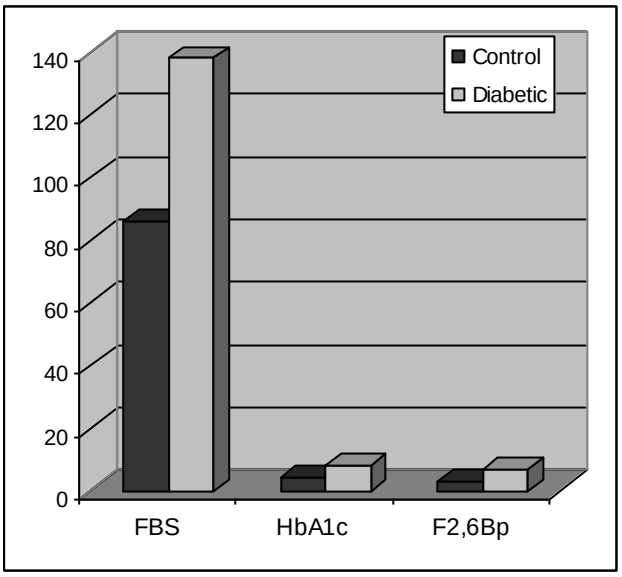


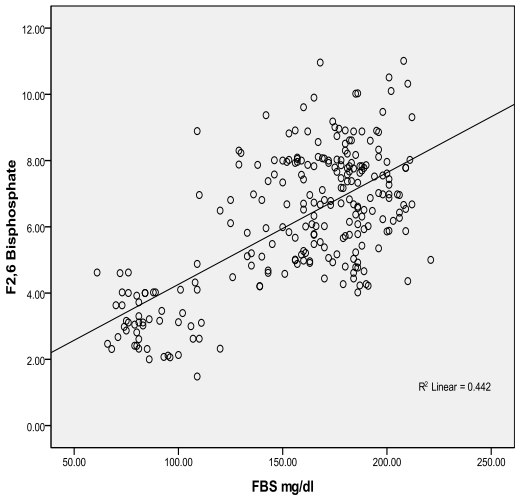
Table 2: Comparison of Fasting Blood glucose, HbA1c and Fructose 2, 6 bisphosphate of control and diabetic subjects The values are expressed as Mean± S.E.M.

Variables	Control n=50	Diabetic n=200
FBS (mg/dl)	86.54±1.95	137.70*** ±1.61
HbA1c (%)	4.33±0.10	8.45***±0.11
Fructose2,6 bisphosphate (pmol)	3.15±0.11	6.91***±0.11

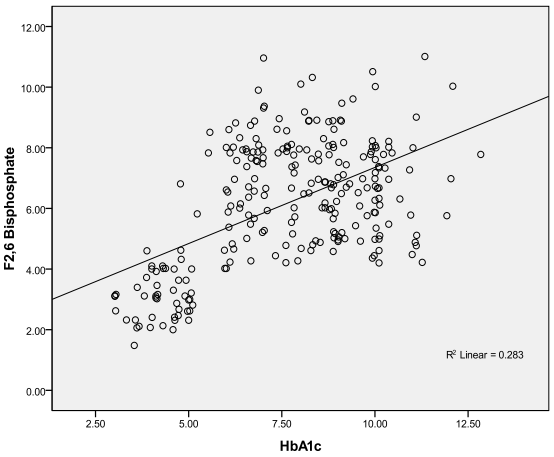
Fig.2: Comparison of fasting blood glucose, HbA1c and Fructose 2,6 bisphosphate of control and diabetic subjects



Correlation (r) of Fructose 2, 6 bisphosphate (pmol) vs FBS



Correlation(r) of Fructose2,6 bisphosphate(pmol) vs HbA1c



DISCUSSION

Diabetes is a chronic disorder that occurs when the pancrease does not produce enough insulin or body can not effectively use the insulin it produce[7]. Infection tends to occur with greater frequency and severity in diabetic patients than in non diabetic. Specific defects in innate and adaptive immune function have been identified in many in vitro studies[16]. Immune cells require glucose uptake and metabolism for normal function. High glucose levels inhibit proliferation of peripheral mononuclear cells. Activated T cells have dramatically increased metabolic requirement, glucose metabolism and aerobic glycolysis fuel this demand[13].

The present study was designed to observe and compare the level of fructose 2,6 bisphosphate in immune cells of diabetics and normal control subjects and to correlate the level of fructose 2,6 bisphosphate and HbA1c for management of diabetes.

It was confirmed decades ago that fasting glucose value is higher in diabetics[4]. In our study we measured fasting blood sugar to determine the control of diabetes and asses the metabolic condition .Our results were in the agreement of the above statement. Group A which consisted of diabetic patients had higher fasting blood sugar values (173.70±1.61mg/dl) than control subjects (86.54±1.95mg/dl) (0.001) as expected. Measurement of HbA1c provides a retrospective index of glycemic control over the 4 to 8 weeks before its determination here it is useful in judging the adequacy of diabetes management and adjust therapies. In a study carried out by[16] it was found that high glycosylated Hb levels are most likely to exhibit close association with particular infection and hyporesponsiveness. In another study8 found significantly higher HbA1c in a group of 1480 subjects with diabetic nephropathy[2] conducted an investigation to see the effect of long term hyperglycemia on intracellular fructose 2,6 bisphosphate in immune cells, which suggested that subjects with higher HbA1c values have in increase intracellular fructose 2,6 bisphosphate in immune cells. Results of our study also showed significant difference in HbA1c ($p<0.001$) in group A and controls actual values being 8.45±0.10 and 4.33±0.11 respectively. Immune cells activation requires an increase in glucose uptake and anaerobic glycolysis. Fructose 2,6 bisphosphate is a powerful activator of rate limiting enzyme of glycolysis. Specific defects in immune function have been identified in diabetic patients. Infection occurs with increased frequency and severity in diabetes. Atsumi et al (2007) showed intracellular fructose 2,6 bisphosphate levels in

peripheral blood mononuclear cells from diabetic subjects were significantly higher than age matched control subjects. Positively significant relationship was observed between fructose 2,6 bisphosphate levels and HbA1c ($r = 0.451, p < 0.001$). Results of[22] indicated that activated thymocytes from diabetic rats showed two fold more fructose 2,6 bisphosphate than cells from normal rats. Results of our study are also in agreement of the above mentioned studies. Significantly high ($p<0.001$) levels of fructose 2, 6 bisphosphate were found in immune cells of diabetics when compared with normal group. We also observed a significant positive correlation between intracellular fructose 2,6 bisphosphate levels and long term glycemic control as assessed by HbA1c. These data suggest that hyperglycemia increases fructose 2,6 bisphosphate in immune cells. These finding may help to clarify the impaired function in immune cells in patients with diabetes. It can be concluded from the above facts that levels of fructose 2,6 bisphosphate in immune cells was increased.

CONCLUSION

It is concluded that in diabetes there is increased fructose 2,6 bisphosphate level in immune cells which is positively correlated to fasting blood glucose and glycated hemoglobin. This could be a cause of reduced immunity in diabetic patients.

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