Levels of Fructose-2,6-Bisphosphate in Lymphocytes of Diabetic Patients

Seemen Ghafoor[1], Azra Saleem[2], Abdullah Abbasi[3], Shaheena[4].

Abstract:

Background: Patients with diabetes mellitus have infections more often than those without diabetes. Several factors predispose diabetic patients to infections, including an alteration in immune defense mechanism. Elevated levels of fructose-2,6- bisphosphate in lymphocyte have been shown in diabetic patients. The activation of glycolysis by fructose -2,6- bisphosphate in peripheral blood mononuclear cells causes the accumulation of glycolytic metabolites and inhibits the activation of immune cells.

Objective: To observe and compare the levels of fructose-2,6-bisphosphate in lymphocytes of diabetics and normal subjects

Material and Methods: 200 diabetic and 50 control subjects were selected for study .The subjects were evaluated for severity of diabetes and their fasting blood glucose, HbA_{1C}, total leucocyte count, lymphocyte count and fructose-2,6-bisphosphate in lymphocytes were estimated.

Results: The results show that mean fasting blood glucose, HbA_{1C}, total leucocytes count and fructose-2,6-bisphosphate levels in lymphocytes were significantly higher ($P<0.001$) while lymphocyte count was significantly lower ($P<0.001$) in diabetic patients as compared to control group.

Conclusions: It was concluded from the facts observed in this study that elevated levels of fructose-2,6-bisphosphate in lymphocytes and decreased number of lymphocytes may have induced chances of infections in diabetic patients.

Keywords: Lymphocytes, Fructose-2,6-bisphosphate, Diabetes, Infections.

Introduction:

Fructose-2,6-bisphosphate is detected in all mammalian tissue[1]. It is powerful allosteric activator of 6 phosphofructose-1-kinase which is the rate limiting enzyme for glycolysis[2]. When levels of fructose-2,6-bisphosphate are high, glycolysis is enhanced and gluconeogenesis is inhibited[3]. 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[4]. The activation of glycolysis by fructose-2,6-bisphosphate in peripheral blood mononuclear cells causes the accumulation of glycolytic metabolites and inhibits the activation of immune cells[5].These altered metabolic products and oxidative stress play a role in the development of diabetic complications[6]. In vitro evidence shows that neutrophil function and humoral immunity may be depressed in people with diabetes[7].

Hyperglycemia increases intracellular fructose-2,6-bisphosphate in immune cells[8].Elevated level of fructose-2,6-bisphosphate in lymphocyte have been shown in diabetic patients. These findings suggest the association between accelerated glycolysis due to hyperglycemia and alteration of the immune system during the diabetic state[9] and may help to determine the impaired function of immune cells in patients with diabetes[10]. Patients with diabetes mellitus have infections more often than those without diabetes. The course of infection is also more complicated in this patient group[11] .Good metabolic control is a major factor in limiting the development and spread of infection[12]

Methodology

This study was carried out in the Department of Biochemistry, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi. The study protocol was approved by the Local Bioethical Committee and informed consent was obtained from all subjects. Total 250 individuals were included in the study, these were divided into two groups. Group A consist of 50 healthy individuals selected as control and group B consist of 200 diagnosed patients of diabetes mellitus of different age and sex with positive history of associated infection. Subjects suffering from anemia, liver diseases, renal diseases, any other endocrine dis-

1. Seemen Ghafoor, Associate Professor of Biochemistry, Sir Sayed College of Medical Sciences for Girls, Karachi
2. Azra Saleem, Assistant Professor of Pharmacology, Shaheed Mohtarma Benazir Bhutto Medical College, Lyari, Karachi.
3. Abdullah Abbasi, Associate Professor of Physiology, Liaquat University of Medical &Health Sciences, Jamshoro
4. Shaheena, Assistant Professor of Biochemistry, Shaheed Mohtarma Benazir Bhutto Medical College, Lyari, Karachi.

eases and pregnant women were excluded. Morning samples were taken after an overnight fast of at least 12-14 hours. About 10 ml blood was drawn from ante-cubital vein after all aseptic measures. 1 ml of blood was used to estimate HbA_{1C} by fast ion exchange resin separation method, using kit supplied by Human Germany Cat No 10658. 6ml of blood was heparinized for the separation of lymphocytes. Serum was separated from rest of the sample and used to estimate blood glucose by enzymatic calorimetric(GOD-PAP)method using kit,No.Cod.1001191 supplied by Spinreact, SA, Spain. Complete blood count(CBC) was done on Sysmex KX 21 automated cell counter, which measures and calculates total leucocyte and lymphocytes counts. Separation of lymphocytes from whole blood was done with lymphocyte separation medium(LSM) catlog no 25-072,CI, 1×100 ml density1.077-1.08 g/ml which is a separation solution made with Ficoll TM is a density gradient media and Dulbecco's phosphate buffered saline without Calcium and Magnesium Cat No 21040-CV.Ficoll TM is a hydrophilic polymer with a molecular weight of 400 Dalton.It is used for the production of density gradients for separation of cells and sub-cellular components, which sediment on centrifugation. Heparinized blood was centrifuged with LSM. Sedimented erythrocytes, polynuclear leukocytes and mononuclear lymphocytes were separated. Superficial lymphocyte layer was aspirated and washed with buffered balanced salt solution and resuspended in the appropriate medium for application.

Determination of fructose-2,6-bisphosphate in lymphocytes was done by chemical method based on the ability of fructose-2,6-bisphosphate to activate pyrophosphate dependent phosphofrutokinase from potato tubers Sigma Chemicals. Statistical analysis was performed using SPSS statistical software by paired student t-test.

Results

A total of two hundred and fifty subjects were studied. Group A consist of 50 healthy individuals as control and group B consist of 200 diabetic patients associated with infections.

Table-1 shows the comparison of the mean values of age, body mass index, total leukocyte count (TLC) and lymphocyte count between control and diabetic patients. It shows that mean body mass index, and TCL of diabetic patients were significantly higher (P<0.001) as compared to control group. The mean value of lymphocyte count was significantly lower (P< 0.001) in diabetic group.

Table-2 Shows the comparison of the mean values of fasting blood glucose, HbA_{1C} and fructose-2,6-bisphosphate levels in lymphocytes between control and diabetic patients. The mean values of these variables were significantly higher (P<0.001) in diabetic patients as compared to control.

Table-1

Comparison of Age, Body Mass Index, TLC and Lymphocyte Count of Control with the Diabetic Subjects.

The values are expressed as Mean± S.E.M. The number of observation and units are given in parenthesis.
n= number of subjects.

Variables	Control n=50	Diabetic n=200
Age(Years)	44.90 ± 1.36	43.26 ±0.56
BMI(kg/m²)	24.12 ±0.45	27.63* ± 0.31
TLC (10^9/L)	7.45 ± 1.13	12.00* ± 0.12
Lymphocyte count (%)	32.33 ± 1.15	27.66* ± 0.32

*‧P <0.001 significant when compared to control.

Table-2

Comparison of Fasting Blood glucose, HbA_{1C} and Fructose-2,6-bisphosphate in control and diabetics.

The values are expressed as Mean± S.E.M.
the number of observation and units are given in parenthesis.

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

n = number of subjects.
*‧P < 0.001 significant when compared to control.

Discussion:

Diabetes is a group of metabolic diseases characterized by hyperglycemia that occurs hen the pancreas does not produce enough insulin or body cannot effectively use the insulin it produces or both[13]. Infections tend to occur with greater frequency and severity in diabetic patients than in non diabetic. Several factors predispose diabetic patients to infections. These factors include: genetic susceptibility to infection, altered cellular and humoral immune defense mechanism, local factors including poor blood supply and nerve damage and alteration in metabolism associated with diabetes mellitus[14]. Specific defects in innate and adaptive immune function have been identified in many in vitro studies[15]. Our study shows that the mean fasting blood glucose, HbA1c and fructose-2,6-bisphosphate levels in lymphocytes were significantly

higher in diabetic group. The results were in agreement with the other studies conducted on similar parameters[4,5,,10,16]. This data suggests that hyperglycemia increases fructose-2,6-bisphosphate in lymphocytes. Also in diabetic patients, significantly increased total leukocyte count and decreased lymphocyte count were observed in present study and this is in agreement with study carried out by earlier workers[17].

It was concluded from the facts observed in this study that elevated levels of fructose-2,6-bisphosphate in lymphocytes and decreased number of lymphocytes may be responsible for the impaired function of immune cells and may have induced increased chances of infections in diabetic patients. Our analysis was based on a single measurement that may not reflect the relation over time. However there is a need for more well designed, randomized studies assessing the value of glycemic control and fructose-2,6-bisphosphate levels for better understanding of frequency of infections in diabetics.

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