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Relationship between Plasma Proteins Glycation and Liver Enzymes, including Level of Glucose, Urea and Creatinine

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ABSTRACT

Diabetes mellitus is a disease that can be defined as the inability to control blood glucose. The primary factor associated with the development of most diabetic complications is the prolonged exposure to hyperglycemia. Glycation of proteins is the most important cause of diabetic complications. Level of protein glycation is considered to be the main symptom and confirmation of diabetes. The present project was designed to study the correlation between level of plasma proteins glycation and liver enzymes (SGOT, SGPT, ALP & AP), including level of glucose, urea and creatinine. A total of 45 blood samples, 25 diabetic and 20 normal individuals were processed for levels of plasma proteins glycation, liver enzymes, glucose, urea and creatinine. Level of glycation was correlated individually with each parameter. In case of glucose, for both diabetic and normal subjects, the results showed the negative correlation between the levels of plasma proteins glycation and plasma glucose. For urea and creatinine, there was negative correlation in both normal and diabetic subjects. Liver enzymes; SGOT has a positive correlation, whereas in case of SGPT and alkaline phosphatase, they were negatively correlated with level of plasma proteins in diabetic as well as normal individuals. Acid phosphatase showed different behaviour in case of normal and diabetic subjects. It was positively correlated in case of diabetic and no correlation or negative in case of normal subjects.

Key Words: Diabetes; Protein glycation; Liver enzymes

INTRODUCTION

Diabetes is one of the oldest recognized disease as it is mentioned as long as 1500 B.C. in the "Ebers Papyrus". It was Greeks who later gave name Diabetes, which means "the passing through of water", obviously referring to the excessive discharge of urine, often observed with diabetic subjects. At that time, it was characterized as "being a melting down of the flesh and limbs into the urine".

Now although there are several forms of the disease, the most common form is diabetes mellitus, the name which has been used since late 18th century. Mellitus is a Latin word meaning, "sweetened with honey". Later on in 1921, the discovery of insulin by Banting, Best and Collip, confirmed the concept that diabetes is due to the lack of insulin, produced in the pancreas by the beta-cells of the islets of langerhans. It is now evident that diabetes is multifaceted and that only a minority (10–20%) of diabetic patients are insulin deficient.

Now-a-days, the disease can be defined as the inability to control blood glucose, chronic hyperglycemia results, characterized by severe thirst, polyuria, weight loss and stupor (WHO, 1985). It is now established that diabetes mellitus is genetically and clinically heterogeneous group of disorders, that are associated with glucose intolerance.

Long term diabetes is associated with a no. of other physical disorders, affecting the retina (retinopathy), the kidney (nephropathy), the peripheral nerves (neuropathy) and the micro-circulation (microangiopathy). Atherosclerosis, leading to heart disease, stroke and foot ulceration are also some manifestations of diabetes mellitus. Generally these complications are due to absence of insulin, or the improper regulatory actions on metabolism of carbohydrates, proteins and lipids (Sims *et al.*, 1996; Berg *et al.*, 1997; Sakata *et al.*, 1999).

The determination of negatively charged adult haemoglobin, designated HbA, as glycated haemoglobin (GHb) by Bookchin and Gallop (1968), led to speculation that other body proteins may be similarly affected. Now it has been established that several plasma proteins, collagen and lens crystallin can also be glycated. The extent of non-enzymic glycosylation (NEG) is affected by pH, temperature, protein and glucose concentration and the time of exposure to glucose (Vlassara *et al.*, 1986; Ryan *et al.*, 1998; Sugiyama *et al.*, 1998).

Up-to-date observations suggest that several types of human and rat serum proteins are subject to non-enzymic glycosylation *in vitro*. It has been concluded that relative extent of glycation of different plasma proteins are a complex function of integrated glucose concentrations overtime, half life and chemical characteristics of each protein (Austin *et al.*, 1987). Free glucose present in the serum or plasma, may interfere with accurate measurement of glycation. Kennedy *et al.* (1980) solved this problem by

dialyzing the serum overnight against normal saline and precipitated the protein with 2.5 M trichloroacetic acid. Ma *et al.* (1981) also precipitated the proteins with trichloroacetic acid. Both groups used thiobarbituric acid for the determination of non-enzymic glycosylation, where free glucose imparts a high value to the results. So removal of free glucose is a necessary prerequisite for TBA method.

Maillard (1912), a French Chemist, established that interaction of sugars with amino groups (solution of glucose & lysine), produced a brown pigments. Later on (1916 & 1917) he extended these studies to different sugars and observed that the reducing group played an important role, and different sugars reacted with different amino acid at different rates. This reaction has since been referred to as either the "Maillard Reaction" or "Non-enzymic browning".

The present project is planned to study the relationship between plasma proteins glycation and liver enzymes, including level of glucose, urea and creatinine. By this study, we will be in a position to use these parameters as biomarkers to check the status of diabetes, just like pentosidine (Advanced Glycation End Products) level used as biomarker of diabetic cardiovascular risk (Sugiyama *et al.*, 1998).

MATERIALS AND METHODS

A total of 45 samples were collected, 25 of which were diabetic and 20 were controlled normal, certified by hospital. A total of 6ml of blood was collected from cephalic vein in a disposable syringes from each individual. Three ml of blood was utilized for plasma and 3ml was used for serum in each case. All the collected serum samples were analyzed for using diagnostic kits (Randox diagnostic & Human diagnostic), for the quantitative estimation of following parameters;

a) Glucose
b) Urea
c) Creatinine
d) Aspartate Amino Transferase-AST (SGOT)
e) Alanine Amino Transferase-ALT (SGPT)
f) Alkaline Phosphatase
g) Acid Phosphatase

Before the determining of plasma proteins concentration by Biuret reagent (Gornall *et al.*, 1949) plasma samples were dialyzed against normal saline. Bovine serum albumin was used for standard curve. For determination of non-enzymic glycosylation, thiobarbituric acid (TBA) method was used (Furth, 1988). The standard curve for hydroxy methyl furfural production, standard fructose solution was used.

TBA technique was used for the determination of both enzymatic and non-enzymatic glycosylation. To one ml of plasma solution, containing 10mg of plasma protein, 0.5 ml of 1N oxalic acid was added and autoclaved at 115 lb/square inch for 15 minutes. Then centrifuged the samples after adding 0.5ml cold trichloroacetic acid. To 1.5ml of supernatant, added 0.5 ml of thiobarbituric acid (0.05M) and incubated for 15 minutes at 37 °C, before taking absorbance at 443 nm. For determination of enzymatic glycosylation, plasma samples were reduced by using 0.1M $NaBH_4$ solution, for the reduction of aldehydic or ketonic linkages The amount of $NaBH_4$ was 400 molar excess of total plasma proteins in samples. $NaBH_4$ was dissolved in 0.01N NaOH solution. After reduction, the same process mentioned above determined the glycation level. Non-enzymatic glycosylation was determined as follows

Non-enzymatic glycosylation= (NE+E Glycosylation)-E Glycosylation

RESULTS AND DISCUSSION

Glycation level. Glycation level of different plasma proteins samples belonging to normal and diabetic subjects, were determined by TBA method. The results of normal samples indicate the values range from 0.0284 to 5.4 mole glucose/mole of plasma proteins, whereas in case of diabetic, the values range from 0.114 to 2.77 mole glucose/mole proteins. The normal value for plasma protein glycation is 0.57 mole glucose/mole proteins (Sheikh, 1991). The level of glycation gave the true picture of the diabetic states. On the basis of protein glycation as independent variable and other parameters as dependent variables, correlations are established.

Effect of glycation on level of glucose. The glucose level in normal individuals, ranges from 77 to 98 mg/dl, whereas in case of diabetic, it ranges from 92 to 389 mg/dl. These values gave a clear cut difference between normal and diabetic individuals. But when level of plasma protein glycation is considered, there is no clear cut difference between normal and diabetic subjects. When correlation was determined between level of glycation and plasma glucose in normal, it is positively correlated (0.1231), and regression line indicates, $y=1.3109x+87.233$ (Fig.1a). In case of diabetic subjects, it is negatively correlated (0.0871), and regression line; $y=-37.75x+212.36$ (Fig.1b). These results indicate that in case of normal, there is positive linkage between level of glycation and glucose but not significantly correlated. In case of diabetic, it is clear

Fig. 1. Effect of glycation on glucose level of blood of (a) normal and (b) diabetic individuals

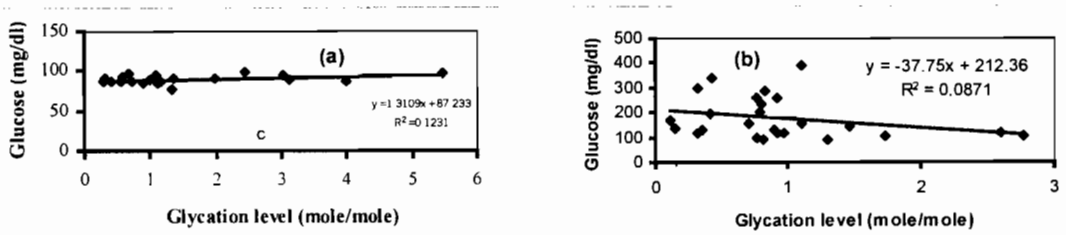


Fig. 2. Effect of glycation on urea level of blood of (a) normal and (b) diabetic individuals

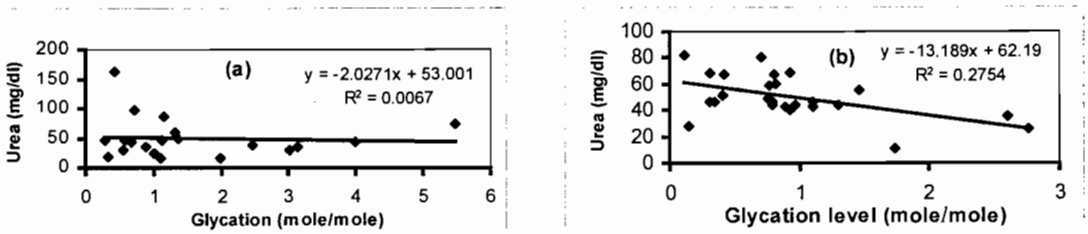


Fig. 3. Effect of glycation on creatinine level of blood of (a) normal and (b) diabetic individuals

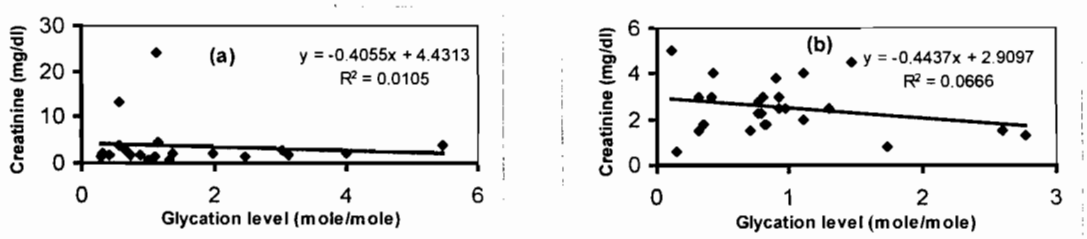


Fig. 4. Effect of glycation on SGOT level of blood of (a) normal and (b) diabetic individuals

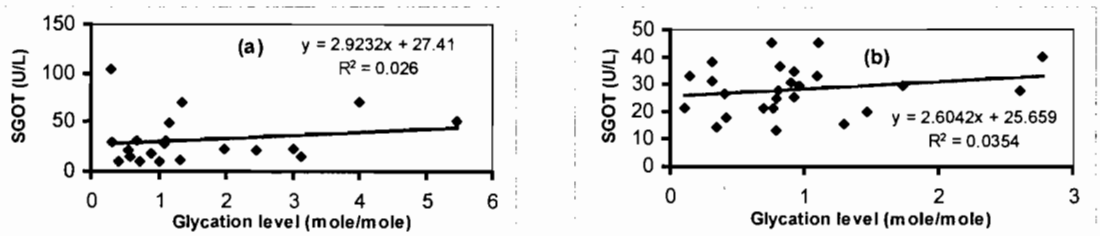


Fig. 5. Effect of glycation on SGPT level of blood of (a) normal and (b) diabetic individuals

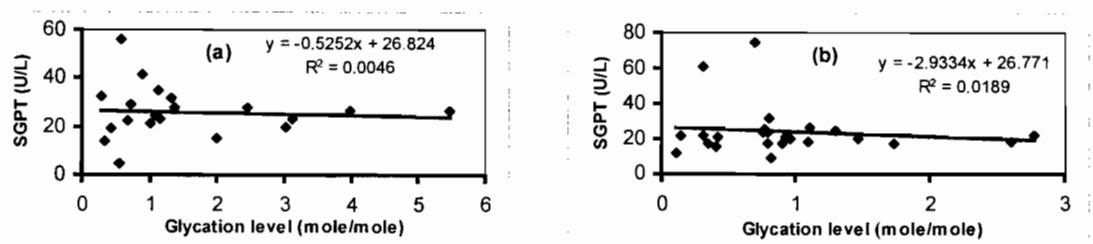


Fig. 6. Effect of glycation on alkaline phosphate level of blood of (a) normal and (b) diabetic individuals

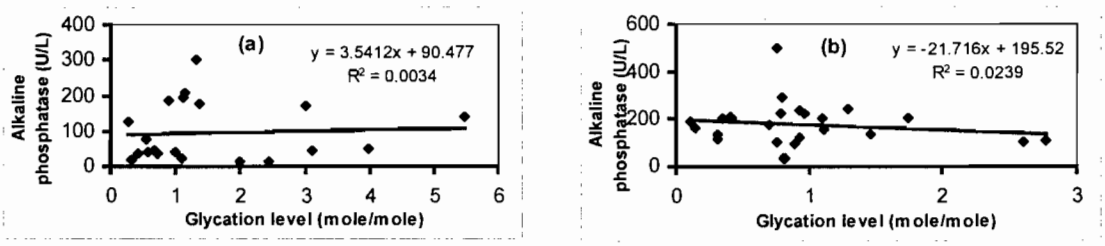
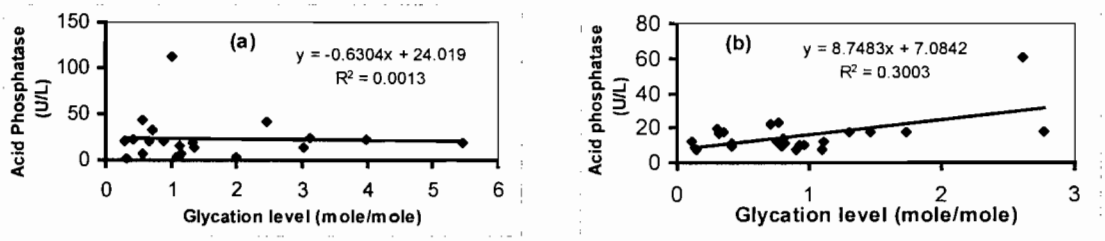


Fig. 7. Effect of glycation on acid phosphate level of blood of (a) normal and (b) diabetic individuals



that level of glucose can not be a good parameter to check the diabetic states. So it is proved that level of glycation is a good parameter as compared to plasma glucose level.

Effect of glycation on urea level. Level of urea for normal individuals show a range of 15.00 to 162.13 mg/dl, while diabetic individuals show the range of 11.40 to 81.7 mg/dl. The level of plasma proteins glycation reveal a negative correlation with level of urea in both normal & diabetic individuals. Correlation coefficients are 0.0067 & 0.2754 respectively. The regression lines indicate; y= -2.0271x+53.001 and y= -13.189x+62.19 respectively (Fig. 2a & 2b). No work has been found on diabetic and level of urea. From the results mentioned already, it can be concluded that level of glycation effects inversely on the level of urea in blood. But this effect among normal and diabetic is different as indicated by different correlation values, that is in order of diabetic>normal. As the urea is produced in liver, so less level of urea production indicates the effected liver by plasma proteins glycation.

Effect of glycation on creatinine. For normal individuals, level of creatinine shows the range of 0.75 to 24 mg/dl, while for diabetic individuals, the range is 0.60 to 5mg/dl. The level of protein glycation shows a negative correlation with level of creatinine in both normal and diabetic individuals. Correlation coefficient for normal and diabetic are 0.0105 &A negative correlation with level of creatinine in both normal and diabetic individuals. Correlation coefficientfor normal and diabetic are 0.015 and 0.0666 respectively. Similarly regression lines indicate; y= -0.4055x+4.4313 & y= -0.4437x+2.9097 respectively for normal & diabetic individuals (Fig. 3a & 3b). According to the work of Langlois *et al.* (1992) and present results, it can be concluded that with glycation, creatinine kinase may be decreased (possibly glycated) in concentration and rate of creatinine formation become low, which results in its decreased concentration in blood.

Effect of glycation on level of SGOT. Normal subjects shows the range of 9.80 U/l to 104.7 U/l for level of SGOT in serum, while diabetic subjects show the range of 12.74 to 45.37 U/l. For both normal and diabetic values, level of glycation shows a positive correlation with level of SGOT. Correlation coefficient are 0.026 & 0.0354 respectively. The regression lines are; y= 2.9232x+24.47& y= 2.6042x+25.659 respectively (Fig. 4a & b). So it is concluded that level of SGOT may increase with increase in level of protein glycation. According to Kumari and Sahib (1993) and above coclusions, it is proved that liver function is disturbed by protein glycation, resulting in the increased concentration of SGOT.

Effect of glycation on level of SGPT. The level of SGPT in normal individuals shows the range of 4.54 to 55.84 U/l, whereas the diabetic group shows the range of 9.31 to 74.45 U/l. With SGPT, for both normal and diabetic subjects, the level of glycation shows negative correlation. The correlation coefficient are 0.0046 and

0.0189 respectively. The regression lines are; y= -0.5252x+ 26.824 & y= -2.9334x+26.771 respectively for both normal & diabetic groups (Fig. 5a & b). The decrease in level with increase in glycation again shows the disturbance of liver functions by protein glycation (Kumari & Sahib, 1993).

Effect of glycation on level of alkaline phosphatase. Normal individuals show the range of 12.00 to 301.76 U/l for level of alkaline phosphatase, while diabetic individuals show the range of 30.36 to 496.80 U/l. In case of normal individuals, there is a positive correlation, which is 0.0034 & regression line; y= 3.5412x+90.477 (Fig. 6a). In case of diabetic individuals, the results are reverse. There is negative correlation with correlation coefficient 0.0239 and regression line; y= -21.716x+195.52 (Fig. 6b). The explanation is only that level of glycation effects the liver in diabetes in greater extent, whereas in normal, there is less effect. According to Ford and Zahra (1993) it can be explained on the possibility of degradation of alkaline phosphatase in diabetic individuals.

Effect of glycation on level of acid phosphatase. Normal subjects show the range of 0.91 to 112.11 U/l for level of acid phosphatase, while diabetic subjects show the range of 6.964 to 60.680 U/l. The level of plasma protein glycation shows a negative correlation (0.0013) and positive correlation (0.3003) with acid phosphatase, in normal & diabetic subjects respectively. The regression lines; y= -0.6304x+ 24.019 & y= 8.7483x+7.0842 respectively in both normal & diabetic subjects (Fig. 7a & b). Until now, no reference has been found on effect of level of glycation on level of acid phosphatase. The results cannot be explained due to complexity of diabetic complications.

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