Original Article

Correlation of Advanced Glycation End Products (AGEs) with the level of Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) Enzymes in Chronic Diabetic Patients

1. Rabbiah Manzoor Malik 2. Sajida Bano 3. Rafique Ahmed

1. Asstt. Prof. of Biochemistry, Wah Medical College 2. Assoc. Prof. of Biochemistry, Hashmat Medical College, Gujrat 3. Assoc. Prof. of Anatomy, Hashmat Medical College, Gujrat

ABSTRACT

Objective: Objective of this study is to find Correlation of Advanced Glycation End products (AGEs) with the level of Alanine amino transferase (ALT) and Aspartate amino transferase (AST) enzymes in chronic diabetic patients.

Study Design: case Control Study

Place and Duration of Study: This study was conducted at the laboratory of Department of Biochemistry, University of Agriculture, Faisalabad from December 2009 to May 2010. For this study blood samples were collected from Pakistan Institute of Medical Sciences, Islamabad.

Materials and Methods: In the present study, the level of protein glycation in human serum samples of healthy older and young control subjects (n = 20), and diabetic patients (n = 45), were investigated. The patients were selected on clinical grounds from Pakistan Institute of Medical Sciences

Results: Serum AGEs were found to be significantly ($P < 0.001$) increased in diabetic patients as compared to healthy older subjects and control subjects. However, no significant difference was found in the level of AST and ALT in diabetic patients and control group. The AGEs distribution in the these groups reveals the hypothesis that the advanced glycation process might play a role in the development of liver diseases, but in this study no correlation has been found in level of glycation and such liver damage which could result in decreased or increased formation of AST and ALT.

Conclusion: No study have been found regarding level of glycation and level of AST and ALT in diabetic patients. From this study we conclude that no correlation is present in level of glycation and level of AST and ALT in blood in diabetes. Since, there is no effect of AGE formation on production of ALT and AST in liver.

Key Words: AGES, ALT, AST

INTRODUCTION

Diabetes is one of the oldest recognized diseases, on the basis of findings in the laboratory, it can be explained as fasting plasma venous glucose concentration greater than 120mg/dl or a concentration of 200 mg/dl or more after a carbohydrate meal or oral ingestion of the equivalent of 75g of glucose, even if the fasting concentration is normal. It is now established that diabetes mellitus is genetically and clinically heterogeneous group of disorders that are associated with glucose intolerance.[1]

Diabetes mellitus is a common endocrine disorder characterized by hyperglycaemia and predisposes to chronic complications affecting the eyes, blood vessels, nerves and kidneys. Hyperglycaemia has an important role in the pathogenesis of diabetic complications by increasing protein glycation and the gradual build-up of advanced glycation end products (AGEs) in body tissues. These AGEs form on intra- and extracellular proteins, lipids, nucleic acids.[2]

Long term diabetes is associated with a number of other physical disorders, affecting the retina, kidney, the peripheral nerves, and the microcirculation. 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 carbohydrate, protein and lipid. The primary factor associated with the development of most diabetic complications is the prolonged exposure to hyperglycemia. The magnitude and duration of target tissue, exposure to abnormally high levels of blood glucose correlate closely with the extent and rate of progression of complications, although genetic determinants of tissue susceptibility and independent accelerating factors such as hypertension also influence the individual clinical course[3].

The determination of negatively charged adult hemoglobin, designated HbA as glycated hemoglobin (GHb) by Bookchin and Gallop[4], led to speculation that other body proteins may be similarly affected. Now, it has been established that several plama proteins,

collagen and lens crystalline 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[5]. According to Cohen[6], the factors that affect the degree of glycation in vivo are: the degree and duration of hyperglycemia, the half life of the protein in circulation or in tissue, the permeability of tissue to free glucose, the number of free amino groups in the protein and the accessibility and pK of the amino groups within the structure of the protein.

The relative reactivities of sugars with protein depend upon the extent to which they exist as open chains, containing a free carbonyl group[7]. Rates of glycation depend on the proportion of sugar present in the carbonyl form, on the amino basicity, and on its stearic accessibility. The thermodynamic stability of the resulting glycosylamine moieties, which presumably depend in part on the protein structure, may, however, be the most important factor in determining the extent of their reaction.

Austin et al.[8] planned to examine the contribution of specific plasma proteins in normoglycaemic and hyperglyacaemic patients. In vivo, glycation for most plasma proteins was very low in non-diabetic patients. On the other hand, in diabetic patients glycation was much greater. Generally it can be ruled that proteins with the longest biological half lives have greater in vivo as well as in vitro glycation. On the other hand, proteins having short half lives showed moderate glycation. More basic proteins showed greater glycation than acidic proteins.

Biological amines react with reducing sugars to form a complex family of rearranged and dehydrated covalent adducts that are often yellow-brown and/or fluorescent and include many cross-linked structures. Food chemists have long studied this process as a source of flavor, color, and texture changes in cooked, processed, and stored foods. During the 1970s and 1980s, it was realized that this process, called the Maillard reaction or advanced glycation also occurs slowly in vivo. Advanced glycation endproducts (AGEs) that form are implicated, causing the complications of diabetes and aging, primarily via adventitious and cross linking of proteins. Long-lived proteins such as structural collagen and lens crystallins particularly are implicated as pathogenic targets of AGE processes[9].

The liver plays a major role in the pathogenesis of type 2 diabetes. It contributes to insulin resistance, along with muscle and adipose tissues, and it has a major impact on the incidence of hyperglycaemia[10]. Hepatic diseases such as cirrhosis, viral hepatitis and non-alcoholic fatty liver disease are associated with altered glucose metabolism and a higher prevalence of diabetes mellitus[11].

The liver enzymes, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), gamma-glutamyl transferase (γGT) and bilirubin, are used routinely for assessing liver function. Although they are present in tissues throughout the body, they are most often elevated in patients with liver diseases or high alcohol consumption[12].

In this study, we have found a correlation between level of protein glycation and level of urea, creatinine and liver enzymes to find out the relation of protein glycation with the development of increased systematic and general complications in chronic diabetic patients.

MATERIALS AND METHODS

This study was conducted at the laboratory of Department of Biochemistry, university of Agriculture Faisalabad. For this study 65 blood samples were collected, 45 of which were from diabetic patients and 20 were from controlled normal individuals. Samples included in this study were from patients presenting in pathological laboratory of Pakistan Institute of Medical Sciences, Islamabad.

Biochemical estimation of protein concentration in blood samples: Plasma samples were first dialyzed and their protein concentration was determined by Biuret method using Biuret Reagent. Biuret Reagent (Gornal) was prepared by dissolving 1.5g copper sulphate pentahydrate and 6.0g of sodium potassium taratrate tetrahydrate in 500 ml distilled water. In this 300 ml of 10% NaOH solution was added with swirling and final volume made upto 1 litre. To 1ml of plasma protein solution (100-500µg) 1ml of biuret reagent was added and incubated for 15 min at 38º C. The tubes were cooled and absorbance at 540nm was measured against an incubated blank. The standard curve was made with bovine serum albumin standard solution.

Determination of Protein Glycation Level of plasma proteins: Thiobarbituric acid (TBA) colorimetric technique was used for the determination of both enzymatic and non-enzymatic glycation. The standard curve was made by using fructose standard solution (Fig2)

Non-enzymatic and enzymatic Glycation (collectively): To 1ml of plasma solution containing 10mg of protein 10.5 ml of 1N oxalic acid was added and autoclaved at 115lb/square inch pressure for 15 minutes. Then centrifuged the samples, after adding 0.5ml of cold trichloroacetic acid. To 1.5 ml of supernatent, added, 0.5ml of thiobarbituric acid and incubated for 15min, at 37º C before taking absorbance at 443nm.

Enzymatic Glycation: For determination of enzymatic glycation, 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 protein present in sample. $NaBH_4$ was dissolved in 0.01N NaOH solution. After resuction the samples were analyzed for determination of glycation level.

Non-enzymatic Glycation: Non Enzymatic glycation was determined as follows:
NE Glycation = (NE + E Glycation) – E Glycation
NE- Non Enzymatic
E- Enzymatic

Biochemical analysis of samples for other parameters: All the samples were analyzed by using diagnostic kits for the quantitative estimation of following parameters:
Alanine amino transferase ALT (SGPT)
Aspartate amino transferase AST (SGOT)
Following kits were used

S.No.	Diagnostic Kit	Parameters
1.	Human Diagnostic Cat. No. 12012	ALT (SGPT)
2.	Human Diagnostic Cat. No. 12011	AST (SGOT)

RESULTS

Alanine amino transferase ALT (SGPT): Normal individual showed the range of 9.8 to 104.70 U/I for level of ALT, while diabetic individuals showed the range of 12.74 to 45.37. U/I. Descriptive statistics gave non- significant result, as shown below in table 1

Table No.1: T-Test for ALT

Subjects	Mean U/I	S.D.	T-Value
Normal	32.05	±25.68	-0.72
Diabetic	27.99	±9.08	

S.D. Standard deviation

Aspartate amino transferase AST (SGOT): Normal subjects showed the range of 4.54 to 55.84 U/l for level of AST, while diabetic subjects showed the range of 9.31 to 36.40 U/l. Descripitive statistics gave a non significant result, as shown in following table2.

Table No.2: T-Test for AST

Subjects	Mean U/I	S.D.	T-Value
Normal	26.20	±10.94	-1.12
Diabetic	22.63	±9.99	

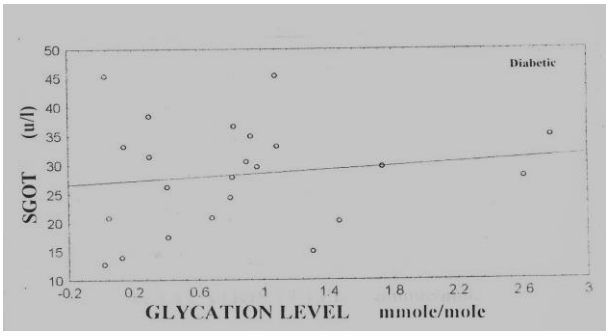

Figure No. 1. Correlation between protein glycation level and level of AST in normal controls

Effect of glycation on level of ALT and AST: For both normal and diabetic ,level of protein glycation revealed a non significant (P>0.05) and positive correlation with level of SGOT. Correlation co-efficient were r= 0.168 and r=0.13 for normal and diabetic respectively, as shown in fig 1and fig.2.

With AST, for both normal and diabetic subjects, the level of protein glycation revealed non-significant and negative correlation. Corelation co-efficients were r= 0.24 for normal and r=-0.182 for diabetic, as shown in fig 3.

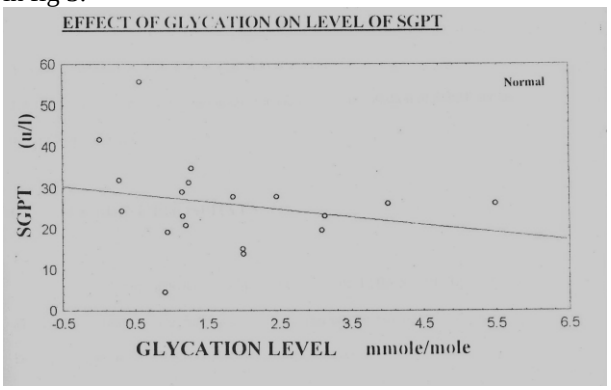

Figure No. 2. Correlation between protein glycation level and level of AST in diabetic patients

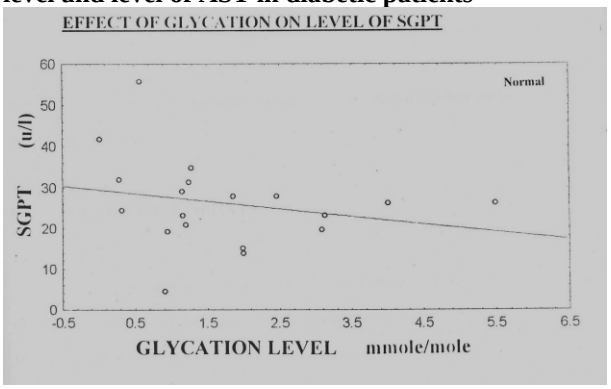

Figure No. 3. Correlation between protein glycation level and level of ALT in normal controls

DISCUSSION

Higher levels of circulating AGEs (as expected in patients with hepatic dysfunction) do not cause a stronger internalisation in hepatocytes, Kupffer cells and/or bile ducts. On the other hand, stronger internalisation could also trigger a higher rate of AGE degradation, keeping internalised levels equal.[13]

Both ALT and AST have been previously found high in diabetic patients14. On the other hand, in 3 recent population-based studies[15,16,17], ALT lost its association with incident diabetes after adjustment for either a minimum[15] or full range of diabetes risk factors[16,17].

In a comparative study among normal individuals and diabetic patients ALT and AST were not found significantly higher in diabetics as compared to normal individuals[18]

In a study based on patients having insulin resistance and non resistant to insulin low levels of AST and ALT have been found[19].

Another study[20] showed that younger diabetic patients were more likely to have high ALT values than the older patients. However the older patients showed elevated AST activity. Supported by earlier studies[21,22,23] this finding suggested that severe steatosis denoted by a higher release of the ALT enzyme in response to hepatocytes derangement, tends to occur earlier in the disease process. As a marker of hepatocyte integrity the ALT activity decreases as steatosis progresses whereas inversely a rise in the AST level has been noticed in the older patients. The latter observation can be attributed to the fact that the clearance of this enzyme is mainly accomplished by the liver sinusoidal cells. While there is no effect from the necro-inflammatory activity on AST level, advancing fibrosis which injures the sinusoidal cells leads to the relative increase in serum AST[24].

CONCLUSION

No study have been found regarding level of glycation and level of AST and ALT in diabetic patients. From this study we conclude that no correlation is present in level of glycation and level of AST and ALT in blood in diabetes. Since, there is no effect of AGE formation on production of ALT and AST in liver.

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