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Impact of Plasma Protein Glycation on Lipid Profile in Diabetics with and without Complications

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ABSTRACT

Plasma protein glycation level and lipid profile were determined in normal group (G1) and in diabetic with and without complications groups (G2-G5). The level of glycation and glucose were high in diabetic with cardiovascular complications than other diabetic groups (G2-G5). Glucose via glycation end products has a central role in the pathogenesis of diabetic complications. The level of protein and high density lipoprotein (HDL-C) were lower in diabetics than normal. The level of cholesterol, low density lipoprotein (LDL-C) and triglycerides were higher in diabetic with complication groups than diabetic without complications followed by normal. All the diabetic subjects with complications groups (G3-G5) had low level of protein but high level of glycation that contributed to the risk of diabetic complication. Cardiovascular patients had lower HDL-C level and high LDL-C level. Diabetes mellitus was accompanied by low HDL-C and high plasma triglyceride levels, which are major cardiovascular risk factor. The results indicated that the prevalence of diabetes increases with age. Females with relatively younger ages were found to be more prone to diabetes than males.

Key Words: Lipid profile; Glycation; Cardiovascular; Diabetes; Pathogensis

INTRODUCTION

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

Diabetes mellitus disease occurs due to lack of insulin produced in pancreas by ß-cells of islets of Langrehans. It is defined as inability to control blood glucose, which results in chronic hyperglycemia, characterized by severe thirst, polyurea weight loss and stupor (Albert & Zimmet, 1998). In short it is genetically and clinically heterogeneous group of disorders that are associated with glucose intolerance. Long term diabetes is associated with number of other physiological disorders such as absence of insulin or improper control on the metabolism of carbohydrates, proteins and lipids (Anonymous, 1985).

The primary factor associated with the development of most diabetic complications is prolonged exposure to hyperglycemia. The magnitude and duration of the target tissue exposure to abnormal levels of blood glucose correlates 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 (Brownlee, 1991; Armando & Morales, 2004). Hyperglycaemia is associated with long-term complications. Two mechanisms are currently proposed: one is non-enzymic glycosylation or glycation (NEG), which is based on the interaction of reducing sugars (primarily glucose) with the amino groups of proteins and nucleic acids. A relatively stable Amadori compound is formed, which can gradually be converted to advanced glycosylation (glycation) end-products (AGE).

$$P + G \Leftrightarrow SB \Leftrightarrow AP \rightarrow AGE$$

(P=Protein, G=Glucose, SB=Schiff base, AP=Amadori product, AGE=Advanced glycosylation end-product).

The other mechanism is that hyperglycaemia causes the accumulation of sorbitol and deficiency of myoinositol in cells. This imbalance is the root cause of complications.

Diabetic complications develop in both insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) and present as complications of eye (retinopathy), kidney (nephropathy), nervous system (neuropathy) and macrovascular disease in later years of diabetes. Due to the nature of these complications, which are identical with disorders, which occur in the elderly (they merely develop earlier). Some researchers now consider diabetes to be a model for the

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normal aging process (Lang *et al.*, 1995). Purpose of this study was to determine the influence of plasma protein glycation on the lipid profiles of diabetic patients.

MATERIALS AND METHODS

A total of 200 blood samples were collected; 160 were of diabetic patients and 40 non-diabetics (normal). The diabetic subjects were certified as diabetic by medical staff of Allied Hospital, Divisional Head Quarter Hospital, Social Security Hospital and National Hospital in Faisalabad, Pakistan. The samples were divided into five groups:

1st group (G1) contained normal males and females

2nd group (G2) contained diabetic males and females without any complication

3rd group (G3) contained diabetic males and females with cardiovascular complications

4th group (G4) contained diabetic males and females with nephropathy

5th group (G5) contained diabetic males and females with retinopathy

The samples were analyzed on the basis of socio-economic and biochemical parameters. The socio-economic parameters included age, sex, marital status, occupation, duration of diabetes, type of diabetes and complications of diabetes. Biochemical parameters included glucose level, protein concentration, plasma protein glycation, liver enzymes, lipid profile and minerals level, SDS-PAGE analysis and Comet assay.

Sample collection. From each of 200 volunteers, 10 mL of whole blood sample at fasting with 0.5 mL EDTA was collected from cephalic vein with sterilized 10 mL capacity plastic disposable syringe and 3 mL blood was used for Comet assay. Remaining 7 mL was centrifuged. Plasma was stored in vials and used to determine different parameters.

All the serum samples were analyzed with various spectrophotometric methods. Diagnostic kits were used for the quantitative estimation of above mentioned biochemical parameters. The biochemical parameters were as under:

1. Glucose (Trinder, 1969).
2. Triglycerides (TG) (Schettler & Nussel, 1975).
3. Low density lipoprotein cholesterol (LDL-C) (Schettler & Nussel, 1975).
4. High density lipoprotein cholesterol (HDL-C) (Warnick & Alben, 1978).
5. Cholesterol (Schettler & Nussel, 1975).

RESULTS AND DISCUSSION

Glycation level in different groups. Normal group (G1) had lesser glycation level as compared to other groups. The glycation value of G3 was greater than that of other groups. There was a significant difference ($P < 0.000$) in glycation values between groups (Table I). Halton *et al.* (1993) who described that non-enzymic glycation occurs when protein is in solution of sugar, which is conformity to our findings. The product of the reaction is a covalently linked glycated protein. Plasma protein usually undergoes glycation, whenever there is hyperglycemic situation especially in diabetic but not the normal subjects. The principle glycated protein was albumin (non-enzymatically glycosylated) with glucose, fructose, galactose and ribose. The difference between the glycation level of females and males was significant ($P<0.01$); males had higher glycation value than females. The interaction between sex and group was also significant ($P<0.05$), which was mainly due to the large difference in G3, G4 and G5.

Table I. Average glycation level, age, glucose, protein and cholesterol levels in different groups

Parameters	Groups	Females	Males	Total
Glycation level	G1	0.297	0.331	0.628 e
	G2	0.909	0.816	1.725 b
	G3	1.350	1.655	3.005 a
	G4	0.675	0.998	1.673 b
	G5	0.942	1.021	1.963 b
Average age	G1	35.70	50.20	42.95 c
	G2	44.75	50.40	47.57 b
	G3	49.85	52.65	47.57 b
	G4	55.20	58.05	56.62 a
	G5	57.35	58.05	57.70 a
Glucose level	G1	114.35	119.25	116.8 c
	G2	255.100	375.75	351.75 a
	G3	356.00	326.40	341.29 a
	G4	263.55	258.25	260.90b
	G5	314.800	317.20	316.00 a
Protein level	G1	7.465	7.605	7.535 a
	G2	7.945	6.362	7.153 a
	G3	4.985	4.760	4.800 c
	G4	7.582	6.950	7.266 a
	G5	5.816	6.050	5.933 b
Cholesterol level	G1	170.650	173.95	172.3 d
	G2	174.839	188.630	181.73 d
	G3	442.55	462.900	452.72 a
	G4	281.700	312.200	269.95 a
	G5	326.400	329.25	327.82 b

Values with same letters in a column differ non-significantly ($P< 0.05$)

Age in different groups. Data indicated significant ($P<0.002$) difference in average ages of males and females ($P<0.000$) in all the four groups. The males were older than females. This gap between ages of females and males in normal group was higher and decreased as the diabetic complications increased. Females with relatively younger ages appear to suffer more as compared to males (Table I). Our results are in agreement to Lang *et al.* (1995) showing that prevalence of diabetes increases with age.

Glucose level in different groups. The level of glucose was lower in normal subjects as compared to diabetics (Table I). Glucose concentration was higher in G2 to G5. In G3 the glucose concentration was higher than other diabetic group. Healthy older adults had higher glucose level than the younger ones. Chronic hyperglycemia does not represent a hallmark of diabetes mellitus but itself is a regulatory factor that contributes to poor metabolic control. Diminished homeostatic control of glucose metabolism is a common

characteristic related to aging. As shown in Table I, there was a significant difference (P<0.04) between the glucose level of females and males. The males have higher value of glucose as compared to females. The interaction between sex and groups was also significant (P<0.001), which was due to large difference in glucose level of normal and diabetic groups. These results are in accordance with Georage and Sivakami (2004), who reported that glucose via glycation end products have a central role in the pathogenesis of diabetic complications.

Protein level in different groups. Protein value decreased as the intensity or complication of diabetes increased, but with significant (P<0.002) difference in females and males. There was a significant difference in protein value within the groups, because of a great variation in the groups. The G1 however had high protein concentration than that of diabetic groups (G2, G3, G4 & G5). Diabetic with cardiovascular complications had smaller protein value than the other groups. This implied that that as the glycation increases protein decreases, which was in harmony with the findings of Luxton (1991). Altered ratio of albumin/globulin is also a pathological indicator towards the abnormalities of liver and immune system. Viktorova *et al.* (1993) found that mean daily glycemia, blood level of glycated Hb and albumin and micro albuminurea was found to contribute to the development of diabetic nephropathy.

The results reported in Table I support the findings of Viktrorova *et al.* (1993), because as the level of glycated protein (glycation level) increases the complication rate increases. All diabetic subjects (G3–G5) had low level of protein but high level of glycation, which contributed to the risk of diabetic complications. Glycogenic amino acids, alanine and glycin, are avidly released from muscle by the liver and used for the formation of glucose. Insulinopenia results in defective ribosomal function, which appears to be the cause of the reduced synthesis of protein. With break down of proteins, urinary excretion of nitrogen increases markedly and severe state of negative nitrogen exist.

Cholesterol level in different groups. Normal group (G1) had lowest cholesterol as compared to other groups. G2 had also lesser cholesterol value as compared to other diabetic group but the cholesterol value was higher than the normal (Table I). There was a significant difference (P<0.001) in cholesterol values due to large differences in the health status of different groups. G3 had higher cholesterol level due to cardiovascular complications. According to Guerci *et al.* (1994) patients with diabetes mellitus were at increased risk of coronary, cerebral and peripheral vascular diseases and frequently had abnormal plasma lipid level. Patients with micro-albuminurea or chronic renal failure showed athrogenic changes of lipoprotein pattern. In diabetes, lipid and lipoproteins were potentially atherogenic although their concentrations were strictly abnormal. These results are in accordance with Borggreve *et al.* (2003) who suggested that type II diabetes is accompanied by low level of HDL and high cholesterol, plasma triglyceride level, which are major cardiovascular risk factor. They also suggested abnormal HDL metabolism and reverse cholesterol transport (transport of cholesterol from peripheral cells back to the liver) and bilary excretion in diabetes mellitus.

Table II. Average values of HDL-C, LDL-C and TG values in different groups

Parameters	Groups	Females	Males	Total
HDL-C values	G1	45.80	43.55	44.675 a
	G2	33.06	48.11	40.585 b
	G3	23.35	24.00	23.175 e
	G4	31.69	29.250	30.470 d
	G5	35.80	36.750	36.275 c
LDL-C values	G1	111.00	122.150	116.555 d
	G2	122.958	117.964	120.461 d
	G3	371.00	397.250	384.125 a
	G4	164.200	130.500	147.350 c
	G5	214.300	221.650	435.950 b
TG values	G1	110.250	109.250	109.75 e
	G2	127.745	133.650	130.55 c
	G3	421.800	391.850	406.65 a
	G4	173.900	207.750	190.825 b
	G5	195.450	204.700	199.725 b

Values with same letters in a column differ non-significantly (P< 0.05)

Table I also indicated significant (P<0.03) difference in the cholesterol values of both the sexes; males showing higher cholesterol level than females. The interaction of sex and groups was however not evident. Our results strongly agree with those of Sobenin *et al.* (1994) who concluded that low density lipoprotein from type I and type II diabetic patients unlike LDL from healthy ones caused 1.5–2.5 fold higher cholesterol level of cell cultured from unaffected human aortic intima, possessing atherogenic potential.

HDL-C level in different groups. The difference between the value of HDL-C in females and males was non-significant (P<0.06), although the difference in the HDL-C value of different groups is significant (P<0.001), which is due to large variation in the health status of different groups (Table II). The interaction between sex and groups is also significant (P<0.001), which implied that response of different female and male groups was not equal and therefore they behave differently.

The averages indicated that the values of HDL-C in G1 was highest but was within the normal range. This value G2 was higher than other diabetic groups but lesser than G1, which indicated some effect of diabetes on HDL-C in females. The level of HDL-C in G5 was lowest of all groups. Cardiovascular patients had lower HDL-C value and higher LDL-C value. The level of HDL-C in diabetic with nephropathy group (G4) was lower than normal and diabetic without any complications showed greater risk of cardiovascular complication.

HDL-C has denser lipoprotein because 50% of its mass is protein. HDL moves cholesterol to the liver and its two antioxidant systems platelet activating factor acetyl hydrolase and paraoxanase are beneficial. Studies further conclude that the reduced HDL-C might be due to enhanced

catabolism due to from increased hepatic triglyceride lipase action of HDL with higher triglyceride contents. The results agree with Borggreve *et al.* (2003) who showed that diabetes mellitus is accompanied by low HDL-C and high plasma triglyceride levels, which are major cardiovascular risk factors. This reflects the nature of hypoglycemic agent used by the patient and the presence or absence of the hypertriglyceri-demia, obesity and of other disorder, such as nephropathy. These data also get support from by Hokanson (1996) who suggests that risk effect of hypetriglyceridemia is independent of HDL, thus both hypertriglyceridemia and low level of HDL-C contribute to coronary risk in diabetes.

LDL-C level in different groups. The LDL-C in G1 was similar to G2, but lower as compared to G3–G5. G3 showed maximum value of LDL-C among the groups because cardiovascular patients show higher LDL-C and lower HDL-C value. G4 and G5 had lesser LDL-C value than G2 and normal group G1 (Table II). The difference between values of LDL-C in both sexes (females & males) was non-significant. The interaction between sex and group was also not significant, which was mainly due to large difference in the groups. The results supported Our results also supported Sobenin *et al.* (1994) who concluded that low density lipoprotein from type I and type II diabetic patients unlike LDL from healthy subjects caused 1.5 to 2.5 fold increase in the cholesterol contents of cell cultured from unaffected human aortic intima i.e., atherogenic potential. The amount of bound LDL was significantly higher in diabetic patients as compared with healthy subjects.

TG level in different groups. There was no difference between the TG level in females and males, but a significant ($P<0.000$) one in the TG level of different groups (Table II). The TG was higher in cardiovascular complications group (G3). The diabetic with nephropathy (G4) and retinopathy (G5) groups showed higher TG level as compared to G1 and G2. Data further showed a non-significant interaction between both the sexes in different groups, which meant that response of diabetes to females and males was similar. According to Fontbonne *et al.* (1989) in males with diabetes or impaired glucose tolerance, death due to coronary artery disease was associated with an increased TG level. The studies by Laakso *et al.* (1993) showed that atherosclerotic cardiovascular disease was associated with both increase in triglyceride rich lipoprotein and decrease in HDL. Our results showed that TG level were higher in G3, which also had low HDL. Our results indicated that LDL level was higher in G2 to G5 than normal healthy subjects. However, the concentration of LDL was higher in G3.

In conclusion, all the four diabetic groups had low level of HDL-C as compared to the normal group but G4 and G5 did not develop cardiovascular complications and also had low level of HDL-C than the normal range. This supported the fact that alone high triglyceride level and low HDL level do not contribute the coronary risk in diabetes.

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