Serum Biochemical Changes in Insulin Dependent and Non-Insulin Dependent Diabetes Mellitus and their Role in the Development of Secondary Complications

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ABSTRACT

Fasting blood glucose, triglyceride, cholesterol, lipoprotein cholesterol, phospholipids, total lipids, uronic acid, total amino acids, glycated serum proteins, fructosamine, copper and zinc levels were determined in insulin-dependent diabetics (IDDs) non-insulin dependent diabetics (NIDDs). Appreciable hyperglycaemia and hypertriglycerideamia was noticed in all diabetics. IDDM females exhibited an increase in total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides, as compared to their age and sex matched controls. In poorly controlled diabetics (fasting blood sugar > 140 mg%) a significant increase in total cholesterol, very low density lipoprotein (VLDL) cholesterol, triglycerides and total lipids was noticed. Glycated serum proteins and fructosamine were significantly higher in diabetics, more so in the poorly controlled group. Serum uronic acid and total amino acids were appreciably higher in diabetics, the latter being much higher in IDDM group. Serum copper was higher in diabetics, female NIDDM group showing highest values. Serum zinc levels were lower in diabetics as compared to controls.

INTRODUCTION

Diabetes mellitus (DM) is characterised by hyperglycaemia due to disturbances in the metabolism of carbohydrate, fat and protein because of abnormalities in the availability of insulin or insulin-action. Even though diabetes mellitus is an endocrine disease in origin, its major manifestations are those of a metabolic disease. The characteristics symptoms are excessive thirst, polyuria, pruritus, and otherwise unexplained weight loss. Diabetes also brings about the progression of secondary complications through the thickening of basement membrane [1].

The most dominant feature of the metabolism in diabetes mellitus is an abnormally high concentration of blood glucose. This can be either due to an abnormally high rate of glucose production or of impaired glucose utilisation. It is now accepted that the high blood glucose level is the result of combination of both these processes.

The secondary complications seen in diabetic patients are found to involve alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products due to over utilisation of glucose in insulin independent tissues [2]. Various authors have shown that hyperglycaemia leads to an increase in serum glycated proteins [3,4,5] alongwith alterations in other atherogenic risk factors.

Further, disturbances in mineral metabolism are also noticed [6] and it is not known whether differences in trace element status are a consequence to the expression of the disease.

In order to understand the underlying pathobiochemical inter-relationships of the late complications of diabetics in more detail, a study was undertaken to look at the serum Cu and Zn levels along with several key metabolities such as serum lipids, glycated proteins, fructosamine, uronic acid and total amino acids.

MATERIAL AND METHOD

All the chemicals used were of analytical grade or the best commercially available grade. Bio-gel P6 was obtained from Bio-Rad USA. All other analytical reagents were obtained from E Merck, SD Fine and BDH firms.

Selection of Subjects:

The study comprised of 31 ambulatory NIDDM patients, of which 18 were males and 13 were females. There were 23 IDDM patients comprising of 12 males and 11 females. The various parameters of both groups were compared with non-diabetic healthy controls.

Collection of blood sample:

After an overnight fast, venous blood samples were collected and the serum was used for lipid profile, total amino acids, uronic acids, glycated serum proteins and fructosamine extra care was taken while treating samples for trace element analysis. Blood was collected with fluoride as preservative and used for blood glucose estimation.

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Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were precipitated from the serum with phosphotungstic acid, magnesium chloride and the supernatent was used for the determination of HDL-cholesterol [7]. LDL-cholesterol in the serum was precipitated by the addition of sodium citrate buffer (pH 5.04) containing heparin [8]. The precipitate obtained was used for LDL-cholesterol estimation.

Assay Methods:

The following determinations were carried out as indicated: Blood glucose [9], uronic acid [10], total amino acids [11], total cholesterol and its fractions by enzymatic kit (Glaxo), triglyceride by enzymatic kit (Glaxo), phospoholipids[12], total lipids [13], GSP (3), fructosamine by enzymatic kit (systems Diagnostics), Cu and Zn by atomic absorption spectrophotometer.

Statistical Analysis:

Students 't' test was used to find out significant differences between two means. All tests were considered significant at $p < 0.05$ level.

RESULTS

Clinical data of the diabetic subjects is presented in Table 1. Significantly elevated levels of FBG were noticed in both types of diabetics as compared to controls (Mean FBG for controls was 87.2 ± 18 mg/dl). Female NIDDs had higher BMI as compared to their male counterparts.

Table-1
Clinical data of diabetic patients

	NIDDM		IDDM	
	Male	Female	Male	Female
Number of patients	18	13	12	11
Mean age	55.5	55.3	24.9	25.0
Mean duration of the disease (yrs)	7.4	7.1	4.9	5.2
Mean FBG (mg/dl)	141.6	154.0	252.7	237.0
Mean BMI*	24.6	26.8	16.0	17.4
Type of treatment	Oral drugs		Insulin	

* Body Mass Index = $\frac{\text{Wt in Kg}}{(\text{Ht in cm})^2} \times 100$

In case of lipids, the values of the patients were compared with corresponding age and sex matched controls. In NIDDs (Table 2), both males and females, the TG and TL levels were significantly elevated as compared to their controls. None of the other parameters exhibited much alteration.

Table-2
Serum lipid profile in NIDDs and controls
(Mean ± SD; mg/dl)

Subject	TC	HDL-C	VLDL-C	TG	PL	TL
Males						
Controls n = 12	189.9 ±24.2	43.4 ±4.1	125.0 ±18.6	21.5 ±3.7	110.8 ±18.9	49.3 ±7.0	377.9 ±45.5
NIDDM n=18	213.0 ±37.9	38.8 ±7.9	130.3 ±27.8	44.7 ±19.3	220.8** ±24.7	43.7 ±8.1	539.2 ±172.9
Females							
Controls n = 11	182.6 ±20.1	50.8 ±4.2	110.8 ±12.2	21.0 ±4.8	105.1 ±23.6	47.5 ±6.7	364.3 ±46.8
NIDDM n=18	208.5 ±37.2	43.6 ±4.9	117.9 ±35.6	47.0 ±4.6	235.6* ±23.5	49.1 ±11.4	506.3** ±65.3

* Significantly different from corresponding control value at P<0.05
** Significantly different from corresponding control value at P<0.01

In IDDs (Table 3), the TG and TL levels were higher than their controls. In female IDDs patients, TG values significantly higher than the male IDDs subjects. Also, LDL-C levels were significantly increased in female IDDs patients as compared to their controls. Mean TC levels were also elevated in female IDDs than their controls and their male counterparts.

Table-3
Serum lipid profile in IDDs patients and controls
(Mean ± SD ; mg/dl)

Subject	TC	HDL-C	LDL-C	VLDL-C	TG	PL	TL
Males							
Controls n = 10	185.2 ±25.0	42.4 ±5.5	107.2 ±15.2	34.5 ±11.7	180.2 ±53.1	43.5 ±9.2	457.3 ±93.1
IDDM n=12	187.4 ±24.3	45.3 ±10.3	110.8 ±23.5	31.7 ±15.6	215.3* ±68.3	39.2 ±4.6	526.0* ±79.2
Females							
Controls n = 11	167.4 ±24.5	50.2 ±9.2	92.5 ±21.6	25.3 ±9.7	124.7 ±46.7	41.7 ±6.3	392.7 ±81.2
NIDDM n=18	206.8 ±32.7	46.8 ±4.9	126.2* ±14.7	33.6 ±13.0	243.6* ±93.8	43.0 ±4.1	570.6* ±106.9

* Significantly different from corresponding control value at P<0.05
** Significantly different from corresponding control value at P<0.05

An altered lipid profile was observed when the NIDDM subjects were grouped according to the FBG levels (Table 4). Serum TC, VLDL-C, TG and

TL were raised in poorly controlled diabetics (FBS>140 mg/dl) as compared to the well controlled group (FBS<115 mg/dl).

Table-4
Influence of fasting blood glucose on lipid profile in NIDDs
(Mean ± SD ; mg/dl)

FBS level	TC	HDL-C	LDL-C	VLDL-C	TG	PL	TL
FBS>140 n+ 20 (A)	209.6* ±21.1	35.3 ±5.3	132.1 ±18.9	43.0* ±6.7	231.8* ±22.6	42.7 ±7.2	584.5* ±53.6
FBS<115 n = 11 (B)	163.7 ±20.7	41.8 ±4.8	101.9 ±19.6	22.3 ±8.6	119.6 ±31.1	41.5 ±6.9	407.2 ±77.1

* Significantly different from (B) at P<0.05

Table 5 gives the levels of GSP and fructosamine in diabetic patients and controls. The mean values were significantly higher in hyperglycaemics as compared to the controls. The levels were much higher in poorly controlled diabetics versus the well controlled group.

Table-5
Glycosylated serum proteins and fructosamine levels in diabetics
(FBG < 115 and > 140 mg/dl) and controls (Mean ± SD)

Subjects	GSP %	Fructosamine UMol/l
Controls n= 20	1.61 ±0.15	244.2 ±29.3
Diabetics n= 31	2.69*** ±0.30	416.4*** ±60.6
FBG>140 mg/dl n= 20 (A)	2.83[aaa] ±0.27	485.6[aa] ±37.8
FBG<115 mg/dl n = 11 (B)	2.37 ±0.18	327.3 ±25.9

*** Significantly different from control value at P<0.001
[aaa] Significantly different from (B) at P<0.001
[aa] Significantly different from (B) at P<0.01

Serum uronic acid and total amino acids were also higher in diabetics than their controls (Table 6). The elevation in total amino acid levels was more in IDDM than NIDDM group.

Table-6
Serum uronic acid and total amino acids level in diabetics and controls (Mean ± SD ; mg/dl)

Subjects	Uronic acid	Total amino acids
Controls n = 20	25.7 ±9.2	36.0 ±15.3
IDDM n = 16	52.3*** ±11.7	118.7*** ±16.4
NIDDM n = 27	55.6*** ±14.0	57.1 ±11.8

*** Significantly different from control value at P<0.001

Serum copper levels were higher in diabetics as compared to controls (Table 7). Female NIDDs showed highest levels of serum cu. Zinc levels in the serum of diabetics were lower as compared to controls (Table 8).

Table-7
Serum Cu levels in diabetics and controls
(Mean ± Sd ; ug/ml)

Subjects	Cu	
	Males	Females
Controls n = 10	1.40 ±0.11	1.30 ±0.12
IDDM n = 16	1.50 ±0.16	1.50 ±0.20
NIDDM n = 18	1.50 ±0.12	1.70* ±0.28

*** Significantly different from corresponding control value at P < 0.05

Table-8
Serum Zn levels in diabetics and controls
(Mean ± SD ; ug/ml)

Subjects	Zn	
	Males	Females
Controls n = 10	1.40 ±0.16	1.40 ±0.26
IDDM n = 16	1.20 ±0.23	0.90* ±0.20
NIDDM n = 18	1.40 ±0.08	1.20 ±0.08

*** Significantly different from our corresponding control value at P < 0.05

Discussion

Diabetes mellitus is a complex disorder affecting the metabolism of carbohydrate, protein and lipids. Hyperglycaemia is known to play a pathogenic role due to the excess formation of glucose-derived end-products [3].

The diabetic patients showed an elevated FBG level, which is the hallmark of diabetes. The IDDM subjects exhibited much higher FBG levels.

An increase in the mean values of lipids was noticed in the diabetic group, which is a secondary manifestation of primary defect in glucose utilisation. In females, increased levels of TG and TL was observed in NIDDM and increase in LDL-C, TG and TL was observed in IDDM group. This makes them more prone to cardiovascular complications.

Significant alterations in the lipid levels were found to be influenced by a glycaemic control. Diabetics with a better glycaemic control maintained near normal lipid values. Although diabetes mellitus is considered to be a risk factor for the development of cardiovascular diseases, this process seems to be accelerated by poor glycaemic control.

Hyperglycaemia leads to higher level of glycated serum protein and fructosamine. Non-enzymatic glycosylation of various body proteins coupled with abnormal collagen metabolism are responsible for the thickening of basement membrane in diabetes. GSP and fructosamine levels were significantly elevated in diabetics, more so in the ones with poor glycaemic control.

The increased levels of uronic acid with poor glycaemic control, suggest that glycosaminoglycan metabolism is altered. This may aggravate the thickening of basement membrane. Higher levels of total amino acids indicate muscle wasting.

There is evidence to show that Copper (Cu) is involved in the regulation of blood cholesterol – Cu deficiency leads to elevated blood cholesterol levels. There is also some evidence to suggest than an inadequate Cu intake is associated with an increased risk of cardiovascular disease due to atherosclerosis[14]. Higher plasma Cu has been found in diabetics with retinopathy, hypertension or cardiovascular disease, as compared to diabetic subjects without complications and as compared to control subjects [6].

The role of zinc (Zn) in diabetes is controversial and is still being examined. Zn is an essential trace mineral directly involved in the physiology and action of insulin. Insulin is stored as Zn crystals in the β cells of the pancreas. It has been suggested that abnormal Zn metabolism may play a role in the pathogenesis of diabetes and some of its complications [15]. A study conducted on 20 adults with stable NIDDM, revealed that 25% had depressed serum Zn concentrations, and all demonstrated heyperzincuria. Such Zn depletion has several potential clinical implications. It is speculated that Zn repletion could improve insulin sensitivity in patients with NIDDM or reduce the severity of certain complications of this disease [16]. In the present study, the serum, Zn level is low in the case of NIDDM and substantially low in the case of IDDM patients, particularly in females. All these observations along with our earlier studies [3,4,5] clearly demonstrate that metabolic alterations are more in female diabetics as compared to male diabetics.

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