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Correlation of Diabetic Retinopathy and Serum Sialic Acid

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SUMMARY

Serum sialic acid (SSA) and fasting blood sugar (FBS) levels were determined in 50 normal and healthy (control) individuals and 100 patients of diabetes mellitus. Diabetic patients were divided into two main groups i.e., with and without retinopathy.

Increase in SSA was found in diabetic patients. The data showed more increase in SSA in diabetic patients with retinopathy than those without it, i.e, 2.59±0.47 mM/L (0.81±0.15 G/L) vs 2.02±0.29 mM/L (0.63±0.09) G/L). Whereas the FBS level in diabetic patients with retinopathy was 10.78±2.05 mM/L (1.94±0.37 G/L) and those without it was 9.94±1.33 in M/L (1.79±0.24 G/L) vs 4.83±0.50 mM/L (0.87±0.09 G/L) in the control individuals. A highly significant relationship of total serum sialic acid was observed in patients with retinopathy where p=0.01 (p=0.0084), whereas no significant relationship of serum sialic acid with blood sugar could be seen in this study, where p > 0.1 (p=0.186).

Moreover our data also depicted a significant relationship of SSA with duration of diabetes mellitus and degree of retinopathy i.e., retinopathy I, whether it is with or without maculopathy. But there was no significant relationship of SSA with age and sex.

INTRODUCTION

Sialic Acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid[1]. They are either n or O-acyl derivatives of neuraminic acid[2], whereas neuraminic acid itself is not found naturally[3]. N-acetyl neuraminic acid (NANA) is the principal SA found in human tissues[2]. A variety of modifications of Sialic acids have been described in nature[4]. There are more than 23 known naturally accruing derivatives of parent NANA molecule, several of which are O-acetylated at the 9 position[5], while others can be O-acetylated at positions 4, 7 or 8. These modifications are developmentally regulated and the addition of a single O-acetyl group can markedly affect the biological properties of the parent molecule[6]. N-acetyl neuraminic acid and one of its modified form that is the N-glycolyl neuraminic acid are very common in nature[7]. NANA constitutes the major carbohydrate component[8] of glycoproteins in animal cell membrane[9]. Sialic acids are widely distributed in animal tissues and micro-organisms as components of oligosacchanide units of polysacharides, mucoproteins, lipids, glycoproteins[10] and apo-B[2] in the cell membranes, body fluids[11] milk oligosaccharides, mucins, gangliosides and in certain microbial polymers[12].

Increased glycosylation of various proteins in diabetic patients has been reported by many authors[13]. Several workers have reported a significant correlation between the degree of glycosylation of proteins like haemoglobin and blood sugar level. Conclusion has been drawn that measurement of gluycosylation of plasma proteins can serve as a sensitive, short term integrator of glucose homeostasis in diabetes mellitus[14]. Patients with either type-I or II diabetes mellitus have raised serum concentration of SA[15]. Jons and Wales (1976) suggested rising levels of certain glycoproteins in the blood of diabetic vascular complications[16]. Korte (1991) observed that the plasma membrane of regenerating retinal pigment epithelium contained SA and N-acetyl glucosamine residues as in normal retinal pigment epithelium. However, the amount of plasma membrane bearing

exposed N-acetyl glucosamine increases during regeneration[17]. Syrbe in 1990 carried out a multivariant analysis on the severity of diabetic retinopathy and its relationship with serum concentration of glucose, SA, HDL cholesterol, proteins, SA per protein and total cholesterol, capillary fragility and the number of large spreading forms of platelets, features of haemostasis. It showed that diabetic retinopathy is characterized by a wide spectrum of different features containing the parameters of haemostasis. Thrombocytic vascular interactions are characterized by platelet spreading and capillary fragility which are significant for the development of diabetic retinopathy[18]. In this study correlation between blood sugar level, diabetic retinopathy and its duration has been investigated in relation to serum sialic acid level (SSA).

MATERIALS AND METHODS

Subjects

A first morning (fasting) blood sample was taken for the determination of fasting blood sugar (FBS) and serum sialic acid (SSA) levels from diagnosed diabetes mellitus patients selected at random from those attending the diabetic clinics of Mayo Hospital, Lahore and Sir Ganga Ram Hospital, Lahore. The ophthalmoscopic examination was carried out by the consultant and three groups of patients of 50 subjects were established; 50 normal subjects from the laboratory and hospital staff as controls, 50 diabetic patients without retinopathy and 50 diabetic patients with retinopathy (Table-1).

5ml blood was drawn with disposable syringes under aspetic measures. Serum was separated within 1 hour and the blood sugar was determined enzymatically and the remaining was transferred to 4cm (5ml) glass tubes for storage in the freezer at –20°C until assayed for sialic acid. Long time storage and repeated thawing did not affect the results of any tested constituents of serum[19].

Estimation of Blood Sugar

Blood sugar was determined by Hexokinase Method using the Blood Glucose Kit of Random Laboratories Ltd., Crumlin United Kingdom based on the method of Stein. MW and following the procedure of the manufacturer of Enzymatic Analysis. Academic Press 197[20].

Assay of Sialic Acid

Sialic acid was determined by Ehrlich's method as given by Shamberger 1984[21].

Reagents

- N-acetyl neunaminic acid (sialic acid) from E. coli from Sigma Chrmical Company St. Lewis MO. USA NOA-2388.
- 4-dimethylamino benzadehydle No. Art 3058 from E. Merck, West Germany.
- Hydrochloric Acid concentrated from E. Merck, West Germany.
- Spectrophotometer 4010; Boehringer Mannheim, Germany.
- Chemistry Analyzer FP 901; Lab System of Finland.

RESULTS

The following are the results of this study.

Table 1 shows that SSA level of controls is 0.45±0.11 g/dl (1.44±0.11 mM/l). It increases in diabetic patients without retinopathy (.63±0.09 g/l) and further increases in diabetic patients with retinopathy (0.81±0.15 g/l). When blood sugar level is taken into account.

Table 1: **Serum sialic acid levels in control and diabetes mellitus (DM) patients both with and without retinopathy (Mean±SD in G/L and mM/L).**

Control/Diabetes Mellitus	*No.*	*G/L*	*mM/L*
Control	50	0.45±0.11	1.44±0.35
DM without retinopathy	50	0.63±0.09	2.02±0.291
DM with retinopathy	50	0.81±0.15	2.59±0.47

There is a significant difference in blood sugar levels of control group and diabetic groups. However, the difference is insignificant, between SSA and fasting blood sugar of diabetic patients with and without retinopathy (P=1.86).

Table 3 shows the duration dependent relationship of SSA with retinopathy, which is highly significant (P=0.0025) as SSA increases with duration of DM in patients without retinopathy i.e. 0.5±0.02 g/l in less than 2 years to 0.68±0.05 g/l after two year. Similarly there is a significant

increase in patients with retinopathy of duration less than 10 years and more than 10 years, P=0.0015. The data shows a progressive rise in SSA with the duration of diabetes with or without retinopathy.

Table 2: Serum sialic acid (SS) and fasting blood sugar (FBS) levels in controls and diabetes mellitus patients both with and without retinopathy were determined. Its Mean & SD are given below.

Control/Diabetes Mellitus	*No. of patients*	*SSA (Mean±SD) in G/L*	*FBS (Mean±SD) in G/L*
Control	50	0.45±0.11	0.87±0.09
DM without retinopathy	50	0.63±0.09	1.79±0.24
DM with retinopathy	50	0.81±0.15	1.94±0.37

Table 3: Serum sialic acid level in patients of diabetes with less than and more than 2Y duration but without retinopathy and less than & more than 10 years with retinopathy were determined. Its Mean and SD are given below.

Duration of DM		*No.*	*G/L*	*mM/L*
Without retinopathy	< 2 Y	25	0.58±0.02	2.16±0.16
	> 2 Y	25	0.68±0.05	2.16±0.16
With retinopathy >	< 10 Y	25	0.76±0.04	2.42±0.13
	> 10 Y	25	0.87±0.06	2.78±0.19

The male to female relationship of diabetic patients without retinopathy or with retinopathy is not significant as shown in Table 4.

The relationship of age in diabetic patients with and without retinopathy is not significant in control groups as shown in Table 5. The P-value are greater than 0.05.

Table 6 shows the results of SSA levels in diabetic patients with grade 1 and grade 2 retinopathy as well as unilateral diabetic retinopathy (UDR) and bilateral retinopathy (BDR). There is a significant difference between retinopathy without maculopathy and retinopathy with maculopathy. On the other hand there is no significant dfference in SSA values of UDR and BDR (P0.66).

Table 4: Results of serum sialic acid levels in male and female diabetic patients both with and without retinopathy (Mean and SD).

DM	*Sex*	*No.*	*G/L*	*mM/L*
Without retinopathy	Male	22	0.61±0.07	1.95±0.22
	Female	28	0.66±0.09	2.11±0.28
With retinopathy	Male	24	0.80±0.03	2.56±0.09
	Female	26	0.82±0.04	2.62±0.13

Table 5: Serum sialic acid levels in diabetic patients with and without retinopathy depending upon age groups were determined. Its Means and SD are given below.

Age (Yrs)	*DM without retinopathy*			*DM without retinopathy*		
	No.	*GL*	*mM/L*	*No.*	*G/L*	*mM/L*
31-40	12	0.59 ±0.06	1.90 ±0.19	07	0.73 ±0.04	2.35 ±0.13
41-50	22	0.63 ±0.05	2.03 ±0.16	19	0.77 ±0.06	2.46 ±10.19
51-60	16	0.65 ±0.03	2.08 ±0.09	16	0.83 ±0.03	2.65 ±0.09
61-70	±0.03	±0.03	±0.03	08	0.94 ±0.02	3.02 ±0.07

DISCUSSION

The mean value of SSA was found by Shamburger (1984) in Germany in normal persons to be 1.74±0.21 mM/l (0.54±00.779 g/l). This was found to be independent of age and sex. In the absence of a proliferative disease the value is very stable in an individual. Alvi and Shaikh found this value to be 1.38±0.20 mM/l (0.43±0.07 g)[1].

Table 6: **Results of serum sialic acid levels in diabetic patients with Grade-I and Grade-II retinopathy as well as Unilateral Diabetic Retinopathy (UDR) and Bilateral Diabetic Retinopathy (BDR). Mean±SD in G/L and m M/L.**

Diabetic Retinopathy	*No.*	*G/L*	*mM/L*
G-I without maculopathy	26	0.72±0.08	2.30±0.25
G-II with maculopathy	24	0.91±0.13	2.91±0.41
Unilateral (UDR)	32	0.86±0.07	2.43±0.22
Bilateral (BDR)	18	0.86±0.12	2.75±0.38

Diabetes mellitus (DM) is a syndrome characterized by chromic hyperglycaemia that is due to relative insulin deficiency or resistance or both. It is usually irreversible and is both a metabolic and vascular disease involving both macro- and micro-vasculature. Microvascular damage is proliferative in nature and causes diabetic retinopathy and nephropathy and contributes to diabetic neuropathy, Kumar and Clark (1996)[23].

SSA in relation to diabetic retinopathy was studied by Crook et al.[1] as a pilot project of small group of 20 patients each whereas in this study there were 50 subjects per group. Evidence has been presented in Table 1. That the SSA is related to the severity of diabetic complications such as retinopathy. There is a significant increase in SSA in the diabetic patients without retinopathy and further increase in association with retinopathy though the difference of SSA between diabetic patients with and without retinopathy is not significant.

DM by definition is a hyperglycaemic state and its complications are rabled to the severity of the disease as indicated by level of hyperglycaemia. Whereas there is a clear cut relationship of blood glucose level and serum sialic acid of controls and diabetic subjects, there was no significant difference between glucose levels of DM with retinopathy and without retinopathy (Table 2). Considering the case of duration of DM it had significant difference in SAA without and with retinopathy (Table 3). As found by Sham burger (1985) the sex and age do not affect SSA (Tables 5 and 6). Whereas there is considerable overlap of values of various groups the mid p value of SSA from age and sex factor makes it a useful indicator of proliferative disease especially carcinogenic growth.

CONCLUSION

In this study we found that serum sialic acid concentrations were elevated in diabetic patients both with and without retinopathy. Increase in serum sialic acid in diabetes mellitus have previously been reported by Rahman and Rahman (1991)[14], Jons & Wales (1976)[16] and Korte (1991)[17]. The relationship of serum sialic acid with diabetic retinopathy has been indicated by Crook et al. (1993)[1] and Syrbe, (1990)[18]. However, in this study we also found a significant relationship of serum sialic acid concentrations with duration of diabetes mellitus and degrees of retinal involvement. Thus a significant correlation of diabetic retinopathy and serum sialic acid has been established.

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