Total Serum Sialic Acid (TSSA) in Selective Patients of Diabetes Mellitus (DM)

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Total serum sialic acid (TSSA) and Fasting Blood Glucose 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. An increase in TSSA was found in all diabetic patients. 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 total serum sialic acid with blood glucose could be seen in this study, $p > 0.1$ ($p = 0.186$). The data showed a greater increase in TSSA 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 Fasting Blood Glucose level in diabetic patients with retinopathy was 10.78 ± 0.94 mM/L (1.94 ± 0.17 G/L) and those without it was 9.94 ± 0.78 mM/L (1.79 ± 0.17 G/L) vs 4.83 ± 0.50 mM/L (0.87 ± 0.09 G/L) in the control individuals. Further more, our data also depicted a significant relationship of TSSA with duration of diabetes mellitus and degree of retinopathy and whether it is with or without maculopathy. But, there were non-significant relationships of TSSA with age as well as sex of the patients with $p > 0.05$ ($p = 0.135$ & 0.102 respectively).

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 neura-minic 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 occurring 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 Sialic Acid component[8] of glycoproteins in animal cell membrane[9]. Sialic acids are widely distributed in animal tissues and micro-organisms as components of oligosaccharide units of polysaccharides, 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 some workers.[13] Several workers have reported a significant correlation between the degree of glycosylation of proteins like hemoglobin and blood sugar level. Conclusion has been drawn that measurement of glycosylation of plasma proteins can serve as a sensitive, short-term integrator of glucose homeostasis in diabetes mellitus.[14] Patients with either type-I or type-II diabetes mellitus have raised serum concentration of SA.[15] Jones and Wales (1976) suggested rising levels of certain glycoproteins in the blood of diabetic patients with 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 multivariate analysis on the severity of diabetic retinopathy and its relationship with serum concentration of glucose, SA, HDL-cholesterol, proteins, and total cholesterol, capillary fragility and the number of large spreading forms of platelets, as features of hemostasis. It showed that diabetic retinopathy is characterized by a wide spectrum of different features containing the parameters of hemostasis. Thrombocytic vascular interactions are characterized by platelet spreading and capillary fragility which are significant for the development of diabetic retinopathy.[18] In this study, blood sugar level and total serum sialic acid level (TSSA) has been estimated in diabetic patients having different criteria like: age, sex, duration of the disease and its complications e.g., retinopathy, maculopathy, etc.

Materials and Methods

Subjects:

A 12 – 14 hrs fasting blood sample was taken for the determination of Fasting Blood Glucose and total serum sialic acid (TSSA) levels from diagnosed diabetic patients selected at random from those attending the diabetic clinics of Jinnah Hospital, Lahore and Sir Ganga Ram Hospital, Lahore. Senior registrar, Jinnah Hospital, carried out the ophthalmoscopic examination. Two groups of diabetic patients with 50 subjects each; one without retinopathy while the other with retinopathy (Table l); and a third group with 50 normal subjects from the laboratory and hospital staff as controls were established.

Serum was separated within 1 hour from the sample of 5ml blood drawn with disposable syringes under aseptic

measures. Later blood glucose was determined by enzymatic method and the remaining serum was transferred to 5rnl 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]

Table 1: *Total serum sialic acid (TSSA) and Fasting Blood Glucose levels in control and diabetes mellitus (DM) patients both with and without retinopathy (Mean ± SD).*

Status of the subjects	No. of subjects	TSSA		Fasting Blood Glucose	
		G/L	mM/L	G/L	mM/L
Control	50	0.45 ± 0.11	1.44 ± 0.35	0.87 ± 0.09	4.83 ± 0.5
DM without Retinopathy	50	0.63 ± 0.09	2.02 ± 0.29	1.79 ± 0.14	9.94 ± 0.78
DM with Retinopathy	50	0.81 ± 0.15	2.59 ± 0.47	1.94 ± 0.17	10.78 ± 0.94

Estimation of Blood Sugar:
Blood Glucose was determined by Hexokinase Method using the Blood Glucose Kit of Randox Laboratories Ltd., Crumlin United Kingdom based on the method of Stein.[20] and following the procedure of the manufacturer of Enzymatic Analysis, Academic Press 1974.

Estimation of Total Serum Sialic Acid:
Total Serum Sialic acid (TSSA) was determined by Ehrlich's method as given by Slamberger.[21]

Table 2: *Total serum sialic acid (TSSA) levels in patients of diabetes with less than and more than 2 yrs duration but without retinopathy and less than & more than 10 years with retinopathy were determined. Its Mean and SD are given below.*

Status	Duration of DM in yrs	No. of subjects	TSSA (G/L)	TSSA (mM/L)
DM without Retinopathy	< 2	25	0.58 ± 0.02	1.85 ± 0.07
	> 2	25	0.68 ± 0.05	2.16 ± 0.16
DM with Retinopathy	< 10	25	0.76 ± 0.04	2.42 ± 0.13
	> 10	25	0.87 ± 0.06	2.78 ± 0.19

Reagents:
* N-acetyl neuraminic acid (sialic acid) from E-coli from Sigma Chemical Company, St. Louis, MO. USA NOA-2388.
* 4-dimethylamino benzaldehyde 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.

Table 3: *Results of total serum sialic acid (TSSA) levels in male and female diabetic patients, both with without retinopathy are given below (Mean and SD).*

DM	Sex	No. of subjects	TSSA (G/L)	TSSA (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

Results

The following are the results of this study:

Table 1 shows that TSSA level of controls is 0.45 ± 0.11 G/L (1.44 ± 0.35 mM/L). This increases in diabetic patients without retinopathy (0.63 ± 0.09 G/L); and further increases in diabetic patients with retinopathy (0.81 ± 0.15 G/L).

Table 4: *Total serum sialic acid (TSSA) levels in diabetic patients with and without retinopathy depending upon different age groups were determined. Its Mean and SD are given below.*

Age Groups (in years)	DM without Retinopathy			DM with Retinopathy		
	No. of Patients	TSSA (G/L)	TSSA (mM/L)	No. of Patients	TSSA (G/L)	TSSA (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 ± 0.19
51 – 60	16	0.65 ± 0.03	2.08 ± 0.09	16	0.83 ± 0.03	2.65 ± 0.09
61 – 70	00	----------	----------	08	0.94 ± 0.02	3.02 ± 0.07

When blood glucose level is taken into account (Table 1), there is a significant difference in blood glucose levels of control group and diabetic groups. However, the difference is insignificant, between TSSA and fasting blood glucose of diabetic patients with and without retinopathy where p = 1.86.

Table-2 shows the duration dependent relationship of TSSA with retinopathy, which is highly significant (p = 0.0025) as TSSA 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 TSSA with the duration of diabetes whether, with or without retinopathy.

The relationships with sex as well as age of diabetic patients both with and without retinopathy are not significant as the p-values are greater than 0.05 (p = 0.102 & 0.135 respectively), which is as shown in Table-3 and Table-4 below.

Discussion

The mean value of SSA was found by Slamburger[21] in Germany in normal persons to be 1.74 ± 0.21 mM/L (0.54 ± 0.79 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 i.e., 0.43 ± 0.07 G/L.[22]

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia 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. Micro vascular damage is proliferative in nature and causes diabetic retinopathy and nephropathy and also contributes to diabetic neuropathy.[23]

SA 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 TSSA is related to the severity of diabetic complications such as retinopathy. There is a significant increase in TSSA in the diabetic patients without retinopathy and further increase in association with retinopathy though the difference of TSSA between diabetic patients with and without retinopathy is not significant.

DM by definition is a hyperglycemic state and its complications are dependent upon the severity of the disease as indicated by level of hyperglycemia. Whereas, there is a clear-cut relationship of blood glucose level and sialic acid of controls and diabetic subjects, there was no significant difference between glucose levels of DM with retinopathy and without retinopathy (Table 1). Considering the case of duration of DM it had significant difference in TSAA without and with retinopathy (Table 2). As found by Slamburger (1985) the sex and age do not affect TSSA (Tables 3 and 4). Whereas, there is considerable overlap of values of various groups the mid p value of TSSA from age and sex factor makes it a useful indicator of proliferative disease especially carcinogenic growth.

Conclusion

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

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