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SIALIC ACID A MARKER OF DIABETIC COMPLICATION

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ABSTRACT

BACKGROUND: The prevalence of diabetes is steadily increasing worldwide particularly in developing countries, it is associated with chronic complications which lead to considerable mortality and morbidity. Type II diabetes is considered as a disease of innate immune response, sialic acid represent as a marker of acute phase protein is found to be elevated in diabetic subjects. MATERIALS AND METHODS: A total of 104 type II diabetic cases were studied. Total serum sialic acid, glycated hemoglobin, lipid profile, urea, creatinine and 24 hrs urinary proteins were estimated in blood samples of both cases and controls. RESULT: Sialic acid, glycated hemoglobin, total protein, albumin and 24 hrs urinary protein concentrations are significantly higher in diabetics associated with complications compared to diabetic without any complications.SUMMARY AND CONCLUSION:ROC analysis showed that 24 hrs urinary protein, sialic acid are good marker for identification of Diabetic complications

KEY WORDS

Sialic acid, Diabetic complication.

INTRODUCTION

Diabetes mellitus long back considered as disease of minor significance to world health is now emerging as one of the main threats to human health in the 21st century. The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide. The world health organization estimated that there were 135 million diabetes in 1995 and this number would increase to 300 million by the year 2025. The prevalence of diabetes is steadily increasing worldwide particularly in the developing countries [1].

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed as the "diabetes capital of the world". According to the diabetes atlas 2006 published by the internal federation the number of people with diabetes in India currently around 40.9 million expected to rises to 69.9 million [2].

Type 2 Diabetes mellitus is the commonest form of diabetes seen worldwide. This form of diabetes is considered as a life style disease. The underlying genetic predisposition gets unmasked in the presence of the environmental factors such as sedentary lifestyle, change in traditional food habits from coarse simple meals to highly refined caloric dense food habits consumption of large amounts of carbohydrate and stress of urban living.

Lack of insulin, whether absolute or relative, affects the metabolism of carbohydrate, protein, fat water and electrolyte. Death may result from acute metabolic decompensation, while long-standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body those of the vascular system being particularly susceptible. These changes lead, in turn to the development of well defined clinical entities, the so called complications of diabetes which most characteristically affect the eye, the kidney the nervous system and cardiovascular system [3]. Chronic complications can be divided into vascular complications and nonvascular complication. The

vascular complications are subdivided into microvascular and macrovascular complications. Micro vascular complications in diabetes contribute to pathologic and functional changes in many tissues, including eye, heart, kidney, skin and neural tissues. Based on the tissues affected these changes are traditionally known as diabetic retinopathy, nephropathy and neuropath. The development and progression of micro vascular complications is associated closely with chronic hyperglycemia, a relationship supported by numerous clinical studies [4,5].

Type 2 diabetes is considered as an acute-phase disease, which is characterized by secretion of a number of proteins called as acute –phase proteins such as fibrinogen, α1-acid glycoprotein, α1-antichymotrypsin, haptoglobin, c-reactive protein and serum amyloid A by liver in reaction to a variety of stresses such as infection, inflammation, tissue injury and malignancies. Increased production of cytokines like IL-1, IL-6 and TNF-α act on liver to produce the characteristic dyslipidaemia of type 2 diabetes (increased VLDL and decreased HDL). They also promote the release from the liver of acute-phase proteins such as fibrinogen, α1-glycoprotein, etc. which contain sialic acid as terminal group. The concentration of sialic acid is more in patients with complications of diabetes and with metabolic syndrome [6].

The present study was taken up to test the hypothesis that type 2 diabetes mellitus is a disease of innate immune response characterized by the presence of increased plasma concentration of sialic acid a marker of acute phase response and to assess the relation of altered sialic acid concentrations, which can also serve as a marker in various complications of type 2 diabetes mellitus.

MATERIAL AND METHODS

A cross sectional study was done with 104 diabetes cases with and without complication between the ages of 27-60 years attending Osmania General Hospital. These cases were divided into five groups, Diabetics without complication (n=22), Diabetics with nephropathy (n=22), Diabetic Neuropathy (n=20), Diabetic Retinopathy (n=20) and Diabetic with coronary artery disease (n=20). These cases were compared with age, sex matched controls (n=30) detailed history was taken regarding diabetic duration associated illness, alcoholism, smoking habit, frequency of mild, moderate and vigorous physical activity was recorded. Detail of the treatment received were also noted. Sitting blood pressure was measured. Hypertension was defined as blood pressure >140/90 mmHg or if the subject was taking antihypertensive medication.

The presence of coronary heart disease (CHD) was assessed by a clinical history of angina pectoris, myocardial infarction or coronary artery bypass graft surgery as well as by 12 lead resting electrocardiogram.

Retinopathy was assessed by fundoscopic examination by a single observer.

The presence of peripheral diabetic neuropathy was assessed by neuropathic symptoms such as tingling and numbness, paraaesthesia, absent tendon reflexes, increased vibration perception threshold.

The presence of peripheral nephropathy was assessed by the presence of proteinuria >330mg/day.

Serum Sialic acid was estimated by resorsinol method [7].

Glycated hemoglobin was done by colorimetric method [8].

Venous blood sample were collected after an overnight fast and serum was separated by centrifugation and analyzed for sialic acid, fasting blood sugar, blood urea, sr. creatinine, triglyceride, cholesterol, HDL cholesterol, Total proteins and Albumin.

Fasting blood glucose, blood urea, serum creatinine, serum triglycerides, total cholesterol, HDL cholesterol, total protein and Albumin was done on automated hitachi 911 instruments

LDL and VLDL were calculated using freidwald's formula.

24 hrs urinary proteins were estimated by turbidimetric method described by Kingsbury et al [9].

Whole blood EDTA sample was used for Glycated hemoglobin estimation.

RESULTS

A total of 104 diabetic patients with and without complications were included in the study, these cases

were compared with 30 normal, healthy, adult controls. They were classed into six groups depending on presence or absence of the disease and or its associated complications.

Table 1: Represent the different groups

Group	Diagnosis	No. of patients	%Males	% Females
1	Normal Controls	30	56.7	43.3
2	DM without complication	22	50	50
3	Diabetic Nephropathy	22	59.1	40.9
4	Diabetic neuropathy	20	55	45
5	Diabetic Retinopathy	20	75	25
6	Diabetes with CAD	20	60	40

Table 2: Representing the mean and standard deviation of different parameter analyzed among various group.

S.No	Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1.	Sialic Acid (mg/dl)	76.67±15.37	114.52±11.85	131.45±23.32	115.8±12.8	130.73±10.99	128.9±17.1
2.	HbA1 (%)	3.59± 0.78	6.53 ± 0.9	7.4 ± 1.57	6.96 ±6.96	6.91 ± 1.08	7.28 ± 0.87
3.	Urea (mg/dl)	22.17±6.17	31.4±8.72	41.72±32.83	27.6±5.66	30.65±7.79	32.75±10.4
4.	Creatinine (mg/dl)	0.73±0.09	0.9±0.22	1.29±1.95	0.845±0.22	0.88±0.25	0.935±0.27
5.	FBS (mg/dl)	73.53±10.8	187.54±82.5	192.73±91.1	155.5±39.2	160±54.49	180±53.29
6.	Triglyceride (mg/dl)	87.27±28.43	177.45±125.5	221.45±144.1	191.45±81.29	164.3±68.96	221.35±70.22
7.	Cholesterol (mg/dl)	148.2±20.8	177.27±61.83	196.55±51.87	198.65±56.29	186.8±56.19	204.8±49.19
8.	HDL –Chol (mg/dl)	38.33±9.08	42.45±7.75	40.77±8.39	45.55±10.5	41.3±9.77	44.05±11.06
9.	LDL –Chol (mg/dl)	91.37±17.37	122.36±51.86	111.32±41.68	103.65±29.99	106.8±43.08	116.4±41.56
10.	VLDL – Chol (mg/dl)	17.17±5.68	35.59±25	44.95±28.41	45.45±26.27	32.9±13.76	44.3±14.07
11.	Total Protein (g/dl)	7.71±0.39	7.73±0.87	7.14±0.6	7.19±0.78	7.01±0.61	7.75±0.86
12.	Albumin (g/dl)	4.28±0.28	4.09±0.46	3.64±0.61	4.17±0.62	3.56±0.58	3.91±0.72
13.	Globulin (g/dl)	3.42±0.39	3.64±0.75	3.5±0.77	3.07±0.72	3.44±0.66	3.83±0.59
14.	24 hrs U.P(mg/day)	30.03±21.33	208.72±63.97	1162.8±942.7	374.2±252.88	887±2003.49	758.05±15
15.	C/HDL ratio	4.02±0.92	4.7±1.25	5.02±0.8	4.47±1.25	4.65±1.41	4.83±1.3

The mean concentrations of all the parameters analyzed are significantly higher and the Albumin values significantly lower in the diabetics as compared to controls. However the significance levels are relatively low for HDL–cholesterol concentrations compared to other analytes. No statistically significant difference in the mean globulin concentrations was present between controls and diabetics.

The mean concentrations of sialic acid, Glycated haemoglobin, urea, FBS, TAG, Total Cholesterol, VLDL-C and 24hr urinary proteins are significantly higher in all groups of diabetics when they are individually compared to controls. The LDL-C values are significantly higher in group 2, 3 and 6. HDL –C is significantly higher in group 4 and globulins are significantly higher in group 6. The serum total protein

concentrations are significantly lower in group 3, 4 and 5 and Albumin concentration are significantly lower in groups 3 and 5 compared to controls. The Mean ± S.D values of cholesterol/HDL – C in diabetics are compared to controls, the Mean ± S.D ration values are significantly higher only in groups 2,3 and 6 compared to controls.

The mean sialic acid concentrations in groups 3 to 6 are higher than those of group 2 this difference is statistically significant only in group 3, 5 and 6 . There is no statistically significant difference in sialic acid concentration between group 2 and 4.

There is no statistically significant difference in the mean concentrations of blood urea, serum creatinine, fasting blood sugar, Triglycerides, serum total cholesterol, HDL cholesterol, Cholesterol/HDL ratio, LDL Cholesterol, VLDL cholesterol and Globulins in groups 3-6 compared to group 2.

The mean glycated hemoglobin concentrations in group 3 and 6 are statistically significantly higher than those of group 2. There is no statistically significant difference in the mean glycated hemoglobin concentrations between group 4 and group 5 compared to group 2.

The mean total protein and Albumin concentrations are significantly lower in groups 3 and 5 compared to group 2. There is no statistically significant difference in the mean concentrations of these parameters in group 4 and 6 compared to group 2.

No statistically significant difference in 24 hrs urinary protein excretion in groups 5 and 6 when compared to group 2. The 24 hr urinary protein excretion is significantly higher in group 3 and 4 compared to group 2.

Statistically significant differences in mean sialic acid concentrations are present between groups 3 and 4, groups 4 and 5, groups 4 and 6.

No statistically significant differences are present in other parameters analyzed between 3, 4, 5 and 6 groups.

The correlation between the altered concentrations of sialic acid and other analytes in various groups studied was found out by subjecting the data to Pearson's correlation method. Significant correlation is present between sialic acid and urea, fasting blood sugar, total cholesterol, LDL cholesterol, Triglyceride, VLDL-C and total proteins in controls. Sialic acid is significantly correlated with FBS, TAG, VLDL-C, globulin and creatinine in group 6, with TAG, 24 urinary protein, TP and globulin in group 4, with urea in group 2. No significant correlation is present between sialic acid and other parameters in different groups.

To assess the diagnostic sensitivity and specificity of the disease the best cut off values are found out by analyzing the data by ROC analysis. The best cut off values, sensitivity, specificity and diagnostic accuracy are presented in Table 3.

Table 3: showing best cut off, Sensitivity, Specificity and Diagnostic efficiency between Diabetic without complication compared with Diabetic with complications

Parameter	Best cut – off	Sensitivity %	Specificity %	D.E
Sialic acid	100.5	95.12	22.72	79.80
HbA1	4.75	99.03	93.33	97.76
Urea	25.5	74.03	86.67	76.86
Creatinine	0.75	71.15	70	70.89
Fasting blood sugar	95.5	98.07	96.67	97.76
Triacylglycerol	117	79.80	90	82.08
Cholesterol	178.5	68.26	93.33	73.88
HDL – Cholesterol	39.5	50.00	70	54.48
LDL – Cholesterol	111	47.11	93.33	57.46
VLDL – Cholesterol	21.5	83.65	86.66	84.33
Total Protein	7.95	27.88	86.66	41.04
Albumin	4.65	11.53	93.33	29.85
Globulin	3.65	45.19	86.66	54.47
24hrs urinary Protein	77.5	99.05	96.66	98.50

In identifying diabetics as a group from controls, sialic acid exhibited lack of specificity. The better markers being 24hr urinary protein, Glycated hemoglobin, FBS, VLDL-C, TAG along with urea, creatinine and cholesterol, which exhibited relatively good characteristics to be used as markers. Other analytes tested exhibited poor sensitivity.

In order to assess the ability of the various analytes in identifying diabetics with complications from those without, the data in analyzed using ROC curves comparing these two groups. The sialic acid along with 24 hrs urinary proteins, HbA1, VLDL-C, TAG and fasting blood sugar exhibited relatively better characteristic to be used as better markers. The other analytes lack either sensitivity or specificity to be used as discriminating markers.

Table 4: showing Area under ROC curve and 95% confidence interval between Diabetic without complication compared with Diabetic with complications

Parameter	AUC	95% Confidence interval	
		Lower bound	Upper bound
Sialic acid	.725	.626	.825
HbA1	.666	.540	.792
Urea	.519	.383	.655
Creatinine	.443	.319	.567
Fasting blood sugar	.479	.335	.624
Triacylglycerol	.623	.494	.751
Cholesterol	.501	.364	.638
HDL – Cholesterol	.518	.403	.633
LDL – Cholesterol	.425	.285	.565
VLDL – Cholesterol	.634	.506	.762
Total Protein	.352	.210	.494
Albumin	.373	.258	.489
Globulin	.457	.323	.591
24hrs urinary Protein	.799	.717	.881

24 hrs urinary proteins is the best marker to discriminate patients with diabetic nephropathy from diabetics without any complications. Sialic acid and urea also are relatively good markers to do so.

24 hours urinary protein, HbA1, TAG, VLDL-C and FBS showed discriminating capacity in identifying neuropathy, Sialic acid along with the other analytes lacked the sensitivity to discriminate the same.

Sialic acid, 24 hr urine protein, HbA1 and TAG are good markers in identifying retinopathy. The other markers lack sensitivity and specificity to act so.

In identifying diabetics with CAD from those diabetics without, sialic acid, VLDL – C, FBS and urea are good markers. Other analytes showed neither required sensitivity nor specificity.

In order to compare the discriminatory capacity of various analytes to be used as markers to identify the various complications associated with type – II diabetes mellitus the areas under curve and 95 percentile values of the various analytes were studied and presented in table 4.

24 hour urinary protein concentrations are the better markers than any other marker in identifying the complications. Sialic acid is a better marker than the remaining in identifying all the diabetic complications studied except diabetic neuropathy. HbA1, TAG and VLDL-cholesterol also showed significant differentiating capacity.

DISCUSSION

The exact cause of Type II DM which affects at least 100 million people throughout the world is not known. Though insulin resistance seems to be central abnormality, the origin of the impaired insulin action and how it explains the many other abnormalities of type II DM is not known. In recent years the evidence is being gathered that in type II DM there is a cytokine-associated acute phase reaction, as a part of the innate immune response.

In the short terms, the acute phase response has survival value and is designed to restore homeostasis after environmental threats [10]. J.C Pickup and M.A. Crook hypothesized that long term activation by life style and environmental stimulants of the innate immune response produces disease instead of repair, probably in those with an innately hypersensitive acute phase response, the subjects who developed type II DM. it has been shown that tissue complications further increase stress reactants in type-II DM [7].

This model is also important as it suggests new therapeutic approaches to type – II DM and its complications, such as modulation of the acute phase response.

Hence the present study is undertaken to test the hypothesis and to establish sialic acid as a marker of acute phase response in diabetes without complications and its utility as a discriminating marker in identifying different complications associated with type II DM compared to other Biochemical markers.

In the present study the reference ranges for serum total sialic acid ranged from 45.91 to 107.41 mg/dL. This is comparable with those studies which followed the same methods for estimation of sialic acid [7].

Sialic acid concentration was found to be significantly higher in female compared to male in control group but not in cases. No fixed trend was found of increase in the sialic acid concentration with increasing age. In the present study, we observed a statistically significant increase in serum total sialic acid concentrations in total diabetic patients studied compared to controls. As the increased serum sialic acid concentration are due to increase in the acute phase reactant proteins and not due to desialyation, we confirm the increased acute phase reactant protein response in Diabetics. This is in consistent with earlier reports [11,12,13] showing increased acute phase reactant protein response in type II diabetics even in the absence of complications which might themselves evoke that response.

The common metabolic syndrome type II DM is associated with characteristic dyslipidaemia [14, 15, 16]. The most common abnormality is increased serum TAG concentration caused by increased VLDL levels [17].

In the present study we observed significantly elevated serum TAG and VLDL in Diabetics, HDL cholesterol was found to be significantly higher in diabetics compared to controls, this we presume to be due to treatment as almost all the patients of the present study are under treatment.

The concentration of LDL and total cholesterol, the powerful predictors of cardio vascular risk in non diabetic population, is shown to be normal or only slightly increased in type 2 diabetes mellitus. The increases are shown to be due to increased production of apo B apoprotein, decreased receptor mediated clearance of LDL and due to TAG enrichment, glycation and oxidation. In the present study we also confirm the presence of significantly higher total and LDL – cholesterol values in diabetics.

In the present study we observe 24 hrs urinary protein concentrations are significantly higher in diabetics even in those who are not identified as having complications, except for one case of all 104 diabetic patients studied urinary protein concentrations are higher.

Type II diabetes is an acute phase disease in which increased concentrations of cytokines are secreted from many cells such as macrophages, adipose tissue and endothelium, under the influence of stimuli such as over nutrition, perhaps in those patients predisposed to having an unregulated response because of increasing age, genetic, fetal metabolic pre-programming. Cytokines mainly IL-1, IL-6 and TNF α act on the liver to produce the characteristic dyslipidaemia of type II diabetes. They also promote the release from the liver of acute phase proteins which are atherosclerotic risk factors, stimulate leptin release from adipose tissue and act on the brain to release ACTH and then coritsol. The latter may contribute to obesity, hypertension and insulin resistance. TNF α also is a major factor in causing insulin resistance, long

term hyper secretion of cytokines may impair beta cell insulin secretion [6].

Our observations of the increased sialic acid concentrations, dyslipidaemia, 24hrs urinary protein excretion, in diabetics in general especially the increases observed in diabetics without any complications, support the theory that the type II diabetes mellitus is a disease of disordered innate immune response. It has been shown by many authors that serum total sialic acid concentrations are significantly higher in type II diabetics with various micro and macro vascular complications compared to controls, as well as to the diabetics without any complications [11, 18, 19, 20, 21, 22]. However in the present study we did not find significant increase in sialic acid concentration in diabetic with neuropathy compared to diabetics without any complications. This is also reported earlier [11, 23]. However the specific reason for this disparity is not clear.

We analyzed the discriminatory characteristics of sialic acid and other analytes. The commonly used markers, glucose, glycated proteins and dyslipidaemia having associations with diabetes with diabetic complications have certain disadvantages.

CONCLUSION

The present study proves the theory that type II diabetes mellitus is a disease of disordered innate immune response. The diabetics with various complications can also be considered as exacerbated response of this disordered immune response. Sialic acid can be used as a marker in identifying the various complications of type II diabetes.

REFERENCES

1. King H, Aubert RE, et al: Global burden of Diabetes, 1995-2025, prevalence, numerical estimates and projects. Diabetes care 1998; 21:1414-31.
2. V.Mohan, C. Varghese et al: Epidemiology of type 2 diabetes : Indian scenario. Indian J Med Res 125, 2007; 217-230.
3. Davidson's Principles and practice of Medicine 22nd edit.
4. The UK prospective diabetes study (UKPDS) group. Intensive blood glucose control with sulphonylureas of insulin copared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS33). Lancet 1998;352:837-53.
5. Thornalley PJ: The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 1990; 269:1-11.
6. Pickup JC, Crook MA :Is type 2 diabetes mellitus a disease of the innate immune system ? Diabetologia 1998: 41:1241-1248.
7. Plucinsky MC, Michael RW et al: Total and lipid Associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement. Cancer 1986; 58:2680-2685.
8. Godkar PB, Textbook of medical laboratory technology: P115-116.
9. Harold Varley, Practical Clinical Biochemistry 4th edit.P-137.
10. Kushner I, Rzewnicki DL: The acute phase response: general aspects. Builliara's Clin Rheumatol 1994: 8:513-530.
11. Abdella K, Akanju AO, et al: Relation of serum total sialic acid concentrations with diabetic complications and cardiovascular risk factors in Kuwaiti Type 2 diabetic patients. Diabetes Res Clin Pract.2000: 50(1):65-72.
12. Akbri MZ, Sheikh AS, et al: Serum sialic acid level in diabetic retinopathy. J Ayub Med Coll 2001; 13(1):29-30.
13. Crook MA, Tutt P, et al:Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. Diabetes Care 1993: 16: 57-60.
14. Barbara VH: Lipoprotein metabolism in diabetes mellitus. J Lipid Research 1987; 28: 615-626.
15. Richard D, Siegel, et al: Lipoproteins, Apolipoproteins, and low Density Lipoprotein size among Diabetics in the Framingham offspring study. Metabolism 1996; 45: 1267 – 1272.
16. Steven M, Haffner: Diabetes, Hyperlipidemia and Coronary Artery Disease. Am J Vardiol 1999; 10: 822-32.
17. Nikkila EA: High density lipoproteins in Diabetes. Diabetes 1981; 2(suppl): 82 -87.
18. Chen J, Gall MA, et al: Raised serum sialic acid concentration in NIDDM patients with and without nephropathy. Diabetes care 1993; 16: 57-60.
19. Crook MA, et al: No difference in serum sialic acid in type 2 diabetics' patients from the United Arab Emirates with and without diabetic retinopathy. Diabetes Res Clin Pract 2000; 47: 147-50.
20. Cooper ME: Pathogenesis, prevention, and treatment of diabetic nephropathy. Lancet 1998; 352:213-9.

21. Pickup JC, Mattock MB, et al: Serum sialic acid concentration and coronary heart disease in NIDDM. Diabetes Care. 1995; 18: 1100-3.
22. Yokoyama H, Jensen JS,eta l : Raised serum sialic acid concentration precedes onset of microalbuminuria in IDDM. A 10 year follow-up study. Diabetes Care 1996;19: 435-40.
23. Powrie JK, Wats GF, eta l : Serum sialic acid and long term complications of IDDM. Diabet med 1996; 13:238-42.

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