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Study on indicators of diabetic nephropathy

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Abstract: *Background:* Microalbuminuria, serum creatinine, albumin/ creatinine ratio & eGFR are currently in use for early changes in DN. Serum SA levels correlate positively with albuminuria which is indicator for DN, hence serum levels are raised even before clinical nephropathy is diagnosed. An elevation in the serum SA concentration has been observed in DN in the study conducted by Jarkko Romppanen. Thus research was undertaken to study indicators of DN like SA, HbA1c, lipid profile, Serum Creatinine & Urine albumin/ Creatinine ratio. *Objectives:* To estimate levels of Serum SA, HbA1c, Lipid profile, Serum Creatinine & Urine albumin/Creatinine ratio in type 2 DM & DN patients. *Methods:* Serum SA by resorcinol-HCl-Copper reagent method, Glycated Hb by Ion exchange resin method, Lipid profile by enzymatic method, Serum Creatinine by Jaffe's method, Urine albumin/ creatinine ratio (UAC)- Urine albumin & creatinine by Immuno-turbidometry. *Results:* Serum SA concentration significantly increased in DN when compared to DM and positively co-related with other factors like glycemic control (HbA1c), lipid profile, Serum creatinine & UAC. Hence, serum SA levels could be used as early indicator of DN. *Conclusion:* SA levels in DN were increased & statistically significant when compared to DM without nephropathy. The mean HbA_{1c}, TC, triglyceride, LDL, serum creatinine, & urine A/C ratio were significantly increased & were correlated positively with SA.

Keywords: SA- Sialic Acid, DN- diabetic nephropathy, HbA1c- Glycated hemoglobin

Introduction

Diabetes mellitus (DM) can be categorized into two types; type I diabetes and type II diabetes. Type II diabetes is mainly due to underlying pathology like insulin resistance, obesity and hyperinsulinemia [1]. The debilitating aspects are the numerous complications that arise from the DM, which includes diabetic retinopathy, diabetic nephropathy (DN) and diabetic neuropathy. The development and severity of the complications are dependent mainly on the good control of blood glucose levels & lipid profile, duration of the diabetes and its management [2].

Glycemic parameter's like fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) are used for the initial diagnosis & prognosis of diabetes. "Interpretation of hemoglobin A_{1c} values" by Saks DB, John WG concluded that HbA1c remains the prime test that can predict the microvascular complications of DM [3], but HbA1c can be misleading in patients with anaemia & in those with chronic kidney disease & cannot predict the underlying DN.

Biological markers are useful in revealing objective information about DN. Microalbuminuria is considered as early indicator in DM patients having underlying DN [4]. Microalbuminuria, serum creatinine, albumin/ creatinine ratio & eGFR are currently in use for early changes in DN. All these markers have some limitations in their clinical efficiency with respect to the time of diagnosis. Hence indicator capable of diagnosing underlying renal endothelial changes as early as possible is necessary to prevent progression of DN.

N-acetylneuraminic acid (sialic acid) is a negatively charged nine-carbon monosaccharide attached to the carbohydrate chain of glycoproteins and glycolipids. The degree of sialylation is responsible for the negative charge of glycoproteins and pathogenesis of atherosclerosis. Due to increased oxidative stress, caused by hyperglycemia and insulin resistance, promote inflammation leading to tissue injury which

stimulates local cytokine secretion from cellular infiltrates, such as macrophages and endothelial cells; this induces an acute phase response with release of acute phase glycoproteins from liver into general circulation leading to their increased levels. The vascular endothelium carries a high concentration of sialic acid (SA) [5-6] thus, extensive microvascular damage sheds SA into the circulation leads to increased vascular permeability and is the factor linking diabetes to the development of atherosclerosis [4].

Thus, elevated levels of SA indicate excessive damage to the vascular cells of retina of the eyes, kidneys, heart and brain. This leads to conditions like retinopathy, nephropathy and neuropathy.

Serum SA levels correlate positively with albuminuria which is indicator for DN, hence serum levels are raised even before clinical nephropathy is diagnosed [7]. An elevation in the serum SA concentration has been observed in DN in the study conducted by JarkkoRomppanen [4]. Thus research was undertaken to study indicators of DN like SA, HbA1c, lipid profile, Serum Creatinine & Urine albumin/Creatinine ratio.

Objectives: To estimate levels of Serum SA, HbA1c, Lipid profile, Serum Creatinine & Urine albumin/Creatinine ratio in type 2 DM & DN patients.

Material and Methods

Study design: A case control study.

Grouping: The study includes total of 100 patients, studied in 2 groups. 50 diabetic patients without any complications were included in group I and group II were 50 diabetic nephropathy patients.

Study was conducted for a period of 1year. Patients were recruited from out-patient department (OPD) and inpatient department (IPD) of medicine and nephrology of tertiary care hospital. The permission of Institutional Ethics Committee (IEC) was taken before starting the study & informed consent was taken from all the participants who were enrolled in the study after explaining the cause of research in their own language.

Inclusion criteria: Patients of both gender, diagnosed as type 2 diabetes mellitus & diabetic nephropathy by clinicians according to American Diabetes Association (ADA) guidelines.

Exclusion criteria: Type 1 diabetes mellitus, albuminuria documented due to causes that are other than diabetes, kidney transplant, acute febrile illness, urinary tract infection, renal calculi, urinary tract obstruction, congestive heart failure or acute coronary syndrome & anti-inflammatory drug (allopurinol).

Methodology: 3ml of fasting blood sample was taken by venepuncture from ante-cubital vein, under aseptic precautions was collected in the vacutainer tube and kept aside for 30 min, centrifuged at 3000 rpm for 5min to obtain clear serum. Serum samples were stored at -20^0 C till further assay. Midstream urine sample was collected in sterile container.

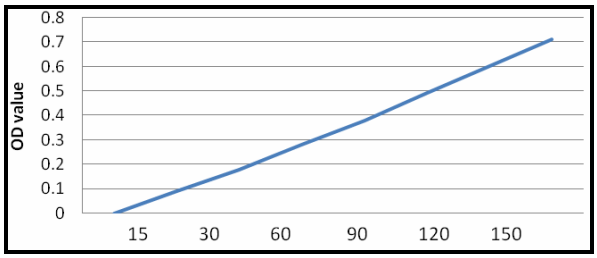
- *Serum SA by resorcinol-HCl-Copper reagent method* [8]: SA was released from glycolipids & glycoproteins on addition of 125mM of sulphuric acid at 80^0C for 1 hour, proteins in the sample is precipitated by adding 5% TCA further centrifuged to obtain clear supernatant containing SA, which is made to react with HCl-resorcinol-copper sulphate reagent at 100^0C for 15 min. The keto group in sialic acid reacts with resorcinol in acidic medium yielding a chromogen (violet colour), copper sulphate acts as a stabilising agent. The intensity of the violet colour is read spectrophotometrically at 580nm which is directly proportional to the concentration of SA in the sample as shown in table no 1 & graph no 1.
  <u>*Normal levels:*</u> 20-60 mg/dl.
  The resorcinol HCl copper method can detect sialic acid as low as 15mg/dl. This method of estimating serum SA was standardised in our laboratory.
- Glycated Hb by Ion exchange resin method [9].
- Lipid profile by enzymatic method [10-12].
- Serum Creatinine by Jaffe's method [13].

- Urine albumin/ creatinine ratio (UAC)-Urine albumin & creatinine by Immuno-turbidometry [14].

Statistical analysis is done through online Graph pad prism.

Table-1: Showing Standardization									
	B	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**T**
Volume of Std (µl)	-	16	30	60	100	130	160	200	-
Concentration of Std (mg/dl)	-	15	30	60	90	120	150	180	-
Distilled water (µl)	250	234	220	190	150	120	90	50	230
Protein free filtrate (µl)	-	-	-	-	-	-	-	-	20
Resorcinol reagent(ml)	250	250	250	250	250	250	250	250	250
nBA:Butanol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
OD value	0	0.09	0.18	0.28	0.38	0.49	0.6	0.74	-

Graph-1: Showing standardization of SA



Results

The subjects in DN group were studied according to the duration of diabetes. Majority of the study subjects included in the group were of 10-13years of diabetes & as the duration of diabetes increased, the incidence of nephropathy also increased as shown in table no 2.

Table-2: Showing Comparison of distribution of duration of diabetes between 2 groups		
Duration of DM (yrs)	**DM group**	**DN group**
1-3	25	-
3-5	18	1
5-8	2	8
8-10	2	11
10-13	3	17
13-15	-	13
Total	50	50

The mean serum SA levels in DM & DN groups were 94.06 ± 26.64 mg/dl and 107.25 ± 35.28 mg/dl respectively as shown in table no 3. The comparison of SA levels between groups (DM – DN) was statistically significant with p value of <0.05. The mean HbA_{1c} levels in DM & DN groups were 7.60 ± 0.51%& 7.83 ± 0.48% respectively. HbA_{1c} level was increased in DM & DN groups with p<0.05 between 2 groups.

Table-3: Showing Serum Sialic acid levels in study groups		
	DM	**DN**
S. Sialic acid (mg/dl)	94.06 ± 26.64	107.25 ± 35.28
Statistical significance p<0.05 between 2 groups		

Lipid profile was estimated in 2 groups. The mean total Cholesterol levels in DM & DN groups were 192.46 ± 49.5 mg/dl & 235.62 ± 53.23 mg/dl respectively. The mean triglyceride levels in DM & DN groups were 194.64 ± 25.95 mg/dl & 249.38 ± 8.92 mg/dl respectively. The mean LDL levels in DM & DN groups were 110.86 ± 28.21mg/dl & 129.78 ± 34.30 mg/dl respectively.

TC, triglyceride levels & LDL levels were increased in DM group & DN group which was statistically significant with a p value of <0.001. The mean HDL levels in DM & DN groups were 24.48 ± 3.97 mg/dl & 19.67 ± 2.99 mg/dl respectively which was statistically significant. The mean Serum Creatinine levels in DM & DN groups were 1.20 ± 0.2mg/dl & 5.39 ± 2.4mg/dl respectively.

The mean UAC ratio was increased in DN group (0.41 ± 0.16 mg/g Cr) when compared to DM group (0.12 ± 0.07 mg/g Cr). SA was correlated [Pearson's correlation (r)] with levels of HBA_{1C}, LDL, TC, serum creatinine, & UAC ratio in DM & DN group, thus had a positive correlation and negative correlate with HDL as the level decreased in DM & DN.

Discussion

DN is diagnosed by the markers like serum Creatinine, UAC ratio & clinical features after proteinuria presenting with edema due to protein loss and reduced urine output. The development and severity of the DN depends on the duration of the diabetes and blood glucose homeostasis. Inflammatory marker SA plays a major role as indicator, as it is released in early stages of DN due to glomerular membrane damage even before signs & symptoms manifests.

As the duration of diabetes increased, the incidence of nephropathy also increased, thus signifiy that as the duration of diabetes increased, risk of complications also increased. The relationship of duration of DM & DN in our study was similar to the findings of Syed Muhammad Shahid et al study [15]. The different mechanisms like desialylation [16] & cytokine induced responses leads to the rise in SA concentration. Thus, SA serves as important indicator, when compared with other markers, in DN screening, disease progression, follow-up and in the monitoring of treatment response [17]. The serum SA concentration increases in type 2 diabetes mellitus patients associated with microvascular complications [18-19].

Crook M *et.al* [20] & Shivanand et al [2] studies have found that serum SA was significantly increased in patients with diabetic complications than without any of the complications, which were similar to the findings in our study. The finding of our study is also in accordance with Syed Muhammad Shahid et al [15], Gavella et al, Crook et al, Powerie & Chen J et al studies [21-24]. Hyperglycemia causes generalized vascular endothelial damage, which reduces functional lipoprotein lipase, leading to higher serum levels of triglycerides and lower serum levels of HDL. The underlying atherosclerosis process in diabetes explains dyslipidemia in DM & DN groups [2, 15, 24]. Serum creatinine is raised in T2DM due to insulin resistance, chronic inflammation, increased vascular permeability, endothelial dysfunction and vascular damage.

KatoreSarika D et al [25], Suchitra MM et al [26] studies explained that insulin has profound effects on HDL metabolism; low levels of HDL cholesterol and a high ratio of total cholesterol to HDL cholesterol & LDL/HDL are strongly related to insulin resistance. DM accelerates dyslipidemia and is further increased in DN. The elevated urinary albumin excretion predicts atherosclerotic vascular changes in T2DM & DN positively co-relating with atherogenic risk factors. Similar finding was observed in Hiroki Yokoyama et al study [7]. Serum SA concentration significantly increased in DN when compared to DM and positively co-related with other factors like glycemic control (HbA1c), lipid profile, Serum creatinine & UAC. Hence, serum SA levels could be used as early indicator of DN.

Conclusion

SA levels in DN were increased & statistically significant when compared to DM without nephropathy. The mean HbA_{1c}, TC, triglyceride, LDL, serum creatinine, & urine A/C ratio were significantly increased & were correlated positively with SA.

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