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ORIGINAL RESEARCH

SERUM TOTAL SIALIC ACID AND HS –CRP (HIGH SENSITIVE C-REACTIVE PROTEIN) AS MARKERS OF DIABETIC NEPHROPATHY

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ABSTRACT:

Background:Diabetes mellitus has become a global pandemic and is estimated that it will be the leading cause of death as a non communicable disease by 2030.Diabetic nephropathy is a major long-term complication of diabetes mellitus (DM). Type 2 DM is frequently associated with an underlying low grade inflammatory mechanism , but less information is available on the relationship explaining the low-grade inflammation process and development of diabetic nephropathy (DN). The aim of this study is to determine the serum level of high sensitivity C-reactive protein (hsCRP) ,an acute phase reactant and Serum Total Sialic acid levels, a marker of excessive glycation in unregulated glycemic control in Diabetes mellitus patients leading to the complication of diabetic nephropathy .And to compare with the normal subjects and also to study the association between serum hsCRP levels and Serum Total Sialic acid levels.

Materials and Methods: 50 patients with Type 2 DM with nephropathy (DN) and 50 patients of Type 2 DM without nephropathy (DM) along with 50 unrelated age and sex-matched healthy controls were included in the study. Plasma fasting and postprandial glucose levels, renal profile (serum creatinine, BUN), and lipid profile, HbA1c and Urinary Microalbumin levels were analysed. Serum TSA test levels and hs-CRP level were evaluated using thiobarbituric acid assay and immunoturbidimetric kit methods respectively.

Results: We observed a higher concentration of Serum Total Sialic acid levels (83.2 ± 6.8 mg/dl) and hs-CRP (3.22 ± 1.48 mg/L) in diabetic nephropathy than the diabetes mellitus group (74.1 ± 6.2 mg/dl and 2.2 ± 1.40 mg/L, respectively). Both Serum Total

Sialic acid levels and hs-CRP levels were found significantly correlated with plasma fasting and postprandial blood sugar, hemoglobin A1c, and urine microalbumin levels in both DM and DN groups. Multiple logistic regression analysis showed that both TSA and hs-CRP was independently associated with diabetic nephropathy.

Conclusion: High serum TSA and hs-CRP levels suggests the underlying inflammatory mechanism in the development of microangiopathic complications of T2DM like diabetic nephropathy.

Keywords: Diabetic nephropathy, type 2 diabetes mellitus, hs-CRP, high sensitive C-reactive protein, total serum sialic acid.

INTRODUCTION:

Diabetes mellitus has become a global pandemic and is estimated that it will be the leading cause of death as a non-communicable disease by 2030.[1]

The prevalence of diabetes was estimated at 171 million in 2000, increasing to 382 million in 2013; and is projected to reach 592 million by 2035. Type 2 diabetes constitutes about 85%–95% of all diabetes cases.[2]

Diabetic nephropathy is a major long-term complication of diabetes mellitus (DM).

Type 2 diabetes mellitus DM is frequently associated with an underlying low grade inflammatory mechanism, but less information is available on the relationship explaining the low-grade inflammation process and development of diabetic nephropathy (DN). The diabetes epidemic has resulted in DN becoming the most frequent cause of end-stage renal disease (ESRD) in most countries.[3] The long-term damage is caused by chronic hyperglycemia and results in dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels.[4]

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics.[5]

Microalbuminuria is the continuous elevation of albumin excretion in urine in a range of 20 mg/L-200 mg/L in early morning urine. Urinary albumin excretion may be then, an indicator of renal diseasein T2DM patients and, in fact, may reflect a state of generalized vascular damage occurring throughout the body. Acute inflammation is the immediate and early response to an injurious agent. T2DM is frequently associated with an inflammatory status; there is a cytokine associated acute phase reaction, part of the innate immune response.[6]

High sensitive C-reactive protein (hs-CRP) is found tobe significant in people with diabetes among various markers of Inflammation.[7] And hs-CRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes mellitus, smoking, and other factors. The progression to DN is highly variable.The main modifiable risks are hypertension, glycemic control, and dyslipidemia.

Incipient nephropathy is defined as the initial presence of low but abnormal amounts of urine albumin, referred to as microalbuminuria (persistent albuminuria at level 30–299 mg/24 hours). And Overt nephropathy or macroalbuminuria (persistent albuminuria at level≥300 mg/24 hours) develops after many years in Type 1 diabetes patients and even may be present at the time of diagnosis of type 2 diabetes. Patients who progress to macroalbuminuria are

more likely to develop End stage Renal Disease ESRD. The naturalhistory depends on the type of diabetes.[8] There is variability in the association of microalbuminuria with Diabetes, in untreated type 1 diabetics, approximately 80% of patients with sustained microalbuminuria increase their albumin excretion by 10%–20% per year until overt nephropathy develops, which normally takes 10–15 years. With the development of overt nephropathy, the GFR declines at a rate of 2–20 mL/minute/year and ESRD develops in 50% within 10 years and in 75% by 20 years.[9]

Structural changes can precede albuminuria and reduced GFR, with glomerular basement membrane thickening and mesangial expansion, can be detected as early as 2–8 years after onset of diabetes. In type 2 diabetics, more patients have DN at the time of diagnosis of diabetes as type 2 diabetes can go unrecognized for years. About 20%–40% of type 2 diabetics with microalbuminuria progress to overt nephropathy; and about 20% will develop ESRD after the development of overt nephropathy.[10,11]

Most guidelines recommend screening with a spot urine albumin/creatinine ratio (ACR; normal >30 mg/g creatinine), from either first morning (preferred) or random specimens. An abnormal result is repeated once or twice over a few months for consistency. This is coupled with an assessment of renal function, using the Modification of Diet in Renal Disease or Chronic Kidney Disease Epidemiology Collaboration formulas for estimated GFR (eGFR) in order to stage chronic kidney disease (CKD).[11]

Screening begins at diagnosis of type 2 diabetes and usually 5 years after onset of type 1 diabetes. Timed collections can also be utilized and will average out diurnal variations in albumin excretion (normal >20 μg/minute). Sialic acid is a generic term for a family of acetylated derivatives of neuraminic acid. It is an essential component of glycoproteins and glycolipids. It is located in the terminal nonreducing ends of carbohydrate chains being linked to other sugars most commonly galactose and N-acetyl galactosamine.[12,13]

Sialic acids are found widely distributed in animal tissues and to a lesser extent in other organisms, ranging from fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides (they occur at the end of sugar chains connected to the surfaces of cells and soluble proteins) That is because it seems to have appeared late in evolution. However, it has been observed in Drosophila embryos and other insects and in the capsular polysaccharides of certain strains of bacteria.[14,15]

Sialic acid acts as a cofactor of many cell surface receptors, e.g., insulin receptor andis positively associated with most of the serum acute phase reactants. In human plasma, large quantity of sialic acid is found as a component of orosomucoid, alpha-1-antitrypsin,haptoglobin, ceruloplasmin, fibrinogen, complement proteins, and transferrin. The negative charge of the glomerular basement membrane is maintained by the sialic acid, and this is important for the membrane permeability. Increased vascular permeability with subsequent release of endothelial sialic acid in the circulation leads to increased serum sialic acid level Acute-phase proteins account for more than 50% of the total sialic acid.[16]

Free sialic acid and creatinine are handled by the kidneys in the same way, being dependant on glomerular filtration and no tubular reabsorption, total sialic acid is elevated in renal disease, diabetes, variety of central nervous system disorders, ovarian cancer, and arthritis.[17-19]

Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycemia and insulin resistance could also promote inflammation, and may be factor linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress.[20]

The vascular permeability is regulated by sialic acid moieties, with increased vascular permeability resulting from the shedding of vascular endothelial sialic acid into the circulation. It is well established that vascular endothelium carries a high level of sialic acid, and the vascular damage leads to its release into the circulation. A relationship between serum sialic acid levels and microvascular complications has been observed before for microalbuminuria and clinical proteinuria in type 1 and type 2 diabetes.

Diabetes patients with albuminuria whatever its type were associated with increased level of serum sialic acid. In DN, there is a greater increase in sialic acid due to the damage of the vascular endothelial cells, and it is considered as a newly established potential risk factor for the development of DN.[17,21] Hypothesis has also been made thata cytokine-induced acute phase response is an integral part of the pathophysiology of T2DM, which leads to elevated serum sialic acid level. In diabetic nephropathy, there is a greater increase in sialicacid and hs CRP with an inflammatory basis and due to the damage of the vascular endothelial cellsof the kidney and such biomarkers are considered as a newly establishedpotential risk factor for the development of diabetic nephropathy.

The hs-CRP is an acute phase reactant which acts as a non-specific systemic marker of inflammation. It is a pentameric, globular protein synthesized by liver and is considered as an effective marker for long term risk assessment.[22,23]

Low grade systemic inflammation may be used to predict the onset of cardiovascular disease and Type 2 diabetes mellitus. Obesity, hypertension and dyslipidemia along with altered level of lipoproteins are associated with glycation, oxidation and insulin resistance in Type 2 diabetes mellitus. Endothelial dysfunction contributes to the development of cardiovascular disease via vascular tone dysregulation, growth, thrombogenicity, and inflammation.

Hemostatic and inflammatory biomarkers of endothelial dysfunction like CRP have been associated with cardiovascular disease and other membrane associated dysfunction.

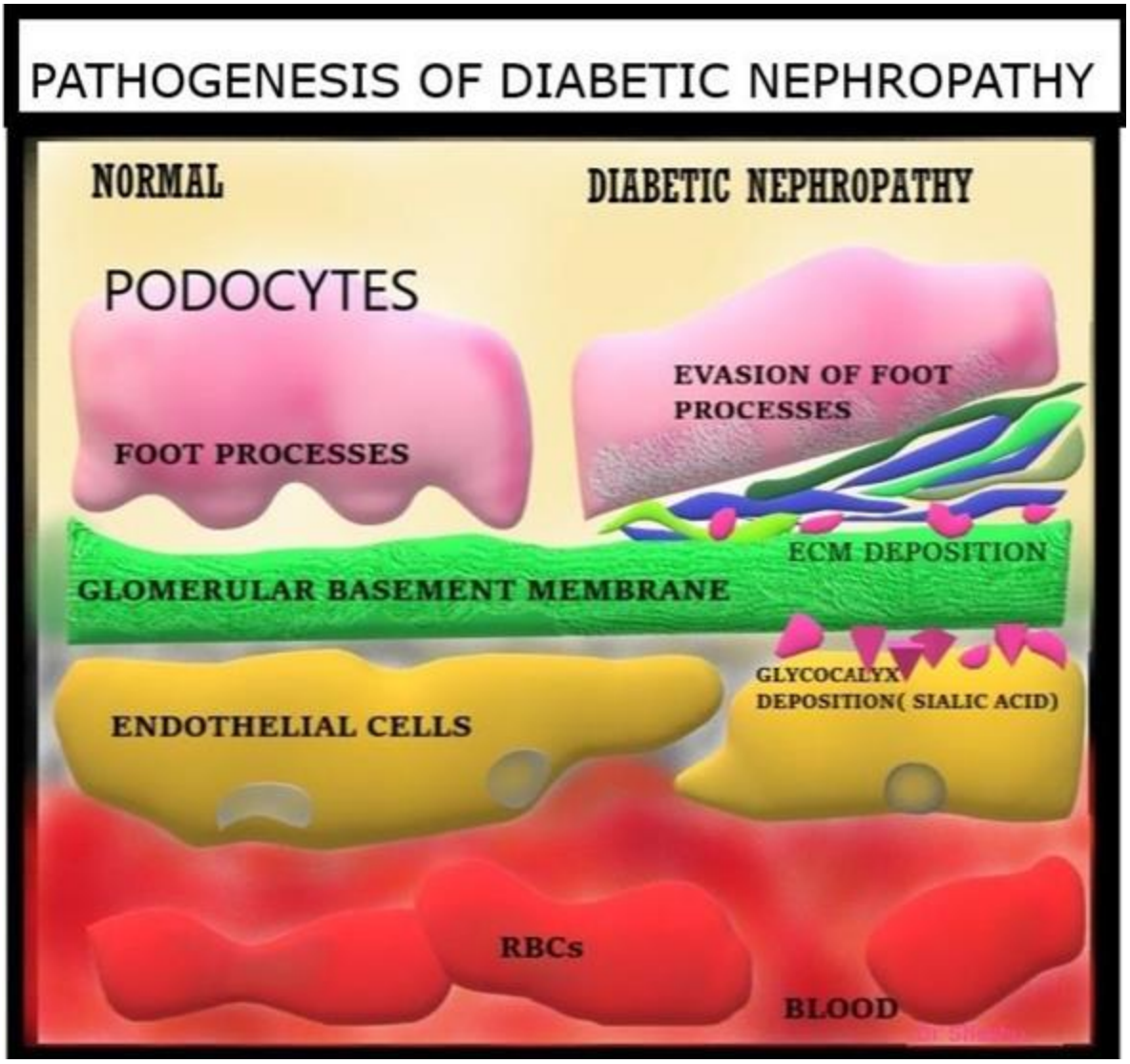

Figure 1: Pathogenesis of Diabetic Nephropathy

Extracellular matrix deposition, evasion of foot processes of Podocytes and glycocalyx deposition (sialic acid) and glomerular basement membrane damage, due to the underlying inflammatory mediators.

The aim of this study is to determine the serum level of high sensitivity C-reactive protein (hsCRP),an acute phase reactant and Serum Total Sialic acid levels, a marker of excessive glycation in unregulated glycemic control in Diabetes mellitus patients leading to the complication of diabetic nephropathy and to compare with the normal subjects and also to study the association between serum hsCRP levels and Serum Total Sialic acid levels.

MATERIALS & METHODS:

50 patients with Type 2 DM with nephropathy (DN) and 50 patients of Type 2 DM without nephropathy (DM) along with 50 unrelated age and sex-matched healthy controls were included in the study. The inclusion criteria for patients were onset duration of of diabetes beyond age 35 years. Exclusion criteria included patients on any drugs like lipid lowering

agents, anti-inflammatory drugs, analgesics, anticoagulants like aspirin, pregnant or lactating women, alcoholics, smokers and individuals with tobacco or drug addiction, past or present history of chronic illness liketuberculosis, rheumatoid arthritis other autoimmune disorders and patients of juvenile and type 1 DM were excluded from the study group.

Plasma fasting and postprandial glucose levels, renal profile (serum creatinine, BUN), and lipid profile, HbA1c and Urinary Microalbumin levels were analysed. Serum hs-CRP was measured by Quantia-CRP-US turbidimetric immunoassay kit supplied by Coral Clinical Systems. Quantia-CRP-US assay kit is based on the principle of agglutination reaction. The test specimen is mixed with Quantia-CRP US® latex reagent, then activation buffer was allowed to react. Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at 540 nm.

Serum TSA test levels and hs-CRP level were evaluated using thiobarbituric acid assay and immunoturbidimetric kit methods respectively.

Fasting venous blood sample collected in EDTA tube (2 ml) and plain tube (5 ml),the serum was carefully separated and transferred to microtubes and stored at −20°C until analysis. Postprandial venous blood sample was collected 2 h after the meal. Total serum cholesterol, triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), fasting, and postprandial glucose were analyzed on the fully automated clinical chemistry analyzer . Serum TSA levels was estimated using thiobarbituric acid assay method as described by Warren.

Statistical analysis:

All the data were entered in SPSS 20.0 (IBM Statistics,SPSS Inc., Chicago, IL, USA). One-way ANOVA test was done to see the difference in means of various biochemical parameters in DM, DN, andcontrol groups. Karl Pearson correlation test was applied to find out the linear correlation of different parameters.The multinomial logistic analysis was performed to see the factors independently associated with diabetic nephropathy.

RESULTS:

The mean age found in the DM and DN patient groups was 55.2 ± 10.8 and 54.1 ± 10.9 years, respectively. There was no significantdifference in age and sex between all groups.

We observed a higher concentration of Serum Total Sialic acid levels (83.2 ± 6.8 mg/dl) and hs-CRP (3.22 ± 1.48 mg/L) in diabetic nephropathy than the diabetes mellitus group (74.1 ± 6.2 mg/dl and 2.2 ± 1.40 mg/L, respectively). Both Serum Total Sialic acid levels and hs-CRP levels were found significantly correlated with plasma fasting and postprandial blood sugar, hemoglobin A1c, and urine microalbumin levels in both DM and DN groups.

Multiple logistic regression analysis showed that both TSA and hs-CRP was independently associated with diabetic nephropathy. We observed a significant higher fasting and postprandialblood glucose, as well as hemoglobin A1c (HbA1c) in DNgroup as compared to DM and control groups. TSA was found significantly raised in DN group (83.2 ±mg/dl) when compared from controls (62.0 ± 5.1 mg/dl). TSA was also found significantlyhigher in DM group as compared to controls ($P < 0.0001$)but it was within the normal limits.

TSA was found significantly correlated with fasting bloodsugar (FBS), postprandial blood sugar (PPBS), HbA1c, urine microalbumin, and hs-CRP in both DM and DN groups.

TG levels were found significantly different in all three groups, and it was highest in diabetic nephropathy. Total cholesterol and LDL levels were similar in all three groups. However, HDL cholesterol levels were found significantly low in DM ($P < 0.0001$) and DN ($P < 0.0001$) group when compared from controls.

No correlation of serum TSA with FBS, PPBS, and HbA1c was observed in thecontrol groups. But hs-CRP levels were also found significantly correlated with FBS, PPBS, HbA1c, urine.

Table 1: Comparison of parameters in controls vs diabetes and diabetic nephropathy groups.

	Control		**DM**		**DN**	
Parameter	**Control MEAN**	**SD**	**DM MEAN**	**SD**	**DN MEAN**	**SD**
Age (years)	55.2	10.8	54.1	10.9	55.1	8.2
Sex (male)	34		35		36	
Sex (female)	16		15		14	
FBS (mg/dl)	86	10	155	52.2	132	65
PPBS (mg/dl)	120	14	242	98.2	290	120
HbA1c (%)	5.6	0.4	8.5	1.8	9.3	1.9
Urea (mg/dl)	24	4.2	25.2	8.2	47	29.1
Creatinine (mg/dl)	0.9	0.15	0.98	0.31	1.62	0.88
Urine microalbumin (mg/day)	10.1	3.6	11.9	4.2	188	120.6
Total cholesterol (mg/dl)	190	38	193.1	44	188.2	53.2
Triglyceride (mg/dl)	130	62.2	156	80.2	178	120
LDL (mg/dl)	118	28.2	114.2	34.1	114.8	42
HDL (mg/dl)	45	7.2	41	8.2	40	7.8
VLDL (mg/dl)	26	14	32	18	36	23
TSA (mg/dl)	62	5.1	74.1	6.2	83.2	6.8
hs-CRP (mg/L)	1.18	0.48	2.2	1.4	3.22	1.48

Table 2: Corelation of hs-CRP and TSA with other parameters in controls vs diabetes and diabetic nephropathy groups.

Correlation of hs-CRP and TSA with other parameters						
	hsCRP			**TSA**		
Parameter	**Control**	**DM**	**DN**	**Control**	**DM**	**DN**
FBS	0.233(0.005)	0.181(0.022)	0.366(<0.001)	−0.018(0.850)	0.198(0.011)	0.292 (<0.001)
PPBS	0.511(0.186)	0.169(0.038)	0.355(<0.001)	−0.025(0.690)	0.206(0.009)	0.255(0.002)
HbA1c	0.078(0.291)	0.351(<0.001)	0.387 (<0.0001)	−0.028 (0.801)	0.333 (<0.0001)	0.226 (0.007)

Urine Microalbumin (MA L)	0.977 (<0.0001)	0.058 (<0.0001)	0.812 (<0.0001)	0.360 (<0.0001)	0.972 (0.000)	0.801 (0.000)
Triglyceride	0.088 (0.290)	0.114 (0.172)	0.228 (0.005)	−0.008 (0.912)	0.138 (0.0882)	0.25 2 (0.002)
Cholesterol	0.046 (0.571)	0.069 (0.410)	0.186 (0.03)	0.006 (0.958)	0.092 (0.288)	0.25 2 (0.001)
HDL	−0.038 (0.658)	0.024 (0.818)	0.029 (0.742)	0.039 (0.650)	0.079 (0.366)	0.08 2 (0.312
LDL	0.068 (0.041)	0.017 (0.810)	0.133 (0.112)	0.150 (0.852)	0.031 (0.696)	0.188 (0.028)
VLDL	0.088 (0.281)	0.112 (0.178)	0.228 (0.0058)	−0.009 (0.912)	0.150 (0.088)	0.25 2 (0.002)
hs-CRP	-	-	-	0.356 (<0.0001)	0.642 (<0.0001)	0.910 (<0.0001)
Sialic acid	0.355 (<0.0001)	0.632 (<0.0001)	0.910 (<0.0001)	-	-	-

Table 3: Corelation of parameters in controls vs diabetes and diabetic nephropathy groups.

	P value		
Control versus DM		**Control versus DN**	**DM versus DN**
Age (years)	0.851	1	0.622
Sex (male)	0.8	0.04	0.095
Sex (female)			
FBS (mg/dl)	<0.0001	<0.0001	<0.0001
PPBS (mg/dl)	<0.0001	<0.0001	<0.0001
HbA1c (%)	<0.0001	<0.0001	<0.0001
Urea (mg/dl)	1	<0.0001	<0.0001
Creatinine (mg/dl)	1	<0.0001	<0.0001
Urine microalbumin (mg/day)	1	<0.0001	<0.0001
Total cholesterol (mg/dl)	1	1	0.93
Triglyceride (mg/dl)	0.018	<0.0001	0.061
LDL (mg/dl)	1	1	1
HDL (mg/dl)	<0.0001	<0.0001	0.081
VLDL (mg/dl)	0.02	0	0.061
TSA (mg/dl)	<0.0001	<0.0001	<0.0001
hs-CRP (mg/L)	<0.0001	<0.0001	<0.0001

Multinomial logistic regression analysis showed that bothTSA and hs-CRP was independently associated with diabetic nephropathy.

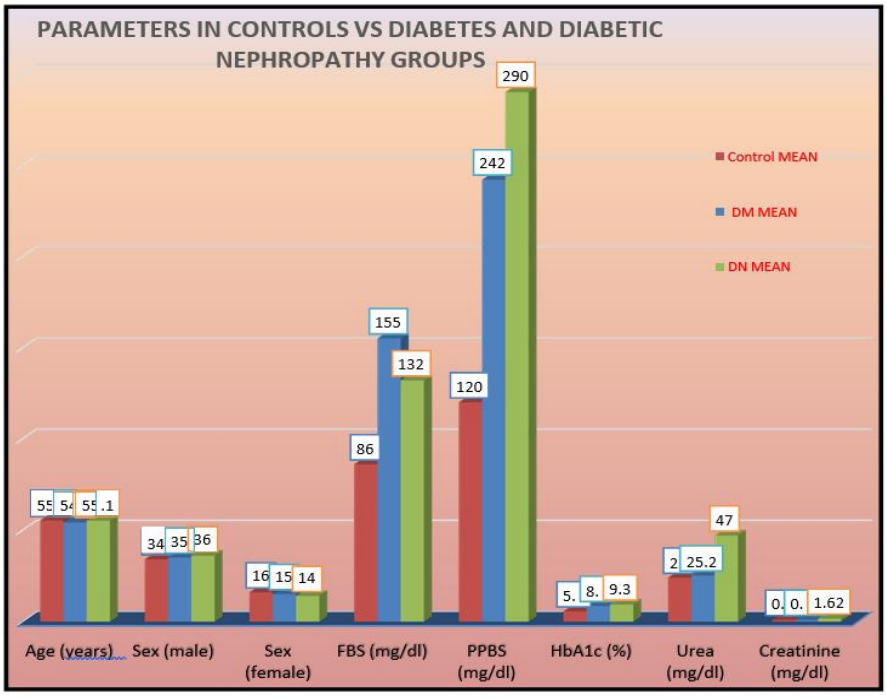

Figure 2: Comparison of parameters in controls vs diabetes and diabetic nephropathy groups.

Table 4: Multinomial logistic regression analysis

Multinomial logistic regression analysis				
Parameter	**B**	**P**	**95 % CI values**	
Age	−0.077	0.251	−0.128	-0.266
FBS (mg/dl)	0.006	0.862	−0.39	-0.092
PPBS (mg/dl)	0.007	0.66	−0.058	-0.029
HbA1c (%)	0.162	0.833	−1.66	-2.1
Urine micro-albumin	1.82	0.001	1.501	-1.812
hs-CRP (mg/L)	−32.99	0.002	−51.02	-26.35
Sialic acid (mg/dl)	2.08	0.017	−1.520	-4.912

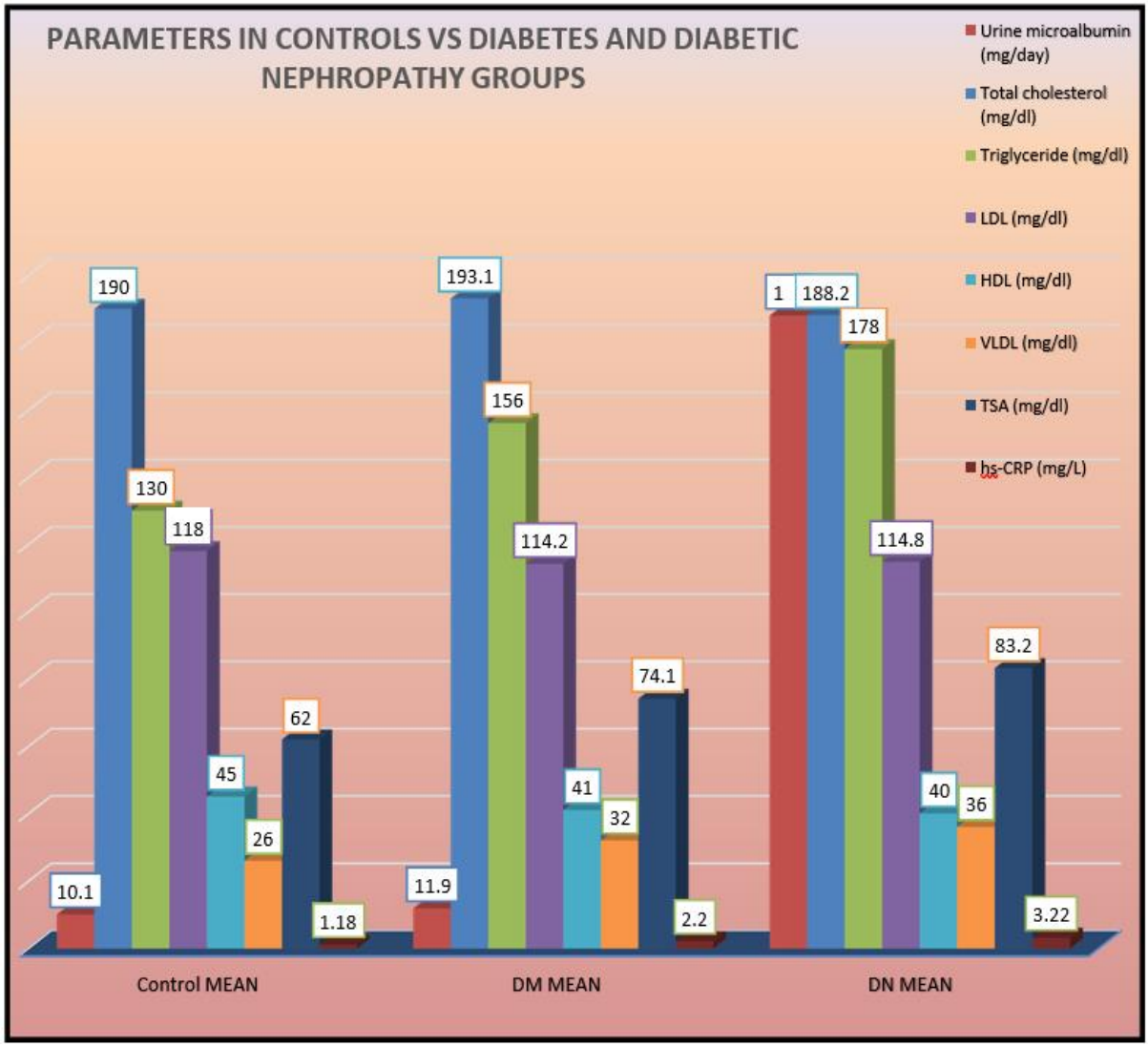

Figure 3: Corelation of parameters in controls vs diabetes and diabetic nephropathy groups.

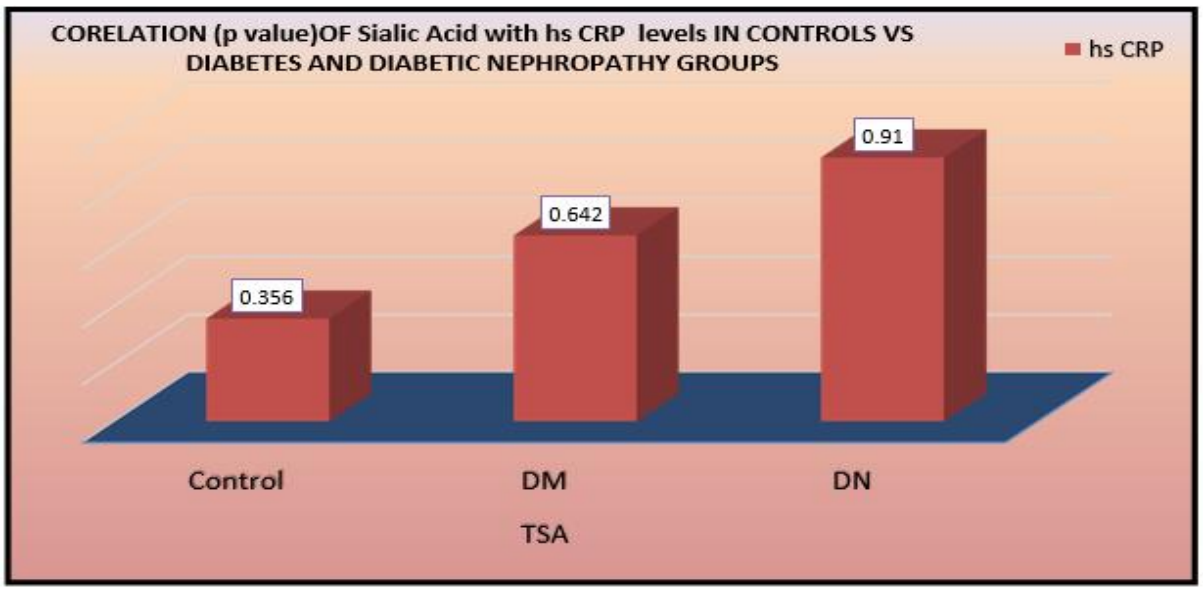

Figure 4: Corelation (P Value) Of Sialic Acid With hs CRP Levels in Controls Vs Diabetes and Diabetic Nephropathy Groups

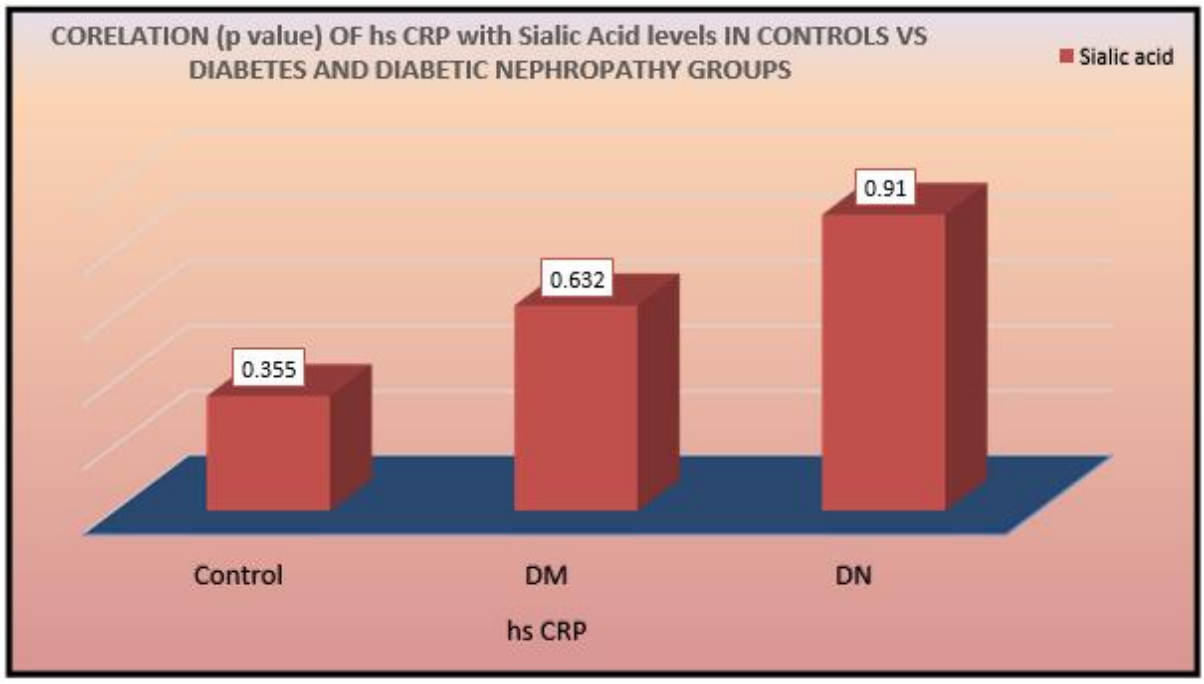

Figure 5: Corelation (P Value) Of Sialic Acid With hs CRP Levels In Controls Vs Diabetes And Diabetic Nephropathy Groups

DISCUSSION:

Inflammation plays a major role in the pathogenesis ofT2DM and its complications. Hence inflammatory markersor acute phase markers have gained the importance as indicators and predictors of the diabetic process. It is perceived that chronic low-grade inflammation as evidenced by elevated hs-CRP might potentially bea cause underlying the etiology and manifestation of T2DM, although the exact mechanisms are still not well understood. The inflammatory response may be due to microvascular or macrovascular complications following Type 2 diabetes mellitus, although the exact mechanisms are still not well understood. Serum hs-CRP level is elevated in Type-2 diabetes and diabetic nephropathy in comparison with non-diabetic healthy controls. The results of the present study showed that the serum hs-CRP concentration is strongly related to Type 2 diabetes and diabetic nephropathy.

Our study showed that on comparing between diabetic patients and control group patients we found that sialic acid is increased in the diabetic group more than the control group and this was statistically significant, sialic acid level and (fasting blood glucose, postprandial blood glucose, HBA1C) were found to be positively correlated and these parameters were considered as predictors of serum sialic acid level.

In study by Divija et al. revealed that serum sialic acid was positively correlated with HBA1c, fasting blood glucose, and postprandial blood glucose in the diabetic cases indicating a linear increase relationship between these parameters.[24] Subzwari and Qureshi revealed a significant increase of serum sialic acid among the subjects with diabetes compared to the

control subjects.[25] Serum and urine sialic acid concentration increased in patients with diabetes as compared to the general population, especially in patients with type 2 diabetes.

A positive significant correlation was found between sialic acid level and DN parameters (creatinine, urea and 24-h urinary albumin), and sialic acid level was considered as a significant predictor of serum creatinine and urinary albuminuria (nephropathic parameters). Poddar and Rayrevealed that a clear-cut elevation in sialic acid levels is evident from the data in diabetics without complications as compared to the healthy controls. Martha and Fernando in their study found that hyperglycemia is an associated factor to the increase of serum CRPlevels; in uncontrolled type 2 diabetes subjects. 26 Lima et al. and Amanullah et al in their study found that hypertensive patients with T2DMhad higher levels of hs-CRP than normal subjects.[27,28]

In a study by Ghosh et al. who revealed that serum sialic acid levels were found to be significantly increased in diabetes with or without nephropathy compared to controls and also revealed a very large positive correlation between serum sialic acid and urinary microalbumin, which showed that as microalbumin excretion increases, serum sialic acid also increases pointing to a contributory role of serum sialic acid toward renal damage. These findings indicate that serum sialic acid increases with the severity of diabetic renal complications.[29]

Abdella et al revealed a progressive elevation in total sialic acid concentrations with increased urinary albumin excretion. The differences in total sialic acid levels among the normoalbuminuric, microalbuminuric, and macroalbuminuric diabetic groups were significant.[30]

Roozbeh et al. revealed that the serum and urine levels of SA and neuraminidase activity were always abnormally higher in diabetic nephropathic patients when compared to diabetic patients with no nephropathy or nephropathic patients, which is expected as both conditions of diabetes and nephropathy may cause an increase in these variables.[31]

Subzwari and Qureshi revealed a significant increase of serum sialic acid among the subjects with diabetes compared to the control subjects. Furthermore, in the subjects with diabetes, urine sialic acid and microalbumin were significantly higher. The sialic acid values were statistically significantly higher with the increased urinary albumin excretion.[25]In their study, Krishnamurthy et al. revealed a progressive rise in serum sialic acid levels with urinary albumin excretion and a significant positive correlation between them. It is noted that there was a significant difference in serum sialic acid levels between controls and normoalbuminuric patients, which was not shown with serum creatinine and albumin excretion levels. Serum creatinine showed a significant increase only in microalbuminuric patients, suggesting its importance only after the onset of nephropathy. Therefore, sialic acid may act as an indicator of the early diabetic process.[32]

Tomino et al found that the levels of sialic acid in sera from DN patients were significantly increased, but they found no significant correlation between the levels of blood urea nitrogen, serum creatinine or proteinuria, and sialic acid. Although an increase of sialic acid in sera is observed in patients with various kinds of inflammatory diseases and cancers, this isconsidered to be nonspecific phenomena.[33] Chen et al. revealed that there was a progressive rise in serum sialic acid concentrations with increasing UAE in non-insulin-dependent diabetes mellitus (NIDDM) patients. Furthermore, normoalbuminuric NIDDM

patients had elevated serum sialic acid concentrations compared with healthy nondiabetic control subjects, suggesting an effect per se of the diabetic state.[20]

These findings suggests that patients with two associated diseaseshave a more active inflammatory state. Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with BMI, family history of DM, smoking, and other factors. Several previous studies hs-CRP levels were found raised in diabetic mellitus with or without nephropathy. Patients with diabetic nephropathy have significant higher hs-CRP than diabetic mellitus.There may be a significant relationship between hs-CRP and complications of Type 2 diabetes mellitus through the acute phase response.

Stehouwer CD et al,[34] Jager A et al,[35] and Samia M et al,[36] reported the relationship between inflammation, Type 2 diabetes mellitus and its complications. Inflammation is linked to the pathogenesis of Type 2 diabetes mellitus. Insulin resistance and hyperglycemia are also promoting inflammation by increased oxidative stress and that may link diabetes mellitus to the development of atherosclerosis.

TSA was also found raised in both diabetes mellitus with or without nephropathy. Similar to our study K Prajna et al, observed significantly increased TSA levels in diabetes mellitus without any complication and in diabetes mellituswith nephropathy as compared to control.It was alsofond positively correlated with blood glucose levels in both the groups. Similar to our study Masuda et al. haveshown that serum TSA reflects the status of blood glucosecontrol and the progression of the ischemic disease of the lower extremities in T2DM.

Crook et al. shows that serum TSA is a newly established potential risk factor for the development of macrovascular and microvascular complications of diabetes.[37]Similar findings have been stated by Chen et al., Nayakand Bhaktha and Krishnamurthy et al.

Shahid and Mahboob in their study found increased serum TSA asa potential risk factor for the development of macro and microvascular complications of diabetes.

Serum sialic acid is considered as a marker of innate immunity, and activated innate immunity is a risk factor for cardiovascular disease mortality in type 2 diabetes. Masuda et al. have shown that SSA reflects the status of blood glucose control and the progression of ischaemic disease of the lower extremities in NIDDM.

Sridevi D et al,[38] found that the CRP levels are elevated in Type 2 diabetic patients with the metabolic syndrome and hence, hs-CRP is added as a diagnostic criterion for metabolic syndrome. A study on hs-CRP levels and glycated haemoglobin by King DE et al., indicate the relationship between hs-CRP and glycaemic control.[39]Mahajan A et al. found a relationship between C-reactive protein and hyperglycemia in urban Northern Indian Type 2 diabetic patients which is an agreement with our study.[40] Lima LM et al., reported that hyperglycemia is an associated factor to the increased serum CRP levels in uncontrolled Type 2 diabetes subjects.[27] Study on inflammatory markers by Waheed P et al,[41] showed the relationship between dyslipidemia, C-reactive protein and low-grade inflammation in diabetic subjects . Mojahedi MJ et al., reported that microalbuminuria is accompanied by elevated hs-CRP, suggesting activation of inflammatory pathways in the progression of cardiovascular and renal disease in Type 2 diabetic patients which is an agreement with our findings.[42]

Clinical and experimental studies have shown that a variety of inflammatory molecules, such as CRP, IL-6, and MCP-1, are involved in the setting of DN. Fujita et al. proposed that IL-18 might have a specific role that contributes more closely to the progression of DN than other

DM complications.[43]Usingatransgenic Leprdb/db mouse model of DN that expresses human CRP, You et al,[44] have recently shown that CRP is pathogenic in type 2 DN and can activated Smad3 signaling directly through the ERK/p38 MAP kinase cross talk pathway and indirectly via a TGF-β1–dependent mechanism. CRP may also promote renal inflammation via the CD32b–nuclear factor-κB signaling mechanism, whereas CRP may enhance renal fibrosis via the CD32b-Smad3- mTOR signaling pathway. CRP was also reported to promote proinflammatory cytokine production, leading to mesangial cell proliferation, matrix overproduction, and increased vascular permeability resulting in albuminuria. An in vitro study suggests that macrophage- produced CRP, in addition to the effect of circulating and liver-derived CRP, could trigger CRP-mediated proinflammatory effects locally. It has been demonstrated that CRP induces macrophage colony–stimulating factor (M-CSF) release via upregulation of nuclear factor- κB, resulting in increased macrophage proliferation.[45]
Macrophages infiltrate the glomeruli and/or interstitium in the kidney in patients with DN, and the intensity of the interstitial infiltrate is proportional to the rate of subsequent decline in renal function.[46] CRP was also reported to promote proinflammatory cytokine production (35), leading to mesangial cell proliferation, matrix overproduction, and increased vascular permeability resulting in albuminuria . Collectively, these findingssupport a pivotal role of CRP in the inflammatory process during development of DN.

CONCLUSION:

Serum hs-CRP and TSA may be used as a predictive biomarkers of diabetic nephropathy. It is important to investigate the relationship between SA and/or other markers and mediators of the acute-phase response (e.g. proinflammatory cytokines) and the development or progression of diabetic microangiopathy.

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