Original article:

A study of serum sialic acid and urine microalbumin in non insulin dependent diabetes mellitus

Dr. Sara Praveen Kumar[1], Dr.J.Madhavi Latha[2], Dr.M.Amarendra[3], Dr.G.V.Benerji[4]

[1] Post Graduate, Department Of Biochemistry,Osmania Medical College,Hyderabad

[2] Associate Professor, Department Of Biochemistry,Osmania Medical College,Hyderabad

[3] Department Of Peadiatrics,KIMS & RF,Amalapuram,E.G dist.,A.P

[4] Professor , Department of Biochemistry, KIMS & RF,Amalapuram,E.G dist.,A.P

Corresponding author : Dr. Sara Praveen Kumar

Abstract:

Background and objectives: Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India. Serum sialic acid, an acute phase reactant and urinary albumin excretion are found to be increased in various conditions like diabetes mellitus, cardiovascular diseases, cancer etc. In diabetes mellitus, acute phase reactants are considered as the indicators of microvascular angiopathy. Microalbuminuria is a predictor of incipient nephropathy and coronary vascular disease in the diabetic patients. Therefore our study was undertaken to understand the association of serum sialic acid levels in incipient diabetic nephropathy patients and to assess the correlation of serum sialic acid and microalbuminuria with glycemic control.

Methods: Present study involved 90participants of which 60 were non insulin dependent diabetes mellitus (NIDDM) patients studied for their urinary microalbumin, serum sialic acid, fasting blood glucose and serum creatinine levels. Analysis was performed by categorized them based on their albumin excretion (normoalbuminuric and microalbuminuric). 30 non diabetic age and sex matched healthy subjects were taken as a control group. Blood samples were drawn and urine samples were collected under aseptic precautions from study subjects. The values were tabulated for cases and controls.

Results: serum sialic acid concentrations found to be elevated in NIDDM of normoalbuminuric (2.247 ±0.2432 mmol/l) patients when compared to controls(1.798 ±0.24 mmol/l) and more so with microalbuminuric patients(2.794 ±0.2122 mmol/l), and that is statistically more significant (P<0.001), whereas serum creatinine shows significant increase only in NIDDM with microalbuminuric patients . There is progressive rise in serum sialic acid levels with increase in urinary albumin excretion in NIDDM patients.

Conclusions: The study concludes that elevated serum sialic acid and microalbumin levels are strongly associated with the progression microvascular complications such as of diabetic nephropathy. Serum sialic acid can be used as a marker of renal dysfunction in various stages of diabetic nephropathy.

 Key words: Sialic acid; Microalbuminuria; Diabetes mellitus.

Introduction:

Diabetes mellitus is a metabolic disorder of multiple etiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1]

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90%of cases globally[2] It is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia.[3] All forms of

diabetes, both inherited and acquired, are characterised by hyperglycemia, a relative or absolute lack of insulin, and development of diabetic specific microvascular pathology in retina, renal glomerulus, and peripheral nerve.[4]

International diabetic federation (IDF) estimates the total number of diabetic subjects in India to be around 40.9million and this is further set to raise to 69.9million by the year 2025.[5] Large prospective clinical studies show a strong relationship between glycemiaand diabetic microvascular complications in both type1 diabetes mellitus and type2 diabetes mellitus. There is a continuous, though not linear, relationship between level of glycemia and risk of development and progression of these complications.[4]

It has been proposed that inflammatory process play an important role in the developmentof diabetes and its late complications. Various acute phase reactants are being studied in diabetic process as indicators or predictors of diabetic microvascular complications.[6]

Serum sialic acid is a newly established potent risk factor for the development of micro andmacro vascular complications of diabetes.[7] Serum sialic acid is a component of glycoproteinssuch as acute phase proteins and several serum acute phase proteins are elevated indiabetes.[8]

Diabetic nephropathy remains a major cause of morbidity and mortality for the persons either T1DM, or T2DM.[4] Diabetic nephropathy occurs in about 25-30% of diabetic patients. However there is an early phase of diabetic renal disease called incipient diabetic nephropathy characterised by increased albumin excretion in the range of 30-300mg/daymicroalbuminuria.[9]Incipient diabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors.[10]

The study was undertaken to correlate serum sialic acid and microalbuminuria which are the markers of early renal damage to estabilish the role of estimation of sialicacid and microalbumin in NIDDM. Incipient diabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors.

The study was undertaken to correlate serum sialic acid and microalbuminuria which is a marker of early renal damage to estabilish the role of estimation of sialicacid in NIDDM.

Materials and methods:

Setting:

A Case control study was conducted during march2013-September 2014, in the Department of Clinical Biochemistry, Osmania General Hospital, Hyderabad; Telangana.

Sources of Samples and Data:

- The established NIDDM patients attending the OPD of Osmania General Hospital, and Department of Clinical Biochemistry Osmania General Hospital,   Hyderabad.

Control group :

 Consists of Age and gender matched healthy controls. None of the patients had a history of Diabetes mellitus, Hypertension, Hepatic, Renal, any other systemic illnesses. Also they were judged to be free of any illness by clinical examination.

 The diabetic patients:

- Age between 40 to 60 yrs of Both Genders.
- The Classification of Subjects in to Diabeticand Non-Diabetic Groups was based on American Diabetes Association Criteria 2011.

urineryalbustic negative

Exclusion Criteria:

- Type 1 diabetic cases.
- Urinary albustic positive cases ofNIDDM
- Cases with inflammatory disorders like eczema,seconderyhyperglycemic states like hypothyroidism,
- Proteinuric conditions like congestive cardiac failure, renal failure, and pregnancy.
- Female patients with menstrual disorder
- Cases with severe combined immune deficiencies.
- **Study Grouping**
- All the study subjects (60 cases + 30 controls) / participants were explained the nature of the study. Informed consent was obtained from all the 90 subjects.
- **The study sample**
- In the present study 90 subjects were selected and divided into two age and sex matched type2 diabetic cases and non diabeticcontrol groups.
- **Group I:** Includes 30 type2 DIABETIC CASES with urine microalbumin negative.
- **Group 2:** Includes 30 type2 DIABETIC CASES with urine microalbumin positive
- **Group 3:** Includes 30 NON DIABETIC CONTROLS.
- **Sample collection:**
- Participants were in overnight fasting status. They were in supine position for 5 to 10 minutes before venipuncture and 3ml to 4ml venous blood was drawn and collected into three tubes. One containing sodium fluoride and potassium oxalate (grey top), and the other was a plain tube (red top). The blood in plain tube was allowed to clot to separate serum. Serum, plasma was separated within one hour after sample collection. Care was taken to avoid Hemolysis. All icteric, hemolysed samples were ignored .Serum for other parameters was stored at -20ºC.
- Blood samples were analysed for fasting blood glucose, serum creatinine,and serum sialicacid.
- Early morning Urine samples was collected under aseptic precautions for estimation of urinary microalbumin.
- **Samples from all 90 subjects were analyzed for the following parameters:**
- 1. Plasma fasting Blood Sugar
- 2.serum creatinine
- 3.urine microalbumin

ESTIMATION PROCEDURES FOR ANALYTES:

 GLUCOSE: (GOD-POD METHOD)

Method: GLUCOSE OXIDASE PEROXIDASE METHOD**.**

ESTIMATION OF SERUM CREATININE:

Method: modified jaffe's reaction

ESTIMATION OF URINE MICROALBUMIN:

Method: TURBILATEX METHOD (immune turbidometric method)

ESTIMATION OF SERUM SIALIC ACID:

Method: MODIFIED THIOBARBTURIC ACID ASSAY OF WARREN (Lorentz and Krass)

Type 2 diabetes usually develops in obese patients who are over 40 years old. Its pathogenesis involves a combination of insulin resistance and impairment of insulin secretion. Insulin resistance in several tissues like skeletal muscle, adipose tissue and liver leads to increased insulin secretion from pancreas. This compensatory hyperinsulinemia maintains glucose levels within normal range but individual is at high risk of developing diabetes. Beta cell function eventually declines and leads to development of impaired glucose tolerance and eventually overt diabetes mellitus.[11]

Environmental influences, such as dietary habits and sedentary life styles, clearly have a role which becomes evident when obesity is considered. Genetic factors are even more important in type 2 than in type 1 diabetes.[12]

Obesity and insulin resistance:

Insulin resistance is the link between obesity and diabetes. The risk for diabetes increases as the body mass index (a measure of body fat content) increases, suggesting a dose response relationship between insulin resistance and body fat.[12]

Results:

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad.

A total of 90 subjects were recruited for the study which included30 healthy individuals as controls, 30 T2DM patients with normoalbuminuria, 30 patientsT2DM with normoalbuminuria.

The following parameters were analysed.

1. Fasting plasma glucose
2. Serum creatinine
3. Urine microalbumin

The results were expressed in milligrams /deciliterfor Fasting plasma glucose, Serum creatinine, mmol/lit for serum sialicacidand milligrams/litre for urine microalbumin.

The data was analysed using GraphPad Prism software version 6.0.

Descriptive results are expressed as mean and SD of various parameters in different groups.

Student's t-test was used for testing the significance difference in mean scores of various bio-chemical parameters between case and control groups. Results indicated that the mean scores on various biochemical parameters differ significantly between cases and controls. Pearson correlations were computed to see the association between different biochemical parameters for case and control groups. Significance of the correlations was indicted with (*) for $p<0.05$ and (**) for $p<0.01$. The statistical significance was set at minimum 5 percent ($p<0.05$). Results were represented in the form of tables and bar diagrams.

Table 1: mean and sd values of parameters in three groups

Parameters	FBS		Microalbumin		Sr.creatinine		Serum sialicacid	
	mean	Sd	Mean	s.d.	mean	s.d	mean	s.d.
Microalbuminurics	145.4	40.24	49.02	23.46	1.206	0.2834	2.794	0.2122
Normoalbuminurics	134.1	43.14	12.49	4.468	1.061	0.2320	2.247	0.2432
Controls	88.90	10.53	9.130	3.724	0.9445	0.1402	1.798	0.2402

Table 1 showing mean ± sd of fasting plasma glucose, serum creatinine, urinemicroalbumin and serum sialic acid levels in controls and NIDDM patients with microalbuminurics and normoalbuminurics. All parameters were found to be increased in NIDDM patients (bothmicroalbuminurics and normoalbuminurics).

Table 2: Comparision of p- values between three groups

Parameter	Controls vs normoalbuminurics	Controls vs microalbuminurics	Normoalbuminurics vs microalbuminurics
FBS	< 0.0001	< 0.0001	0.3013
Serum creatinine	0.2917	0.0759	0.0572
Microalbumin	0.062	< 0.0001	< 0.0001

Table 2 shows serum sialic acid levels showed significant difference between controls and normoalbuminurics (p<0.0001), in between controls and microalbuminurics (p<0.0001), in between normoalbuminurics and microalbuminurics (p<0.0001). And all are highly significant.

Urine microalbumin levels showed significant difference between controls and microalbuminurics (p<0.0001) and in between normoalbuminurics and microalbuminurics(p<0.0001) whereas not significant between controls and normoalbuminurics.

Fasting glucose levels showed no significant difference in their levels levels when compared between normoalbuminurics (134.1 ±43.14) and microalbuminurics (145.4 ± 40.24) and p value (0.301) also not significant.

Table 3:Pearsons Correlation between different parameters in control group

Parameter	FBS	MICROALBUMIN	SR.CREATININE	SERUM SIALICACID
FBS		0.148	0.142	0.126
		0.428	0.446	0.499
MICROALBUMIN	0.148		0.092	-0.013
	0.428		0.624	0.944
SR.CREATININE	0.142	0.092		-0.037
	0.446	0.624		0.842

Table 4: Pearsons correlation between different parameters in normoalbuminurics group

Parameter	FBS	MICROALBUMIN	SR.CREATININE	SERUM SIALICACID
FBS		0.395	0.192	-0.024
		0.031	0.311	0.898
MICROALBUMIN	0.395		0.283	0.492
	0.031		0.130	0.006
SR.CREATININE	0.192	0.283		0.273
	0.311	0.130		0.144

In table 4 there is significant positive correlation between microalbumin excretion and sialic acid (p 0.003 and r 0.395); microalbumin excretion and and FBS in normoalbuminurics(p 0.006 and r 0.492).

Table 5 : Pearsons Correlation between different parameters in microalbuminurics group

Parameter	FBS	MICROALBUMIN	SR.CREATININE	SERUM SIALICACID
FBS		0.032	0.111	0.219
		0.866	0.559	0.244
MICROALBUMIN	0.032		0.176	0.717
	0.866		0.353	0.0001
SR.CREATININE	0.111	0.176		0.360
	0.559	0.353		0.050

In table 5 shows there is significant positive correlation microalbumin excretion (p<0.0001 and r 0.71); serum creatinine levels (p 0.05 and r 0.36).

Discussion:

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90%of cases globally.[2] All forms of diabetes, both inherited and acquired, are characterised by hyperglycemia, a relative or absolute lack of insulin, and development of diabetic specific microvascular pathology in retina, renal glomerulus, and peripheral nerves.Incipient diabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors.[10] Olmesartan increses the time to the onset of microalbuminuria in patients with micralbuminuria in patients with type 2 diabetes, even when blood pressure control is excellent according to current recommendations.

Blood glucose:

Diabetes mellitus is a group of metabolic disorder of carbohydrate metabolismin which glucose is underutilized producing hyperglycemia. The diagnosis of DMsolely depends on the demonstration of hyperglycemia. The criteria for the diagnosisof diabetes mellitus is when the fasting blood glucose level is ≥126mg/dl or 2hr postload plasma glucose concentration of ≥ 200mg/dl during the oral glucose tolerancetest or the classic symptoms of diabetes mellitus with casual plasma glucoseconcentration ≥ 200mg/dl.

Hyperglycemia is a causative factor in the pathogenesis of diabeticnephropathy. Glucose reacts non enzymatically with primary amines of proteinsforming glycatedcompounds. Hyperglycemia exerts toxic effects and results in kidney damage by directly altering intracellular signaling pathways and via many biochemical pathways.

In the study the mean FBS values were in controls (88.90 ±10.53), in cases with normoalbuminurics(134.1 ±43.14) and microalbuminurics (145.4 ± 40.24) which are statistically highly significant (P<0.001). FBS values were higher than the cutoff value of 110mg/dl in cases which correlated well with theclinical diagnosis.

The potential biochemical pathways leads to dibetc nephropathy are polyol pathway, nonezymatic glycation, glucose auto-oxidation and denovo synthesis of diglycerol leading to protein kinase C and phospholipase A2 activation.

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.

Serum creatinine

Serum creatinine is the most important indicator of renal function. Creatininelevels in blood and urine may be used to calculate the creatinine clearance (CrCl),which reflects the glomerular filtration rate (GFR). The measurement of GFR isclinically important as it is a measure of renal function.

In the present study the mean serum creatinine values were 0.944±0.140 in controls, in normoalbuminutics is 1.061±0.232 and in microalbuminurics is 1.206±0.283 which are showing the progressive increase but are statistically not significant, indicating that gradual progression of kidney damage with increased albumin excretion.

In the study of Krishnamurthy et al showing that serum creatinine increased, in microalbuminurics and normoalbuminurics but significant increase only in microalbuminuric patients when compared with normoalbuminurics and controls, but not in between normoalbuminurics and controls suggesting its importance only after the onset of nephropathy.

In the study of Ashok kumar et al showing serum creatinine levels were found to be increase in type2 DM without any complications and type 2 DM with nephropathy when compared to controls.

In the study of Shahid at al showing that serum creatinine levels were found to be increase in type2DM without any complications and with nephropathy and showing p value showing significant only with diabetic nephropathy patients.

In the study of Divija showing that serum creatinine levels were increased and statistically significant in diabetic nephropathy patients when compared with healthy individuals.

In another study, Shahid SM and Mahaboob T, showed significant increase inserum creatinine levels in diabetic nephropathy patients as compared to diabetes without nephropathy and controls.

Our study is in accordance with several studies, which have shown increase inserum creatinine levels in type2 DM with normoalbuminurics and microalbuminuric type2 diabetic patients compared to healthy controls.

Urine microalbumin

Microalbuminuria is an important risk factor for cardiovascular disease andprogressive renal impairment. Microalbuminuria arises from the increased passage ofalbumin through the glomerular filtration barrier which results from ultrastructuralchanges rather than alterations in glomerular pressure or filtration rate alone.[88] Microalbuminuria predicts the development of overt diabetic nephropathy intype 1 and type 2 DM but the relationship is less clear in type 2 because ofheterogeneity and presence of other risk factor for microalbuminuria in these elderlypatients.Glomerular structural changes typical of diabetic nephropathy are establishedby the time microalbuminuria becomes apparent.

In the study of Prajna K. et al showed that the urine microalbumin levels were found to increased, in type 2 diabetics without any complications and type 2 diabetics with nephropathy when compared to controls, which was statistically significant. The increase in urine albumin in the diabetics can be interpreted as an early sign of nephropathic changes in those individuals. Increase in urine albumin seen with diabetic nephropathy can be attributed to degradation of the glomerular basement membranes and hypertension, both characteristic of diabetic nephropathy. The presence of microalbuminuria is a marker of endothelial dysfunction, and indices an the rate of progression of increased risk of generalized atherosclerosis and increased mortality from cardiovascular disease.

With the increase in protein excretion there is a tendency of GFR to fall to lower level but not below the normal range during the 2nd and 3rd stages of diabetic nephropathy. With the onset of persistent proteinuria, GFR progressively falls and culminates in the

end stage renal disease (ESRD) in months to a year if left untreated. Raised albumin excretion is also associated with HbA1c in patients with incipient nephropathy. The rate of progression of nephropathy is correlated is correlated with metabolic control.

For a predictor of diabetic nephropathy to be optimally useful, it should identify individuals at an increased risk of the development of serious diabetic renal disease early enough in the natural history of the disorder that the evolution of the process can be influenced by interventional strategies. Microalbuminuria in terms of predictive power is still the strongest broadly available marker or predictor of diabetic nephropathy. In diabetic pregnancy, an increase of microalbuminuria predicts complications.

In the present study the mean values of urinary microalbumin were 9.13±3.72 incontrols, in patients of diabetes with normoalbuminurics12.49±4.46 and in patients of diabetes with microalbuminurics 49.02±23.46 in cases which is statistically highly significant (P<0.001).Urinary microalbumin values were higher than the cut off value of 20mg/l in cases with microalbuminurics which is statistically correlated well.

In a study done by Melidonis A, Tournis S, it was shown that urinary albuminlevels were higher in type 2 diabetic patients with signs of nephropathy compared tothose without signs of nephropathy and control group.

Chen JW, Gall MA in their study, demonstrated increase in urinary albumin levels in NIDDM patients with microalbuminuria and diabetic nephropathy patientscompared to controls. But the increase was far more significant in diabeticnephropathy patients compared to diabetic with microalbuminuria.

In the study of shivananda nayak, Heidi Duncan, sunita increased urinary albumin excretion levels diabetes without nephropathy and diabetes with nephropathy when compared with healthy control group.[89]

Our study is in accordance with Krishnamurthy U, Halyal SS,jayaprakash murthy, who demonstrated increase in urinarymicroalbumin levels in NIDDM patients with microalbuminuric when compared tonormoalbuminuric and healthy controls and found significant positive correlationbetween microalbumin excretion and sialic acid.

Correlation between serum sialic acid and other study

There is a very small correlation between urine microalbumin excretion and FBS in cases with microalbuminurics group (r=0.032) but moderate correlation in cases with normoalbuminurics (0.395) and small correlation in controls (r=0.148) showing that FBS levels are more important in developing towards albumin excretion than the developed microalbuminuria. There is a mild correlation between urine microalbumin excretion and serum creatinine in cases with microalbuminurics (r=0.176), moderate correlation in cases with normoalbuminurics (r=0.283) when compared with control group (r=0.142).

In the present study is in accordance with the study done by Krishnamurthy U, Halyal SS,jayaprakash murthy, who also showed significant positive correlation between serum sialic acid and microalbumin excretion, FBS, serum creatinine in NIDDM patients with microalbuminurics, normoalbuminurics compared to healthy control group. Also showing that progressive rise of serum sialic acid levels with increasing microalbumin excretion.

Summary and conclusion:

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, and the most common endocrine disorder characterized by metabolic abnormalities and long term complications such as retinopathy, nephropathy and neuropathy. Diabetic nephropathy remains a major cause of morbidity and mortality for thepersons either T1DM, or T2DM. Diabetic nephropathy occurs in about 25-30% of diabetic patients.

As the progression of diabetic nephropathy is slow, it is possible to be detected at an early stage. Microalbuminuria is an early indicator of diabetic nephropathy.'Prevention is better than cure' holds good for diabetic nephropathy as thebest way of treatment for

this disease is to control the risk factors such as increase inblood glucose and blood pressure level. Incipientdiabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors.The pathophysiology of the disease has to bewell known, in order to prevent a disease. Various hypotheses have been proposed tilldate on how diabetic nephropathy progresses in human subjects. These include theinvolvement of renin- angiotensin system, advanced glycation end product formation(AGE), endothelial dysfunction and oxidative stress.

The present study was undertaken to study the levels microalbuminuria to assess whether there is a relationship between these parameters with FBS and serum creatinine in diabetic patients towards the development of diabetic nephropathy, before landing into the stage of macroalbuminuria and end stage renal disease. 30 clinically diagnosed cases of diabetes with microalbuminuria and 30 clinically diagnosed cases of diabetes with normoalbuminuria who attended outpatient department at Osmania general hospital were taken for case study. 30 age and sex matched healthy persons were taken ascontrols.

A statistically significant difference was observed in values of FBS, serum creatinine, serum sialic acid and urinary microalbumin levelsin cases with microalbuminuria when compared to cases with normoalbuminuria and controls. In our study positive correlation was observed between serumsialic acid and urinary microalbumin in cases. It was also observed that serum sialicacid concentrations were strongly associated with several risk factors like glycemic status, renal dysfunction (creatinine) and urine albumin eaxcretion for thedevelopment of micro and macrovascular complications. These markers wereclinically correlated with increasing concentration of sialic acid. It was also observed that urine microalbumin excretion was positively associated with glycemic status and serum creatinine.

It is concluded that increase in circulating serum sialic acid is an earlymanifestation of diabetic renal disease (micro vascular complications) and hence estimation of both microalbuminuria and serum sialicacid levels in NIDDM is helpful in assessing the progress of disease and identifying the risk category for complications, such as diabetic nephropathy which are main causes for mortality and morbidity among diabetes mellitus patients. Further studies would be helpful to clarify the role of sialic acid in the pathogenesis of diabetic renal disease.

References:

1. American diabetes association. Diagnosis and classification of diabetes mellitus. Diabetes Care January 2006;29(1):s43-s48.

2. Kronenberg HM, Melmed S, Kenneth S, Polonsky, Larsen PR, editors. Williams textbook of endocrinology. Type 2 diabetes mellitus. 12th ed. Saunders Elsevier publishing Division; 2008:1371-1435

3. Kumar R, Abbas A, Fausto N, Aster JC, editors. Robbins and cotron "Pathologic basis of disease" 8th Ed. Saunders Elsevier Publishing Division; 2010.1131-1146.

4. Kronenberg HM, Melmed S, Kenneth S, Polonsky, Larsen PR, editors. Williams textbook of endocrinology. Complications of diabetes mellitus. 12th ed. Saunders Elsevier publishing Division; 2011:1462-1551

5. Sicree R, Shaw J and Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D, editor. Diabetes Atlas. In-ternational Diabetes Federation. 3rd ed. Belgium: In-ternational Diabetes Federation; 2006 p. 15-103.

6. Taniuchi K, Chifu K, Hayashi N, Nakamachi Y, Yamaguchi N and Miyamato Y: A new enzymatic method for the determination of sialic acid and its application as marker of acute phase reactants. Kobe J Med Sci 27:91–102, 1981 #.

7. Shahid SM, Mahaboob T. Clinical correlation between frequent risk factors of diabetic nephropathy and serum sialic acid. Int J Diabetes and Metabolism 2006;14:138-142

8. Mohammad JS, Muhammad TM, Ahmad M, Riaz M, Umair M. Serum Sialic acid concentration and type 2 DM. Professional Med

J Dec 2006;13(4):508-510.

9. Powers AC. Diabetes Mellitus, Chapter 338. In: Fauci AS, Braunwal E, and   Eds. Harrison's Principles of In-ternal medicine, 17th edn. New York:MGrawHill2008: 2275 – 2304.

10. Zelmanovitz T, Gerchman F, Balthazar AP, Thomazelli FC, Matos JD, Canani LH. Diabetic nephropathy. DiabetolMetabSyndr. 2009; 1:10

11. Medical text book of Biochemistry. Dominiczak. Third Edition

12. Salzler M, Crawford JM, Maitra A, Kumar V. Robbins Basic Pathology. 8thed. Elsevier publishers; 2009.

13. Kumar R, Abbas A, Fausto N, Aster JC, editors. Robbins and cotron "Pathologic basis of disease" 8th Ed. Saunders Elsevier Publishing Division; 2010.2197-2200.

14. Kahn SE, Hull RL: mechanisms linking obesity to insulin resistance and type 2 diabetes: nature 2006; 444:840

15. Long YC, Zierath JR: AMP-activated protein kinase signalling in metabolic regulation. J Clin Invest 2006; 116:1776

16. Vasudevan DM, Srekumari S. Text Book of Bio-chemistry for Medical Students. 5th ed. Jaypee brother's medical publishers (P) Ltd; 2007.

17. Bender DA, Mayes PA. Gluconeogenesis & the Control of blood Glucose. Harper's Illustrated Biochemistry 27th ed. Mc Graw-hill publisher; 2006.

18. Berg JM, Tymoczko JL, Stryer L Biochemistry 4th ed. W. H. Freeman and Company. New York; 1995.

19. Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ, editors. Joslin's Diabetes mellitus. 14th ed. South Asian: Lippincott Williams and Wilkins 2010:796-808, 854-866.

20. Colledge NR, Walker BR, Ralston SH editors. Davidson's principles andpractice of medicine 21st ed. Churchill Livingstone Elsevier;2010.

21. salah R. ben salehhamed, pajikadiabetologia 1993;36;948-984

22. diabetoligia. 1993;36:1017-1020

23. diabetologia. 1994; 37:1251-1258

24. nature. 2001; 414:782-787

25. cooper ME. Pathogenesis, prevention and treatment of diabetic nephropathy. Lancet 1998;352:213-219.

26. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease: with emphasis in the stages of incipient diabetic nephropathy. Diabetes.1983;32:64-78

27. prajna k, ashokkumar j, shobhithkumar Shetty; journal of clinical and diagnostic research. 2013 nov, vol-7(11): 2435-2437

28. Evans TC, Capell P. Diabetic Nephropathy. Clinical Diabetes 2000;18(1).