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SERUM OSTEOGLYCIN ASSAY AS AN EARLY MARKER IN DIAGNOSIS OF DIABETIC NEPHROPATHY AND ITS CORRELATION WITH DIFFERENT CKD STAGES

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ABSTRACT

Context: Glomerular impairment is also evident in diabetic normoalbuminuric patients, which might progress to advanced stages of CKD. Studies on osteoglycin which is an SLRP suggested that it has a role in pathogenesis of Nephropathy and its level could be assessed as a viable marker for diagnosing and identifying progression of nephropathy. **Aims:** To Assess and correlate the relation between levels of serum osteoglycin as a potential novel biomarker in the early diagnosis of Diabetic Nephropathy and correlating it with different CKD stages. **Settings and Design:** Cross sectional observation study. **Material and Methods:** It was conducted on 120 eligible subjects, who are divided into control and diabetes groups, subjects in diabetes group are further divided into 3 groups based on urine microalbumin creatinine ratio as normoalbuminuric, microalbuminuric and microalbuminuric and they are further divided into 5 CKD stages based on their eGFR calculated by CKD EPI equation. Serum osteoglycin levels were estimated in all the groups using an ELISA kit. **Statistical analysis used:** Comparison among different diabetes and control group was done using ANOVA (Analysis of variants) followed by post hoc comparison, ROC curve, multivariate analysis. **Results:** Our study showed that Serum osteoglycin levels can detect microalbuminuria in diabetes group with a sensitivity of 83.78% and specificity of 86.67% and its levels were low in stage 1 CKD they increase up to stage 3b CKD and as the disease progresses, they are decreased in stage 4 and 5 CKD. **Conclusion:** Osteoglycin has the potential to be an early marker for screening diabetic patients who are at risk for developing nephropathy and might also be helpful in assessing the progression of nephropathy as the changes in its levels were evident in different CKD stages However, as studies on osteoglycin levels in diabetic nephropathy are limited understanding the exact mechanism requires further research to establish it as a novel biomarker.

KEYWORDS: Osteoglycin, diabetic nephropathy, microalbuminuria, Chronic kidney disease, Biomarker, SLRP-small leucine rich peptide.

INTRODUCTION

Diabetic kidney disease is a complex and heterogeneous disease, which has various superimposed etiopathological pathways including changes in glomerular hemodynamic, oxidative stress, inflammation, and interstitial fibrosis and tubular atrophy.[11] Microalbuminuria is considered to be an early marker of diabetic nephropathy. However glomerular impairment is also evident in diabetic normoalbuminuric patients as shown by some contemporary studies[8] which might progress to advanced stages of CKD before the onset or sometimes without even development of albuminuria. moreover, evidence has shown that albuminuria may regress even from the severely increased range. Lately studies on osteoglycin which is a small leucine-rich proteoglycan suggested that it has a role in pathogenesis of Nephropathy, and that osteoglycin levels could be assessed as a viable marker for diagnosing and identifying diabetic nephropathy in its early stages.[1,2]

Recent data also revealed that osteoglycin is associated with the development of atherosclerosis, neovascularization, and angiogenesis.[6] Renal artery atherosclerosis and damage to vascular endothelium are important pathological mechanisms leading to diabetic nephropathy. Hence it is hypothesized that Osteoglycin has a role in pathogenesis and progression of diabetic nephropathy. However only limited research and data is available on assessing the relationship between

Osteoglycin levels and diabetic nephropathy. Therefore, it is needed to further investigate and study the correlation between Osteoglycin and diabetic nephropathy, and to determine the relevance of Osteoglycin levels in the early diagnosis and monitoring of diabetic nephropathy.

SUBJECTS AND METHODS

Type of Study: Hospital based cross-sectional case control study.
Source of Data: Patients attending JSS Hospital General medicine / Nephrology OPD or admitted in general medicine or nephrology departments during the study period from January 1st 2019 to July 2020 fulfilling the inclusion and exclusion criteria.
Study Setting: JSS Hospital and Medical College.
Duration of Study: 18 MONTHS.

NUMBER OF SAMPLES

Taking the prevalence of diabetic nephropathy as 8% in a general population with sample size of 120, this is a comparative study with a purposive sampling. This study includes a total of 120 subjects contained those with type 2 diabetic mellitus (T2DM, n = 97), and controls (n = 23). Based on urine albumin/creatine ratio diabetic mellitus group was classified into patients with normoalbuminuria (n=30), Patients with microalbuminuria (n=37), Patients with macroalbuminuric (n=30). Based on their eGFR the subjects were further classified into 5 subgroups depending on their CKD stage.

Sample selection criteria

Inclusion criteria

- All subjects above the age of 18 years of who have been diagnosed as T2DM According to American diabetic association criteria (FBS ≥126mg/dl, PPBS ≥ 200mg/dl, RBS ≥ 200mg/dl)
- Patients with diabetes with albuminuria who are classified based on urine albumin creatinine ratio into normoalbuminuria, microalbuminuria and macroalbuminuria group.
- Healthy nondiabetic patients without albuminuria

Exclusion Criteria

- Patients who have or admitted with acute renal or hepatic or cardiac dysfunction.
- Patients with history of trauma to the bony or bony fracture within the past 3 months
- Patients diagnosed to have an autoimmune disease.
- Patients diagnosed with malignant cancer.
- Patients admitted with acute severe infection.
- Patients who have metabolic disturbances like ketoacidosis.
- Patients not giving consent.

Study setting and Method of collection of data

Patients presenting to JSS Hospital General medicine / Nephrology OPD or admitted under General medicine / Nephrology departments and fulfilling the inclusion and exclusion criteria. Such patients were followed up clinically and through lab investigations. Based on urine albumin and creatinine ratio patients were assigned to normal/ micro/ macroalbuminuria groups. Serum osteoglycin concentration was determined using an ELISA kit.

The study was carried out after approval from the institutional ethical committee and with fully informed written consent from the subjects.

Clinical Data and Laboratory Investigations

The medical history of subjects like duration of diabetes and hypertension were recorded,

Blood pressure is measured by mercurial sphygmomanometer in the resting state,

Fasting blood sugars, post prandial blood sugars, Hba1c levels

Renal function tests
Urine albumin levels / spot urine microalbumin/creatinine ratio

eGFR was estimated by using the CKD-EPI formula to the Serum creatinine levels.

Serum osteoglycin concentration was determined using an ELISA kit in accordance with the protocols issued by the manufacturer.

Data Analysis

The collected data entered into MS Excel followed by the analysis using SPSS version -23.

The demographic characters are represented using analytical means with standard deviation and percentages. Bar diagram, pie diagram is used wherever necessary.

Comparison among different albuminuria and CKD categories was done using ANOVA (Analysis of variants) followed by post hoc comparison, the test will be fixed only after finding out the normality of data.

All the possible association between the demographic characteristics and the outcome was found out using Chi square test, a p value < 0.05 will be considered as statistically significant.

Estimation of serum osteoglycin levels

Serum osteoglycin concentration was determined using an ELISA kit following the manufacturer's protocols (Elabscience Biotechnology Co, Limited, 14780 Memorial Drive, Suite 216, Houston, Texas 77079.) and a TECAN Infinite F50 enzyme mark analyser (Tecan, Männedorf, Switzerland). OGN levels were calculated based on the corresponding absorbance values of the standard curve generated using the ELISA kit OGN.

RESULTS

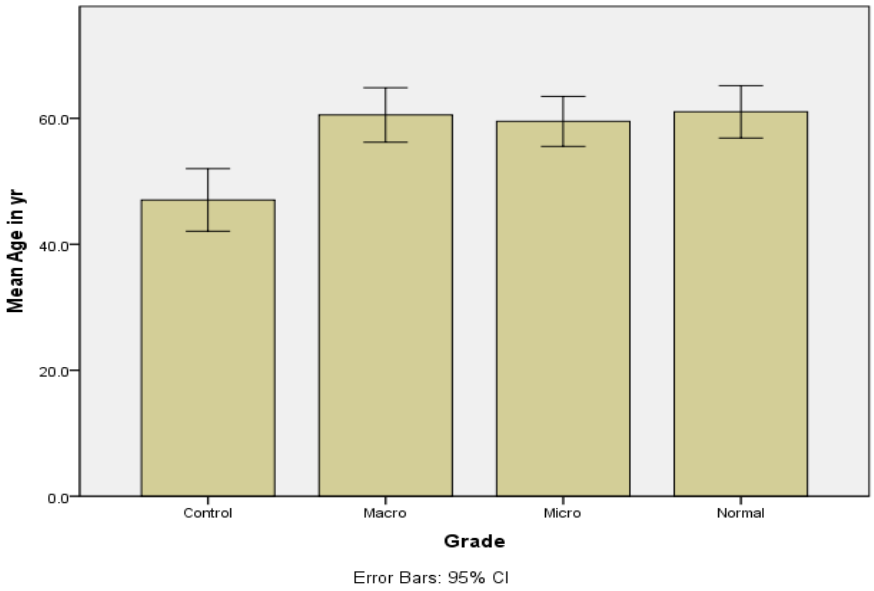

Figure I: Demography Data Representing Age Groups.

eGFR of the subjects was estimated by using the CKD-EPI formula to the Serum creatinine levels, and are grouped into following as shown.

Table I: Data Showing Classification of Subjects by egfr. (in ml/min/1.73m^2).

		Group			
		Control (Non-Diabetic)		DM	
		Tally	N %	Tally	N %
CKD Stage	1	17	73.9%	30	30.9%
	2	6	26.1%	23	23.7%
	3A	0	.0%	13	13.4%
	3B	0	.0%	11	11.3%
	4	0	.0%	13	13.4%
	5	0	.0%	7	7.2%

P=0.002

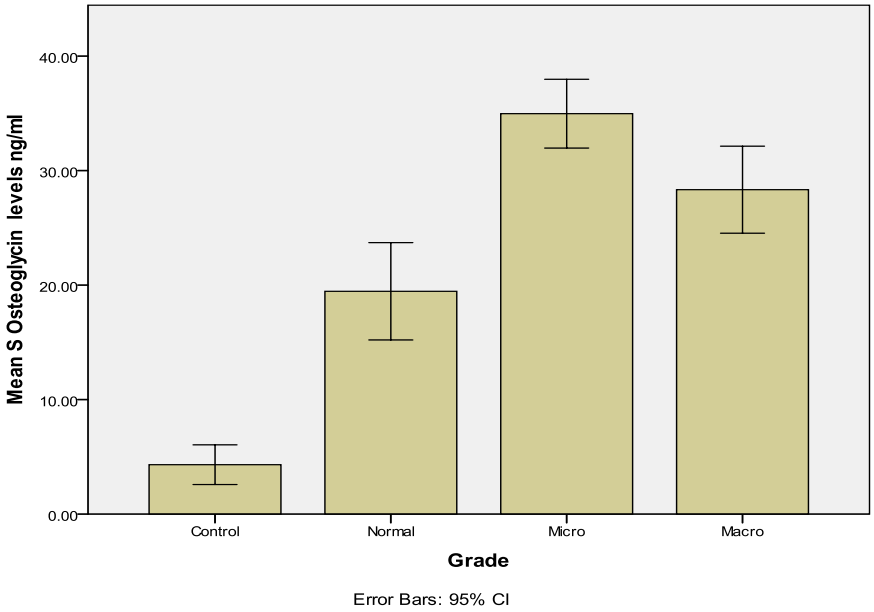

Figure II: data showing comparison of s.ogn levels with albuminuria.

Table Ii: Data Showing Comparison of S.Ogn Levels With Albuminuria.

		S Osteoglycin levels in ng/ml			
		Count	Column N %	Mean	Standard Deviation
Grade	Control	23	19.2%	4.31	4.01
	Normal	30	25.0%	28.33	10.18
	Micro	37	30.8%	34.97	9.01
	Macro	30	25.0%	19.46	11.39

P<0.0001

As per this study it shows that mean S.OGN levels were highest in microalbuminuria group reduced in macroalbuminuria and control groups and elevated in normoalbuminuria group and elevation is less as compared to micro group. The p value is <0.0001 which is statistically significant.

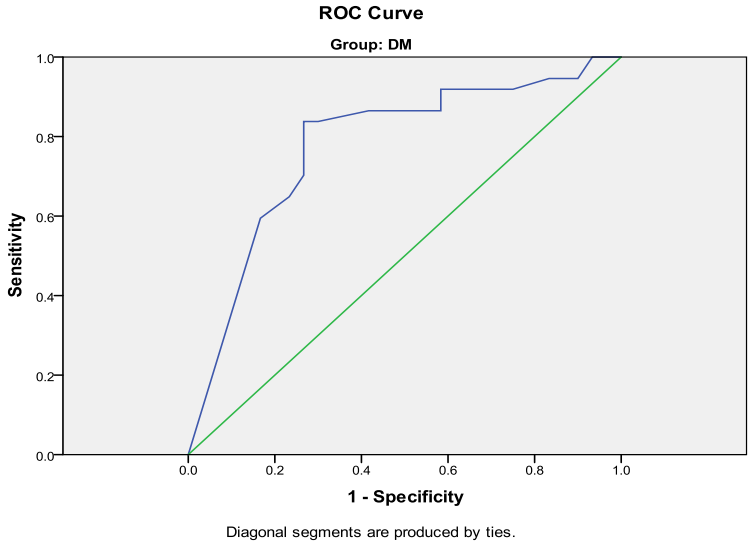

Figure Iii: Roc Curve To Descriminate Microalbuminuira From Normoalbuminuira In Diabetes Group.

Table iii: Cut off To Discriminate Microalbuminuria And Normoalbuminuria.

				Micro albuminuria	Normoalbuminuria
				Count	Count
	DM,	S.OGN LEVELS	>32ng/ml	31	4
			<32ng/ml	6	26

ROC curve analysis showed that a cut off of 32.0ng/ml and it demonstrates that in diabetes group, Subjects with Serum Osteoglycin levels more than 32ng/ml are likely to be having microalbuminuria. This is with a sensitivity of 83.78% and specificity of 86.67%.

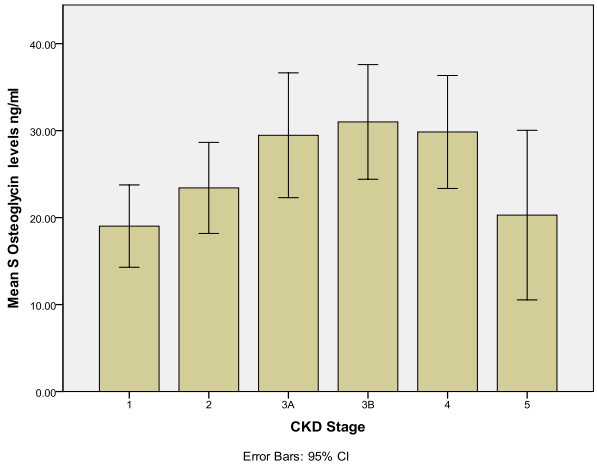

Figure Iv: Data Comparing S.Ogn Levels in Each Ckd Stage.

Table IV: Data Comparing S.Ogn Levels In Each Ckd Stage.

		S Osteoglycin levels in ng/ml			
		Tally	N %	Mean	Standard Deviation
CKD Class	1	47	39.2%	19.02	16.11
	2	29	24.2%	23.41	13.76
	3A	13	10.8%	29.46	11.87
	3B	11	9.2%	31.00	9.81
	4	13	10.8%	29.85	10.75
	5	7	5.8%	20.29	10.55

P=0.023

As per this study it shows that OGN levels rise until CKD stage 3b and is decreased in stage 4 and stage 5 CKD.

DISCUSSION

While the gold standard for diagnosis of diabetic nephropathy[3] is defined by histology of the kidney, it is unnecessary to undergo invasive procedure that may not alter treatment, the limitations of albuminuria as the "earliest" marker of diabetic kidney disease have led many experts to propose and determine an earlier and more sensitive marker. Lately studies on one SLRP-osteoglycin (OGN)[4] suggested that it has a role in pathogenesis of Nephropathy, and that osteoglycin levels could be assessed as a viable marker for diagnosing and identifying diabetic nephropathy in its early.

Our present study is conducted primarily in diabetic subjects, and it is aimed to measure and correspond the levels of Serum osteoglycin with albuminuria and we further correlated its levels with CKD stage (which was calculated using CKD-EPI equation) and the following observations were made as per our study.

Mean Serum Osteoglycin levels in our study were higher in diabetes group as compared to control group. When comparing Serum Osteoglycin levels with albuminuria in this study it is evident that Serum osteoglycin levels were highest in microalbuminuria group when compared to other groups. In subjects with normoalbuminuria group Serum osteoglycin levels were elevated but not as much as in microalbuminuria group. When an ROC curve was plotted and a cut off of 32ng/ml was taken Serum osteoglycin levels as a biomarker can detect microalbuminuria in diabetes group with a sensitivity of 83.78% and specificity of 86.67%. However, ROC curve analysis plotted for detecting macroalbuminuria in this study showed Serum Osteoglycin as not a worthy marker for detecting macroalbuminuria.

When correlating Serum Osteoglycin levels with CKD stage it was observed in this study that Serum osteoglycin levels were low in stage 1 CKD they were elevated up to stage 3b CKD and its levels were decreased in stage 4 and stage 5 CKD. Thus, as per this study as eGFR decreases Serum Osteoglycin levels increase until stage 3b CKD and with further decline in eGFR Serum Osteoglycin levels also decrease, hence it can be understood that Osteoglycin levels are closely related with renal function and that change in osteoglycin levels and decline in the late CKD stages can be helpful in predicting the progression of diabetic nephropathy in such patients.

In a study of diabetic mice decrease of Osteoglycin levels was described in the kidneys of diabetic mice with advancement of complications and development of late stages of diabetic nephropathy, showing that Osteoglycin may be associated with the pathogenesis progression and development of diabetic fibrotic complications.[6]

From the above data it can be hypothesized that in the initial stages of diabetic nephropathy with inflammation, tissue remodeling and renal ultrafiltration there might be elevation in osteoglycin levels and when fibrosis and late complications set in there is decrease in osteoglycin levels which is in accordance with our study. However, the exact mechanism, clear cut demarcation and association of osteoglycin in pathogenesis of diabetic nephropathy is not known and further research is required in this aspect.

In one prior study WEI's study[1] done in a tertiary care hospital in Fujian province of china showed that serum Osteoglycin can be a Prominent diagnostic marker of diabetic microalbuminuric patients, In this study in subjects with microalbuminuria osteoglycin levels were >275.00pg/ml and <367.50pg/ml and cut off of 343.4pg/ml is taken for predicting microalbuminuria, and there was positive correlation between osteoglycin levels and eGFR and osteoglycin levels were reduced in end stage kidney disease, These findings were similar to our study.

There were certain differences with our study no of controls in our study were less as compared to WEI's is study and mean Serum Osteoglycin levels were more in control group in WEI's study. CKD stage at which osteoglycin levels were decreased was not specified in WEI's stage, the differences might be due to because of selecting different racial populations and lab errors.

In other study Wang's study[2] done in a tertiary care hospital in Henan province of china stated that Osteoglycin was elevated in patients with diabetic nephropathy and it is more increased in macroalbuminuria group followed by microalbuminuria and then normoalbuminuria, which is contrary to outcome of our study. There were differences between the two studies like the duration of diabetes and diabetes nephropathy was different in the patients in the two studies, it was carried out in Chinese population and this was on Indian population and in Wang's study overall eGFR in macroalbuminuria group was higher and wang dint specify among which CKD stage groups Osteoglycin levels were elevated.

The small-scale cross-sectional case control study, carried out at a single center and with a comparatively smaller number of subjects, tells that a study with prolonged follow-up and larger metanalysis studies will be needed to assess the long-term predictive use of Osteoglycin. Furthermore, research is necessary to determine the mechanism of association of osteoglycin with diabetic nephropathy and establishing it as a novel marker for early diagnosis of diabetic nephropathy.

LIMITATIONS

1. The sample size of this study was limited due to the constraints in the possible study period that could be undertaken. This precluded us from getting statistical significance when it came to certain variables associated the severity of diabetic nephropathy.

2. All study subjects were sourced from a single tertiary care hospital.
3. It is cross sectional study with no follow up involved.
4. The ELISA kit used has the detection range of only 0.63-40 ng/mL and any osteoglycin level >40ng/ml is detected as 40ng/ml

RECOMMENDATIONS

We discern that more extensive studies be conducted on the Indian population with regard to better and novel markers for early diagnosis of diabetic nephropathy, with larger sample sizes and follow up which would greatly advance our knowledge in the therapeutic management of this disease and its prognostication.

Serum Osteoglycin levels could be a vastly helpful marker in the early diagnosis, prognosis and monitoring the patients with diabetic nephropathy which will aid in appropriate therapeutic management.

CONCLUSION

Osteoglycin has the potential to be a novel marker for screening diabetic patients and might also be helpful in assessing the progression of nephropathy as per our study the changes in the osteoglycin levels were evident in different CKD stages However, as studies on osteoglycin levels in diabetic nephropathy are limited understanding the exact mechanism requires further research to establish it as a novel biomarker.

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