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BIOMARKERS FOR EARLY DETECTION OF KIDNEY INJURY AMONG TYPE 2 DIABETES MELLITUS PATIENTS.

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ABSTRACT: Chronic diabetes is relative with damage, dysfunction, and failure of several organs, especially the eyes, nerves, foot, blood vessels, kidneys, and heart. The microvascular complications of diabetes induce to renal damage known as diabetic nephropathy (DN), the most common complication of type 2 diabetes mellitus.

AIM: Biomarkers For Early Detection Of Kidney Injury Among Type 2 Diabetes Mellitus Patients.

METHODOLOGY: 89 patients with Type 2 DM who fulfil the inclusion and exclusion criteria

RESULTS :89 subjects with Type 2DM are enrolled in the present study to evaluate early biomarkers in the detection of kidney injury. Female predominance observed. Mean levels NGAL , Cystatin C, KIM1 and B2MG are elevated in all patients . NGAL , Cystatin C, KIM1 showed high sensitivy with low specificity but B2MG had high specificity than sensitivity. There is correlation among biomarkers and eGFR , albuminuria .

CONCLUSION: The present study proves none of the biomarkers can be considered superior to the others in terms of diagnostic ability . So all these Bio markers predicts early renal involvement and also progression of illness beyond serum creatinine and urinary albumin.
KEYWORDS: Type 2 DM, NGAL, B2MG, KIM1,Cystatin C

INTRODUCTION:

Type 2 diabetes mellitus is a pathology of heterogeneous etiology characterized by hyperglycemia resulting from defects of insulin action, insulin secretion, or both, [1] and the population with diabetes mellitus is predicted to be about 439 million worldwide by 2030. [2] Chronic diabetes is relative with damage, dysfunction, and failure of several organs, especially the eyes, nerves, foot, blood vessels, kidneys, and heart . [3] Prolong diabetes has been related with microvascular complications such as retinopathy, neuropathy, and nephropathy. [4]

The microvascular complications of diabetes induce to renal damage known as diabetic nephropathy (DN), the most common complication of type 2 diabetes mellitus, [5] and it is the leading cause of end-stage renal disease worldwide, which is associated with high morbidity and mortality. [6] It develops in approximately 40% of patients with diabetes, [7] after

10 years of type 2 diabetes mellitus were diagnosed. [4] DN is characterized by persistent albuminuria (or albuminuria excretion rate of >300 mg/d or 200 μg/min) measured at least twice within three to 6 months interval, progressive decreasing in glomerular filtration rate (GFR), [8] which often occur in association with an elevate in blood pressure, ultimately leading to end-stage renal disease. [9]

It is critical to diagnose patients who are more sensible to develop DN for better control of the process of disease. Several factors and mechanisms enhance to the development and outcome of diabetic nephropathy. Albuminuria has been one of the biomarkers to screen renal function and it has generally been examined to primarily reflect glomerular injury and increased glomerular permeability to macromolecules. However, it may not be detectable in early stage. Albuminuria has lots of limitations such as larger variability and low sensitivity, it cannot predictably lead to a renal outcome nor are specific for DN. There are several significant kidney damage and disease biomarkers which helps in early detection of DN. [7]

An early biomarker may allow earlier diagnosis, treatment reduces DN prevalence and slows DN progression, thereby raising life expectancy among people with diabetes while increasing healthcare spending by less than one percent. [10]

Cystatin C, a 13 kDa cysteine protease inhibitor, a small protein that freely filtered by the renal glomeruli, is a novel biomarker of kidney failure. Cystatin C has been associated with the decrease of eGFR and predictors of the progression of type 2 DN. [11]

Neutrophil gelatinase-associated lipocalin (NGAL)L is a 25-kDa molecule which was produced in the distal nephron and increased in response to kidney injury within a few hours after damaging. [11] NGAL excretion in serum and urine were found to be early predictive biomarkers of acute kidney injury. [12] The appearance of NGAL in the urine may indicate early glomerular injury, and this has been showed at earlier stage than the appearance of microalbuminuria, the gold standard marker for early DN. [13]

The best cut-off value of NGAL for early detection of DN is 77.72 ng/mL with sensitivity 96%, specificity 80%, PPV 82.6%, NPV 95.24%, and accuracy 88%. [14]

Kidney injury molecule-1 (KIM-1) is a Type I transmembrane glycoprotein expressed on renal proximal tubule epithelial cells and plays an important role in renal tubulointerstitial damage.[15] Studies indicate that KIM-1 is a sensitive and specific marker of kidney injury as well as a predictor of prognosis.[16] There are many studies that have shown that urinary KIM-1 is an early marker of acute kidney injury (AKI)/CKD, but very few studies have evaluated the usefulness of blood KIM-1 as an early marker of renal damage is inconclusive.[17,18] Thus, the present study evaluated the usefulness of serum KIM-1 as a marker of DN.

Serum β2 microglobulin is the light chain in the major histocompatibility complex (MHC) class I molecule [19]. It is widely distributed in all nucleated cells in the body [20]. Under

normal physiologic conditions, β2 microglobulinis produced at a constant rate and normal β2 microglobulin concentration is 1.5-3 mg/L [21]

While, Inker et al. established that serum β2 microglobulin is a novel endogenous filtration marker and developed a glomerular filtration rate estimating equation by using serum β2 microglobulin [22]. Recently it was reported that serum β2 microglobulin is an early predictor of renal function [23]. Serum β2 microglobulin exclusively eliminated by glomerular filtration and has been used to determine the estimated glomerular filtration rate (e-GFR) [24]. It was reported that, high level of serum β2 microglobulin has the higher prevalence of diabetic nephropathy compared with low level of β2 microglobulin with normal renal function [24].

With this background our study is taken up the role of different biomarkers in establishing early renal dysfunction and the predictive value of each biomarker and relation with each one in establishing renal disease in Type2 DM.

Materials and Methods:

Study Design: Prospective analytical study.

Study Period : 1year between Dec 2023 – Dec 2024

Sample Size: 89 subjects

Place of Work: Department of General Medicine, SVRRGGH,SVMC,Tirupati.

Sample selection:

- **INCLUSION CRITERIA :**
  Patients with Type II Diabetes Mellitus who are willing to give written informed consent .
- **EXCLUSION CRITERIA :**
  1. Patients diagnosed previously with any cancer, MI, stroke, peripheral arterial disease, thyroid disorders, UTI.
  2. Patients who are in sepsis and critically ill.
  3.Patients on steroids or any drugs which are prone to cause proteinuria.
  4.Patients previously diagnosed with nephropathy and CKD.

Methodology:

- Detailed History and Physical examination and GFR calculation is done according to cockcroft-gault formula. All patients are subjected to do– FBS/PPBS, urine albumin level ,urine 24 hour protein levels , serum creatinine ,serum total protein and serum albumin, USG abdomen ,thyroid profile, serum Cystatin C , Beta 2 microglobulin, NGAL and KIM-1 .

RESULTS : 89 subjects with Type 2DM are enrolled in the present study to evaluate early biomarkers in the detection of kidney injury.

1. **AGE AND GENDER DISTRIBUTION**:

63% of subjects are between the age group of 51-70 years followed by 14.6% in 41-50 and above 70 years age group. 2.2% subjects are less than 30 years of age. 64% are female and 36% are male population with female to male ratio of 1.7:1

2. **MEAN SERUM LEVELS OF BIOMARKERS**:

KIM1,NGAL and B2 Microglobulin levels are elevated in all patients. 44% of patients had raised Cystatin C levels. The mean values of all parameters are higher in females compared to males.

3. **e GFR IN RELATION WITH BIOMARKERS:**

There is a lenior relation between eGFR and mean values of NGAL, Cystatin C and KIM1. These levels are decreased with decreasing e GFR but not statistically significant. There is no similar patterns associated with B2 microglobulin where B2 microglobulins are elevated with decreasing eGFR.

post hoc test analysis shows with in the groups of eGFR also there is no significant change between the biomarkers .

4.URINARY PROTEIN LEVELS WITH BIOMARKERS:

There is no statistical significance between various levels of urinary protein and each biomarker. Post hoc analysis shows with in the group also there is no significance in biomarker levels.

5. **CUT OFF VALUES OF BIOMARKERS:** NGAL, KIM1 and Cystatin C had more sensitivity with low specificity . B2MG had high specificity with low sensitivity.

6.ROC STATISTICS:

AUC **Value**s range from 0 to 1, with higher **Value**s indicating better diagnostic performance. For all biomarkers it is in the mid range (**NGAL1**: AUC = 0.528, **Cystatin_C1**: AUC = 0.514,**B2Microglobulin1**: AUC = 0.503,**KIM1**: AUC = 0.512). A smaller SE suggests that the AUC estimate is more precise. The SE AUC **Value** for Cystatin_C1 is 0.06473, having less SE AUC **Value** compared to other bio marker.

Cystatin_C has a confidence interval from 0.38695 to 0.64068, which has the narrower confidence interval as compared to others. Narrower confidence intervals indicate more precise estimates. The Z-scores for all the markers are close to zero, suggesting no significant deviation from the mean. The p-**Value**s for all the biomarkers are above 0.05, indicating that the results are not statistically significant.

7. MULTIPLE COMPARISONS OF BIOMARKERS:

The p-**Value**s for all the biomarkers are above 0.05, indicating that the results are not statistically significant. So this indicates none of the biomarker are statistically significant which means none of the biomarkers can be considered superior to the others in terms of diagnostic ability, as the differences are not statistically significant.

DISCUSSION : 63% of subjects are between the age group of 51-70 years. 2.2% subjects are less than 30 years of age. In the present study female to male ratio is 1.7:1. KIM1,NGAL and B2 Microglobulin levels are elevated in all patients. 44% of patients had raised Cystatin C levels. There is a lenior association between eGFR and mean values of NGAL, Cystatin C and KIM1. These levels are decreased with decreasing e GFR but not statistically significant. There is no similar patterns associated with B2 microglobulin.

KIM1,NGAL and B2 Microglobulin levels are elevated in all patients. 44% of patients had raised Cystatin C levels. The mean values of all parameters are higher in females compared to males. The sensitivity of biomarkers are 85.7% for NGAL, 89.3% for both Cystatin C and KIM1. 96.2% specificity is observed with B2MG.

Study done by KIM S.S et al , they observed both serum and urinary Cystatin C levels were associated with both decreasing in eGFR and progression of kidney disease.[25]

JEON et al observed in their study Cystatin C levels increased with increasing stages of CKD and also seen increased with albuminuria patients [26]

Kaul A et al demonstrated that NGAL allows the early detection of Diabetic nehropathy and predicts the appearance of albuminuria.[27]

A recent meta-analysis that included 19 studies found that serum NGAL had a pooled sensitivity of 0.79 (95% confidence interval [CI] 0.60–0.91) and a specificity of 0.87 (0.75–0.93). These results indicate that NGAL can be useful for classifying DKD and can provide an added diagnostic value in the group of patients with normoalbuminuric kidney disease [28].

According to Nowak N et al , 462 type 1 DM patients were included , of whom 259 were normoalbuminuric and 203 of had microalbuminuria, plasma KIM-1 levels predicted an early reduction in eGFR and the progression of kidney disease independent of other variables[29].

In a study done by SABBISETTI V.S et al , observed patients with type 1 diabetes mellitus and proteinuria, baseline serum KIM-1 levels were a strong predictor of eGFR loss and ESKD during the 5 to 15 years of follow-up after adjusting their values for baseline urinary albumin-to-creatinine ratio levels, eGFR, and Hb1Ac [30]

KAMAL M *et al* The mean serum β2 microglobulin level was significantly higher in patients with diabetic nephropathy. There was significant positive correlation between serum β2 microglobulin with serum creatinine and with urinary microalbumin), but a significant negative correlation with e-GFR . The best cut-off point of serum β2 microglobulin for diabetic nephropathy was 4.35 μg/ml with 93.3% sensitivity and 80.0% specificity[31]

Zainab A. Hussein et al observed that, the serum β2M of normoalbuminuria group was (2.86±0.95 μg/mL), microalbuminuria group was (5.06±1.97 μg/mL) and macroalbuminuria group (3.6±1.59 μg/mL). The results showed significant increase (p<0.05) in the β2M level of microalbuminuria group when compared with that of normoalbuminuria and macroalbuminuria groups. In addition, a highly significant increase (p<0.01) in β2M concentration was observed in microalbuminuria group when compared with that of the control group.[32]

According to MK KIM et al The prevalence of diabetic retinopathy and nephropathy were significantly higher with a high B2M than with a low B2M. The multiple adjusted OR for diabetic nephropathy was 2.29 (95% CI: 1.11–4.72) per 1 mg/L increase of B2M.[24]

CONCLUSIONS: Novel biomarkers that detects early tubular interstitial changes of kidney may prove to be better predictors of early renal involvement in Diabetic patients. The present study proves high levels of serum Biomarkers KIM1,NGAL, Cystatin C and B2MG in patients with Type2 DM with out increasing urinary albumin and changes in the egfr. Cystatin C has more precise when compared to others, but the study proves none of the biomarkers can be considered superior to the others in terms of diagnostic ability . So all these Bio markers predicts early renal involvement and also progression of illness beyond serum creatine and urinary albumin.

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Statement and declarations:

Authors' Contribution:

1. Study concept and design: Dr.M.Ramadevi
2. Acquisition of data: Dr. M.Ramadevi
3. Analysis and interpretation of data: Dr.M.Ramadevi
4. Drafting of the manuscript: Dr,M.Ramadevi
5. Critical revision of the manuscript for important intellectual content: Dr.S. Suneetha
6. Statistical analysis: DR. Vishnu vardhan, Mr.Kutti kumar
7. Administrative, technical, and material support: Dr.P.Suresh, Dr.C.Madhusudhana
8. Study supervision: MRU, SVMC

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Competing Interest: Nil

Data sharing and Data availability: Data collected ,analysed and stored in the system.

Ethical Approval statement: Obtained Institutional Ethical Committee approval

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