DOI: 10.7860/JCDR/2021/48085.15100

Original Article

Biochemistry Section

Association of Diabetic Kidney Disease Markers and Urinary Beta-CrossLaps in Type 2 Diabetes

LALITHAMBIGAI ARUMUGASAMY[1], HETAL G PATEL[2]

ABSTRACT

Introduction: Diabetic Kidney Disease (DKD) is a chronic complication in Type 2 diabetes. The Chronic Kidney Disease (CKD273) peptide classifier has been found to predict development of DKD even before microalbuminuria develops. Seventy four percent of peptides in the CKD273 classifier are Collagen degradation fragments. The Beta-CrossLaps (β-CTx) Enzyme Llinked Immunosorbent Assay (ELISA) assay detects the specific collagen degradation product, C terminal telopeptide of Type 1 collagen. In light of the Capillary Electrophoresis/Mass Spectrometry (CE-MS) findings, linking collagen degradation fragments excretion to early detection of DKD, the significance of urinary β-CTx levels as a DKD biomarker needs to be evaluated.

Aim: To study the urinary excretion of β-CTx in type 2 diabetes patients and to evaluate its relation to microalbuminuria status and estimated Glomerular Filtration Rate (eGFR) of the patients.

Materials and Methods: This descriptive cross-sectional study was undertaken at a tertiary care hospital, with enrollment of 82 type 2 diabetes patients from the diabetes Out Patient Department (OPD). Participants were divided into groups based on their Urinary Albumin Creatinine Ratio (UACR) and eGFR levels. The study participants were tested for Urinary β-CTx level, UACR and eGFR. Mean or median was calculated for the parameters with normal and non-normal distribution, respectively. All statistical testing was performed on online calculators available at the site; https://www.socscistatistics.com/.

Results: The median urinary β-CTx level observed was 100.6 ng/mmol of creatinine. Among the 82 participants, 15 participants had urinary β-CTx level 15 pg/mL, the sensitivity of the kit. Among the remaining 67 participants, the minimum Urinary Beta-CrossLaps: Creatinine ratio observed was 2.6 ng/mmol and the maximum value observed was 2071 ng/mmol (i.e., 2.1 µg/mmol). The median urinary β-CTx level was highest (100.6 ng/mmol creatinine) in the patient group with eGFR in the normal range. The urinary β-CTx level was found to decline with decline in eGFR, with median urinary β-CTx 65.5 ng/mmol creatinine in the patient group with mildly decreased eGFR and 7.2 ng/mmol creatinine in the patient group with moderately decreased eGFR.

Conclusion: The Urinary β-CTx concentration in type 2 Diabetes patients is dispersed over a wide range. The Urinary β-CTx concentration correlates with the eGFR of the patient and is not influenced by age, gender or duration of diabetes. This parameter is a potential early DKD biomarker.

Keywords: Collagen degradation products, Diabetic nephropathy, Urinary biomarker

INTRODUCTION

The worldwide prevalence of diabetes is 8.8% (95% confidence interval 7.2-11.3%) [1]. In India the prevalence of diabetes is 20% in urban areas and 10% in rural areas [2]. DKD is a complication of diabetes with prevalence of 20 to 40% among diabetes patients and is responsible for 50% of End Stage Renal Disease (ESRD) [3].

The American Diabetes Association (ADA) guidelines [4], recommend annual screening of diabetic patients for micro-albuminuria. The dependence on albuminuria as a marker of nephropathy is being debated now, due to observation of progressive deterioration of renal function and progress of chronic DKD, in significant proportion of diabetic patients in the absence of albuminuria [5,6].

The urinary proteome of diabetes patients has been assessed using mass spectrometric analysis to identify new biomarkers of DKD. The urinary proteome profile obtained is used to develop a classifier which may clearly differentiate between diabetes patients at low risk and high risk of progression to DKD [7-9].

Good DM et al., have described a 273 peptide classifier for prediction of development of CKD, irrespective of underlying pathology [10]. This classifier was applied to a cohort of diabetes patients, in whom it showed similar ability to predict development of DKD [8]. It resulted in early prediction of microalbuminuria by 3.4±2.1 years and macroalbuminuria by 4.9±2.2 years. The CKD273 classifier was identified by application of SVM methods on the urinary peptidome data obtained from healthy subjects and subjects with different types of biopsy proven kidney diseases. There are 273 peptides derived from 30 different proteins, in the CKD273 classifier. Of these 273 peptides, the majority (196 peptides) were derived from collagen type α 1(I), collagen type α 2(I) and collagen type α 1(III) [10]. The only other proteins contributing more than 10 peptides to the classifier are Uromodulin and alpha-1 Antitrypsin. The CKD classifier revealed that as kidney disease progresses the fragments of serum proteins in urine increases whereas the collagen degradation fragments decreased. Since the proportion of collagen fragments is significantly higher in the CKD classifier than the other peptides, the quantity of these collagen fragments in urine do contribute more to the diagnostic ability of the classifier [10].

The collagen fragments observed in urine are the product of degradation of collagen. The degradation of type 1 collagen results in the generation of two important bone turnover biomarkers CTX and NTX, representative of the C-terminal and N-terminal telopeptides of Type I Collagen [11,12]. As part of normal bone aging, the alpha form of aspartic acid in the C-terminal telopeptide of Collagen undergoes isomerisation to beta form and gives rise to β-CTx biomarker, also known as Beta-CrossLaps [13]. The CKD273 classifier relies significantly on Type I collagen degradation fragments, which are normally excreted in urine and the C-terminal telopeptide (CTx) or Beta-CrossLaps is one such fragment of collagen degradation [11,12].

The use of Capillary Electrophoresis-Mass Spectrometry (CE-MS) for predicting DKD in diabetics, in the routine clinical setting would

be costly and would require new equipment, which are not routinely available in clinical chemistry laboratories. Urinary β-CTx represents the renal handling of collagen degradation fragments, which make upto 74% of the peptides included in the CKD273 classifier [10]. So, this study was planned to evaluate the potential of urinary β-CTx as a DKD marker. Urinary β-CTx was chosen due to the ready availability of ELISA kits for testing their level in urine.

MATERIALS AND METHODS

This was a descriptive cross-sectional study conducted from March 2019 to October 2019. The study participants were selected from the patients attending the Diabetes OPD of Medicine Department, at GMERS Medical College and General Hospital, Gotri, Vadodra, India. The ethical approval for conducting the study was obtained from the Institutional Human Ethics Committee (IHEC). (The IHEC approval number is IHEC, 182/2017: Biochemistry 06/2017, dated 18/08/2017).

Inclusion criteria: The inclusion criterion was the patients diagnosed with Type 2 Diabetes as per the ADA diagnostic criteria [14], without any age or gender restriction.

Exclusion criteria: The exclusion criteria were existing advanced DKD (eGFR <30 mL/min), uncontrolled hypertension, history of Myocardial Infarction, hematuria, malignancy and pre-existing renal, liver and bone pathology. The reason for exclusion of patients with these conditions was to keep the study population homogenous and prevent presence of confounders and effect modifiers.

Study Procedure

The Type 2 diabetes patients attending the Diabetes OPD were invited to participate in the study. The details of the study were explained to them, including the samples which will be collected and the tests which will be performed. Informed consent was obtained from those willing to participate in the study.

This was the first instance of evaluation of Urinary Beta-CrossLaps in type 2 diabetes patients, so baseline data was unavailable to perform sample size calculation. Since the standard Beta-CrossLaps in ELISA kit contains 96 wells, it was decided to enroll patients on first come first serve basis. Eighty-two Type 2 diabetes patients were enrolled in the study. The demography variables of all study participants were recorded in the case record form, with a unique ID, to ensure patient confidentiality. The study participants were examined in the OPD and their Systolic and Diastolic blood pressure was recorded. The Mean Arterial Pressure (MAP) was calculated from this data. The participants were then sent to the central laboratory for collection of blood and urine samples. Two mL of blood was collected in plain vacutainer (red cap) for serum creatinine estimation. Wide mouth urine collection containers (volume=25 mL) were given to the patient for urine collection (done in the premises). The serum creatinine estimated was used to calculate eGFR value of each patient using the CKD-EPI equation [15].

The patients' urine samples were processed as described: An aliquot of 5mL of urine sample was transferred from the primary sample container into a labelled test tube (the enrollment serial number was used as the identifier). After centrifugation of the sample, urinary albumin estimation was performed using Immunoturbidimetry method, kit manufactured by Erba Diagnostics Ltd. Urine Beta-CrossLaps was estimated using ELISA method, kit manufactured by Immunotag Diagnostics. All chemistry analyses were performed in the clinical chemistry laboratory, on the Erba XL360 fully automated analyser.

Serum Creatinine estimations were done with reagent based on Jaffe kinetic principle, manufactured by Erba Diagnostics Ltd. The system was standardised with Erba Multical, ensuring traceability of creatinine estimations to Isotope Dilution-Mass Spectrometry (IDMS).

Urine creatinine concentrations were also estimated by Jaffe kinetic principle, using a reagent kit from Erba Diagnostics Ltd. The manufacturer's instructions were followed for the estimation process. The UACR was calculated using the values of urine albumin (mg/dL) and urine creatinine (g/dL), to obtain ratio as mg/g creatinine.

Urinary β-CTx was estimated by ELISA method, using kit manufactured by ImmunoTag Diagnostics. The process described in the kit insert was followed for the ELISA process. The urinary β-CTx values were adjusted for variable urine concentrations, by expression as urinary β-CTx creatinine ratio.

The correlation of urinary β-CTx excretion with UACR was studied by categorising the study population into three groups, Normoalbuminuric, Microalbuminuric and Macroalbuminuric based on the UACR cut-offs, <30 mg/g creatinine, 30 to 300 mg/g creatinine and >300 mg/g creatinine respectively [4].

Subsequently, the correlation of urinary β-CTx excretion with eGFR was studied by categorising the study population into three groups, normal renal function, mildly decreased renal function and moderately decreased renal function, based on eGFR cut-offs, >90 mL/min/1.73 m^2, 60-89 mL/min/1.73 m^2 and 30-59 mL/min/1.73 m^2, respectively [16].

STATISTICAL ANALYSIS

In case of parameters which showed normal distribution (age, MAP and eGFR) the mean and Standard Deviation (SD) were calculated and used as measure of central tendency and dispersion, respectively. In case of parameters which showed non normal distribution (Duration of diabetes and urinary Beta-CrossLaps) the median and Interquartile Range (IQR) were calculated and used as measure of central tendency and dispersion, respectively. The urinary Beta-CrossLaps: Creatinine ratio correlation with age of individuals, duration of diabetes and MAP, was tested using Spearman's Rho coefficient calculator. It's correlation with sex of individual was tested using Point Biserial Correlation Calculator, with urinary β-CTx as the continuous variable and the sex of the patient as the dichotomous variable. After division of the study population into sub-groups based on UACR and subsequently eGFR levels, ANOVA, Kruskal Wallis test and chi-square tests were used to determine significance of differences in mean, median and proportion, respectively, between the study groups.

RESULTS

The demographic data of the 82 study participants is given in [Table/Fig-1].

Sr. No.	Demographic parameter	Frequency (n=82)
1.	Mean age (in years) (SD)	51 (6.9)
2.	Gender distribution (M:F)	35:47
3.	Mean duration of (diabetes (in years) median and IQR)	3 (1-6)

[Table/Fig-1]: Demographic data of study population.
SD: Standard deviation; IQR: Inter-quartile range; M: Male; F: Female

Among the 82 study participants, 15 patients had urinary Beta-CrossLaps levels less than 15 pg/mL. The remaining 67 participants had Urinary Beta-CrossLaps concentration spread over a wide range, with minimum value of 19 pg/mL to the maximum observed value of 26 ng/mL. The median observed was 991 pg/mL.

To overcome the limitations of using a random urinary sample to estimate Beta-CrossLaps excretion, the Urinary Beta-CrossLaps concentration was converted into Urinary Beta-CrossLaps Creatinine ratio (ng/mmol). The minimum Urinary Beta-CrossLaps Creatinine ratio observed was 2.6 ng/mmol and the maximum value observed was 2071 ng/mmol (i.e., 2.1 µg/mmol). The median Urinary Beta-CrossLaps Creatinine ratio observed in the study population was 100.6 ng/mmol.

Statistical analyses showed no correlation of urinary Beta-CrossLaps Creatinine ratio to age, gender, duration of diabetes and MAP.

The association of urinary Beta-CrossLaps: Creatinine ratio with UACR was studied by categorising the patients into three groups based on the UACR levels. The results of this comparison are tabulated in [Table/Fig-2].

Parameter	Normo-albuminurics (n=48)	Micro-albuminurics (n=28)	Macro-albuminuric (n=6)	p-value
Age (in years) Mean (SD)	52 (6.6)	49.4 (6.7)	51 (10.1)	0.28
M:F	18:30	15:13	2:4	0.35
Duration of diabetes (in years) Median (IQR)	3 (5.7)	3 (4)	5.5 (14.25)	0.19
Mean arterial pressure (mm of Hg) Mean (SD)	100.9 (5.5)	100.3 (5.4)	98.3 (5.9)	0.54
eGFR (CKD-EPI) Mean (SD)	80.7 (17.3)	87.1 (17.8)	65.9 (24.9)	**0.03**
Urine β-CTx (ng/mmol creatinine) Median (IQR)	60.3 (166.9)	65.9 (205)	44 (125.6)	0.5

[Table/Fig-2]: Comparison of demographic parameters, eGFR and urine β-CTx values between Normoalbuminuria, Microalbuminuria and Macroalbuminuria patients.
SD: Standard deviation; IQR: Interquartile range
p-value less than or equal to 0.05 was considered statistically significant

The association of urinary beta-cross laps: Creatinine ratio with eGFR was studied by categorising the patients into three categories based on the eGFR levels. Individuals with eGFR >90 mL/min/1.73 m^2, were categorised as normal kidney function group (group 1), eGFR between 60-89 mL/min/1.73 m^2 were categorised as mild loss of kidney function (group 2) and individuals with eGFR between 30-59 mL/min/1.73 m^2 were categorised as moderate loss of kidney function (Group 3). There were no participants in our study with eGFR <30 mL/min/1.73 m^2, as it was an exclusion criteria. The urinary Beta CrossLaps: Creatinine ratio was compared between the three groups. The results of this comparison are tabulated in [Table/Fig-3].

Parameter	Group 1 normal eGFR (n=32)	Group 2 Mild decrease in GFR (n=42)	Group 3 Moderate decrease in GFR (n=8)	p-value
Age (in years) Mean (SD)	46.8 (7.2)	53.9 (5.0)	51.6 (6.4)	0.00002
M: F	12:20	14:28	1:7	0.4
Duration of diabetes (in years) Median (Range)	2 (4)	3.5 (5)	3 (5)	0.16
Mean arterial pressure (mm of HG) Mean (SD)	100.8 (5.3)	100.2 (5.7)	100.8 (5.7)	0.88
Urine β-CTx (ng/mmol creatinine) Median (IQR)	101.2 (267.1)	65.5 (119.8)	7.2 (58.8)	0.04

[Table/Fig-3]: Comparison of demographic parameters and urine β-CTx values between patients with normal renal function, mildly decreased renal function and moderately reduced renal function.
SD: Standard deviation; IQR: Interquartile range; p-value less than or equal to 0.05 was considered statistically significant

Age, MAP and eGFR were compared between the groups using ANOVA. The gender ratio between the groups was compared by Chi-square test. The duration of diabetes and Urine β-CTx concentration was compared using Kruskal-Wallis test. Age and MAP was compared between the groups using ANOVA. The gender ratio between the groups was compared by Chi-square test. The duration of diabetes and Urine β-CTx concentration was compared using Kruskal-Wallis Test.

The correlation between the eGFR values and urine β-CTx concentration was also evaluated considering both the parameters as continuous variables, using Spearman's Rho Correlation calculator. The rs value of 0.25 with p=0.025, indicates weak but statistically significant correlation between eGFR and urine β-CTx concentration.

DISCUSSION

The clinical management of DKD is still beset with the problems of late diagnosis and non-availability of definitive drugs for treatment [17]. The identification of new biomarkers is not only essential for detection or prediction of DKD development earlier than microalbuminuria, but also for evaluation of effectiveness of new drugs treatments [17]. The CKD273 profile has helped DKD research in both the above fields. It helps early prediction of DKD development, approximately four years before onset of microalbuminuria [18]. It has also been found to be useful in evaluating effectiveness of new treatment protocols, as in the Priority trial, in which an expand, randomized control rial was embedded to evaluate efficacy of Spironolactone, in delaying development of microalbuminuria [19]. However, it will take a few years or even decades before CE-MS equipment become more widely available and before urinary peptidome estimation protocols by CE-MS can be used in routine practice for Diabetes patients.

Most other studies of urinary proteome profiling have used Tandem MS or LC-MS [20,21]. The proteome profile obtained from all these studies is different. So, to reproduce the effectiveness of CKD273, CE-MS use is mandatory. However, widespread availability of CE-MS equipment and protocols is unlikely in the near future. In the meantime, the peptides of the CKD273 profile may be studied to understand the pathological process behind the profile and to develop novel biomarkers. The peptides in CKD273 belong to 30 different proteins [22]. Of these the peptides corresponding to collagen degradation are found to be decreased in the urine of DKD patients [18,23]. As per Pontillo C et al., the reduction of collagen fragments may indicate a decrease in the degradation of collagen within the renal parenchyma, which may result in collagen accumulation and fibrosis [24]. This hypothesis is supported by the observation of fibrosis in CKD patients [25].

Since 74% of the peptides were from Collagen α-I, we evaluated the Collagen C terminal peptide (β-CTx) in this study in the hope to gather some information about its potential to be a surrogate marker for early prediction of DKD. Serum Beta-CrossLaps (β-CTx) is clinically used as a bone turnover marker [13]. In the clinical laboratory, it is used for monitoring postmenopausal osteoporosis patients on anti-resorptive therapy [26,27]. Serum Beta-CrossLaps levels have been reported to be elevated in patients of Diabetic Nephropathy and serve as a better indicator of osteoporosis than bone scan [28]. Urinary Beta-CrossLaps is predominantly a research tool and is not used routinely in the clinical setting for patient management.

In pubmed, only one research publication of urinary Beta-CrossLaps in diabetes patient was found. The authors were evaluating the potential of urine Beta-CrossLaps as a marker of osteoporosis. They found statistically significant correlation of urine Beta-CrossLaps with urine deoxypyridinoline (another bone collagen degradation marker). However, the absolute levels of Beta-CrossLaps in urine were not much different from the healthy population, hence discouraging its use as a osteoporosis marker [29].

This study shows that urinary Beta-CrossLaps β-CTx excretion has significant correlation with eGFR. This observation of progressive decline in Beta-CrossLaps excretion in urine, with decline in eGFR may be interpreted as the dependence of excretion of collagen degradation fragments on glomerular filtration. This hypothesis is supported by the fact that Beta-CrossLaps are sufficiently small in size and their excretion is only via renal filtration [12]. Further the CKD273 classifier is also found to predict decline in eGFR [30]. The deterministic role played by Type 1 collagen fragments in CKD [24] and DKD prediction [23], as part of CKD273 proteomic profile, along with the observation of this study, points to a novel role for urinary Beta-CrossLaps, as a marker for glomerular function and DKD progression.

Further prospective studies should be conducted to evaluate whether it can be used as indicator of future microalbuminuria onset.

Another avenue of investigation should be diagnostic value of β-CTx in conjunction with the other proteins, whose peptides were found in CKD273. This study gives proof of concept for the transferability of findings of CE-MS and other proteomic studies, to other testing methods for estimation of same or similar proteins.

Limitation(s)

Being a preliminary study, the sample size of this study was small. This may be the reason why no correlation was observed between urinary Beta-CrossLaps excretion and age or gender of the patient. Collagen degradation rate increases with age, and is higher in postmenopausal women we need to further evaluate urinary Beta-CrossLaps excretion in different age groups and specifically postmenopausal women.

CONCLUSION(S)

Urinary Beta-CrossLaps excretion correlates well with kidney function (eGFR) in type 2 diabetes patients. The considerations of cost, equipment and expertise required, to perform CE-MS, to estimate CKD273 peptide profile, presently preclude its wide application in type 2 diabetes patients. Under these circumstances, it is useful to further explore the potential of alternate markers of DKD, such as urinary Beta-CrossLaps, which can be processed on any immunoassay platform.

Acknowledgement

The authors would like to thank Mr.Vijay Vasava for technical support provided by him as Technician at Department of Biochemistry.

REFERENCES

[1] International Diabetes Federation. IDF Diabetes Atlas, 8th edn. Brussels, Belgium: International Diabetes Federation, 2017. http://www.diabetesatlas.org; last accessed on August 25, 2019.
[2] Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al., ICMR–INDIAB Collaborative Study Group. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdiaDIABetes (ICMR-INDIAB) study. Diabetologia. 2011;54:3022-27. PMID: 21959957.
[3] Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, et al. Diabetic kidney disease: A report from an ADA Consensus Conference. Diabetes Care. 2014;37:2864-83.
[4] Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes- 2018. Published by American Diabetes Association. Diabetes Care. 2018;41(Suppl 1):S105-18; Doi: 10.2337/dc18-S010.
[5] Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. JAMA. 2003;289:3273-77.
[6] Molitch ME, Steffes M, Sun W, Rutledge B, Cleary P, de Boer IH, et al. Development and progression of renal insufficiency with and without albuminuria in adults with type 1 diabetes in the Diabetes Control & Complications Trial. Diabetes Care. 2010;33:1536-43.
[7] Ben Ameur R, Molina L, Bolvin C, Kifagi C, Jarraya F, Ayadi H, et al. Proteomic approaches for biomarkers of Diabetic Nephropathy. NDT. 2012;25(9):2866-75.
[8] Merchant ML, Perkins BA, Boratyn GM, Ficociello LH, Wilkey DW, Barati MT, et al. Urinary Peptidome may predict renal function decline in Type 1 Diabetes & microalbuminuria. JASN. 2009;20(9):2065-74.
[9] Zurbig P, Jerume G, Hovind P, Macisaac RJ, Mischak H, Nielsen SE, et al. Urinary proteomics for early diagnosis in diabetic nephropathy. Diabetes. 2012;61:3304-13.
[10] Good DM, Zürbig P, Argilés A, Bauer HW, Behrens G, Coon JJ, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol Cell Proteo-mics. 2010;9:2424-37.
[11] Seibel MJ. Biochemical markers of bone turnover: Part I: Biochemistry and variability. Clin Biochem Rev. 2005;26(4):97-122.
[12] Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. Clinical Chemistry. 2017;63(2):464-74.
[13] Seibel JM. Biochemical markers of bone turnover part II: Clinical applications in the management of osteoporosis. Clin Biochem Rev. 2006;27:123-38.
[14] Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2018. Published by American Diabetes Association. Diabetes Care. 2018;41(Suppl 1):S13-27. Doi: 10.2337/dc18-S002.
[15] Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: More accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis. 2010;55(4):622-27.
[16] Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of Chronic Kidney Disease: A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney International. 2005;67:2089-100.
[17] Doshi SM, Friedman AN. Diagnosis and management of type 2 diabetic kidney disease. Clinical Journal of the American Society of Nephrology: CJASN. 2017;12(8):1366-73. https://doi.org/10.2215/CJN.11111016.
[18] Lindhardt M, Persson F, Zurbig P, Stalmach A, Mischak H, de Zeeuw D, et al. Urinary proteomics predict onset of microalbuminuria in normoalbuminuric type 2 diabetic patients, a sub-study of the DIRECT-Protect 2 study. Nephrol Dial Transplant. 2017;32(11):1866-73.
[19] Tofte N, Lindhardt M, Adamova K, Bakker SJL, Beige J, Beulens JWJ, et al. PRIORITY investigators. Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): A prospective observational study and embedded randomised placebo-controlled trial. Lancet Diabetes Endocrinol. 2020;8(4):301-12. Doi: 10.1016/S2213-8587(20)30026-7. Epub 2020 Mar 2. PMID: 32135136.
[20] Lewandowicz A, Bakun M, Kohutnicki R, Fabijańska A, Kistowski M, Imiela J, et al. Changes in urine proteome accompanying diabetic nephropathy progression. Pol Arch Med Wewn. 2015;125(1-2):27-38. Doi: 10.20452/pamw.2640. Epub 2015 Jan 12. PMID: 25578432.
[21] Moresco RN, Sangoi MB, De Carvalho JA, Tatsch E, Bochi GV. Diabetic nephropathy: Traditional to proteomic markers. Clin Chim Acta. 2013;421:17-30. Doi: 10.1016/J.Cca.2013.02.019. Epub 2013 Feb 26. PMID: 23485645.
[22] Argilés Á, Siwy J, Duranton F, Gayrard N, Dakna M, Lundin U, et al. CKD273, a new proteomics classifier assessing CKD and its prognosis. PloS One. 2013;8(5):e62837. https://doi.org/10.1371/journal.pone.0062837.
[23] Alkhalaf A, Petra Z, Bakker S, Bilo H Cerna M, Fischer C, et al. Multicentric validation of proteomic biomarkers in urine specific for diabetic nephropathy. PLoS ONE. 2010;5(10):e13421.
[24] Pontillo C, Mischak H. Urinary peptide-based classifier CKD273: Towards clinical application in chronic kidney disease. Clinical Kidney Journal. 2017;10(2):192-201.
[25] Duffield JS. Cellular and Molecular mechanisms in Kidney Fibrosis. J Clin Invest. 2014;124:2299-306.
[26] Shetty S, Kapoor N, Bondu JD, Thomas N, Vizhalil T. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian J Endocrinol Metab. 2016;20(6):846-52.
[27] Kawana K, Takahashi M, Hoshino H, Kushida K. Comparison of serum and urinary C-terminal telopeptide of type I collagen in aging, menopause and osteoporosis. Clin Chim Acta. 2002;316(1-2):109-15.
[28] Mohamed A. Serum β crosslaps as a predictor for osteoporosis in postmenopausal women with early Diabetic Nephropathy. The Egyptian Journal of Internal Medicine. 2019;31:52-56.
[29] Fassbender WJ, Gödde M, Brandenburg VM, Usadel KH, Stumpf UC. Urinary bone resorption markers (deoxypyridinoline and C-terminal telopeptide of type I collagen) in healthy persons, postmenopausal osteoporosis and patients with type I diabetes. Adv Med Sci. 2009;54(1):01-06. Doi: 10.2478/v10039-009-0003-x. PMID: 19482729.
[30] Pontillo C, Jacobs L, Staessen J, Schanstra JP, Rossing P, Heerspink HJL, et al. A urinary proteome-based classifier for the early detection of decline in glomerular filtration. Nephrol Dial Transplant. 2017;32(9):1510-16.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, GMERS Medical College and General Hospital, Vadodara, Gujarat, India.
2. Associate Professor, Department of Medicine, GMERS Medical College and General Hospital, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Lalithambigai Arumugasamy,
Assistant Professor, Department of Biochemistry, GMERS Medical College, Gotri, Vadodara, Gujarat, India.
E-mail: drlalitha1981@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 17, 2020
- Manual Googling: Apr 07, 2021
- iThenticate Software: Apr 24, 2021 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Dec 15, 2020**
Date of Peer Review: **Mar 06, 2021**
Date of Acceptance: **Apr 07, 2021**
Date of Publishing: **Jul 01, 2021**