Original Article

Noninvasive Method of Differentiating Diabetic Nephropathy and Nondiabetic Renal Disease Using Serum Bone Morphogenetic Protein-7 and Transforming Growth Factor-Beta 1 Levels in Patients with Type-2 Diabetes Mellitus

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ABSTRACT. Diabetic nephropathy (DN), one of the major complications of diabetes mellitus, is diagnosed by the presence of pedal edema, 24-h proteinuria >300 mg/day, and retinopathy. However, in view of variable clinical presentations and deviation from the above-said clinical features, it has become difficult to diagnose DN or the presence of nondiabetic renal disease (NDRD). Many biomarkers have been identified which could predict the progression of DN. Despite such advancement in science, it is still difficult to differentiate between DN and NDRD. Diabetes is a state of chronic inflammation. Among the pro-inflammatory cytokines, it has been shown that transforming growth factor-beta (TGF-β) and bone morphogenetic protein-7 (BMP-7) play a key role in the development and progression of DN. We assessed whether the levels of serum BMP-7 and TGF-β can help differentiate between DN and NDRD, thus serving as surrogate markers of DN.

Introduction

Diabetic nephropathy (DN) is one of the major complications of diabetes mellitus (DM).1 DN is defined as progressive rise in urinary albumin excretion of >300 mg in a 24-h collection along with progressive decline in renal function as evidenced by an abnormality in serum creatinine and a decline in glomerular filtration rate (GFR).2 Apart from DN, nondiabetic renal disease (NDRD) is known to occur in 25%–50% of patients with Type-2 diabetes. Clinically, it is difficult to differentiate DN and NDRD and hence the need for biopsy. Renal biopsy is performed only in those with indications that are well described in literature. One of the main indications for biopsy remains the absence of DN. Few studies have shown that in the absence of diabetes retinopathy, renal biopsy is essential to make the diagnosis of DN and thus, the presence or absence of diabetic retinopathy would not help in the differentiation of DN from NDRD.3-5 In the review by Prakash et al, it was observed that the presence of diabetic
retinopathy would concur with DN but does not exclude NDRD and hence, in a proteinuric diabetic patient, renal biopsy needs to be done to differentiate DN from NDRD.  

Many circulating factors have been identified to predict the diagnosis of DN and its progression, such as uric acid, tumor necrosis factors 1 and 2, fibroblast growth factor, and Vitamin D.  

It needs to be studied whether these markers are useful in the experimental and interventional studies. Till date, there are no studies which have been performed in correlation with the histological grading.

Biomarkers such as bone morphogenetic protein-7 (BMP-7) and transforming growth factor-beta (TGF-β) were assessed for the prediction and progression of DN in experimental studies. There is sparse literature regarding the utility of these biomarkers in humans. Urinary TGF-β was identified as one of the markers although the reproducibility and reliability have been doubtful.

Therefore, we undertook this study to differentiate DN from NDRD with the assessment of serum levels of BMP-7 and TGF-β and their correlation with the histological grading of DN.

**Objectives**

In this study, we aimed to determine the usefulness of serum BMP-7 and TGF-β levels as screening markers to distinguish DN and NDRD in patients with Type 2 DM, assess the correlation between the circulating levels of serum BMP-7 and TGF-β with interstitial fibrosis and tubular atrophy (IFTA) on renal biopsy, and assess various factors affecting serum BMP-7 and TGF-β levels.

**Patients and Methods**

This is a prospective observational study conducted from December 2014 to December 2016 in our hospital, a tertiary care referral center. This study was conducted after obtaining approval from the ethics committee. Patients with a history of DM presenting to the nephrology department with renal insufficiency were included in the study. The study was performed on 100 patients with Type 2 DM who presented with renal insufficiency with or without proteinuria. Patients with isolated proteinuria without renal insufficiency were not included in the study.

**Inclusion criteria**

Patients with Type 2 DM presenting to the department of nephrology with renal insufficiency and proteinuria were included in the study, who were further categorized into the following three groups:

1. Patients with presumed DN: Patients with Type 2 DM with a history of pedal edema, facial puffiness, DN, and presence of proteinuria of >300 mg are presumed to have DN

2. Patients who underwent renal biopsy and had DN on renal biopsy: Type 2 DM patients underwent biopsy for indications such as massive proteinuria, absence of DN, presence of active urine sediment, and whose histopathology is suggestive of DN

3. Patients who underwent renal biopsy and had NDRD on biopsy: Type 2 DM patients who underwent biopsy for the indications mentioned earlier and whose histopathology was suggestive of NDRD.

**Exclusion criteria**

Exclusion criteria included the following: DM with acute kidney injury, DM with pregnancy, and Type 1 DM and Type 2 DM without renal insufficiency

In the present study, at the time of enrollment, we could not find Type 2 DM patients without renal insufficiency in our nephrology outpatient department and hence, they could not be included in the present study.

**Study protocol**

Out of the 100 patients with Type 2 DM enrolled in the study, 45 patients were diagnosed to have presumed DN based on the presence of 24-h urinary protein of >300 mg along with the presence of diabetic retinopathy. Renal biopsy was performed in 55
patients who had conventional indications for biopsy in Type 2 DM, which included the following: massive proteinuria (10 patients), active urine sediment (20 patients), absence of diabetic retinopathy (15 patients), and unexplained deterioration in renal function (10 patients).

Based on the clinical and laboratory features, the patients were categorized into the following three groups:
1. Group 1 (presumed DN): Those with proteinuria and diabetic retinopathy
2. Group 2 (NDRD): Those with biopsy suggestive of findings other than DN
3. Group 3 (biopsy-proven DN): Those with biopsy-proven DN.

After obtaining approval from the institutional ethics committee, patients were included in the study. Informed consent was taken from the patients at the time of renal biopsy.

Demographic data of the patients along with the laboratory investigations were noted in the pro forma. Renal biopsy was performed in those with standard indications as mentioned in the literature. Patients with presumed DN were not subjected for renal biopsy unless a specific indication for biopsy was present as mentioned above. The renal histopathological findings were graded based on the Trevart classification.

Assessment of serum bone morphogenetic protein-7 and transforming growth factor-beta levels

Lithium heparin anticoagulated blood samples were collected for the estimation of serum BMP-7 and TGF-β1 levels. The blood samples were immediately subjected to centrifugation at 2000 ×g for 10 min at 41°C. The supernatant plasma was aliquoted and stored at −80°C for biomarker assay.

The total plasma TGF-β1 protein concentration was measured using an isoform-specific TGF-β1 enzyme-linked immunosorbent assay according to the manufacturer’s protocol [TGF-β1 enzyme-linked immunosorbent assay (ELISA) – IBL International]. TGF-β1 ELISA kit is a solid-phase ELISA based on the sandwich principle.

Circulating BMP-7 levels were determined using immunoassay kit (IBL International). Heparinized plasma (150 mL) without assay diluent was used. The absorbance values at 450 nm, corrected by readings at 540 nm, were obtained and plotted against the standard to determine the circulating level of BMP-7.

Serum BMP-7 and TGF-β1 levels correlated with the amount of IFTA on renal biopsy. Factors influencing serum BMP-7 and TGF-β1 are analyzed.

Statistical Analysis

Statistical analysis was performed by utilizing the Statistical Package for the Social Sciences (SPSS) software version 18.0 (SPSS Inc., Chicago, IL, USA). Initially, frequency tables were made to estimate the frequency and percentage of each parameter analyzed. Descriptive statistics were expressed in terms of mean and standard deviation. The probability of association between two discrete attributes was made by Chi-square test. Means of the various parameters were compared by using Student’s t-test (for two groups) and by analysis of variance (ANOVA), for more than two groups. P <0.05 was considered statistically significant.

Spearman’s correlation coefficient “r” was calculated to see the correlation between BMP-7 and TGF-β1 levels and the amount of IFTA and proteinuria. Scatter plot graphs were drawn to estimate the correlation between serum BMP-7 and TGF-β1 levels with the amount of IFTA and the degree of proteinuria. Box plot graphs were drawn using ANOVA between serum BMP-7 and TGF-β1 levels and the amount of IFTA on renal biopsy.

Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated for BMP-7 and TGF-β1. The cutoff value for BMP-7 was taken as 200 ng/mL, which is the mean BMP-7 of the study population, for calculating the accuracy of sensitivity, specificity, positive predictive value, and negative predictive value. The cutoff value for TGF-β1 was taken as 600 pg/mL, which is the upper limit as given by
Results

One hundred patients satisfied the inclusion criteria and were included in the study. Of these, 45 patients had presumed DN (Group 1), 30 patients had biopsy-proven NDRD (Group 2), and 25 patients had biopsy-proven DN (Group 3).

The mean age of the study population was 50 ± 9.2 years. Patients with biopsy-proven DN were younger compared to other groups although this was not statistically significant (P >0.05). There were 67 males and 33 females. Baseline characteristics of all the three groups are tabulated in Table 1. Duration of diabetes, number of males, presence of proteinuria and diabetic retinopathy, mean degree of proteinuria, mean GFR, presence of macrovascular disease, and hepatitis C seropositivity showed statistically significant difference among the three groups.

Duration of diabetes, the mean degree of proteinuria, the mean GFR, and the mean level of hemoglobin were higher in Group 2 compared to Group 1 and Group 3 (P <0.05). Degree of hypertension (HTN), associated macrovascular disease, and presence of diabetic retinopathy were more severe in Group 1 (P <0.05). The degree of hyperglycemia and the number of patients with hepatitis C seropositivity were higher in Group 3. Of the laboratory parameters that were studied, severe hypoalbuminemia and severe hypocalcemia were noted in Group 3 (Table 1).

Serum levels of bone morphogenetic protein-7 and transforming growth factor-β

Levels of BMP-7 and TGF-β in all the three groups are tabulated in Table 2. Patients in Group 2 had better levels of both BMP-7 and TGF-β. Very low levels of BMP-7 were observed in Group 3 with biopsy-proven DN. TGF-β was near normal in patients with NDRD (P <0.05) (Table 2).

It was observed that lower degree of proteinuria was associated with low levels of BMP-7 and high levels of TGF-β. Higher degree of proteinuria was found to be associated with better levels of BMP-7 and lower levels of TGF-β. To avoid confounding, only the group

| Variable                                | Group I Presumed DN (45) | Group II NDRD (30) | Group III Biopsy-proven DN (25) | P value |
|-----------------------------------------|--------------------------|--------------------|---------------------------------|---------|
| Mean age (years)                        | 51.71±10.7               | 51.03±7.61         | 49.6±8.27                      | 0.67 (NS) |
| Males                                   | 37 (82.2%)               | 17 (56.6%)         | 13 (52%)                       | 0.01    |
| Females                                 | 8 (17.7%)                | 13 (43.3%)         | 12 (48%)                       |         |
| Duration of DM (years)                  | 3.8±1.13                 | 10.3±2.51          | 3.8±1.15                       | <0.0001 |
| HTN                                     | 45 (100%)                | 18 (60%)           | 23 (92%)                       | <0.0001 |
| Mean proteinuria (g)                    | 1609.9±250               | 3099.4±120         | 1516.6±202                     | <0.0001 |
| Mean GFR (mL/min/1.73 m²)               | 29.75±11.4               | 34.6±17.8          | 8.52±2.29                      | <0.0001 |
| Presence of macrovascular disease       | 25 (100%)                | 0                  | 10 (48%)                       | <0.0001 |
| Diabetic retinopathy                    | 45 (100%)                | 7 (23.3%)          | 3 (12%)                        | <0.0001 |
| Hepatitis B+                            | 3 (6.6%)                 | 2 (6.6%)           | 1 (4%)                         | 0.8     |
| Hepatitis C+                            | 0                       | 2 (6.6%)           | 5 (20%)                        | 0.0007  |
| Mean blood sugar level (mg/dL)          | 172±70.4                 | 113±27.2           | 251±76.5                       | <0.0001 |
| Mean Hb (g/dL)                          | 9.4±1.63                 | 10.2±1.09          | 7.5±0.82                       | <0.0001 |
| Mean albumin (g/dL)                     | 2.8±0.46                 | 2.92±0.74          | 2.7±0.39                       | 0.01    |
| Mean calcium (mg/dL)                    | 8.4±0.76                 | 8.09±0.82          | 7.73±0.59                      | 0.0008  |

DN: Diabetic nephropathy, NDRD: Nondiabetic renal disease, DM: Diabetes mellitus, GFR: Glomerular filtration rate, Hb: Hemoglobin, HTN: Hypertension.
with DN was assessed, wherein a positive correlation was observed with TGF-β and negative correlation with BMP-7.

The serum levels of BMP-7 were low in both Stage III and Stage IV DN ($P = 0.47$), and the levels of TGF-B were high in Stage IV compared to Stage III DN. The mean TGF-β1 level in DN II was 900 pg/mL, in DN III was 912 ± 173 pg/mL, and in DN IV was 1072 ± 170 pg/mL. Overall, the serum TGF-β1 levels were higher in patients with DN and the values progressively increased with increasing histopathological class of DN (Table 3). However, this difference was not statistically significant ($P = 0.07$).

The degree of IFTA with the serum levels of BMP-7 and TGF-β is tabulated in Table 4. The mean BMP-7 level in patients with IFTA <25% in NDRD was 474.89 ± 58 ng/mL, in biopsy-proven DN, it was 12 ± 5.8 ng/mL, and the difference was statistically significant ($P <0.0001$). The mean BMP-7 level in patients with IFTA 25%–50% in NDRD was 460 ng/mL and in biopsy-proven DN, it was 21.7 ±12.8 ng/mL, and the difference was statistically significant ($P = 0.02$). Three patients had IFTA >50%, and all of them had DN with mean BMP-7 values of 8 ± 1.73 ng/mL. There was a strong negative correlation between the levels of BMP-7 and IFTA as estimated by Spearman’s correlation coefficient ($r = −0.83$).

The mean TGF-β level in patients with IFTA

| Table 2. Serum levels of BMP-7 and TGF-β in the study population. |
|-------------------|-------------------|-------------------|---------------|---------------|
| Serum             | Group 1            | Group 2            | Group 3       | $P$            |
|-------------------|-------------------|-------------------|---------------|---------------|
| BMP-7 (ng/mL)     | 196±32            | 474±100           | 19.76±12.6    | 0.001         |
| TGF-β (pg/mL)     | 1001±102          | 46.2±27           | 1033±56       | 0.001         |

TGF-β: Transforming growth factor-beta, BMP-7: Bone morphogenetic protein-7.

| Table 3. Various factors affecting both BMP-7 and TGF-β. |
|-------------------|-------------------|---------------|---------------|
| Variable          | BMP-7 (ng/mL)     | $P$           | TGF-β (pg/mL) | $P$           |
|-------------------|-------------------|---------------|---------------|
| Gender            |                   |               |               |               |
| Males (67)        | 247.38±220        | 0.45          | 728±223       | 0.88          |
| Females (33)      | 211.5±231.8       |               | 711±290       |               |
| Duration of DM    |                   |               |               |               |
| <6 years          | 134±150*          | <0.0001       | 999.3±200     | <0.0001       |
| >6 years          | 484±90            |               | 46.7±27.3     |               |
| HTN               |                   |               |               |               |
| Yes               | 206±115*          | <0.001        | 812.12±252    | <0.0001       |
| No                | 412±197           |               | 517±150       |               |
| Proteinuria       |                   |               |               |               |
| <2.5 g            | 191.5±129*        | 0.19          | 832±150       | 0.22          |
| >2.5 g            | 357.6±150         |               | 411±250       |               |
| Diabetic retinopathy |               |               |               |               |
| Yes               | 225±12            | 0.6           | 875±55*       | 0.001         |
| No                | 247±15            |               | 536±52        |               |
| Macrovascular disease |               |               |               |               |
| Yes               | 19.63±70*         | 0.0001        | 1000+28*      | 0.001         |
| No                | 228.88±62         |               | 600±98        |               |
| DN                |                   |               |               |               |
| III               | 18±7.3            | 0.0001        | 912±173       | <0.0001       |
| IV                | 15.4±9            |               | 1072±170      |               |
| Hyperglycemia     | 17.42±90*         | 0.00001       | 1186±150*     | 0.00001       |
| Hb <10 g/dL       | 132±52            | <0.0001       | 930±125       | <0.0001       |

DM: Diabetes mellitus, DN: Diabetic nephropathy, TGF-β: Transforming growth factor-beta, BMP-7: Bone morphogenetic protein-7, Hb: Hemoglobin, HTN: Hypertension.
<25% in NDRD was 45.44 ± 20 pg/mL, and in biopsy-proven DN, it was 885 ± 120 pg/mL, and the difference was statistically significant ($P < 0.0001$). The mean TGF-β1 level in patients with IFTA 25%–50% in NDRD was 69 pg/mL, in biopsy-proven DN, it was 1055 ± 104 pg/mL, and the difference was statistically significant ($P < 0.0001$). Three patients had IFTA >50%, and all of them had DN with mean TGF-β1 level of 1100 ± 100 pg/mL. There was a strong positive correlation between the amount of IFTA and TGF-β levels as estimated by Spearman’s correlation coefficient ($r = 0.86$).

The receiver operating characteristic curve of BMP-7 and TGF-β in DN and NDRD was assessed (Figures 1 and 2). It was observed that both the markers were sensitive and specific in identifying DN from NDRD (AUC of BMP-7 = 0.9 and AUC of TGF-β = 1). Hence, these markers may be assessed in differentiating DN from NDRD.

### Discussion

Even after many years of description of the entity of DN, the definitive diagnosis remains elusive, short of renal biopsy. Most of the times, an assumptive diagnosis is made based on clinical features supported by biochemical findings. Many biomarkers have been mentioned in literature, but none has been found to be an alternative to pathological diagnosis.
In majority of the patients, DN may be associated with significant chronicity changes on histopathology. TGF-β has a major role in renal fibrosis. Several TGF-β have been identified as novel markers in the prediction of progression of DN.\textsuperscript{12}

BMP-7 is a member of the BMP family that forms together many protein families including TGF-β family and has a significant role in organogenesis. Although selectively expressed, its role in kidney is highest. It is expressed in the tubules of outer medulla and in podocytes. BMP-7 is known to be anti-fibrogenic which involves smad signaling. TGF-β, on the other hand, has a major role in renal fibrosis. It is known that decrease in BMP-7 would favor renal fibrogenesis.

The present study was performed to assess the serum levels of BMP-7 and TGF-β as markers of identification of DN and NDRD in patients with Type 2 DM presenting with renal insufficiency. The study population was divided into three groups as follows: those with presumed DN based on classical definition, those with NDRD, and those with biopsy-proven DN. Serum BMP-7 and TGF-β showed an inverse relation in DN. Serum BMP-7 levels were better in those with NDRD compared to DN. In contrast, the levels of TGF-β were very high in DN compared to those of NDRD.

In our study population of 100 patients of Type 2 DM, 30 patients had NDRD, 25 patients had biopsy-proven DN, and 45 patients had presumed DN. Levels of serum BMP-7 and TGF-β were assessed and correlated with histopathological changes of DN. More than half of the study group (55%) underwent renal biopsy, of which 30% were NDRD group and 25% were DN with indications for renal biopsy, all of which were proven histologically to be DN.

Among the baseline characteristics of all the three groups, gender, duration of diabetes, HTN, degree of proteinuria, presence of macrovascular damage, glycemic status, and degree of anemia showed a statistically significant difference. Longer duration of diabetes, high degree of proteinuria, and higher GFR were noted in the NDRD group. None of the NDRD group had macrovascular disease. DN was more in those with presumed DN group. A worse glycemic status, low levels of hemoglobin, low serum albumin, and low serum calcium were observed in the biopsy-proven DN group.

Regarding the levels of BMP-7 and TGF-β, it was observed that patients with NDRD had preserved levels of BMP-7 and low levels of TGF-β, and both the presumed DN and biopsy-proven DN groups had low levels of BMP-7 and high levels of TGF-β, HTN, presence of higher histological changes of DN, and macrovascular disease. Factors influencing the serum levels of these factors showed a statistically significant association with hypertension, diabetic retinopathy, macrovascular disease, progressing class of DN, hyperglycemia, and anemia. Preserved levels of BMP-7 and low TGF-β levels were observed in those without HTN, diabetic retinopathy, macrovascular disease, hyperglycemia, and anemia and low levels of BMP-7 and high levels of TGF-β were noted in those with the above morbidities. Factors such as proteinuria and duration of diabetes were associated with high TGF-β and low levels of BMP-7 compared to those without proteinuria and longer duration of diabetes. This observation is contrary to other findings in the study, explaining the fact that there are increased levels of fibrogenic factors and decreased levels of anti-fibrogenic factors with the progression of disease and presence of comorbidities.

Serum BMP-7 and TGF-β levels also correlated with the degree of IFTA. With increasing IFTA, in those with DN, increased levels of TGF-β and decreased levels of BMP-7 were noted. However, in NDRD, the levels of BMP-7 were preserved though the levels of TGF-β did not increase. Hence, it may be surmised that the levels of TGF-β may be used to differentiate between DN and NDRD although large-scale studies need to be undertaken to prove the usefulness of TGF-β levels.

A study done by Shaker\textsuperscript{13} on 72 patients of diabetes with different degrees of albuminuria
Table 5. Comparison of the present study with other studies.

| Variable                          | Shaker\textsuperscript{13}                  | Hefini et al\textsuperscript{14}                  | Present study                  |
|-----------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------|
| Number of patients                | 72 Type 2 DM and 30 controls                | 45 males with Type 2 DM and 15 controls        | 100 patients with Type 2 DM  |
| Duration of DM                    | Longer duration of DM correlated with the presence of macroalbuminuria | Longer duration of DM correlated with the presence of macroalbuminuria | Longer duration of DM correlated with the presence of NDRD |
| Presence of HTN                   | Correlated with the presence of DN          | Correlated with the presence of DN             | Correlated with the presence of DN |
| eGFR                              | Lower in patients with macroalbuminuria     | Lower in patients with macroalbuminuria       | Lower in patients with DN    |
| Presence of DR                    | N/A                                         | N/A                                           | Correlated with the presence of DN |
| Presence of macrovascular disease | N/A                                         | N/A                                           | Correlated with the presence of DN |
| Hepatitis C positivity            | N/A                                         | N/A                                           | Correlated with the presence of DN |
| Serum BMP-7                       | N/A                                         | Decreased in macroalbuminuric patients (79–527 pg/mL) | Decreased in DN (19–196 ng/mL) compared to NDRD |
| Serum TGF-β1                      | (1) Increased in patients with macroalbuminuria ($r=0.9$)  (2) Sensitivity, specificity, and accuracy are 100%, 73.5%, and 87%, respectively | Increased in macroalbuminuric patients (36–60 ng/mL) | (1) Increased in DN (980–1033 pg/mL) compared to NDRD ($r=0.62$).  (2) Sensitivity, specificity, and accuracy are 82.86%, 100%, and 88%, respectively |
| IFTA correlation with serum BMP-7 and TGF-β1 | N/A                                         | N/A                                           | IFTA is inversely proportional to the levels of serum BMP-7 and directly proportional the serum TGF-β1 levels in patients with DN |

DM: Diabetes mellitus, NDRD: Nondiabetic renal disease, HTN: Hypertension, DN: Diabetic nephropathy, IFTA: Interstitial fibrosis and tubular atrophy, eGFR: Estimated glomerular filtration rate, BMP-7: Bone morphogenetic protein-7.
showed a significant correlation between macroalbuminuria and serum TGF-β levels. In this study, both urine and serum TGF-β were assessed. It was shown that both urinary and TGF-β were elevated in those with macroalbuminuria compared to those with microalbuminuria or normoalbuminuria. Duration of diabetes and presence of HTN were other factors which showed a significant correlation with macroalbuminuria in their study. It was suggested that urinary and serum TGF-β may be used as markers for the presence of DN in patients with Type 2 diabetes. This study did not include histopathological findings. Our study has shown elevated levels of serum TGF-β in patients with DN and also showed a positive association with increasing degree of IFTA.

Hefini et al.14 studied serum BMP-7 and TGF-β in 45 patients with Type 2 diabetes and showed a significant correlation of development of macroalbuminuria with duration of diabetes. Low levels of serum BMP-7 and high levels of TGF-β were noted in those with macroalbuminuria (Table 5).

The above two studies were the only studies performed on human subjects, hence the paucity of literature. Both the studies were conducted on those with DN to correlate with the levels of markers with the progression of DN as evidenced by high levels in macroalbuminuria compared to normo/microalbuminuria. In both the above studies, all the patients were included based on the definition of DN. None of them underwent renal biopsy.

In our study, diagnosis of DN was made histopathologically in Group 3 patients unlike in other groups where it was a clinical and laboratory-based diagnosis. It was also observed that in both the groups of DN, high levels of TGF-β were noted, which correlated with the histopathological findings. Whereas, in NDRD, TGF-β levels were not high and BMP-7 levels were preserved.

Thus, a high level of TGF-β and a low level of serum BMP-7 in a patient of Type 2 DM with proteinuria suggest an underlying DN and in those with already existing DN, it suggests the severity of IFTA, thus the chronicity. Thus, NDRD patients may be identified by low serum levels of TGF-β and preserved levels of serum BMP-7.

It may be concluded that serum TGF-β may be used as a marker to distinguish DN from NDRD. Though the levels of serum BMP-7 were preserved and were higher in NDRD compared to DN, the sensitivity and specificity were high with TGF-β than that with BMP-7. In addition, high serum TGF-β in known DN suggests severe IFTA and thus progressing CKD.

**Limitations**

The present study is done in smaller study population which included only patients with Type 2 DM. The present study did not include follow-up evaluation of the study population. Age-, gender-, and glycemic index-matched BMP-7 and TGF-β levels are not available in the literature and hence, arbitrary cutoffs of BMP-7 and TGF-β in known patients of diabetes and presence of nephropathy in Type 2 diabetic patients. Int J Diabetes Metab 2005;13:1-9.

**References**

1. El Mesallamy HO, Gad MZ, Sallam AM. The association of TGF-β 1, angiotensin II and oxidative stress with diabetic nephropathy in type 2 diabetic patients. Int J Diabetes Metab 2008;16:63-8.
2. Obineche EN, Adem A. Update in diabetic nephropathy. Int J Diabetes Metab 2005;13:1-9.
3. Li XQ, Zheng X, Chen M, Zhao MH. Characteristics of diabetic nephropathy patients without diabetic retinopathy: A retrospective observational study. Medicine (Baltimore) 2017;96:e6805.
4. Prakash J, Lodha M, Singh SK, Vohra R, Raja R, Usha. Diabetic retinopathy is a poor predictor of type of nephropathy in proteinuric type 2 diabetic patients. J Assoc Physicians India 2007;55:412-6.
1. Prakash J. Non-diabetic renal disease (NDRD) in patients with type 2 diabetes mellitus (type 2 DM). J Assoc Physicians India 2013;61:194-9.
2. Jalal DJ, Maahs DM, Hovind P, Nakagawa T. Uric acid as a mediator of diabetic nephropathy. Semin Nephrol 2011;31:459-65.
3. Agarwal R. Vitamin D, proteinuria, diabetic nephropathy, and progression of CKD. Clin J Am Soc Nephrol 2009;4:1523-8.
4. Isakova T. Comparison of mineral metabolites as risk factors for adverse clinical outcomes in CKD. Semin Nephrol 2013;33:106-17.
5. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol 2012;23:516-24.
6. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol 2012;23:507-15.
7. Wang SN, LaPage J, Hirschberg R. Role of glomerular ultrafiltration of growth factors in progressive interstitial fibrosis in diabetic nephropathy. Kidney Int 2000;57:1002-14.
8. Wong TY, Poon P, Chow KM, Szeto CC, Cheung MK, Li PK. Association of transforming growth factor-beta (TGF-beta) T869C (Leu 10Pro) gene polymorphisms with type 2 diabetic nephropathy in Chinese. Kidney Int 2003;63:1831-5.
9. Shaker YM. Serum and urinary transforming growth factor beta 1 as biochemical markers in diabetic nephropathy patients. Beni-Suef Uni J Basic Appl Sci 2014;3:16-23.
10. Hefini S, Kamel A, El-Banawy H, Refai W, Khalil G. The role of BMP-7 and TGF-ß1 in diabetic nephropathy. J Med Res Inst 2007;28:235.

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