ORIGINAL ARTICLE

# mRNA Expression of Podocyte Associated Proteins in Peripheral Blood Mononuclear Cells of Type 2 Diabetes Mellitus Patients with and without Nephropathy

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**Abstract:**

*Background:* Diabetic nephropathy is the leading cause of End-Stage Renal Disease (ESRD) emerging in developed as well as developing countries, with the complicated pathogenesis. The study of expression of the genes related to kidney cells e.g. podocytes has been shown to be associated with the condition, helping in the elucidation of pathogenesis of the disease. Previously the gene expression associated was studied in urine samples. *Material and Methods:* In the present study, it was attempted to analyze the mRNA expression of podocyte related genes viz. podocalyxin, podocin and synaptopodin in Peripheral Blood Mononuclear Cells (PBMCs) in patients with diabetes with and without nephropathy in comparison with healthy controls by reverse transcriptase Polymerase Chain Reaction (PCR), followed by semi-quantitative PCR. *Results:* The expression of Synaptopodin (SYNPO) was increased in diabetics than the controls, while no significant difference was found for Podocalixyn (PODXL) and Podocin (NPHS2). The expression of PODXL and NPHS2 was significantly up-regulated; SYNPO was unaltered in microalbuminuric patients than healthy controls. PODXL and SYNPO were increased significantly in nephropathy subjects than controls, with no significant change in NPHS2. The expression of only PODXL was found to be up-regulated in microalbuminuric patients as compared to T2DM patients without nephropathy. PODXL, SYNPO were significantly up-regulated and NPHS2 was significantly down-regulated in nephropathy subjects as compared to T2DM patients without nephropathy. A significant down-regulation was found for NPHS2 expression in nephropathy patients than micro-albuminuric patients of T2DM with nephropathy. *Conclusion:* The detection of gene expression of these proteins can be used as an early marker for the detection of development of nephropathy in T2DM patients and preventive measures can be taken to prolong the onset of nephropathy in these patients, increasing the life expectancy.

**Keywords:** Diabetic Nephropathy, Podocytes, Synaptopodin, Podocalixyn, Podocin, Gene Expression

## Introduction:

In United States, the leading cause of End-Stage Renal Disease (ESRD) is Diabetic Nephropathy (DN), which is also showing higher trend for ESRD in developing countries like India [1]. DN pathogenesis is complex and still not completely evaluated [1-2]. The glomerular filtration barrier has three important components: endothelial cells, the Glomerular Basement Membrane (GBM) and the podocytes [3].

The integration of gene expression data into their functional context is one of the greatest challenges to employ this information towards "personalized molecular medicine" of DN. Genes influenced by a common environmental challenge or genetic predisposition are assumed to show co-regulation in the examined tissue resulting in similarities of their patterns of expression in these patients [4].

The highly specialized cells podocytes also called glomerular visceral epithelial cells give rise to primary, secondary and foot processes. The filtration slits are formed due to interlocking of foot processes in between neighboring podocytes. The extracellular substance 'slit diaphragm' bridges the filtration slits, and plays an important role by creating a barrier for protein on the basis of size. The podocytes are also known to synthesize matrix molecules to GBM, including type IV collagen, laminin, entactin, and agrin. Growth factors, the mediators in DN pathogenesis viz. transforming growth factor-beta and angiotensin II, along with hemodynamic factors directly or indirectly enhance the processes of hypertrophy, apoptosis and/or structural changes; thereby the synthesis of collagen type IV is increased by podocytes [5].

As podocyte proteins are specifically expressed on podocyte cells, analyzing their expression help in understanding of development of proteinuria in nephropathy patients. The podocyte protein podocin present in slit diaphragm is closely associated with synaptopodin and alpha actinin-4 of cytoskeleton; this enables progressive podocyte architecture rearrangements. The filtration of negatively charged albumin is restricted by the phenotypic marker and apical membrane protein of podocyte cells i.e. podocalyxin [6-7].

The quantitative measurement of mRNA levels as an expression marker for podocyte injury has been investigated in renal tissue using immunostaining or molecular analysis in glomerular diseases. But for the diagnosis of DN renal biopsy is not preferred as other non-invasive methods are available. The usefulness of measurement of mRNA levels of podocyte associated proteins in urine is emerging in DN patients and also in patients with other glomerular diseases, a non-invasive tool by which a signal of podocyte injury and disease i.e. podocyturia can be assessed [7].

Peripheral Blood Mononuclear Cells (PBMCs) generally refer to monocytes and lymphocytes, representing cells of the innate and adaptive immune systems. PBMCs are a promising target tissue in the field of nutrigenomics, to identify the genes that influence diet related diseases such as Type 2 Diabetes Mellitus (T2DM) and Cardio-vascular Diseases (CVD) because they seem to reflect the effects of dietary modifications at the level of gene expression. The use of transcriptomics can help to provide more information about eventual biomarkers of certain diseases or physiological changes related to the pathogenesis of the disease. PBMCs are convenient because they can be easily collected in sufficient quantities. PBMCs have been used for exploring gene expression in various diseases and to predict clinical outcomes. [8]

Therefore, it was hypothesized that there may be differences in the expression levels of podocyte proteins viz. podocalyxin, podocin and synaptopodin, in T2DM patients with nephropathy as compared with T2DM patients without nephropathy and healthy individuals from PBMCs.

**Material and Methods:**

The present study was carried out in Department of Biochemistry, Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital and Interactive Research School for Health Affairs (IRSHA), Pune, Maharashtra. The study protocol was approved by Institutional Ethics Committee, BV (DTU) Medical College, Pune. The sample size for T2DM was calculated at 95% confidence interval, from the prevalence of T2DM i.e. 9.3% [9]. And for T2DM patients with nephropathy was calculated to be 34, from the prevalence of overt nephropathy i.e. 2.2% [10]. As the sample size calculated for T2DM was 101, equal number of T2DM and T2DM with nephropathy, were included, and with finite population correction we included 110 patients in each group.

Total of 220 clinically diagnosed T2DM patients were recruited from Department of Medicine, BVDU Medical College and Hospital. Of which 110 T2DM patients were recruited with nephropathy as study group, and 110 T2DM patients without nephropathy were recruited as disease control group. The T2DM with nephropathy patients again divided into two groups viz. i. T2DM patients with microalbuminuria (n=55) (Urine albumin level between 30 to 300 mg/24 hours) ii. T2DM patients with proteinuria (n=55) (Urine albumin level more than 300 mg/24 hours). One hundred ten age and gender matched healthy volunteers, without any clinical and biochemical evidence of Diabetes Mellitus (DM), chronic kidney or liver diseases, and other endocrine disorders, were selected as healthy controls. The patients with any other complications of diabetes like hypertension were excluded from the study. An informed consent was obtained from every patient after complete explanation of procedure. Venous blood samples were collected from anticubital vein under aseptic conditions. The blood samples were collected in EDTA vacutainers, and were used for RNA isolation from PBMCs for gene expression study.

**Isolation of PBMCs:**

To a 15-mL conical centrifuge tube, 3 mL of histopaque solution (Histopaque-1077, Sigma Aldrich, Missouri, United States) was drawn and kept at room temperature for 10 min. Three mL whole blood was layered onto the Histopaque-1077. The tubes were centrifuged at 1550 rpm for exactly 30 minutes at room temperature. Erythrocytes and granulocytes were settled at bottom, mononuclear cells formed a band at the interface between the Histopaque-1077 and the plasma, as shown in Fig. 1.

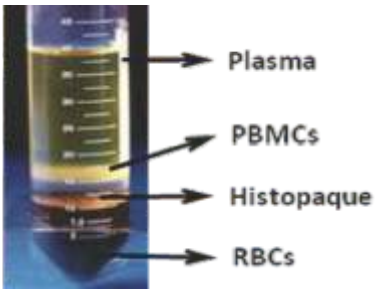


**Fig. 1: Isolation of PBMCs Using Histopaque-1077**

The opaque interface was carefully transferred with a pipette into a clean conical centrifuge tube. The cells were washed by adding 10 mL isotonic phosphate buffered saline solution, and mixed gently. Again the tubes were centrifuged at 1250 rpm for 10 minutes. The supernatant was discarded. The cell pellet was re-suspended with 5 mL isotonic phosphate buffered saline solution, and mixed gently and centrifuged at 1250 rpm for

10 minutes. Total cellular RNA from PBMCs was extracted by using phenol-chloroform method [7, 11].

**RNA Extraction from PBMCs:**

RNA extraction was performed by Chomczynski method. Total RNA was extracted using QIAzol Lysis Reagent (Qiagen). PBMCs were added in 1 mL of QIAzol lysis reagent and vortexed vigorously for 15 seconds. Chloroform (200 µl) was added to tubes and the contents were gently mixed by inverting. The tubes were incubated for 2 to 3 minutes at room temperature. The mixture was centrifuged at 12000 rpm for 15 minutes at 4°C. The aqueous phase was transferred carefully to a new tube, without disturbing the interphase. Chilled isopropyl alcohol was added to the aqueous phase in a new tube and incubated overnight at -20°C. Next day, kept for 10 minutes at room temperature. The mixture was centrifuged at 12000 rpm for 15 minutes at 4°C. The pellet was washed with 500 µl of 75% chilled ethanol (Freshly prepared) and centrifuged at 7500 rpm for 5 minutes at 4°C. The supernatant was discarded and pellet was suspended in 30 µl Diethylpyro-carbonate (DEPC) treated water. RNA samples (2 µl) were loaded on 0.8% agarose gel for quality check [12].

**RNA Quantification and Quality Check:**

Quantification of RNA was performed with UV spectrophotometer (NanoDrop; Eppendorf, Hamburg, Germany). RNA quantification and purity were checked at 260 nm, 230 nm and 280 nm using a spectrophotometer. RNA concentration was determined by the measurement of absorbance at 260 nm, while the ratios 260/280 and 260/230 were recorded to detect contamination by phenols, proteins and other organic compounds. RNA samples with 260/280 nm ratio > 1.8 were used for gene expression analysis of podocyte related proteins viz. podocalyxin, podocin and synaptopodin. Image of the gel with resolution of total RNA is shown in Fig. 2.

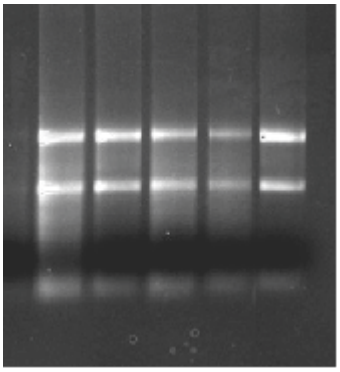

**Fig. 2: RNA Electrophoresis for Quality Check**

**cDNA Synthesis:**

First strand synthesis of complementary DNA (cDNA) was done by reverse transcription, in a reaction volume of 20 µl. Briefly, 2 µg RNA was mixed with 3 µl of random hexamer (50 ng/µl) and 1 µl of dNTPs (10 mM) in a total volume of 12 µl with DEPC water. The mixture was incubated at 65°C for 5 minutes to melt secondary structure within the template. After the incubation, the reaction was cooled rapidly on ice for 1 minute, followed by addition of 4 µl 5x first strand buffer (Promega), [250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2], 2 µl 0.1 M DTT (Invitrogen, USA) and 1 µl RNaseOUT™ Recombinant ribonuclease inhibitor (VWR, Life science, Solon). The tubes were incubated at 37°C for 2 minutes followed by addition of 0.5 µl M-MLV RT (200 units/µl, Promega). The contents of the reaction were mixed gently by pipetting up and down. Reverse transcription included the following three phases: the reaction was incubated at 25°C for 10

minutes for RT enzyme activation followed by 50 minutes at 37°C for reverse transcription and the reaction was inactivated by heating at 70°C for 15 minutes. Products of the reaction were appropriately diluted and subjected to end point PCR using house-keeping gene Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH). The synthesized cDNAs were stored at -80°C.

**Semi-Quantitative Polymerase Chain Reaction:**

The cDNA was diluted 40 times with Tris buffer ($T_{10}E_1$ buffer) (10 mM, Tris (pH 8.0), 1mM EDTA (pH 8.0) and used for semi-quantitative Polymerase Chain Reaction (PCR). The diluted cDNAs were used for normalization using gene specific primers for amplification of house-keeping gene GAPDH. For semi-quantitative PCR, normalized concentration of 1:40 diluted cDNA was used for amplification in a final reaction volume of 25 µl consisting of 12.5 µl of 2 x Taq PCR Master mix (Taq DNA Polymerase, 2 x QIAGEN PCR Buffer, 3 mM MgCl2, and 400 µM of each dNTP) (Qiagen), 0.5 µl each of forward and reverse, KiCqStart® primers (10 pM/µl stock) (Sigma-Aldrich, USA). The temperature profile for semi-quantitative PCR was as below: Initial denaturation at 94°C for 10 minutes, followed by 35 cycles, each comprising 1 minute denaturation at 94°C, 30 seconds annealing at 60°C and 1 minute extension at 72°C with final extension at 72°C for 5 minutes followed by incubation at 4°C. GAPDH gene was used as a control (housekeeping gene) for normalization. Expression analysis of Podocalyxin (PODXL), Podocin (NPHS2) and Synaptopodin (SYNPO) was done from all the samples. Sigma KiCqStart® primers were used to study modulation of gene expression. The primer sequences are listed in below Table 1. The amplified 25 µl PCR products were resolved by electrophoresis on 1.5% agarose gel (Low EEO, Bangalore Genei), resolved and photographed under UV transilluminator (GelDoc™ XR+, Bio-Rad). The bands were quantified by densitometry using Image Lab™ software V 4.1. Gene expression levels were normalized to those of GAPDH (Fig. 3).

**Table 1: KiCqStart® primers used to study modulation of gene expression of PODXL, NPHS2 and SYNPO**

| Gene | Primer sequence | Annealing temperature |
|---|---|---|
| GAPDH | F 5'-AGTTCAACGGCACAGTCAAG-3'<br>R 5'-TACTCAGCACCAGCATCACC-3' | 60℃ |
| PODXL | F 5'-CTACTAGAGACAGTGTTTCAC-3'<br>R 5'-GAGGTCTGTTGAGTTCTTTG-3' | 45℃ |
| NPHS2 | F 5'-CTTTTCATGAGATCGTGACC-3'<br>R 5'-GACTGCTTAGGAGAAGAGAG-3' | 48℃ |
| SYNPO | F 5'-CTGTGATTCCTGTGGTATTG-3'<br>R 5'-GCTGAGACGAAAGATAAGTC-3' | 45℃ |

**Statistical Analysis:**

The quantitative variables were presented as Mean ± Standard Error (SE) and differences in means between mRNA expression of podocalyxin, podocin and synaptopodin in T2DM patients with and without nephropathy and controls were compared using students' unpaired 't' test. The p values of <0.05 were considered significant. Statistical analysis was done using SPSS for Windows, version 21.

**Results:**

The mean ages of healthy controls, T2DM without and with nephropathy were 49.3 ± 7.28, 50.94 ± 7.92 and 51.0 ± 7.7 years, respectively. The gender distribution among the groups viz. healthy controls, T2DM without and with nephropathy was 59, 50 and 66 were males, while 51, 60 and 44 were females. The differences in mean age (p=0.1746) and gender distribution (p=0.0956) when compared among the groups found non-significant. The biochemical analysis of these patients including the duration of diabetes is published previously [13]. The expression analysis of relevant genes from podocytes in T2DM nephropathy patients was evaluated in present study. PBMCs were isolated from blood samples of all subjects, followed by extraction of total RNA from PBMCs. The gene expressions of podocyte associated proteins viz. SYNPO, PODXL and NPHS2 were studied. The expression of SYNPO (p=0.0289) was significantly increased in diabetics than the controls, while no significant difference was found for PODXL and NPHS2. The expressions of PODXL (p<0.0001) and NPHS2 (p=00410) were significantly up-regulated, SYNPO was unaltered in microalbuminuric patients than healthy controls. PODXL (p=0.0002) and SYNPO (p=0.0091) were increased significantly in nephropathy subjects than controls, with no significant change in NPHS2. Comparison of gene expressions of podocalyxin, podocin and synaptopodin between T2DM, T2DM with nephropathy and healthy controls, are shown in Table 2. Figs 2A, 2B and 2C show the comparison of gene expressions of PODXL, NPHS2 and SYNPO in T2DM patients with and without nephropathy, and controls respectively.

Expressions of PODXL, NPHS2 and SYNPO genes were compared between diabetics without nephropathy, microalbuminuric and proteinuric T2DM patients. The expression of only PODXL (0.0014) was found to be up-regulated in microalbuminuric patients as compared to T2DM

**Table 2: Comparison of Gene Expression of Podocalyxin, Podocin and Synaptopodin between T2DM, T2DM with Nephropathy and Control Groups**

| Gene | Controls Vs DM | Controls Vs MAU | Controls Vs Nephropathy |
|---|---|---|---|
| **Podocalyxin** | 0.0634 (0.2639) | <0.0001* (0.8656) | 0.0002* (0.9645) |
| **Podocin** | 0.1210 (0.1502) | 0.0410* (0.2294) | 0.1503 (-0.1452) |
| **Synaptopodin** | 0.0289* (-0.1311) | 0.2665 (0.1543) | 0.0091* (0.3398) |

*DM: T2DM without nephropathy; MAU: Microalbuminuria*
**statistically significant (p<0.05)*

patients without nephropathy. PODXL (p=0.0031), SYNPO (p<0.0001) were significantly up-regulated and NPHS2 (p=0.0005) was significantly down-regulated in nephropathy subjects as compared to T2DM patients without nephropathy. A significant down-regulation was found for NPHS2 (p=0.0004) expression in nephropathy patients than microalbuminuric patients of T2DM with nephropathy, and no significant difference was found for podocalyxin and synaptopodin. Comparison of gene expressions of podocalyxin, podocin and synaptopodin between diabetics without nephropathy, microalbuminuric and proteinuric (nephropathy) T2DM patients, is shown in Table 3.

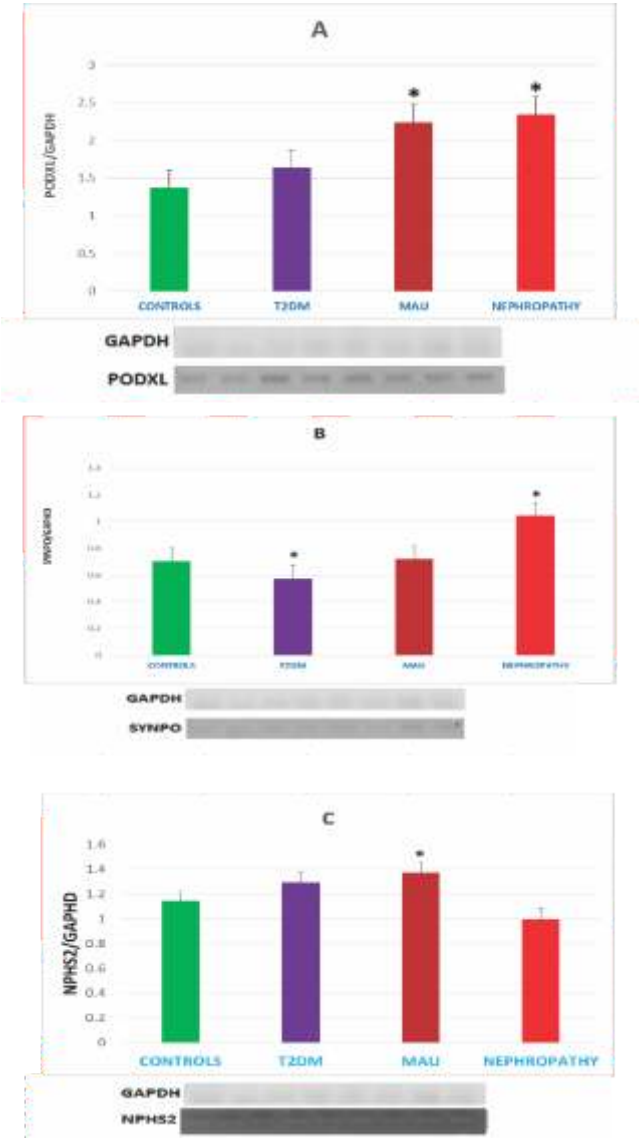


**Fig. 3: Gene Expression of PODXL (A), SYNPO (B) and NPHS2 (C) from Peripheral Blood Mononuclear Cells of T2DM Patients with and without Nephropathy and Healthy Control Groups**

**Table 3: Comparison of Gene Expression of Podocalyxin, Podocin and Synaptopodin between T2DM, Microalbuminuria and Nephropathy Patients**

| Gene | DM Vs MAU | DM Vs Nephropathy | MAU Vs Nephropathy |
|---|---|---|---|
| **Podocalyxin** | 0.0014* (0.6017) | 0.0031* (0.7007) | 0.5449 (0.09897) |
| **Podocin** | 0.2588 (0.07923) | 0.0005* (-0.2955) | 0.0004* (-0.3147) |
| **Synaptopodin** | 0.0710 (0.2854) | <0.0001* (1.7710) | 0.2384 (0.1855) |

*DM: T2DM without nephropathy; MAU: Microalbuminuria*
**statistically significant ($p<0.05$)*

**Discussion:**

The chronic hyperglycemia, a characteristic of diabetes mellitus, with other risk factors shows long term consequences including microvascular and macrovascular complications which may lead to damage to several organs. The macrovascular complications of diabetes comprise of CVD and stroke, and diabetic nephropathy, neuropathy and retinopathy are the microvascular complications [14]. Uncontrolled hyperglycemia and hypertension are established causal factors for the development of DN [15] whose natural history has been changed most as a consequence of the scientific advances over the last 20 years [16]. DN is the major single cause of ESRD [17], a life-threatening complication resulting in a poor prognosis for patients as well as high medical costs [18].

ESRD requires dialysis and is becoming a staggering challenge to public health care systems due to the prohibitive costs of renal replacement therapy. About 20 to 40% of all diabetic patients are prone to develope kidney failure [19]. DN manifests as a clinical syndrome that is composed of albuminuria, progressively declining GFR, and increased risk for CVD [19-20]. The identification of microalbuminuria as an early biomarker/ predictor for both renal and cardiovascular diseases has allowed patients at high risk to be identified and treated [16].

In mammalian tissues, mRNA expression, related to the transcriptional activity of various genes is in a state of dynamic turnover during different developmental and pathophysiological states. In such a process, various genes are differentially up- or down-regulated depending on a given disease process [21]. Podocytes are highly specialized visceral epithelial cells which are important for maintenance of dynamic functional barrier and regulate glomerular function by covering GBM from outside. As podocyte proteins are specifically expressed on podocyte cells, analyzing their expression help in the understanding of development of proteinuria in nephropathy patients. The podocyte protein podocin present in slit diaphragm is closely associated with synaptopodin and alpha actinin-4 of cytoskeleton; this enables progressive podocyte architecture rearrangements. The filtration of negatively charged albumin is restricted by the phenotypic marker and apical membrane protein of podocyte cells i.e. podocalyxin [6-7].

The association of proteinuria with number of genes and podocyte associated proteins has been proved already, which become suggestive of use of markers related to podocytes can be used for monitoring DN progression [1]. In the past few years, an increased number of podocyte-expressed genes have been identified, stimulating a growing interest in studying their role in the development of proteinuria and glomerular disease [22].

In this study, the investigation of gene expression of podocyte related proteins viz. podocalyxin, podocin, and synaptopodin in T2DM patients with and without nephropathy from PBMCs was done. The expression of podocalyxin was up-regulated significantly in microalbuminuric and overt nephropathy patients than the T2DM patients without nephropathy and controls. Podocin expression was found to be up-regulated in microalbuminuric patients than T2DM without nephropathy patients, and it was down-regulated in overt diabetic nephropathy patients than T2DM without nephropathy and microalbuminuric patients. The gene expression of synaptopodin was down-regulated in T2DM patients without nephropathy than controls, and it was up-regulated in overt diabetic nephropathy patients than controls and T2DM patients without nephropathy. Previously, researchers focused on the detection of these podocyte related proteins and expression of their genes in excreted podocytes from urine samples, we have made an attempt to study the gene expression of these podocyte associated proteins from PBMCs.

It is widely accepted that podocyte injury may trigger a sequence of events through epithelial-mesenchymal transition and apoptosis or detachment, to ultimately contribute to glomerulosclerosis and decline of renal function [23]. It was detected by Hara *et al.* [2] in patients with diabetes that, urinary podocalyxin was higher in 53.8% patients at the normoalbuminuric stage, in 64.7% at the microalbuminuric stage and in 66.7% at the macro-albuminuric stage. Zheng *et al.* [23] determined the significantly increased mRNA expression of podocyte associated genes in urine viz. synaptopodin, podocalyxin, CD2-AP, α-actinin 4, and podocin from patients with varying stages of DN compared with controls.

Wang *et al.* [24] in DN patients found the higher urinary mRNA expressions of nephrin, podocin, synaptopodin, WT-1 and α-actinin 4 in comparison to controls. Proteinuria and/or renal function were also shown to be associated with urinary nephrin and synaptopodin expressions, while degree of histological damage was found to be related to WT-1 expression. In a study by Wang *et al.* [25], intra-renal expression of podocyte-associated molecules was found to be correlated with glomerular podocyte number, renal function, and tubulo-interstitial scarring, and suggested that intra-renal, but not urinary expression of podocyte-associated molecules might be used to assess the degree of podocyte loss in DN.

Aaltonen *et al.* [26] studied the expression levels of nephrin-specific mRNA in streptozotocin model of rats and non-obese diabetic mouse model and up to 2 fold increase in nephrin specific mRNA expression during follow up after several weeks was noted, with the conclusion of connection of loss of glomerular filtration function, early changes in DN with a podocyte protein nephrin. Toyoda *et al.* [27] examined the expression of nephrin mRNA in the kidneys of T2DM with DN, and suggested that low expression of nephrin mRNA may be closely linked to development and/or progression of proteinuria in human DN.

Baelde *et al*. [28] evaluated mRNA expression profiles of glomeruli from DM and healthy individuals. Oligonucleotide microarray analyses on control and diabetic glomeruli were presented and discussed in their relation to vascular damage, mesangial matrix expansion, proliferation, and proteinuria. Their findings suggest that progression of DN might result from diminished tissue repair capability. do Nascimento *et al*. [7] demonstrated that nephrinuria was correlated with diabetic nephropathy stage and predicted pathological albuminuria but they could not find significant difference in urinary mRNA levels of podocyte markers in pre-diabetic subjects than controls.

The evaluation of mRNA profile of podocyte related proteins in urine and renal tissues of patients with proliferative or non-proliferative glomerulopathies by Rodrigues *et al*. [29], found in non-proliferative glomerulopathy, pointed higher degree of intra-renal podocytopenia and of podocyturia in the proliferative pathologies. The expressions of mRNAs of proteins related to podocytes were decreased effectively after immunosuppressive therapy.

Inhibition of podocyte-associated mRNAs in kidney tissue suggests that podocyte injury occurs regardless of the severity of lupus nephritis. Increased urinary excretion of podocyte mRNAs, mostly in patients with moderate to severe lesions, may reflect a greater burden of glomerular damage with detachment of podocytes into the urine. According to dos Santos *et al*. [30] Numerous factors have been implicated in the pathophysiology of podocyte injury in diabetic nephropathy. Among them, high glucose, angiotensin II, TGF-$\beta$, and mechanical stress have widely been studied to explore the precise mechanisms of podocyte injury under diabetic conditions [5].

The potential mechanisms of podocyte injury include foot process effacement, hypertrophy, detachment, apoptosis, and perhaps Epithelial to Mesenchymal Transition (EMT), and these mechanisms are believed to be associated with either type 1 or T2DM in the early phases of DN [1, 3]. Several studies have been performed on the angiotensin II activity which is involved in podocyte injury in diabetes. Angiotensin converting enzyme inhibitors prevented loss of podocytes and podocyte injury in streptozotocin induced diabetic rats. In addition to angiotensin converting enzyme inhibitors, angiotensin II type 1 receptor antagonists attenuated podocyte foot process broadening in the streptozotocin induced diabetic rats [19].

**Conclusion:**

To our knowledge, this is the first study from India to note the gene expression of podocyte related proteins in patients of T2DM with and without nephropathy. The use of the detection of altered or modulation in gene expression of these proteins can be used as an early marker for the detection of development of nephropathy in T2DM patients. The preventive measures can be taken to prolong the onset of nephropathy in these patients, this increases the life expectancy. It will also lead to identification of candidate disease susceptible or resistant genes, but also would delineate novel mechanisms and pathways involved in various diseases.

**Acknowledgment**

The authors would like to thank technical staff of central clinical laboratory of Bharati Hospital, Pune.

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**How to cite this article:**

Bhoite GM, Bulakh PM, Kuvalekar AA, Padwal MK, Momin AA. mRNA Expression of Podocyte Associated Proteins in Peripheral Blood Mononuclear Cells of Type 2 Diabetes Mellitus Patients with and without Nephropathy. *J Krishna Inst Med Sci Univ* 2020; 9(3):10-21

***Submitted:*** *06-May-2020* ***Accepted:*** *27-May-2020* ***Published:*** *01-July-2020*