
Review article

Nephrin and Podocalyxin - New Podocyte Proteins for Early Detection of Secondary Nephropathies

Irena Kostovska¹, Katerina Tosheska Trajkovska¹, Svetlana Cekovska¹, Goce Spasovski² and Danica Labudovic¹

¹Department of Medical and Experimental Biochemistry, ²Department of Nephrology, Medical Faculty, Skopje, University "Ss Cyril and Methodius", Skopje, Republic of Macedonia

Abstract

In the last two decades a great progress was observed in understanding of podocytes, their specific structure and function identifying many specific podocyte proteins, such as nephrin and podocalyxin. Podocytes form the final barrier to plasma proteins leakage. Nephrin as a main component of the filtration diaphragm forms a physical barrier while podocalyxin as sialoglycoprotein forms an electrostatic barrier. Podocyte damage, i.e. podocytopathies and their loss through urine-podocyturia, are crucial in pathogenesis and progression of nephropathies with proteinuria as main clinical manifestation. In podocytopathies, nephrin and podocalyxin appear in the urine before proteinuria and microalbuminuria which were previously considered as earliest markers of nephropathies. Nephrinuria and podocalyxuria indicate damage of the podocytes on glomerular level and/or presence of apoptotic and necrotic podocytes in urine. These urinary markers are also important in early

diagnosis of secondary nephropathies such as diabetic, lupus and hypertensive nephropathy as the most common causes of end-stage renal failure (ESRF). These markers are also important in the prediction of preeclampsia, which is the most common complication in pregnancy. In this review we elaborate in dept the main structural and functional features of podocytes and their specific proteins, nephrin and podocalyxin, summarizing the recent literature data on their importance in the early diagnosis of the most common secondary nephropathies.

Keywords: nephrin, podocalyxin, podocytes, podocytopathies, secondary nephropathies

Introduction

Nephrin and podocalyxin are specific podocyte proteins. Podocytes are terminally differentiated cells creating

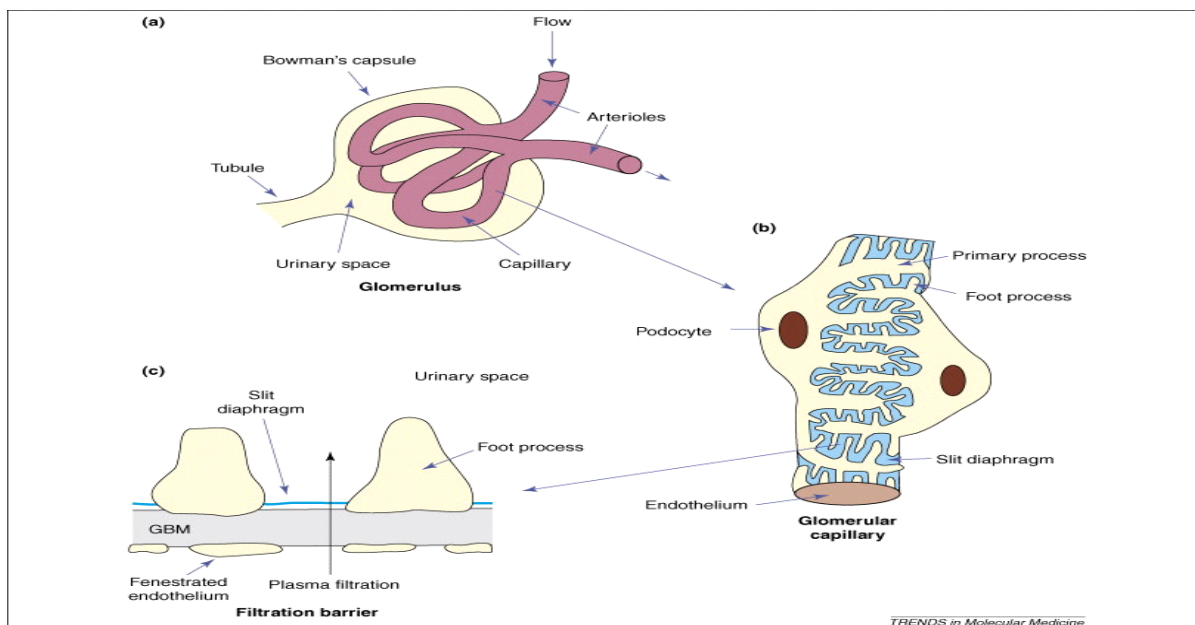


Fig. 1. a) Structure of the glomerulus b) structure of the glomerular filtration barrier c) structure of the slit diaphragm [3]

the visceral epithelial layer of the glomerulus. The glomerulus is a network of capillary loops surrounded by the Bowman's capsule and performs the first step of blood filtering. As a selective filter, based on its size and charge, the glomerulus allows passage of materials that circulate through blood, creating primary ultrafiltrate. This selectivity is based on the structural integrity of the three main components of the glomerular filtration barrier: the fenestrated vascular endothelium, the glomerular basement membrane (GBM), and the visceral epithelium overlying the GBM. Podocytes as a part of glomerular filtration barrier have foot processes that encircle the GBM. The interdigitating foot process of podocytes is joined by a slit diaphragm. Slit diaphragm is described as a zipper-like interaction of membrane proteins such as nephrin molecules between neighboring podocyte foot processes. The slit diaphragm has an essential role in size selectivity of the glomerular filtration barrier [1,2]. Figure 1 illustrates the structure of the glomerulus, glomerular filtration barrier and filtration diaphragm also called slit diaphragm.

Nephrin - (NPH), structure and function

Nephrin was first discovered in 1998 as a mutant product of NPHS1 gene, which was first cloned by Kestila and colleagues in children with Finnish type of congenital nephrotic syndrome-(Congenital nephrotic syndrome of the Finnish type-CNF). CNF is an autosomal recessive disease characterized by massive proteinuria in utero and symptoms of nephrotic syndrome (hypoalbuminemia, hyperlipidemia and swelling) that occur in the first days after birth. Renal biopsy in these children shows obliteration of podocyte foot processes and lack of slit

diaphragm [4]. In mice inactivation of NPHS1 gene causes massive proteinuria and death in the first 24 hours after birth [5]. This suggests the importance of nephrin in the process of glomerular filtration as a structural component of the slit diaphragm. Nephrin is exclusively expressed by podocytes but also may be expressed in brain, lymphoid tissue, heart, testis, placenta and β cells of the Langerhan's islets of the pancreas [6]. NPHS1 gene is located on chromosome 19 (19q13.1), organized in 29 exons [7]. Nephrin has 1241 amino acids with molecular weight of 180 kDa (135 kDa without posttranslational modification). It is a transmembrane glycoprotein belonging to the immunoglobulin superfamily of cell-adhesion receptors. It contains eight extracellular Ig like domains, followed by a fibronectin type III-like module, a short transmembrane domain and a cytoplasmic C-terminus. Cytoplasmic segment contains nine tyrosine residues which are phosphorylated in interaction with other nephrin molecules, a process which is very important in the intracellular signaling. Nephrin has three cysteines in the extracellular segment that are important in the podocyte foot process interaction, described as zipper interaction in the center of the slit diaphragm. As a major component of the slit diaphragm, nephrin forms the physical barrier to plasma proteins [8]. Nephrin is important in organization and maintenance of integrity of podocyte cytoskeleton and as a signal molecule, through several signaling pathways, regulates the shape and structure of the podocytes and slit diaphragm [9,10]. Nephrin is located laterally on the foot processes and it is a major component of the slit diaphragm (Figure 2).

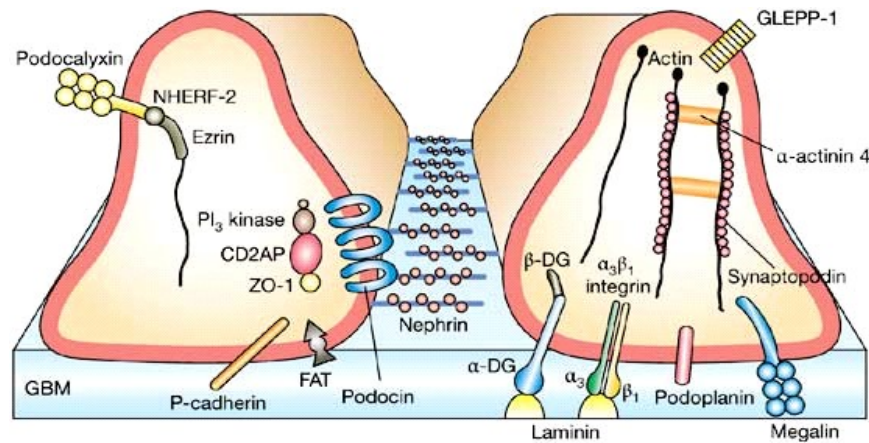


Fig. 2. Arrangement of the podocyte foot process and slit diaphragm proteins. Localization of nephrin and podocalyxin in podocytes [11]

Podocalyxin - (PODXL), structure and function

Podocalyxin is an anionic transmembrane protein localized at the apical surface of the podocytes (Figure 2); it may also be expressed on the surface of hematopoietic progenitor cells, vascular endothelial cells, neurons and numerous tumor cells [12]. Podocalyxin is a main sialoglycoprotein of the podocyte glycocalyx, which primarily has been identified in mice, and later in the humans. As sialoglycoprotein, podocalyxin forms the electrostatic barrier to plasma proteins. Podocalyxin is a member of the CD34 (Cluster of Differentiation 34) family with a molecular weight of 140 kDa. The extracellular part of podocalyxin is rich in serine, threonine and proline, containing O-glycosylated, sialysed and N-glycosylated domain. The intracellular part has several phosphorylation sites along one protein interaction domain, through which domain, podocalyxin interacts with Na / H exchanger regulatory factor 1 and 2 (NHERF1 and NHERF2) [13-16]. Podocalyxin interacts with ezrin molecules that are part of the podocyte cytoskeleton [17]. Podocalyxin is important in the development of glomeruli and mice that do not express podocalyxin were found to have disrupted architecture of the podocytes and showed absence of foot processes and slit diaphragm [18]. Since sialomucin has cell-cell antiadhesive effect, which is important to keep the filtration pores open and prevent conglomeration of the parietal and visceral epithelial layer of Bowman's capsule. All these processes are important to keep the normal glomerular filtration [14]. Podocalyxin is a major hallmark of podocyte phenotype and preferred protein marker for the detection and identification of podocyte with immunofluorescence technique in bioptic material and urine.

Podocytopathies

In recent years the attention of scientists, especially nephrologists and pathologists, has been focused on the role of podocytes in the pathogenesis of glomerulopathies or nephrotic syndrome. Furthermore, a great prog-

ress has been achieved in the study of the biology of podocytes, their function and mechanisms of their impairment. The response to injury of podocytes as highly differentiated cells is not typical and once they are damaged, there is a progression towards glomerulosclerosis [19,20]. The etiology of podocytopathies may be different: immunological, mechanical, infectious, metabolic, toxic, genetic etc. Reaction of podocytes to etiological factors can be different:

1. foot process effacement without changes in the number of podocytes,
2. apoptosis and loss of podocytes,
3. changes in development of podocytes and their proliferation,
4. de-differentiation.

Hence, based on the histological changes there are four types of podocytopathies:

1. minimal change nephropathy with normal number of podocytes,
2. focal segmental glomerulosclerosis with podocytopenia,
3. diffuse mesangial sclerosis with low proliferative index,
4. collapsing glomerulopathy with high proliferative index [21].

Diagnosis of podocytopathies includes morpho-pathological examination by light and electron microscopy of bioptic kidney material, immunohistochemistry-identification of specific proteins of podocytes in the bioptic material, detection and quantification of circulating biomarkers, detection and quantification of urinary biomarkers (Enzyme-linked immunosorbent assay (ELISA), Western blot, immunofluorescence, flow cytometry, mass spectrometry and Reverse transcription polymerase chain reaction (RT-PCR) for detection of mRNA of specific podocyte proteins), genetic analyses in hereditary podocytopathies [2,22].

Diagnostic relevance of nephrin and podocalyxin in secondary nephropathies

Specific podocyte proteins, nephrin and podocalyxin are relatively new urinary markers for detection of nephropathies. The diagnostic advantages of these markers are: high specificity, non-invasive detection, monitoring of nephropathies, and they can be measured by relatively simple and sensitive methods such as ELISA. This is in agreement with the statement of Walter Piering, MD: "Urine is the liquid biopsy of the kidney". Relevance as an early diagnostic marker is reserved for secondary nephropathies such as diabetic, lupus, hypertensive and preeclampsia. Early nephropathy detection may allow timely treatment and prevention for the need of renal replacement therapy as well as significant reduction of complications and mortality in these patients.

Nephrinuria and podocalyxuria in diabetic nephropathy

The podocytopathies play a critical role in the early functional and structural changes of diabetic kidney disease [23]. In diabetic nephropathy (DN) there is a decreased podocyte number and/or density as a result of apoptosis or detachment, GBM thickening and a reduction in nephrin protein in the slit diaphragm with podocyte foot process effacement [24]. Pathohistologically, DN begins with hypertrophy and hyperactivity of podocytes which lead to damage of the slit diaphragm. In advanced stage there is an ensuing atrophy of podocytes, narrowing of the foot processes, fragmentation and detachment of podocytes from GBM. All these changes lead to proteinuria [24,25]. Thus, a significant increase of foot processes width is noted in histomorphological studies in diabetic patients with advanced nephropathy and proteinuria [26]. Diabetic Pima Indians with clinical nephropathy have fewer glomerular epithelial cells compared to those with less-advanced renal disease and also there is a correlation between the number of podocytes and the degree of proteinuria. In this study, it has been shown that the number of glomerular podocytes is the best predictor of glomerular damage in diabetics [27]. In another cohort study including patients with type 2 diabetes a significant reduction was found in the number of glomerular podocytes even in the normoalbuminuric patients [28]. In one Japanese study podocytes were detected in the urine in 53% of microalbuminuric patients and 80% of macroalbuminuric patients with type 2 diabetes. In the same study, trandolapril reduced urinary albumin excretion, as well as urinary podocytes in patients with DN. This study showed that podocyturia can be a useful marker for disease activity and trandolapril can be useful drug in DN [29]. All these studies suggest that morphological changes in podocytes are present before appearance of proteinuria. In the study of Patari, nephrinuria was present in 30% of normoalbuminuric, 17% of microalbuminuric, 28% of macroalbuminuric, 28% of new-microalbuminuric patients and 0% in control subjects. This

study reconfirmed that nephrinuria may have a prognostic value in DN [30]. It was also observed that the number of urinary podocalyxin-positive elements (PCX+EL) may be significantly increased in the early course of DN compared to health controls and correlated well with the clinical diagnosis of DN, especially in the stage of normoalbuminuria [31]. Hara *et al.* found that urinary podocalyxin was significantly higher in 53.8% of normoalbuminuric, 64.7% of microalbuminuric and 66.7% of macroalbuminuric patients with DN. Thus, podocalyxin measured in urine by the ELISA method can be used as a marker for early detection of diabetic nephropathy [32].

Nephrinuria and podocalyxuria in preeclampsia

Preeclampsia is a pregnancy-specific disorder associated with significant maternal-fetal morbidity and mortality. Hypertension (>140/90 mm Hg) and proteinuria (>300 mg in a 24-hour urine) are the main clinical manifestations of preeclampsia, which usually occurs after 20 weeks of gestation. Preeclampsia is a secondary nephropathy that includes damage to podocytes and their loss on glomerular level that leads to proteinuria. One study demonstrated that podocyturia was present in pregnant women who developed preeclampsia, at a time when hypertension and proteinuria were absent, suggesting that podocyturia may serve as a predictive marker in preeclampsia. In addition, there was a positive correlation between the number of podocytes and the degree of proteinuria, suggesting that podocyte loss may be related to the onset and severity of proteinuria [33]. In a study of Garovic *et al.* podocyturia exhibited 100% sensitivity and 100% specificity in the diagnosis of preeclampsia [34]. On the other hand, in the study of Wang urine nephrin and podocalyxin levels were found significantly higher in women with preeclampsia compared to those in normal pregnant [35]. Son *et al.* also found a positive correlation between urinary nephrin and proteinuria, creatinuria and diastolic blood pressure in preeclamptic women, which indicate the importance of nephrin in the pathogenesis of proteinuria in preeclampsia and the ability to be a reliable indicator of renal damage [36]. On the other hand, Jim *et al.* found that nephrinuria had 57% sensitivity and 58% specificity as a diagnostic tool in preeclampsia [37]. In pregnant women with preeclampsia and eclampsia in Paraguay elevated concentrations of podocalyxin in urine were found regardless of the presence of proteinuria. In fact, podocalyxin concentration in urine correlated with the degree of damage to podocytes [38]. In the same study the quantification of urinary podocalyxin was made by the ELISA method as a relatively inexpensive, simple and also a useful method for detecting damage of podocytes.

Nephrinuria and podocalyxuria in hypertensive nephropathy

Hypertensive nephropathy or hypertensive nephrosclerosis is a medical condition referring to damage to the kidney due to the high blood pressure. This is the second most common cause of ESRF [39]. Podocyte damage is important in the pathogenesis of hypertensive nephropathy, but human data on the mechanisms of damage to podocytes in hypertension are yet limited. However, it is supposed that mechanical damage of podocyte cytoskeleton is crucial in pathogenesis of the hypertensive podocytopathy [40]. Pathohistological examination of bioptic material in hypertensive adult Africans showed that 13% of them presented with typical focal segmental glomerulosclerotic lesions [41]. The study of Wang demonstrated the presence of podocytopenia on glomerular level and reduced intrarenal gene expression of podocyte-associated molecules in patients with hypertensive nephropathy. These findings were in correlation with renal function and the degree of renal fibrosis, suggesting that podocyte loss may play an important role in the pathogenesis of hypertensive nephropathy [42].

Nephriuria and podocalyxuria in lupus nephropathy

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease characterized by the production of antinuclear antibodies. As a multi-organ disease SLE involves the kidney. Lupus nephritis (LN) patients present with proteinuria that has generally been associated with immune complex deposition in the glomerular capillary wall and endo-capillary proliferation and inflammation [43]. A decreased number of glomerular podocytes, an association between proteinuria and decreased podocyte numbers in lupus glomerulus and an increased excretion of urinary podocytes in patients with lupus nephritis have been recently found [44]. Interestingly, it has also been found that in patients with SLE there are increased urinary levels of podocyte proteins, nephrin and podocalyxin. Thus, urinary podocalyxin/creatinine ratio may be used as a non-invasive marker for pathological impact of SLE on the kidney [45].

Conclusion

Nephrin and podocalyxin are relatively new markers for detection of podocytopathies. They appear in urine before microalbuminuria and proteinuria and therefore may be useful in the early diagnosis of secondary nephropathies. They have a great importance as auxiliary tools in the diagnosis, differential diagnosis and prognosis in primary nephropathies, reducing the requirement of indications for renal biopsy. The meaning of early diagnostic markers is reserved for secondary nephropathies because in the primary nephropathies proteinuria is often the first clinical manifestation. Nephrin and podocalyxin are important as markers for early detection of secondary nephropathies such as diabetic, lupus, and hypertensive, which are the most common causes of end-stage

renal disease. These markers are also important in the prediction of preeclampsia as a leading cause of complications during pregnancy. Early detection of these secondary nephropathies will allow timely treatment and reduce complications and mortality. The advantage of these urinary markers is high specificity and sensitivity for secondary nephropathies and ability to be measured by relatively inexpensive, simple and non-invasive methods. Nephrin and podocalyxin have a promising potential as diagnostic and prognostic markers for clinicians and researchers, but more extensive clinical research is required to assess their true diagnostic and prognostic value. These markers are also new potential therapeutic targets in renal diseases.

Conflict of interest statement. None declared.

References

1. Kumagai T, Mouawad F, Takano T. Pathogenesis of common glomerular diseases-role of the podocyte cytoskeleton. *Cell Health and Cytoskeleton* 2012; 4: 103-118.
2. Sekulic M, Pichler Sekulic S. A compendium of urinary biomarkers indicative of glomerular podocytopathy. *Pathol Res Int* 2013; 2013: 782395.
3. Patrakka J, Tryggvason K. Nephrin-a unique structural and signaling protein of the kidney filter. *Mol Med* 2007; 13(9): 396-403.
4. Kestila M, Lenkkeri U, Mannikko M, et al. Positionally cloned gene for a novel glomerular protein-nephrin-mutated in congenital nephrotic syndrome. *Mol Cell* 1998; 1: 575-582.
5. Putaala H, Soinen R, Kilpelainen P, et al. The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death. *Hum Mol Genet* 2001; 10(1): 1-8.
6. Li XZ, He JC. An update: the role of Nephrin inside and outside the kidney. *Sci China Life Sci* 2015; 58: 649-657.
7. Lenkkeri U, Antignac C, Kashtan CE, et al. Structure of the gene for congenital nephrotic syndrome of the Finnish type (NPHS1) and characterization of mutations. *Am J Hum Genet* 1999; 64: 51-61.
8. Ruotsalainen V, Ljungberg P, Wartiovaara J, et al. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 1999; 96: 7962-7967.
9. Lehtonen S, Lehtonen E, Kudlicka K, et al. Nephrin forms a complex with adherens junction proteins and CASK in podocytes and in Madin-Darby canine kidney cells expressing nephrin. *Am J Pathol* 2004; 165(3): 923-936.
10. Benzing T. Signaling at the slit diaphragm. *J Am Soc Nephrol* 2004; 15(6): 1382-91.
11. Alain Meyrier. Mechanisms of Disease: focal segmental glomerulosclerosis. *Nat Clin Pract Nephrol* 2005; 1: 44-54.
12. Doyonnas R, Nielsen JS, Chelliah S, et al. Podocalyxin is a CD34-related marker of murine hematopoietic stem cells and embryonic erythroid cells. *Blood* 2005; 105 (11): 4170-4178.
13. Orlando RA, Takeda T, Zak B, et al. The glomerular epithelial cell anti-adhesin podocalyxin associates with the actin cytoskeleton through interactions with ezrin. *J Am Soc Nephrol* 2001; 12(80): 1589-1598.
14. Kershaw DB, Beck SG, Wharram BL, et al. Molecular cloning and characterization of human podocalyxin-like protein. Orthologous relationship to rabbit PCLP1 and rat podocalyxin. *J Biol Chem* 1997; 272: 15708-15714.

15. Kerjaschki D, Sharkey DJ, Farquhar MG. Identification and characterization of podocalyxin-the major sialoprotein of the renal glomerular epithelial cell. *J Cell Biol* 1984; 98 (4): 1591-1596.
16. Li Y, Li J, Straight SW, *et al.* PDZ domainmediated interaction of rabbit podocalyxin and Na⁺/H⁺ exchange regulatory factor-2. *Am J Physiol Renal Physiol* 2002; 282(6): F1129-F1139.
17. Doyonnas DB Kershaw, Duhme C, *et al.* Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. *J Exp Med* 2001; 194(1): 13-27.
18. Takeda T, Go WY, Orlando RA, *et al.* Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. *Mol Biol Cell* 2000; (11): 3219-3232.
19. Pollak MR. Inherited podocytopathies: FSGS and nephrotic syndrome from a genetic viewpoint. *J Am Soc Nephrol* 2002; 13: 3016-3023.
20. Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol rev* 2003; 83(1): 253-307.
21. Barisoni L, Schnaper HW, Kopp JB. A proposed taxonomy for the podocytopathies: A reassessment of the primary nephrotic diseases. *Clin J Am Soc Nephrol* 2007; 2: 529-542.
22. Wągrowska-Danilewicz M, Stasikowska O, Danilewicz M. Immunorexpression of podocyte-associated proteins in acquired human glomerulopathies with nephrotic syndrome. *Pol J Pathol* 2006; 57: 17-21.
23. Wolf G, Chen S, Ziyadeh FN. From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. *Diabetes* 2005; 54: 1626-1634.
24. FN Ziyadeh, G Wolf. Pathogenesis of the Podocytopathy and Proteinuria in Diabetic Glomerulopathy. *Current Diabetes Reviews* 2008; 4: 39-45.
25. Mandache E, Penescu M. Nanostructural features of diabetic podocytopathy. *Rom J Morphol Embryol* 2012; 53: 23-27.
26. Bjorn SF, Bangstad HJ, Hanssen KF, *et al.* Glomerular epithelial foot processes and filtration slits in IDDM patients. *Diabetologia* 1995; 38: 1197-1204.
27. Meyer TW, Bennett PH, Nelson RG. Podocyte number predicts long-term urinary albumin excretion in Pima Indians with type II diabetes and microalbuminuria. *Diabetologia* 1999; 42: 1341-1344.
28. Vestra MD, Masiero A, Roiter AM, *et al.* Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. *Diabetes* 2003; 52: 1031-1035.
29. Nakamura T, Ushiyama C, Suzuki S, *et al.* Urinary excretion of podocytes in patients with diabetic nephropathy. *Nephrol Dial Transplant* 2000; 15: 1379-1383.
30. Patari A, Forsblom C, Havana M, *et al.* Nephrinuria in diabetic nephropathy of type 1 diabetes. *Diabetes* 2003; 52: 2969-2974.
31. Ye H, Bai X, Gao H, *et al.* Urinary podocalyxin positive-element occurs in the early stage of diabetic nephropathy and is correlated with a clinical diagnosis of diabetic nephropathy. *J Diabetes Complicat* 2014; 28: 96-100.
32. Hara M, Yamagata K, Tomino Y, *et al.* Urinary podocalyxin is an early marker for podocyte injury in patients with diabetes: establishment of a highly sensitive ELISA to detect urinary podocalyxin. *Diabetologia* 2012; 55(11): 2913-2919.
33. Iasmina M Craici, Steven J Wagner, Kent R Bailey, *et al.* Podocyturia predates proteinuria and clinical features of preeclampsia: A longitudinal prospective study. *Hypertension* 2013; 61(6): 1289-1296.
34. Garovic VD, Wagner SJ, Turner ST, *et al.* Urinary podocyte excretion as a marker for preeclampsia. *Am J Obstet Gynecol* 2007; 196: 320 e321-e327.
35. ang YW, Zhao S, Loyd S, *et al.* Increased urinary excretion of nephrin, podocalyxin, and β ig-h3 in women with preeclampsia. *Am J Physiol Renal Physiol* 2012; 302(9): F1084-F1089.
36. Son GH, Kwon JY, Lee S, *et al.* Comparison of serum and urinary nephrin levels between normal pregnancies and severe preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2013; 166(2): 139-144.
37. Jim B, Mehta S, Qipo A, *et al.* A comparison of podocyturia, albuminuria and nephrinuria in predicting the development of preeclampsia: prospective study. *PloS One* 2014, 9: e101445. 10.1371/journal.pone.0101445.
38. Palacios de Franco Y, Velazquez K, Segovia N, *et al.* Urinary podocalyxin as a marker of preeclampsia in a Hispanic population. *Int J Physiol Pathophysiol Pharmacol* 2014; 6(2): 115-124.
39. Rishi Sharma, Surineni Kamalakar, Ellen McCarthy, *et al.* Proteinuria in Hypertensive Nephropathy: A Review. *Open Journal of Nephrology* 2014; 4: 92-99.
40. Endlich N, Kress KR, Reiser J, *et al.* Podocytes respond to mechanical stress in vitro. *J Am Soc Nephrol* 2001; 12(3): 413-422.
41. Fogo A, Breyer JA, Smith MC, *et al.* Accuracy of the diagnosis of hypertensive nephrosclerosis in African Americans: a report from the African American Study of Kidney Disease (AASK) trial. AASK pilot study investigators. *Kidney Int* 1997; 51: 244-252.
42. Wang G Lai, Kwan FM, Lai BC, *et al.* Podocyte loss in human hypertensive nephrosclerosis. *Am J Hypertens* 2009; 22: 300-306.
43. Gao J, Cai GY, Liu SW, *et al.* Characteristics and influence factors of pathologic transformation in the subclasses of class IV lupus nephritis. *Rheumatol Int* 2012; 32(6): 1751-1759.
44. Bollain-y-Goytia JJ, Gonzalez-Castaneda M, Torres-del-Muro F, *et al.* Increased excretion of urinary podocytes in lupus nephritis. *Indian J Nephrol* 2011; 21: 166-171.
45. Ahmed T, Abou Ghanima, Mohammed F. Almaghraby, Hossam M. Elsaadany *et al.* Urinary podocalyxin and nephrin levels as biomarkers in lupus nephritis patients:Relation to renal involvement and disease activity. *The Egyptian Rheumatologist* 2015; doi.org/10.1016/2015.11.002.