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Podocytes in urine: what have we found so far?

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Abstract

Chronic kidney disease is currently one of the leading causes of death worldwide, with obesity being a risk factor for the development of chronic kidney disease. Proteinuria, which occurs when kidney damage has already been established, is one of the parameters par excellence for diagnosing kidney disease. For this reason, the determination of a diagnostic method for early kidney damage is fundamental in the management and prediction of kidney disease. For this reason, the detection of podocytes, key cells in the glomerular filtration barrier, in urine (or podocyturia) is one of the main challenges. So far, several attempts have been made to determine the presence of whole podocytes, some of their components or podocyte-derived structures in urine. In this paper we have compiled the published information on this subject in order to have a comprehensive overview of the current state of the art.

Keywords:

- Podocyte
- Podocyturia
- Renal damage renal
- Urine

Introducción

Chronic kidney disease is currently one of the leading causes of death worldwide. One of the factors compromising kidney health is obesity, as the kidneys of a patient with CKD are overworked due to the obese condition.[(1)].

Obesity is associated with other comorbidities, such as hypertension, which is also observable at the glomerular level within the kidney. In these patients, dysregulation of the Renin-Angiotensin-Aldosterone System (RAAS) occurs because the expanding adipose tissue can synthesize and release all the factors involved in the alteration of this system and, therefore, raise blood pressure. This, together with poor sodium management, increased dilatation of the glomerular afferent arteriole and vasoconstriction of the glomerular efferent arteriole leads irremediably to the development of glomerular hypertension and thus to an increase in glomerular size or glomerulomegaly, described in patients with obesity[(2)].

This increase in intraglomerular pressure generates mechanical stress on podocytes. Podocytes are highly specialized cells that form part of the glomerular filtration barrier. They are located hugging the glomerular blood capillaries through protrusions known as podocyte processes, which interdigitate with the podocyte processes of neighboring podocytes (glomerular filtration diaphragm) forming a selective filter to molecules present in the blood circulation. These podocyte processes never come into direct contact in a physiological state but communicate via filtration slit proteins such as podocin and nephrin.

When the mechanical stress resulting from glomerulomegaly and intraglomerular hypertension increases, podocytes initially react by hypertrophying to cover the increased bare area in the blood vessels [(2)]. The effort made to activate this compensatory mechanism leads to podocyte death. This constant loss of podocytes leaves regions devoid of podocytes in the glomerulus, compromising the integrity of the glomerular filtration barrier. In this way, molecules that had previously been retained in the blood plasma, such as albumin and other proteins, will escape into the primary filtrate and subsequently into the urine. This renal damage,

if it continues to progress, will eventually lead to chronic kidney disease and subsequently to end-stage renal failure that will force the patient to undergo transplantation or renal replacement therapy.

For this reason, early detection of kidney damage, when podocytes (podocyturia) rather than protein (proteinuria) are still being lost, becomes vitally important to prevent irreversible progression of kidney disease. This is one of the main goals of the nephrology community. To date, several attempts have been made to characterise podocyturia in the different renal pathologies, which will be summarised below.

Material and methods

SA literature search was performed in the PubMed database using the search criteria "Podocyte AND Urine" and "Podocyturia". All articles unrelated to the diagnosis of early kidney disease, obesity and its comorbidities were discarded.

Results

Detection of complete Podocytes

The detection of complete podocytes in urine, viable or dead, has been one of the main goals of researchers in the field of nephrology. To date, several attempts have been made to detect them. Studies using the cytospin immunofluorescence technique have shown that healthy individuals excrete less than 400 podocytes/mg creatinine in urine. In contrast, patients with kidney disease may have a higher urinary excretion, when selected by the podocalyxin marker. Unfortunately, podocalyxin is not a protein exclusive to podocytes as it is expressed by other cell types, both outside and inside the glomerulus [5]. Nevertheless, podocalyxin has been used for podocyte detection in urine from patients with obesity, alone or in combination with other proteins that were not podocyte markers [3]. Podocalyxin labelling also revealed an increase in podocalyxin-labelled cells in the urine of patients with renal pathology, as well as in the urine of healthy controls.[4].

Furthermore, not only the detection of the complete podocyte in urine is crucial for the early diagnosis of kidney disease, but also its characterization. For this reason, attempts have been made to identify the presence of binucleated podocytes as an indicator of glomerular damage [5,6]. Identification of binucleated podocytes has been performed both in renal biopsies [6] and in urine samples, using fluorescent labelling and microscopic observation. [5].

In all the above-mentioned cases, the correction of podocyturia data has been standardized by means of the parameters traditionally used for this purpose, such as milligrams of creatinine in urine [4] or the total number of podocytes in 24 hours [5].

Our group has contributed to the detection of podocyturia by establishing a protocol for the detection of binucleated podocytes in the urine of patients with obesity and without renal disease. This protocol has been carried out by detecting podocytes labelled with an anti-nephrin antibody, a podocyte-specific marker, and anti-lamin A, a nuclear membrane protein that allows the detection and classification of large and small binucleated podocytes by flow cytometry. Furthermore, the correction of the data presented in this work is novel, as it is carried out using the total number of cells excreted in the urine, and not through factors such as urine volume or the milligrams of creatinine excreted in the urine.[7].

Detection of podocyte Proteins

Tagging and detection of podocyte-specific proteins, such as nephrin, has also been identified in urine samples, a phenomenon referred to as nephrinuria[8]. The presence of nephrin was detected in the urine of all patients with diabetic nephropathy who had albuminuria, and in 54% of those who did not have albuminuria [8]. In this study, nephrin levels appeared to correlate directly with albuminuria levels and inversely with renal function [8]. Likewise, in the same study, podocalyxin detection in patients with diabetic nephropathy preceded the onset of microalbuminuria and correlated directly with HbA1c levels.[8].

Podocyte specific urinary RNAm and microARNs

Another approach implemented to date for the detection of podocytes or their components in urine has been based on messenger RNA (mRNA) extracted from the sediment after centrifugation of urine samples. This technique has certain advantages as it is easily quantifiable, sensitive and specific, while allowing the expression of several markers to be measured at the same time by RT-PCR (real-time polymerase chain reaction) [9].

Thus, it has been shown that nephrin mRNA levels, for example, show a direct correlation with podocyte counts

in urine [8]. Podocyte-related mRNA shows a significant 79-fold increase in patients with glomerulopathy, who show a 50% reduction in renal function [9]. Furthermore, nephrin mRNA has been shown to decrease relative to podocin mRNA in a model of progressive glomerular damage, and the ratio of podocin to nephrin mRNA correlates directly with hypertension and with the progression of renal damage observed by histological techniques [8].

Similarly, microRNAs (miRNAs) present in urine can be quantified by RT-PCR. Although podocytes appear to be only a minority contributor to the total pool of miRNAs present in urine and therefore, changes detected in these miRNAs may be due to alterations in other renal cell types[10].

Fragments derived from podocytes

The constituent renal cells of different segments of the nephron, including podocytes, can release extracellular vesicles, with apical or intracellular membrane content [8]. The researchers in this study again used podocalyxin to detect these vesicles from podocytes in both renal biopsies and urine samples and observed that levels are increased in murine models of diabetes or under high glucose conditions [8]. In the case of podocytes, the extracellular vesicles, always detected by podocalyxin labelling, transmission electron microscopy or urine centrifugation and subsequent flow cytometry, appear to originate from the apical membrane [11] or microvilli.[12].

Discusion

Podocytes are highly differentiated cells that form part of the renal filtration barrier. When these podocytes are detached from the glomerulus, the selective filter is broken and molecules that should be retained in the blood, such as proteins, escape into the urine. Proteinuria is therefore one of the parameters of diagnosis of kidney damage, but in many cases, when proteinuria is detected, it means that we are already too late for this patient and that the progression of the disease over time will be progressive and irreversible. For this reason, the detection of podocyte loss through urine is one of the main goals in the diagnosis of early kidney damage.

To this end, several attempts have been made to determine the presence of podocytes or their components in urine. Thus, detection of both complete podocytes and vesicles excreted by podocytes has been carried out mainly by podocalyxin labelling [3,4,8,11,12]. This type of approach poses the problem that podocalyxin is a sialoprotein present not only in podocytes, but also in other cell types, even within the glomerulus, so that podocalyxin-based podocyte identification is not guaranteed to be very specific for this cell type, even within the glomerulus [8].

Podocyturia, although primarily associated with patients with glomerular damage, has also been observed in healthy subjects [4,5]. Studies have extrapolated podocyturia values in healthy subjects from urine podocyte counts and by spot counting these cells in fields under the light microscope. This inaccuracy means that further work is needed to determine a reliable value for podocyturia in healthy subjects.

In addition, the determination of podocyte-related parameters (podocyturia, protein loss or podocyte-specific elements) has been difficult due to the confusion that has arisen from normalizing these parameters to 24-hour urine volume [8], as this can be altered by other factors.[13].

On the other hand, other approaches have been performed by detecting podocyte-related proteins or mRNAs [8,9]. It has been observed that these molecules could originate from extracellular vesicles or fragments of podocytes, and not necessarily testify to the presence of complete podocytes in urine.[8,9].

Although numerous attempts have been made to establish adequate and reliable podocyturia detection techniques suitable for different renal diseases, patients with obesity still lack a comprehensive characterization. So far, three studies have shown podocyte loss in patients with obesity, using podocalyxin labelling [3], mRNA labelling [14] and the one that seems most reliable and promising, using a double labelling of nephrin and lamin A and correcting the values obtained by total cell count [7]. On this basis, it appears that podocyturia may be a process affecting patients with obesity, although more trials with podocyte-specific markers and a larger cohort of patients are needed to gain a better understanding of this population group.

Conclusions

Podocyturia represents a promising future in the early detection of kidney damage. However, there are many renal pathologies that need to be addressed, including obesity. The need for a protocol for podocyturia determination

based on podocyte-specific markers and the determination of podocytes excreted by healthy patients remains a relevant goal in the fight against renal pathologies.

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