An ebook on Type 2 Diabetes

Chapter 3

The old and the new risk factors for chronic renal disease in type 2 diabetics

Ana Paula Silva, MD, MSc[1,2*]; Filipa Mendes, MD[1]; Pedro Leão Neves, MD, PhD[1,2]

[1]Nephrology, University Hospital Center of Algarve, Faro, Portugal

[2]Department of Biomedical Sciences and Medicine,University of Algarve, Faro, Portugal

***Corresponding Author:** Ana Paula Silva*

Hospital de Faro. Rua Leão Penedo, 8000-386 Faro, Portugal

Telephone: +351289891220; Fax:+351 289891221; E-mail:anapassionara@gmail.com

Abstract

The pathophysiological mechanisms associated with chronic renal disease in type 2 diabetic are complex and still remain to be fully understood. It is well known that there are traditional factors responsible for kidney disease such as poor metabolic control, time of evolution of diabetes, age, gender, uncontrolled hypertension, ethnical background and genetic factors. Nevertheless, recent evidence also points to non-traditional risk factors that might trigger glomerular barrier changes, leading to an increase durinary album in excretion along side with the progression of diabetic nephropathy to end-stage chronic kidney disease. Among these factors are inflammation and oxidative stress; alterations in mineral metabolism and homeostasis might also contribute to the clinical manifestation and/or progression of diabetic nephropathy.

Our proposal is firstly to discuss the traditional risk factors associated pathogenesis and progression of chronic kidney disease associated with diabetes and secondly we will consider the new risk factors or new signs including the Klotho-FGF23 axis, magnesium and vitamin D.

Keywords: Chronic Kidney Disease; Diabetes; Mineral Metabolism

1. Introduction

Diabetes mellitus is one of the major epidemics in the modern world, with a world wide increase in incidence by around 50% over the past 10 years [1].

Diabetes is a chronic and multifactorial disease. There are 2 major forms of diabetes that vary in their aetiology, type I and type II, although both are clinically characterised as hyperglycaemia due to chronic and/or relative insulin in sufficiency [1].

Type II diabetes mellitus is characterised by insulin resistance concomitant with insulin deficiency. The increasing peripheral resistance to insulin is off set over the course of years by an increase of insulin secretion by the pancreatic β-cells, the biological source of this hormone. The progressive decline of pancreatic function as age advances and chronic hyper stimulation result in an increasing hyperglycaemic state [2].

The most devastating consequences of diabetes are vascular complications, which can be organised into micro vascular and macro vascular and can occur in both forms of diabetes (type I or type II). The most common microvascular complications are nephropathy, retinopathy and neuropathy, with myocardial in farction and stroke being the most frequent macrovascular complications [1].

The aim of this work is to summarise current scientific knowledge on factors that contribute to the urinary excretion of albumin in type II diabetics.

2. Old Risk Factors for Chronic Renal Disease in Type 2 Diabetic Patients

Diabetic nephropathy (DN), besides being a major risk factor for cardiovascular complications perse, is the major cause of end-stage renal disease in Western societies.

DN develops in 35-40% of diabetic patients as the result of intra renal metabolic, hemodynamic and structural changes [1,2]. DN is a complex phenotype caused by the combined effects of susceptibility alleles and environmental factors which contribute to poor glycaemic-control and hypertension [1].

The nephropathy does not necessarily develop in a significant proportion of diabetic patients, suggesting the involvement of specific genes [1].

2.1. Pathophysiology of Diabetic Nephropathy

It is established that hyperglycaemia is the triggering factor of tissue damage in diabetes [1,3].

But why are only some cells in the body affected? The answer is obtained by studying the mechanisms of glucose transport in the cells of the different tissues. In contrast to tissues damaged by hyperglycaemia (capillary endothelial cells in the retina, mesangial cells in the glomerulus, neurons and Schwann cells in the peripheral nerves), the other cells have the ability to regulate the transport of glucose to inside the cell, reducing it when exposed to hyperg-

lycaemia and consequently keeping intracellular glucose levels constant [1,3].

The glomerulus is the location of histological lesions associated with DM, where mesangial cell proliferation, the excessive production of extracellular matrix (fibronectin, laminin, collagen type IV), occurs due to increased levels of intracellular glucose.

The mechanisms associated with DN are not fully understood, and many of the molecular factors associated with the renal lesion are under investigation.

However, it is known that there are four mechanisms responsible for the onset of DM complications that are triggered by the increase of intracellular glucose.

These mechanisms are:

Increased flow through the poliospathway, with consequent increase of sorbitol in the tissues, including the renal tubules and glomeruli. This increase in sorbitol causes tissue lesion by changing the cellular osmo regulation. This is the primary mediator of protein kinase C (PKC) cellular activation [3].

The increase in the non-enzymatic advanced glycosylation end products (AGEs) causes an increase in plasma proteins and extracellular matrix. When AGEs bind to specific receptors, identified in the macrophages of endothelial and mesangial cells, they induce the synthesis and secretion of cytokines (ILTNF-α, IL -1,IL-6) and insulin growth factor (IGF1), stimulating mesangial cells proliferation and collagen IV production. Moreover, by cross linking with collagen, AGEs can induce an increase in extracellular matrix synthesis by stimulating growth-factors (TGF-β1,CTGF,VEGF,PDGF) [3-5];

Increase of the flow through the hexos amine pathway, causing changes in gene expression of glomerular cells and endothelial cells [3].

These mechanisms are, among others, responsible for the changes in the glomerular filtration barrier whose manifestation is the emergence of proteinuria.

High intracellular glucose concentrations in susceptible tissues lead to the dysfunction of several cell signalling pathways with the consequent increase in the mitochondrial production of ROS (reactive oxygen species) that damage the cell itself. This fact explains the microvascular damage caused by hyperglycaemia [1,3].

Regarding the macrovascular damage hyperglycaemia is not a major direct determinant, being insulin resistance the “culprit” of this phenomenon. Insulin resistance promotes an increase in the release into the blood stream off ree fattyacids by the adipocytes, which will be oxidized by the mitochondria of ROS-producing endothelial arterial cells. Again it is the increase in ROS production that causes cellular damage [1,3] (**Figure1**).

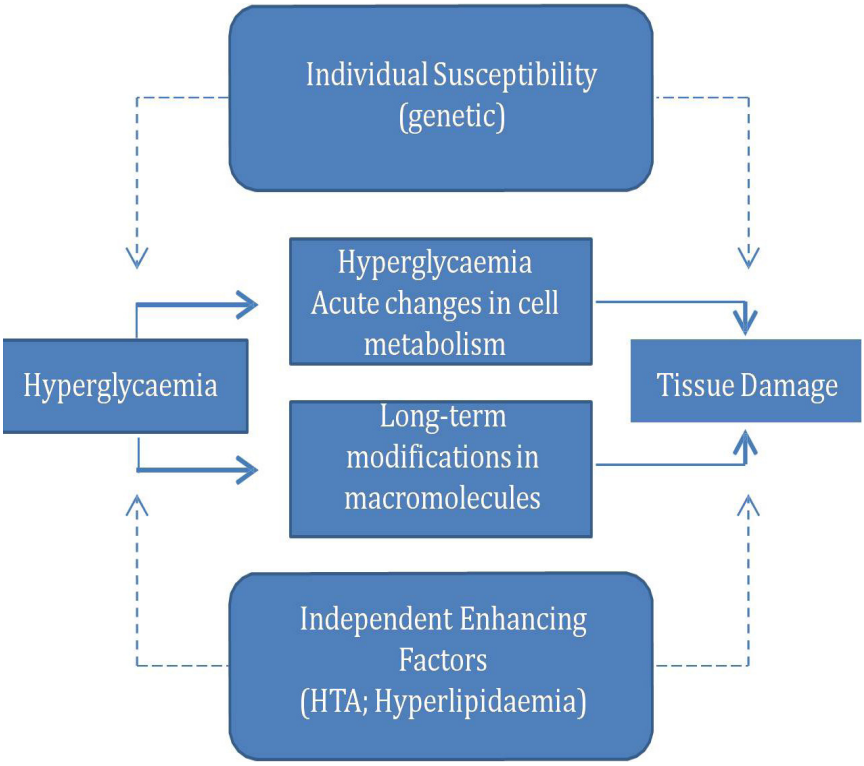

Figure 1: Mechanism of tissue damage resulting from hyperglycaemia.

Several authors have found that the excessive production of advanced glycation end products and free radicals resulting from chronic hyperglycaemia with consequent imbalance of the anti-oxidative mechanisms culminates in endothelial dysfunction [4-5]. Oxidative stress has been increasingly recognised as an important agent causing kidney damage. Infact, it is thought that the underlying factor of renal damage is a disturbance of the balance between oxidative and anti-oxidative mechanisms and that this alteration precedes renal injury [4,6]. It is also believed that the oxidative mechanisms gradually increase and in parallel with the progression of the renal disease [6-7].

In addition to direct tissue damage, these glycation metabolites promote the increase of angiotensin II, apro-fibrotic, pro-angiogenic and pro-inflammatory agent that plays an important role in the renal diabetic pathology [8-10]. Angiotens in II it is the main mediator of TGFβ1 and connective tissue growth factor (CTGF) production at the level of mesangial and tubular cells, leading to an increased production of extracellular matrix and contributing to the development and progression of glomerulosclerosis and tubulo interstitial fibrosis and sclerosis [10,11]. Moreover, it was described the possible directaction of saturated fatty acids on podocytes contributing to their dysfunction and resulting in albuminuria [12] (**Figure 2**). These damaging stimuli manifest themselves, at a nearly stage, by an increase in systemic blood pressure and consequent increase of glomerular pressure and glomerular hyperfiltration [13]. The kidney under goes hypertrophy and hyperplasia. Glomerular and tubular hypertrophy, thickening of the glomerular basement membrane and mesangial expansion are identified [1]. This transformation leads to an increase of the glomerular filtration rate (GFR) and increased filtration of glucose, fattyacids, proteins, and aminoacids, growth factors and cytok-

ines, responsible for the dysfunction of anti-oxidative mechanisms, inflammation and fibrosis [1].

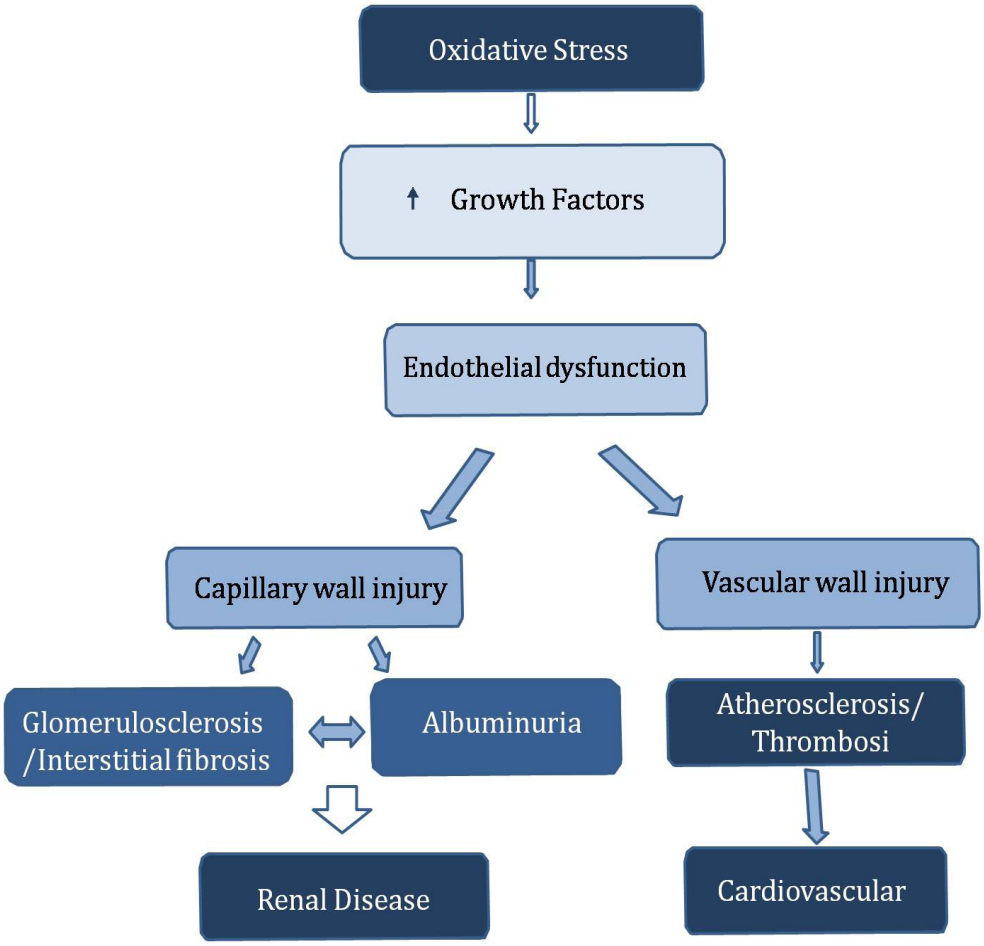

Figure 2: Pathophysiology of oxidative stress in renal and cardio vascular disease

The renal repercussions induced by these damaging stimuli manifest themselves, at a nearly stage, by an increase in systemic blood pressure and consequent increase of glomerular pressure and glomerular hyperfiltration [13]. The kidney undergoes hypertrophy and hyperplasia. Glomerular and tubular hypertrophy, thickening of the glomerular basement membrane and mesangial expansion are identified [1]. This transformation leads to an increase of the glomerular filtration rate (GFR) and increased filtration of glucose, fatty acids, proteins, and aminoacids, growth factors and cytokines, responsible for the dysfunction of anti-oxidative mechanisms, inflammation and fibrosis [1].

The progression to proteinuria and subsequent progressive decrease of GFR reflectsthe chronic damage of the glomerular filtration barrier, in particular the glomerular and podocyte epithelial cells, thickening of the basement membrane and mesangial expansion [1] Extra cellular matrix deposition (tubulo interstitial fibrosis) is hypothesized to be the major determining fact or in the progression of renal disease in diabetes [1].

An underlying inflammatory process is common to all pathophysiological mechanisms. Countless studies back there levant role of inflammation in renal disease and the development of micro albumin uria in the general population [14].

Stuveling et al. demonstrated the association between high CRP (C-reactive protein) values and the change in the relationship between blood pressure and micro albuminuria, i.e. inflammation increases the likelihood of glomerular losses of albuminina situation of increased blood pressure [14]. The role of inflammation in nephropathy had already been pointed out by Navarro et al. who demonstrated a dose-dependent increase of micro and macro albuminuria in type II diabetics in response to raised CRP and TNF-α (tumour necrosis factor alpha) [14-15].

2.2. Risk factors for the development of Diabetic Nephropathy in Type II Diabetics

Genetic susceptibility should be regarded as a non-modifiable risk factor that influences both the incidence and the severity of diabetic nephropathy [16].

The prevalence of diabetic nephropathy also varies with ethnicity. It is relatively high in African Americans, Latin Americans from Mexico, Australian Aborigines and Indo-Asian immigrants in the United Kingdom, when compared with Caucasian individuals [1].

Concerning modifiable risk factors, metabolic syndrome,which of ten precedes the development of type II diabetes, is characterised by several metabolic aterations that contribute to the development and/or progression of diabetic nephropathy [17-18].

Also high blood pressure which can be "transferred" to the glomerular system is associated with the progression of renal disease. Several studies have shown that the control of the blood pressure decreases albuminuria and the progression of renal disease [8,12,14].

A high body mass index (BMI) and, therefore, obesity have been strongly associate with an increased risk of developing renal disease in diabetic individuals [8,12].

Dyslipidaemia has a potential role in triggering and progression of chronic kidney disease, particularly low HDL values, hyper triglyceridemia and increased insulin resistance [12,19].

Finally several studies have demonstrated the adverse effects of tobacco on diabetic kidney disease such as worsening of albumin uria, increased risk of developing end- stage renal disease, and decreased survival of haemodialysis patients [8].

2.3. Urinary excretion of Albumin

The term proteinuria refers to the presence of proteins in the urine. Its main component is albumin which, due to its small size, more easily passes through the fenestrated glomerular capillaries. A sarule, this proteinuria reflects changes in renal haemodynamics underlying a pathological modification of the characteristics of the glomerular filtration barrier, particularly the podocytes, glomerular endothelial cells [1].

The presence of proteins in the urine can be classified qualitatively and quantitatively. In relation to the former, proteinuria can be defined as selective or non-selective depending on the type of proteins that are lost in the urine. In selective proteinuria, also called albuminuria, only albumin is lost, where as in non-selective proteinuria there is the loss of albumin as well as other larger plasma proteins, and in this case a greater degree of glomerular damage is implied [1].

Urinary protein losses areal so classified according to the amount of losses. Micro albumin uria is defined as the urinary excretion of small amounts of albumin, more specifically 30 to 300mg/day of albumin. Below this range we have normoalbuminuria and above it there is macro albuminuria and proteinuria when it includes the excretion of proteins other than albumin [20].

Proteinuria is one of several renal disease markers that are used, particularly in subjects with diabetes for screening for diabetic nephropathy [20].

The gold standard for album in quantification is to measure the albumin concentration in urine collected over 24 hours [20]. Given the inconvenience of this type of sample collection, the urinary albumin/creatinine ratio in a urine sample, expressed as mg/g, is typically used. The first urination in the morning is preferably used. The latter method has a sensitivity and specificity level of approximately 95% when compared to the gold standard [20].

The variability obtained led Chenetal. to recommend albumin quantification in 2 to 3 urine collections, atleast two of which are separated by 3 to 6 months [20].

The importance of microalbuminuria is that it is frequently identified in subjects with an established diagnosis of diabetes and it is the simple stand most sensitive prognostic factor to assess the risk of developing nephropathy in diabetics, representing the initial stages of progressive diabetic renal disease [20]. It also allows micro-and macro vascular complications and cardiovascular mortality to be predicted in type II diabetics [20].

Micro albuminuria is often associated with poor glycometabolic control and a higher incidence of chronic complications, such as diabetic retinopathy, peripheral vascular disease and diabetic neuropathy. Moreover, several studies have observed that the increase in the amount of protein in the urine is strongly related to the development of renal and cardio vascular disease, regardless of other markers of structural or functional renal damage [20].

2.4. Diabetic Nephropathy and Albuminuria

Micro albuminuria is a nearly biomarker of renal disease and vascular dysfunction. It is associated with the development of end-stage renal disease, cardiovascular morbidity and increased mortality, in both therenal and the general population [20-23].

The relationship is linear since there is already an increase of the cardiovascular risk for values of urinary albumin excretion that are now a days accepted as normal [20,24]. Currently, it is discussed whether the microalbuminuria value presently accepted, below 30 mg/day, may have a cutoff that is too high due to this apparent increase in cardiorenal risk [25].

Several authors have demonstrated that the extent of microalbuminuria affects the long-term prognosis [26]. Urinary albumin appears to be pro-inflammatory and, therefore, being filtered and reabsorbed at the level of the proximal renal tubules induces there lease of pro-fibrotic cytokines, in particular TGF-β, responsible for the process of glomerulosclerosis and tubulo interstitial fibrosis [24]. Shao et al. also report that although microalbuminuria is the best non-traumatic predictor currently available for early stage diabetic nephropathy, important changes in renal structure may have already occurred before microalbuminuria is present [26].

Karalliedde et al. demonstrated that the reduction of albuminuria led to a delay in the progression of renal disease. In relation to normoalbuminuria and microalbuminuria, there is no evidence that preventive or reversal measures would lead to a better clinical outcome [24].

According to the study by McFarlane et al. the therapies that reduce micro albuminuria reduce the risk of mortality and morbidity, though not primarily directed towards this goal, but the rapies aimed at the reduction of albuminuria seem to have little benefit in this field and have even been associated with an increased number of adverse effects [25].

Classically, diabetic nephropathy is characterised by persistent microalbuminuria that-progresses over time and through the various phases of nephropathy described above. Nevertheless, about 50% of subjects with diabetes and concomitant renal disease do not present the classical changes of diabetic nephropathy in the renal biopsy [25].

In the study developed by Afghahi et al. only one-third of patients who developed renal disease also developed albuminuria [12]. The development of nephropathy with out albuminuria has already been described in subjects with typeII diabetes and without diabetes [27-32]. One of the proposed hypotheses is the fact that the development of renal damage results from glomerular and extra-glomerular changes, where as albuminuria would only result from modifications related to the glomerulus itself [12,28]. More recent studies support this hypothesis, as they have demonstrated a higher prevalence of micro and macro angiopathy in type II diabetics and non-albuminuric nephropathy [12,32-33].

More over, these verity of nephropathy in the biopsy is not always related to the degree of microalbuminuria, especially for values within the micro albuminuria range [25].

Further more, it should be considered that diabetic individuals may suffer from conditions that lead to transitory albuminuria, which are therefore not necessarily related to future-cardio-renal events. Nevertheless, the usefulness of measuring the presence of albumin in urine is extremely high [25].

2.5. Risk factors contributing to urinary excretion of albumin

According to Shao et al. there is a significant correlation between the extent of microalbuminuria in type II diabetic subjects and the markers of oxidative stress studied by this author. Increased synthesis and release of ROS leads to increased oxidative stress and consequent damage of the local tissues, such as proteins [26]. The author highlights the particularly important role of oxidative stress and oxidative damage of lipids in the pathogenesis and progression of microalbuminuria in the early stages of diabetic nephropathy [26].

Chen et al., like many other authors, have observed that ageing, changes in glucose tolerance, insulin resistance, arterial hypertension, obesity and, consequently, high BMI, dyslipidaemia, cardiovascular history and metabolic syndrome are independent risk factors for the presence of albuminuria [12,20,34-36]. Hyperinsulinemia increases glomerular hydro static pressure and renal vascular permeability, aggravating glomerular hyper filtration and enhances renal reabsorption of sodium [35]. Poor glycaemic control, smoking and low HD Lare also risk factors for the development of albuminuria [12].

It is imperative, given the high prevalence of diabetic nephropathy and the absence of detectable symptoms in the early stages of the disease, to study and explore modifiable risk factors, the use of sensitive and specific markers for this condition in order to be able to implement renal disease prevention measures [20]. It is advocated that it is especially important to identify and educate individuals at high risk of developing microalbuminuria not only through diabetes, but also by interacting with other concomitant risk factors [34].

3. New risk factors for chronic renal disease in type 2 diabetics

The central role of albuminuria in the pathogenesis and progression of diabetic nephropathy justifies research for new factors that shape it.

3.1. What is FGF23?

The fibroblast growth factor (FGF) family includes a large number of polypeptides that share a common region. FGF23 is a hormone primarily synthesized by osteocytes and osteoblasts in response to hypercalcaemia and hyperphosphatemia, increased calcitriol and parathyroid hormone. This glycosylated peptide is responsible for phosphate homeostasis. It induces an increase in the urinary excretion of this ion by reducing sodium-phosphate co-transporters in the proximal tubule cells. It also indirectly limits its intestinal absorption [37-40]. FGF23

also plays a keyrole in vitamin D metabolism. It not only inhibits the synthesis of calcitriol in the kidney but also stimulates the catabolism of the active form of vitamin D [37]. Studies conducted on animals have demonstrated a potent inhibition of them RNA expression of 1α-hydroxylase (25(OH)D-1α-hydroxylase) in the proximal renal tubule, responsible for the conversion of vitamin D to its active form, mediated by FGF23 [37-40]. It concomitantly stimulates the expression of 24-hydroxylase (CYP24A1) responsible for the inactivation of calcitriol [37,39-40]. Calcitriol, at the same time, stimulates the synthesis of FGF23 and Klotho expression, increasing Klothom RNA synthesis [37].

The Klotho protein forms a complex with the FGF23 receptor, significantly increasing its affinity for FGF23. This protein is expressed on the surface of various organs, dominating in the kidney, and it seems to be extremely important in the action of FGF23 [37,40]. This "composite" receptor appears to be involved in the pathophysiology of various conditions.

3.2. FGF23-Klotho

The Klotho protein may play a critical role in the survival of subjects with CKD. Its over expression in rats significantly increased their survival [37]. Zanchi et al. demonstrated that ramipril exerts its renoprotective action through a significant increase in Klothom RNA levels and there is no apparent action on FGF23 levels in rats with diabetic nephropathy. Nevertheless, a significantly reduced expression of FGF23 mRNA was documented [40]. This study demonstrated that ramipril prevents the aggravation of renal disease, with significant attenuation of glomerular and tubular alterations, interstitial inflammation and a reduction in proteinuria that reached 55%. It was also showed a positive correlation between renal expression levels of FGF23 and proteinuria, glomerulosclerosis, tubular damage and interstitial inflammation [40].

According to Vervloet et al. there is a significant association between the FGF23 value, proteinuria and smoking in subjects with CKD. Although this relationship is well established, the mechanism by which this relationship develops has not yet been clarified. It is known that tobacco contributes to oxidative stress. It is believed that the FGF23 levels are increased by this means, contributing to the dysfunction of the respective receptors, inducing a state of resistance to FGF23 that is compensated by the increase in its production [41].

Several mechanisms are proposed to justify the relationship between FGF23 and proteinuria. Direct action by FGF23 on the glomerular endothelium is proposed, although there is little scientific evidence supporting this theory. Also proposed is an elevation of FGF23 secondary to a hyperparathyroidism resulting from a vitamin D deficiency. This latter fact would justify proteinuria. Lastly, the most consensual theory is based on the fact that proteinuria *per se* is harmful to the tubular nephron cells, with consequent disruption of the FGF23 operating sites and subsequent compensatory increase in FGF23 secretion. Nevertheless, we cannot

discard the hypothesis that both these disorders, proteinuria and elevated FGF23 levels, may result from the same damaging mechanism to the kidney, such as the aforementioned oxidative stress [41].

FGF23 serum levels are related to the risk of progression of CKD in subjects with diabetic nephropathy in the macroalbuminuria spectrum [38,42]. It is important to clarify whether FGF23 contributes to the progression of nephropathy or if it is just a risk biomarker [38,42].

3.3. FGF23–vitamin D

Calcitriol (1.25-dihydroxyvitamin D_3), a biologically active form of vitamin D_3, has several functions in addition to mineral metabolism. There is evidence that this vitamin is an important immunomodulator, inhibitor of the proliferation of cell differentiation and inhibitor of the renin angiotensin-aldosterone axis [11]. It acts by activating the VDR and modifying the transcription of several genes [11].

Vitamin D synthesis begins in the epidermis, where vitamin D_3(cholecalciferol) is synthesized by the action of UVB rays. It then undergoes two hydroxylations, the first on carbon-25 that occurs in the liver and is not dependent on hormonal regulation and the second hydroxylation is on the first carbon made by 1α-hydoxylase [37]. This enzyme is strongly expressed in the proximal renal tubule. It is the primary source of circulating calcitriol, vitamin D in its biologically active form [37].

Observational studies mention the association between VDR, serum levels of calcitriol, glucose in tolerance and insulin sensitivity [11,43]. It is also accepted that vitamin D, being the main regulator of calcium metabolism, facilitates the production of insulin by acting on pancreatic β cells [11]. This factis strengthened by the identification of VDR in the pancreas and hypovitaminosis D as a riskfactor for the development of type II diabetes and metabolic syndrome as a consequence of pancreatic β-cell dysfunction and increased peripheral insulin resistance [44-45].

Hyperglycaemia decreases the expression of VDR and 1α-hydroxylase in the proximal tubule cells with consequent decrease in vitamin D reabsorption and increased proteinuria [11]. The role of calcitriol in the pathogenesis of proteinuria is mediated through the regulation of cell proliferation, apoptosis, angiogenesis and anti-inflammatory action [11,46]. On the other hand, the combination of hyperglycaemia and decreased VDR results in a greater activation of the renin-angiotens in-aldosterone axis [11].

Recently, the existence of VDR in podocytes was documented. An association between the quantity of this vitamin D and the number of podocytes has also been demonstrated. Considering the important role that the decrease in the number of podocytes has in micro albu-

minuria, this relationship may be potentially important in the prevention and reduction of mesangial cell proliferation and proteinuria [11,47].

There is as low down in the development of glomerulosclerosis and in the progression of albuminuria in rats subjected to subtotal nephrectomy and treated with calcitriol [11,48] and it was also demonstrated that calcitriol reduces mesangial cell proliferation, glomerular hypertrophy, and progression of glomerulosclerosis [11,49].

Similar results were found with paracalcitol: its administration reduced protein excretion in subjects with renal disease and macroproteinuria [50-51] and albuminuria in type II diabetic patients [52]. Several other studies have supported these findings. Therapeutics with calcitriol or VDR activators have shown beneficial effects in reducing the risk of cardiovascular morbidity and mortality, diabetes, auto-immune diseases and cancer in subjects undergoing renal replacement therapy [37]. Paricalcitol in end-stage renal disease promotes a reduction of FGF23 and suppresses the parathyroid hormone [39].

In subjects with CKD, high FGF23 and low vitamin D values are associated with similar adverse reactions, suggesting that the potential toxicity of FGF23 may be partially mediated by there duction in FGF23-induced calcitriol levels [37]. Both disorders, high FGF23 and vitamin D deficiency, are predictors of a rapid progression of renal disease [37] (**Figure 3**).

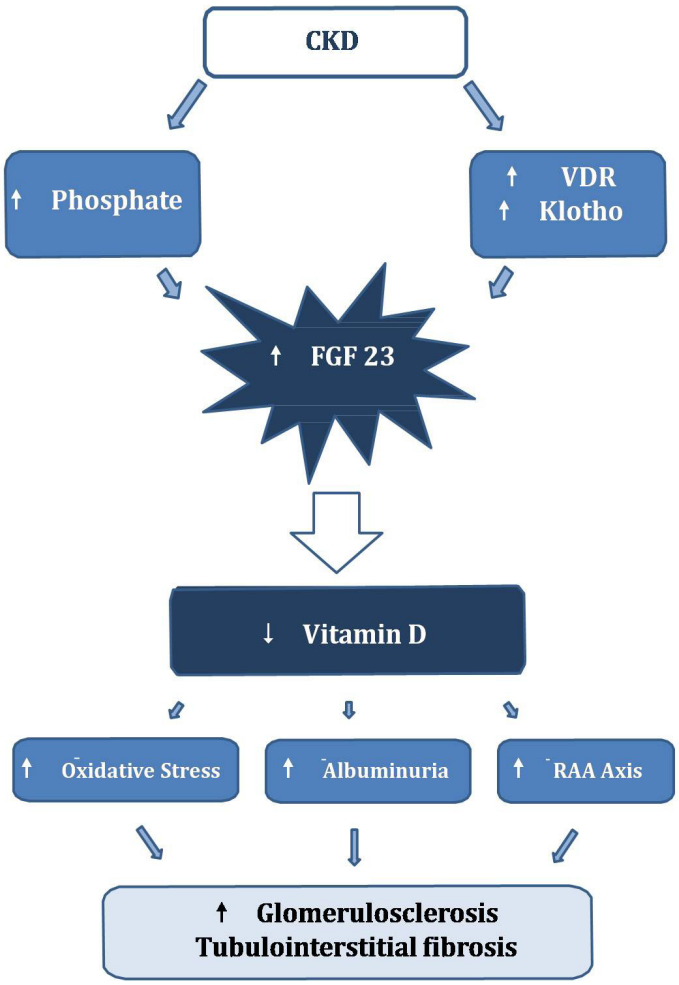

Figure 3: Role of FGF23 in Diabetic Nephropathy

3.4. Klotho and urinary excretion of albumin

Klotho was mainly associated with urinary excretion of albumin. Interstitial inflammation induced by proteinuria may down regulate Klotho expression [53]. There lease of inflammatory cytokines such as tumour necrosis factor (TNF) - like weak inducer of a poptosis'(TWEAK) and TNF-α were responsible for the down regulation of Klotho expression through a nuclear factor kappa-B-dependent mechanism [54]. The observations from More noetal, were supported by the fact that Klotho circulating levels increased in type 2 diabetic patients whose proteinuria was a meliorated with losartan. According to the authors, the use of renin-angiotens in system antagonists in patients with diabetic nephropathy was able to reverse Klotho's down regulation induced by angiotens in II, and therefore retarding disease progression [55].

Another direct mechanism thought to be part of the relationship Klotho/urinary excretion of albumin is the one involving the vascular endothelial growth factor A(VEGF-A). It is believed that VEGF-A plays a pathogenic role indiabetic nephropathy, particularly on angiogenesis and vascular permeability. In their study on diabetic patients, Kacsoetal observed a consistent positive relationship between soluble Klotho and VEGF-A, (both factors having low levels in the presence of microalbuminuria). It is still to be understood if they both respond to the same pathogenic trigger or if it is are active action to the other. Nevertheless, we may hypothesize that the down regulation Klotho/VEGF-A can lead to and urinary excretion of albumin worsening through their impact on endothelial dysfunction [56].

In addition to these mechanisms, Klotho has also an endogenous anti-fibrotic function via antagonism of Wnt/β-catenin signalling, which promotes fibrino genesis. Therefore, it is reasonable to argue that a loss of Klotho may be associated with the progression of diabetic-nephropathy and urinary excretion of albumin worsening by accelerated fibrinogenesis [57-58].

Some studies have also speculated that the correlation between Klotho and urinary excretion of albumin may be due to indirect mechanisms as well. Within the Klotho/FGF-23/ Vitamin D axis, it is already known that low Klotho levels are associated with increased FGF-23 and decreased Vitamin D levels. FGF-23 is then able to indirectly increase proteinuria by diminishing calcitriol synthesis and inducing endothelium dysfunction [59-60]. It is also negatively correlated with Vitamin D levels in diabetic nephropathy patients with microalbuminuria [11,51]. Thus, it is not unreasonable to hypothesize that Klotho and ACR levels might be correlated through indirect, albeit unclear but possibly FGF-23-mediated, mechanisms.

3.5. Magnesium and urinary excretion of albumin

Magnesium is the fourth most abundant cationin ourbody. About 99% is in the intracellular compartment and only 1% in the extracellular fluid [61-62]. It is an essential cofactor in

more than 300 enzymatic reactions, playing an important role in several biological processes, such as cardiac excitability, transmembrane ionic flux, neurotransmitter release, calcium channel regulation and it may even have the role of physiological antagonist of calcium [61,63].

It should also be noted that the total serum value of magnesium does not reflect the intracellular concentration of this ion or its ionized fraction. The small intestine is the primary absorption site of magnesium, while excretion is primarily performed by the kidney, and in healthy subjects serum magnesium levels are extremely constant [62,64].

Some hormones play an active role in the regulation of magnesium metabolism in ourbody, such as insulin [62,64]. Insulin stimulates the transport of magnesium from the extracellular to the intracellular compartment contributing to increase its concentration in the latter, although the specific mechanisms by which this happens are not yet clear [62,64]. The action of insulin on ionic regulation is specific, dose-dependent and independent of glucose up take [62,64]. It is important to note that not only does insulin play an important role in the homeostasis of magnesium, but magnesium itself is an important modulator of insulin action and insulin sensitivity and, therefore, of glucose metabolism [62,64].

Magnesium concentration is determinant in tyrosine kinase phosphorylation of the insulin receptor, as well as other proteinkinases found in the cell membrane and in the endoplasmic reticulum [62,64]. Low concentrations of intracellular magnesium result in impaired tyrosine kinase activity and the consequent development of "post-receptor" insulin resistance associated also with reduced cellular utilization of glucose. In other words, the lower the concentration of magnesium, the less responsive is the cell to stimulation by insulin and a greater quantity of insulin is thus required to metabolize the same amount of glucose [62,64].

These findings confirm the strong dependence on the action of insulin relative to the intracellular concentration of magnesium, and the findings are consistent with the fact that magnesium deficiency is the potential cause and not merely a consequence of peripheral insulin resistance [62].

Following on from what has been stated, an increase in insulin resistance in tissues where the entry of glucose into cells is insulin dependent, such as musculoskeletal, cardiac, and fat, would be expected in the presence of decreased magnesium values. Taking into account the fact that the ''uptake" of magnesium by the cells is an insulin-regulated process, it will also be a process affected by insulin resistance,which may be responsible for the intracellular magnesium deficiency or aggravating an existing deficiency [62].

Glucose also appears to contribute to ionic cellular homeostasis independently of insulin. *In vitro* studies have shown direct suppression of magnesium resulting from hyperglycaemic states [62]. Since hyperglycaemia *per se* significantly contributes to insulin resistance

in diabetesmellitus and because the reduction of magnesium promotes vasoconstriction, it is hypothesized that these changes in magnesium induced by glucose are the cause of the vasoconstriction seen in chronic diabetes states [62,64-66].

Epidemiological studies have shown a high prevalence of hypomagnesemia in subjects with type II diabetes, especially in those with poor glycaemic control [62,64]. This cellular and extracellular magnesium depletion appears to be more severe in subjects with recent diagnosis [61].

Among the mechanisms in diabetes that may favour the depletion of magnesium found in these subjects are poor dietary intakes and increased urinary excretion of magnesium and calcium. Their absorption and retention is not altered in type II diabetes [62].

There is a negative correlation between serum magnesium concentration and microalbuminuria in middle-aged and elderly Chinese subjects with diabetes [61]. I was also found-that subjects with microalbuminuria or clinical proteinuria had a significant decrease inserum magnesium concentration compared to the non-microalbuminuria group [67].

The actual pathophysiological mechanism that explains this association is not well understood. Nevertheless, there are some mechanisms that potentially explain this relationship. One of these is the aforementioned link of hypomagnesemia to the insulin resistance state. Magnesium has a number of biological actions, including that of moderately potent calcium antagonist. In a situation of hypomagnesaemia, the relative intracellular increase of calcium may compromise adipocyte and skeletal muscle response to insulin with consequent development of insulin resistance [61]. Other studies report that insufficient serum levels of insulin or insulin resistance can affect the renal absorption of magnesium, decreasing it and contributing to the hypomagnesemia reported in diabetics. This vicious cycle may contribute to the increased risk of microalbuminuria in this population [53,61] (**Figure 4**).

Another proposed mechanism is related to oxidative stress, recognised as one of thecausative agents of microalbuminuria. Given the antioxidant properties of magnesium, Xu et al. suggest that this may be the mechanism that links hypomagnesemia and microalbuminuria [61].

On the other hand, there is evidence that serum magnesium concentration and the concentration of systemic inflammatory markers, whose role in the pathogenesis of microalbuminuria is crucial, have an inversely proportional relationship [61]. It is to be noted that several studies have shown the antioxidant and anti-inflammatory properties of magnesium [63].

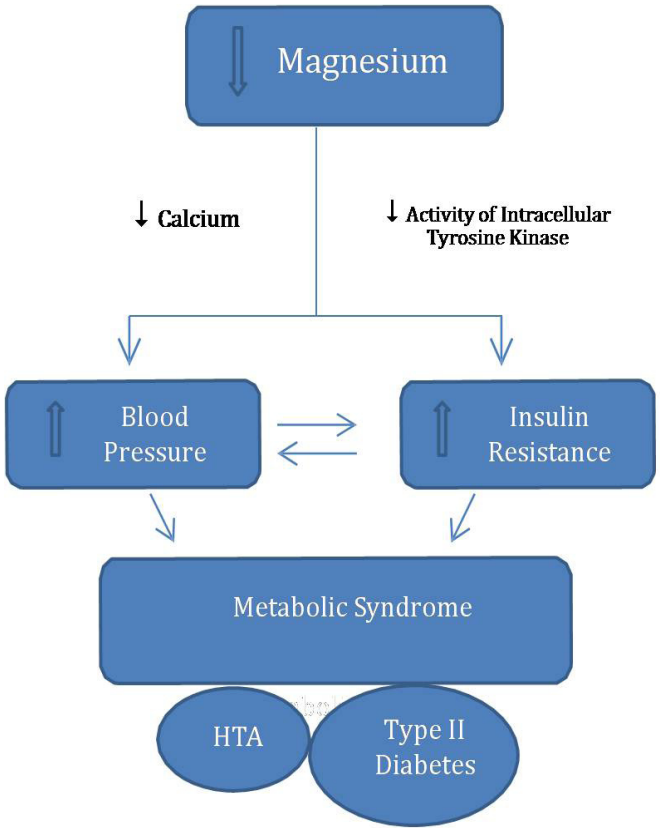

Figure 4: The contribution of magnesium to the metabolic syndrome

Magnesium deficiency may not only be secondary to type II diabetes but it may precede and cause insulin resistance *per se*, impaired glucose tolerance, and even type II diabetes [62,65-66]. Magnesium supplementation may improve the metabolic profile of diabetics but other studies did not show any benefit [62]. Once Magnesium is filtered by the kidney it should be administered with caution in patients with decreased glomerular filtration [63].

4. Conclusions

Diabetic nephropathy is the main cause of end-stage renal disease in the Western Countries. We should insist in the reduction of the incidence of diabetic nephropathy and in the delay of the progression of the renal insufficiency. The pathophysiology of the disease is complex and multifactorial. We must continue using the classical useful treatments such as the antagonists of renin-angiotens in system. However new players have been identified in the pathophysiology of the disease and new possibilities of intervention are possible.

5. References

1. Forbes J.M, Cooper M.E. Mechanisms of Diabetic Complications. Physiol Rev. 2013; 93: 137-188.

2. Baynes H.W. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. J Diabetes Metab. 2015; 6: 541.

3. Brownlee M. The Pathobiology of Diabetic Complications. A Unifying Mechanism.Diabetes. 2005; 54: 1615-1625.

4. Singh A, Fridén V, Dasgupta I, et al. High glucose causes dysfunction of the human glomerular endothelial glycocalyx. Am J Physiol Renal Physiol. 2011; 300: F40–F48.

5. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. Biomolecules. 2015; 5(1): 194–222.

6. Shao N, Kuang H.Y, Wang N, et al. Relationship between Oxidant/Antioxidant Markers and Severity of Microalbuminuria in the Early Stage of Nephropathy in Type 2 Diabetic Patients. Journal of Diabetes Research. 2013.

7. Piarulli F, Sartore G, Ceriello A, et al. Relationship between glyco-oxidation, anti oxidant status and microalbuminuria in type 2 diabetic patients. Diabetologia. 2009; 52: 1419–1425.

8. Bakris G .L. Recognition, Pathogenesis, and Treatment of Different Stages of Nephropathy in PatientsWithType 2 Diabetes Mellitus. Mayo ClinProc. 2011; 86(5): 444-456.

9. Duran-Salgado M.B, Rubio-Guerra A.F. Diabetic nephropathy and inflammation. World J Diabetes. 2014; 5(3): 393-398.

10. Macia-Heras M, Del Castillo-Rodriguez N, Navarro González J.F. The Renin-Angiotensin-Aldosterone System in Renal and Cardiovascular Disease and the Effects of its Pharmacological Blockade. J Diabetes Metab. 2012; 3:171.

11. Silva A.P, Fragoso A, Neves P.L. Relationship of Vitamin D with Diabetes Mellitus and Diabetic Nephropathy. Port J Nephrol Hypert. 2014; 28(2): 108-118.

12.Afghahi H, Cederholm J, Eliasson B, et al. Risk factors for the development of albuminuria and renal impairment in type 2 diabetes-the Swedish National Diabetes Register (NDR). Nephrol Dial Transplant. 2011; 26(4): 1236-1243.

13.O'Bryan G.T, Hostetter T.H. The renal hemodynamic basis of diabetic nephropathy. Seminars in Nephrology. 1997; 17: 93-100.

14. Stuveling E.M, Bakker S.J.L, Hillege H.L, Jong P.E, Gans R.O.B, Zeeuw D. Biochemical risk markers: a novel area for better prediction of renal risk? Nephrol Dial Transplant. 2005; 20: 497–508.

15. Navarro-Gonzalez J.F, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. Clin J Am Soc Nephrol. 2008; 19: 433-442.

16. Freedman B.I, Bostrom M, Daeihagh P, Bowden D.W. Genetic Factors in Diabetic Nephropathy. Clin J Am Soc Nephrol. 2007; 2: 1306-1316.

17.Maria C, HallJ E. Obesity, metabolic syndrome and diabetic nephropathy. Contrib Nephrol. 2011; 170: 28-35.

18. Li X.H, Lin H.Y, Wang S.H, et al. Association of Microalbuminuria with Metabolic Syndrome among Aged Population. Bio Med Research International. 2016; 9241278: 7.

19. Lennon R, Pons D, Sabin M.A, et al. Saturated fatty acids induce insulin resistance in human podocytes: implications for diabetic nephropathy. Nephrol Dial Transplant. 2009; 24: 3288-3296.

20. Chen W.Z, Hung C.C, Wen Y.W, et al. Effect of glycemic control on microalbuminuria development among type 2 diabetes with high-normal albuminuria. Ren Fail. 2014; 36(2): 171–175.

21. Xiao J, Xing X, Lu J, et al. Prevalence and associated factors of microalbuminuria in Chinese individuals without diabetes: cross-sectional study. BMJ Open. 2013; 3: 1-10.

22. Yan L, Ma J, Guo X, et al. Urinary albumin excretion and prevalence of microalbuminuria in a general Chinese population: a cross-sectional study. BMC Nephrol. 2014; 15:165.

23. Viberti Gand Karalliedde J. Commentary: The birth of microalbuminuria: a milestone in the history of medicine. Int J Epidemiol. 2014; 43(1):18-20.

24. Karalliedde J, Viberti G. Proteinuria in Diabetes: Bystander or Pathway to Cardiorenal Disease? J Am Soc Nephrol. 2010; 21: 2020–2027.

25. McFarlane P.A. Testing for albuminuria in 2014. Can J Diabetes. 2014; 38: 372-375.

26. Shao N, Kuang Y.H, Wang N, et al. Relationship between Oxidant/Antioxidant Markers and Severity of Microalbuminuria in the Early Stage of Nephropathy in Type 2 Diabetic Patients. J Diabetes Res. 2013; 232404: 6.

27. MacIsaac R.J, Tsalamandris C, Panagiotopoulos S, et al. Non albuminuric renal insuficiency in type 2 diabetes. Diabetes Care. 2004; 27: 195-200.

28. Nosadini R,Velussi M, Brocco E, et al. Altered transcapillary escape of albumin and microalbuminuria reflects two different pathogenetic mechanisms. Diabetes. 2005; 54(1): 228–233.

29. MacIsaac R.J, Ekinci E.I, Jerums G. Progressive diabetic nephropathy. How useful is microalbuminuria?: Contra. Kidney International. 2014: 86; 50–57.

30. Thomas M.C, Macisaac R.J, Jerums G, et al. Non albuminuric renal impairment in Type 2 diabetic patients and in the general population (national evaluation of the frequency of renal impairment cO-existing with NIDDM [NEFRON] 11). Diabetes Care. 2009; 32: 1497–1502.

31. Robles N.R, Villa J, Gallego R.H. Non-Proteinuric Diabetic Nephropathy. J Clin Med. 2015; 4: 1761-1773.

32. Robles N.R, Villa J, Felix F.J, et al. Non-proteinuric diabetic nephropathy is the main cause of chronic kidney disease: Results of a general population survey in Spain. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2017.

33. Laranjinha I, Matias P, Mateus S, et al Diabetic kidney disease: Is there a non-albuminuric phenotype in type 2 diabetic patients? Nefrologia. 2016; 36: 503-509.

34. Rossi M.C.E, Nicolucci A, Pellegrini F, et al. Identifying patients with type 2 diabetes at high risk of microalbuminuria: results of the DEMAND (Developing Education on Microalbuminuria for Awareness of renal and cardiovascular risk in Diabetes) Study. Nephrol Dial Transplant. 2008; 23: 1278–1284.

35. Hsu C.C, Chang H.Y, Huang M.C. Association Between Insulin Resistance and Development of Microalbuminuria in Type 2 Diabetes. Diabetes Care. 2011: 34; 982–987.

36. Pilz S, Rutters F, Nijpels G. Insulin Sensitivity and Albuminuria: The RISC Study Diabetes Care. 2014: 37; 1597–1603.

37. Prie D, Friedlander G. Reciprocal Control of 1,25-Dihydroxyvitamin D and FGF23 Formation Involving the FGF23/Klotho System. Clin J Am Soc Nephrol. 2010; 5: 1717–1722.

38. Titan S.M, Zatz R, Graciolli F.G, Reis L.M, Barros R.T, Jorgetti V, Moysés R.M.A. FGF-23 as a Predictor of Renal Outcome in Diabetic Nephropathy. CJASN. 2011; 6: 241-247.

39. Quarles L.D. Role of FGF 23 in Vitamin D and Phosphate Metabolism: Implications in Chronic Kidney Disease Exp Cell Res. 2012; 318(9): 1040–1048.

40. Zanchi C, Locatelli M, Benigni A, et al. Renal Expression of FGF23 in Progressive Renal Disease of Diabetes and the Effect of Ace Inhibitor. PLoS One. 2013; 8(8): e70775.

41. Vervloet M.G, Van Zuilen A.D, Heijboer A.C, et al. Fibroblast growth factor 23 is associated with proteinuria and smoking in chronic kidney disease: Analysis of the MASTERPLAN cohort. BMC Nephrol. 2012; 13:20: 10.1186/1471-2369-13-20.

42. Silva A.P, Mendes F, Fragoso A, Jeronimo T, et al. Altered serum levels of FGF-23 and magnesium are independent risk factors for an increased albumin-to-creatinine ratio in type 2 diabetics with chronic kidney disease. J Diabetes Complications. 2016; 30: 275-280.

43. Harinarayan C.V. Vitamin D and diabetes mellitus. Hormones. 2014; 13: 163-181.

44. Al-Daghri N.M, Alkharfy K.M, Al-Othman A, et al. Vitamin D supplementation as an adjuvant therapy for patients with T2 DM: an 18-month prospective interventional study. Cardiovasc Diabeto. 2012; 11(1): 85.

45. Peterson C.A, Tosh A.K, Belenchia A.M. Vitamin D insufficiency and insulin resistance in obese adolescents. Ther-Adv Endocrinol Metab. 2014; 5(6): 166–189.

46. Alborzi P, Patel N.A, Peterson C, et al. Paricalcitol reduces albuminuria and inflammation in chronic kidney disease. A randomized double-blind pilot trial. Hypertension. 2008; 52: 249-255.

47. Kriz W, Lemley K.Y. The role of the podocyte in glomerulosclerosis. Curr Opin Nephrol Hypertens. 1999; 8: 489-497.

48. Schwarz U, Amann K, Orth S.R, et al. Effect of 1,25(OH)2 vitamin D3 on glomerulosclerosis in subtotally nephrectomized rats. Kidney Int. 1998; 53:1696 -1705.

49.Weinreich T, Merke J, Schonermark M, et al. Actions of 1,25-dihydroxy vitamin D3 on human mesangial cells. Am J Kidney Dis. 1991;1 8: 359-366.

50. Fishbane S, Chittineni H, Packman M, et al. Oral paricalcitol in the treatment of patients with CKD and proteinuria: a randomized trial. Am J Kidney Dis. 2009; 54: 647–652.

51. Aperis G, Paliouras C, Zervos A, et al. The role of paricalcitol in proteinuria. J Ren Care. 2011; 37: 80- 84.

52. deZeew D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITALstudy). A randomised controlled trial. Lancet. 2010; 376: 1543-1551.

53. Silva A.P, Mendes F, Pereira L et al. Klotho levels association with insulin resistance and albumin-to-creatinine ratio type 2 diabetic patients. Int Urol Nephrol. 2017.

54. Moreno J.A, Izquierdo M.C, Sanchez-Niño M.D, et al. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFkB.J Am Soc Nephrol. 2011; 22: 1315-25.

55. Lim S.C, Liu J.J, Subramaniam T, Sum C.F. Elevated circulating alpha-klotho by angiotensin II receptor blocker losartan is associated with reduction of albuminuria in type 2 diabetic patients. J Renin Angiotens in Aldosterone Syst. 2014;15: 487-490.

56. KacsoI. M, Bondor C.I, Kacso G. Soluble serum Klotho in diabetic nephropathy: relationship to VEGF-A. Clin Biochem. 2012; 45: 1415-20.

57. Haruna Y, Kashihara N, Satoh M, et al. Amelioration of progressive renal injury by genetic manipulation of Klotho-gene. Proc Natl Acad Sci USA. 2007; 104: 2331-23316.

58. Lindberg K, Amin R, Moe O.W, et al. The kidney is the principal organ mediating klotho effects. J Am Soc Nephrol. 2014; 25: 2169-2175.

59. Kocełak P, Olszanecka-Glinianowicz M, Chudek J. Fibroblast Growth Factor 23–Structure, Function and Role in Kidney Diseases. Adv Clin Exp Med. 2012; 21: 391–401.

60. Yilmaz M.I, Sonmez A, Saglam M, et al. FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. Kidney Int. 2010; 78: 679-685.

61. Xu B, Sun J, Deng X, Huang X, et al. Low Serum Magnesium Level Is Associated with Microalbuminuria in Chinese Diabetic Patients. Int J Endocrinol. 2013, Article ID580685.

62. Barbagallo M, Dominguez LJ. Magnesium and type 2 diabetes. World J Diabetes 2015; 6(10): 1152-1157.

63. Mirrahimi B, Hamishehkar H, Ahmadi A, et al. The efficacy of magnesium sulfate loading on micro albuminuria

following SIRS: One step forward in dosing. DARU Journal of Pharmaceutical Sciences 2012, 20: 74.

64. Barbagallo M, Dominguez L.J. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch Biochem Biophys. 2007; 458: 40-47.

65. Barbagallo M, Dominguez L.J, Galioto A, Ferlisi A, et al. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med 2003; 24: 39–52.

66. Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. Am J Hypertens 1997; 10: 346-355.

67. Corsonello A, Ientile R, Buemi M, et al. Serum ionized magnesium levels in type 2 diabetic patients with microalbuminuria or clinical proteinuria. American Journal of Nephrology 2000; 20: 187-192.