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NORMOALBUMINURIC DIABETIC KIDNEY DISEASE - AN OFTEN MISSED ENTITY

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ABSTRACT One of the serious complications of diabetes is diabetic kidney disease (DKD), linked with renal impairment and cardiovascular disease. There is an increasing prevalence of DKD in diabetic patients and is the principle cause for chronic kidney disease globally. Proteinuria, especially microalbuminuria was identified as an early marker preceding decline in renal function.It was proposed that DKD typically follows a sequence of hyperfilteration, microalbuminuria, and decrease in renal function with gross proteinuria and finally renal shutdown. The advent of better treatment modalities and contribution of other pathological factors, have altered this sequence with identification of many subsets of which normoalbuminuric diabetic kidney disease (NADKD) is one. This review attempts to summarize the clinical presentation and etiopathogenesis of patients having normoalbuminuric diabetic kidney disease as well as identification of novel biomarkers, which can provide critical diagnostic and prognostic information and facilitate proper treatment and thus reduce this disease burden from society.

KEYWORDS : Diabetic Kidney Disease, Microalbuminuria, Normoalbuminuric Diabetic Kidney Disease

INTRODUCTION

The incidence of diabetes mellitus (DM) is increasing rapidly worldwide, it is predicted that diabetes will be the seventh leading cause of mortality in the world (Roshan and Stanton, 2013). Diabetic nephropathy (DN) is one of the main complications of DM and is detected in about 40% of Type 1 and Type 2 diabetic patients (Gluhovschi et al, 2016). In most of the countries, diabetes is the leading cause of chronic kidney disease (CKD) that often proceeds to end stage renal disease (ESRD), which requires expensive renal replacement therapy in the form of dialysis or renal transplantation (Gluhovschi et al, 2016; Laranjinha et al, 2016; Fiseha, 2015). Records indicate that as many as 26 million adults in US, 13% and 16% population in China and Australia respectively suffer from CKD (Weiner, 2009). CKD in diabetic patients is linked with substantial morbidity, mortality, and increased health care expenses leading to unavoidable health burden to the patients and their families (Roshan and Stanton, 2013; Weiner, 2009). Hence, CKD is a major public health concern worldwide.

Stages of classical diabetic kidney disease

Diabetic nephropathy is a type of renal microvascular complication (Uwaezuoke, 2017). The concept of Diabetic kidney disease (DKD) was first recommended by American Kidney Association (Uwaezuoke, 2017). The clinical features of DKD include increasing albuminuria with urinary albumin excretion rate (UAER)> 300 mg/day or 200 ug/min, recorded at least twice within a 3-to 6- month interval and urinary albumin creatinine ratio (UACR)>300mg/g, gradual decrease in glomerular filtration rate (GFR), hypertension, reduced renal function, and in extreme cases kidney failure (Uwaezuoke, 2017; Chao etal, 2017; Silva et al, 2017; Cao and Cooper, 2011). During early microalbuminuria, GFR is usually normal or slightly elevated (deJong and Curhan, 2006). Reduction of GFR is mostly associated with severely increased albuminuria (macroalbuminuria), although decreased GFR was reported in several normoalbuminuric DM patients (Gluhovschi et al, 2016; Uwaezuoke, 2017). Until recently, DKD was thought to progress through normoalbuminuria to microalbuminuria to macroalbuminuria and finally to ESRD. This linear progression of DKD is classified into 5 stages. The first stage is the normoalbuminuria or pre-nephropathy with UACR<30mg/g and GFR≥30mL/min/1.73m², which is linked with glomerular hyperfiltration and hypertrophy. The second stage is the microalbuminuria or incipient nephropathy with UACR 30-300mg/g and GFR ≥30mL/min/1.73m² and alterations of renal structures including glomerular capillary basement membrane thickening. The third stage called overt nephropathy is characterized by consistent proteinuria≥0.5g/day or macroalbuminuria with UACR≥300mg/g and GFR≥30mL/min/1.73m². The fourth stage involves any level of albuminuria or proteinuria and GFR ≤30mL/min/1.73m² leading to renal failure. In the final stage there is uremia that requires dialysis therapy and even renal transplantation in extreme cases(Silva et al, 2017).

Albuminuria is the earliest sign of vascular damage in the kidney and renal function loss in Type1 and Type2 DM patients and is mostly considered as the predictor for progression of DN (Ruggenenti and Remuzzi, 2006). The concept of albuminuria dates back to 200 years and its correlation with kidney disease was envisioned by Richard Bright in 1827 (Koroshi, 2007). Normally, the filtered albumin is endocytosed, directed to lysosome, gets denatured and then returns to blood. During microalbuminuria there is increased glomerular permeability, which raises the levels of filtered albumin through glomerular filtration barrier. Eventually, the endocytic pathway gets saturated and elevates UAE (Silva et al, 2017). Proteinuria or macroalbuminuria is the universal finding in progressive renal disease and is a significant risk factor for DKD and ESRD.

Pathogenesis of Typical DKD

The pathophysiology of DKD is quite complicated and is dependent on multifactorial interaction between metabolic (involving glucose-dependent pathways) and hemodynamic pathways (renin-angiotensin system). The interactions are anticipated to occur via shared molecular and signaling pathways linked with the generation of reactive oxygen species (ROS). These interactions might result in structural and functional changes of kidneys, which are clinically manifested as DN (Cao and Cooper, 2011). The major pathogenic components of DN include renal fibrosis, mesengial expansion, glomerular hypertrophy, oxidative stress, and tubular inflammation (Figure 1).

Figure 1: *A schematic representation of the pathogenesis of diabetic nephropathy*
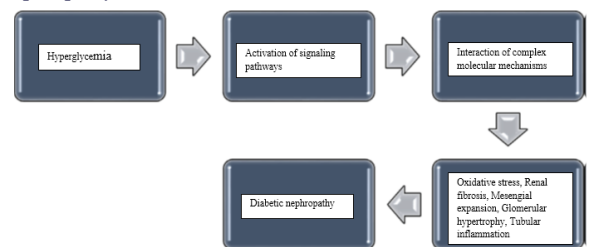
(*Source:* Uwaezuoke SN. The role of novel biomarkers in predicting diabetic nephropathy: a review. *Int J Nephrol Renovasc Dis.* 2017;10: 221-231.)

Even though the exact mechanism of development of DKD is not clarified, a possible correlation between renin-angiotensin-aldosterone system (RAAS) and inflammatory cytokines such as interleukin (IL)-1β, IL-6, and IL-8 and tumor necrosis factor (TNF)-α was detected. Aldosterone and angiotensin II were assumed to be the principal mediators of DKD pathogenesis(Uwaezuoke, 2017).TNF- α is supposed to reduce glomerular blood flow directly and elevates glomerular vasoconstriction, thus lowers GFR. This represents the role of immune inflammation in renal dysfunction (Chao et al, 2017). Type 2 DM patients with elevated levels of circulating TNF receptors are found to be at higher risk of developing ESRD (Uwaezuoke, 2017). Additionally, protein kinase C (PKC) and its isoforms (PKC-α, PKC-β, and PKC-Є) initiate DN and facilitates it progression via complex

mechanisms. PKC isoforms upregulate the expression of vascular endothelial growth factor (VEGF) in mesengial cells and transforming growth factor- β (TGF- β), laminin, and fibronectin in the glomeruli, thereby cause mesengial expansion and renal fibrosis. Besides, mesengial expansion and albuminuria are triggered by renal oxidative stress, which boosts fibronectin and collagen-1 expression (Uwaezuoke, 2017). Loss of renal function in diabetic patients involves nephrotoxic pathway that is stimulated by reabsorbed albumin from glomerular filtrate through cubulin-megalin complex in renal tubule, which causes tubule-interstitial atrophy and fibrosis and ultimately complete loss of renal function (Bolignano and Zoccali, 2017).

Normoalbuminuria- the potent non-proteinuric phenotype of DKD

DKD, believed to progress through microalbuminuria is one of the key causes of ESRD. Lately, many studies had challenged the classical route of DKD progression since microalbuminuria has shown regression to normoalbuminuria (presence of normal levels of albumin in the urine) or remains constant, instead of progression to macroalbuminuria (Gluhovschi et al, 2016). In their study with 216 Type2 DM patients, after a 6-year follow-up, Araki et al noticed regression of microalbuminuria to normoalbuminuria in 51% patients and progression to macroalbuminuria in 28% patients (Gluhovschi et al, 2016; Araki et al, 2007). Study by Gaede et al with a follow – up of 7.8 years detected out of 151 Type2 DM patients, 46 showed remission to normoalbuminuria, 47 progressed to macroalbuminuria, and 58 remained microalbuminuric. Regression to normoalbuminuria prevent GFR decline during the follow-up period (Gaede et al, 2004). Proper glycemic (ideally HbA1c ≤ 6.5%) and blood pressure control (under130/80mmHg) are thought to play a role in the remission to normoalbuminuria (Chang, 2008). Different researches detected that many DM patients despite having normoalbuminuria suffer from significant renal dysfunction with reduced estimated GFR (eGFR) level (Laranjinha et al, 2016), indicating the existence of an alternative pathway of DKD progression. Multiple studies reported about the presence of another entity which progressed to ESRD without having albuminuria (Silva et al, 2017). DKD in this cohort of patients is referred to as normoalbuminuric diabetic kidney disease (NADKD). The proposed diagnostic criteria for NADKD include: i) agreement with the diabetes diagnostic criteria set by American Diabetes Association (ADA) or WHO, ii) $eGFR<60mL/min/1.73m^2$, iii) UAER<30mg/day (under use of normal hypertensive drugs), or UACR<30mg/g, and iv) omission of other kidney diseases (Chao et al, 2017). The presence of this entity has been shown to be having few unique features of its own, which opens up a new dimension in the diagnosis and management of DN.

Prevalence of NADKD

A multitude of epidemiological studies in last few years revealed that NADKD is a common occurrence with many patients suffering from this condition. UK Prospective Diabetes Study (UKPDS), a longitudinal study over a median of 15 years of follow up after diagnosis of Type 2 diabetes detected 28% with normoalbuminuria among 1132 DM patients (Retnakaran et al, 2006). Swedish National Diabetes Register (NDR), another prospective cross-sectional study with a 5-year follow up with 3660 patients found 6-7% normoalbuminuric patients with renal insufficiency (Afghahi et al, 2011).

Numerous cross-sectional studies worldwide detected a considerable proportion of normoalbuminuric DM patients with renal insufficiency and low GFR value <60ml/min/1.73m². A national cross-sectional study by National Health and Nutrition Examination Survey (NHANESIII) consisting of a nationally representative sample of the total US civilian, non-institutionalized population) with 2798 diabetic patients, found 56.3% NADKD (95% CI 51.7-60.9) patients (Mottl et al, 2013). The PERCEDIME2 study in Spain was a national cross-sectional study on the prevalence of CKD in patients with Type 2 diabetes. The study involved 1145 DM patients with renal impairment in 206, of which 69.4% had NADKD (Rodriguez-Poncelas et al, 2013). The Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular Risk in Diabetes (DEMAND) study determined 17% NADKD patients, who were in stage 3 – 5 of CKD (Dwyer et al, 2012). NEFRON, another cross-sectional study with 3893 patients, spotted 55% patients with normoalbuminuria (Thomas et al, 2009). RIACE, a multicenter study with DM patients, reported 18.8% normoalbuminuric patients (Pugliese et al, 2014). In their study on Korean Type2 diabetes patients, An JH et al (2009) noticed 29.1% normoalbuminuric renal

insufficiency patients among the total study population. A Japanese cross-sectional study with 3297 Type 2 DM patients with $eGFR<60ml/min/1.73m^2$ in 15.3% patients, identified 11.4% normoalbuminuric patients (Yokoyama et al, 2009). Kramer et al (2007) detected 36% normoalbuminuric patients among 9737 DM Type 2 patients with $eGFR<60ml/min/1.73 m^2$ among12.3% patients. Other studies by Maclsaac et al with 625Type 2 DM patients and Boronat et al (2016) with 78 DM Type2 patients determined 39% and 21.8% normoalbunimuric patients respectively (MacIsaac et al, 2004). In addition, The Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) identified 22.47% patients with Type 1 DM having $eGFR<60mL/min/1.73m^2$ and UAER< 30m/24hr, signifying normoalbuminuric phenotype (Molitch et al, 2010). Table 1 summarizes the results of the studies, which detected the existence of NADKD in patients with renal impairment. Findings of all these studies designated that a substantial proportion of diabetic patients (both Type1 and Type2) does not follow the classical DKD pathway from normoalbuminuric to microalbuminuric to macroalbuminuric and ultimately renal failure, rather presented impaired renal function even in the absence of an enhanced UAER. Hence, normoalbuminuria directly leads to renal insufficiency and low eGFR (Ekinci et al, 2013). However, prevalence of NADKD varies across studies, which can be due to use of diverse methods to measure proteinuria or prior treatments to reduce proteinuria.

Table 1: *Prevalence of normoalbuminuric diabetic kidney disease observed in different studies*

Author name	Type of study	Total number of type 2 diabetes mellitus patients (n)	Percentage of patients with eGFR less than 60mL/min/1.73 m²	Percentage of normoalbu minuria patients (%)
Retnakaran et al[17]	Prospective 15 years of follow up (UK Prospective Diabetes Study)	7642	16%	28%
Afghahi et al[18]	Prospective 5 years follow up (Swedish national diabetes register)	3660	11%	6 – 7 %
Rodriguez-Ponceles et al[20]	Cross sectional study (PERCEDIME 2) study in Spain	1145	18%	69.4%
Dwyer et al[21]	Cross sectional (DEMAND) study	11573	22.3%	20.5%
Thomas et al[22]	Cross sectional (NEFRON Survey)	3893	23.1%	55%
Pugliese G et al[23]	Cross sectional (RIACE database)	15773	37.5%	18.8%
Yokoyama et al[25]	Cross sectional	3297	15.3%	11.4%
Kramer C et al[26]	Cross sectional	9737	12.3%	36%
Maclsaac et al[27]	Cross sectional	625	36%	39%
Boronat et al[28]	Cross sectional	78	100%	21.8%

Prevalence of NADKD has been known to be dependent on race with Caucasian Type1 and Type 2 DM patients suffering more from NADKD compared to Asian and Australian people (Gheith and Al-Otalbi, 2016). Motl et al (2013) revealed that non-Hispanic whites have higher probability to develop NADKD than Hispanic blacks, who get albuminuric DKD mostly. Moreover, cross-sectional study by Parving et al (32) among 32,208 DM patients across 33 countries exhibited highest prevalence of microalbuminuria (43.2%, 43.8%) and macroalbuminuria

(12.3% and 10.3%) among Asian and Hispanic patients respectively, whereas Caucasians have lowest microalbuminuria (33.3%) and macroalbuminuria (7.6%) indicating genetics and environmental factors play important role in the development of NADKD (Gheith and Al-Otalbi, 2016).

Besides, recent findings suggested that females are at a higher risk of developing NADKD than males, who are more vulnerable towards albuminuria excretion rate progression (Silva et al, 2017). In a study with 526 patients of which 151 had renal insufficiency (eGFR< 60mL/min/1.73m^2), An et al (2009) detected, the correlation of normoalbuminuric renal insufficiency with female predominance (76%) compared to microalbuminuric renal insufficiency. Similar to this data, Ekinci et al (2013) noticed 5 out of 8 (62.5%) subjects with normoalbuminuria (eGFR<60mL/mim/1.73m^2) were females compared to 1 out of 6 (16.7%) in microalbuminuria and 3 out of 17 (17.65%) in the macroalbuminuria groups. In a study by Kramer et al (2007) with total 660 normoalbuminuric Type 2 DM patients with low eFGR (eGFR<60mL/mim/1.73m^2), 77.4% were older females of average age 63 years. Moreover, a multicenter study performed by Molitch et al (2010) with a follow up of 19 years noticed that among 89 Type1 DM patients with eGFR<60mL/min/1.73m^2, 81% normoalbuminuric patients were women, whereas, 64.3% and 51.85% of women had microalbumiuria and macroalbuminuia respectively. Rigalleau et al (2007) also detected among 89 DM patients with renal impairment, 66% normoalbuminuric and 40% albuminuric with the average age of normoalbuminuric women above 60 years. A clear association between normoalbuminuria and female prevalence was observed by Motl and colleagues and the age of the NADKD patients was mostly 69 to 73 (Afghahi et al, 2011). A meta-analysis study revealed that rapid decline of eGFR occurs in women with NADKD than in men (Chao et al, 2017). All these data indicate normoalbuminuric phenotype of DN is more prevalent among postmenopausal women of age 60-80 years (suffering from Type 2 DM) than younger women (Silva et al, 2017; Kramer et al, 2007) suggesting that female hormonal changes around menopause might affect NADKD pathogenesis (An et al, 2009) by lowering GFR and causing renal insufficiency. Few studies even indicated contradictory effects of estrogen on GFR among diabetic women (Chao et al, 2017). Additionally, age-related reduction of GFR due to the loss of nephron might account for reduced GFR in aged normoalbuminuric DM patients (Laranjinha et al, 2016).

Pathophysiology of NADKD
Pathophysiology of NADKD can be explained by several mechanisms:

1. More of macro and less of microangiopathy: Various studies have shown that the typical microangiopathic lesions like retinopathy associated with classical DN with albuminuria is not common in NADKD patients. Accordingly, Penno et al (2012) exhibited in their multicenter study that diabetic retinopathy (DR) was more common in albuminuric DKD group (29.72%) compared to NADKD group (21.66%). An et al (24) found DR in 16% NADKD patients (7/44), 56% (28/50) in microalbuminuric and 81% (46/57) in macroalbu minuricpatients. Likewise, Kramer et al (2003) spotted 28% DR in NADKD patients and 45% in albuminuric DKD patients. Several studies denoted the macroangiopathic pathway rather than microangiopathic pathway to be the underlying factor for NADKD (Silva et al, 2017). In an observational study by Lee et al (2016) with 1136 DM patients followed up for 44 months, the incidence of cardiovascular disease (CVD) was similar between NADKD and albuminuric DKD patients. Nonetheless, study by Boronat et al (2016) with 78 DKD patients detected coronary heart disease in 47.1% and retinopathy in 52.9% NADKD group, whereas, 29.5% coronary heart disease and 59% retinopathy in the albuminuria group. The prevalence of CVD, but not DR, was found to be higher in patients with normoalbuminuric renal impairment (stages 3-5) than in micro and macroalbuminuric subjects as observed by Pugliese and his colleagues (Pugliese et al, 2014). Hence, mechanism other than DR is involved in the pathogenesis of NADKD.

2. More atypical glomerular changes: Majority (90%) of the renal tissue consists of the tubulointersitium, which contains tubular epithelium, vascular structures, and intersititium. Conventional interstitial pathological changes found in DN are tubular basement membrane thickening, interstitial fibrosis, tubular atrophy, and arteriosclerosis (Chao et al, 2017; Bader et al, 1980). Renal biopsy of DKD patients in Ekinci et al (2013) study revealed interstitial and vascular lesions in 3 out of 8 (37.5%) NADKD patients and 1 out of 23 (4.3%) in proteinuria patients. For microalbuminuric and macroalbuminuric patients, the renal biopsies were

mainly classified as Class II (as per Fioretto stage) demonstrating typical renal structure changes and for normoalbuminuric patients, the renal biopsies presented heterogeneous changes (Silva et al, 2017). Another study conducted renal biopsies of 111 normoalbuminuric and microalbuminuric Japanese DM patients and followed up for 11 years for renal function changes in 37 patients, showed that the renal histological pattern had severe atypical changes (Class III as per Fioretto stage) in NADKD patients (Moriya et al, 2014). Study by Budhiraja et al (2013), using kidney biopsies of normoalbuminuric patients (eGFR< 60mL/min/1.73m^2) detected capillary thickening in 10 patients, of them two had severe diffuse mesengial thickening without nodules and eight had Kimmelstiel-Wilson nodules. Moreover, the well preserved tubules and tubule interstitium were observed along with afferent and efferent arteriolar hyalinosis (Budhiraja et al, 2013). Presence of well-preserved tubule facilitates considerable reabsorption of albumin from glomerular filtrate thus UAE remains in the normal range. In fact, glomerular changes are much more heterogeneous in NADKD than albuminuric DKD (Silva et al, 2017). Additionally, interstitial fibrosis was found to be responsible for further decline of kidney function rather than glomerular injury, and this decline is not dependent on albuminuria (Chao et al, 2017).

3. Elevated intrarenal arteriosclerosis: In contrast to classical glomerulosclerosis changes in albuminuric subjects, hemodynamic changes such as increased intrarenal arteriosclerosis, or arterial hyalinosis were viewed in normoalbuminuric patients with DN. However Maclsaac et al concluded from their study that diabetic patients with renal impairment had same level of intrarenal vascular disease irrespective of AER status assessed via intrarenal arterial resistance index (MacIsaac et al, 2004), suggesting that renal vascular lesions might be responsible for NADKD pathogenesis.

4. Repeated episodes of acute kidney injury (AKI)- Diabetic patients are found to be susceptible for AKI repeatedly and this might cause long term CKD, even though renal function can be recovered to some extent. The progression of AKI to CKD might be explained by certain mechanisms including nephron loss by endothelial injury, inflammation, epigenetic changes, and arrest of cell cycle in epithelial cells. Repeated episodes of AKI declines renal function irrespective of urinary albumin level due to limited regeneration capacity of tubular progenitor (Chao et al, 2017; Silva et al, 2017). Different studies had established that AKI is an important risk factor for CKD in NADKD patients (Silva et al, 2017).

5. Association with Metabolic syndrome: A positive correlation between metabolic syndrome and NADKD was identified by some researchers (Mattock et al, 1998). Laranjinha et al (2016) in their 1 year retrospective observational single center study showed that out of 146 patients, 46.6% had NADKD and metabolic syndrome was present in 50% NADKD patients. Luk et al (2008) in their 5 year prospective analysis of Hong Kong diabetes registry spotted a positive correlation between CKD and metabolic syndrome (p value 0.001). In another study, Kramer et al (2003) reported about high level of triglyceride and metabolic syndrome in Type 2DM patients with normoalbuminuria and renal insufficiency. They explained that increased triglycerides and metabolic syndrome resulting from renal microvascularatherlosclerotic complications lowered renal plasma flow and thus reduced GFR (Kramer et al, 2007).

6. Use of Renin-angiotensin-aldosterone-system (RAAS) blockage: RAAS blockers are a traditional class of drugs for eliminating or lowering proteinuria in DKD patients (Chao et al, 2017). Most of the NADKD patients were found to use RAAS blockers and also clinical researches showed that discontinuation of RAAS blockers induces the progression of normoalbuminuria to microalbuminuria. The widespread use of RAAS inhibitors like angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin 2 receptor blockers (ARBs) seems to be also responsible for the emergence of NADKD group as they have a propensity of decreasing albumin excretion (Robles et al, 2015). An et al (2009) studied 151 patients with renal insufficiency and detected 29.1% normoalbuminuric, 33.1% microalbuminuric, and 37.8% macroalbuminuric patients before treatment with RAAS blockers. Following treatment with a RAAS inhibitor, the proportions were 35.3%, 41.2%, and 23.5% respectively. A recent study by Porrini et al (2015) recommended that treatment of DM patients with RAAS inhibitors might be partially involved in the progression of NADKD as they are able to reduce proteinuria but unable to improve the GFR.

7. Genetic factors- Genetic factors are thought to play role in the

development of renal insufficiency in normoalbuminuricType 2 DM patients. In a study with Japanese diabetic patients, polymorphism of PKC-β gene was found to be associated with eGFR decline in normoalbuminuria (An et al, 2009). However, the exact causative or susceptibility genes responsible for the pathogenesis and progression of NADKD need to be determined.

Clinical manifestations of NADKD

The clinical features of NADKD and DKD with microalbuminuria and macroalbuminuria vary. The pathological changes of NADKD patients with low eGFR mostly include higher glomerular fibrosis, mesengial matrix proliferation, and hyaline degeneration of renal arteries. The salient feature of altered renal structure in NADKD includes tubular and interstitial damage. An experiment on Cohen diabetic-resistant rat and Cohen diabetic-sensitive rat models (which develops normoalbuminuric DKD) detected mesengial matrix proliferation, higher level of type IV collagen in the glomeruli and interstitium, and thickening of glomerular basement membrane (Chao et al, 2017). Compared to DKD with macroalbuminuria, NADKD with low GFR used to follow a more benign course (Mottl et al, 2013) with lower burden of diabetic complications as depicted in Figure 2.

Figure 2: *Frequency of complications in different DKD groups: a) Normoalbuminuria group (UAER <30mg/24h), b) Microalbuminuria group (UAER 30-300 mg/24h), iii) Macroalbuminuria group (UAER>300mg/24h)*
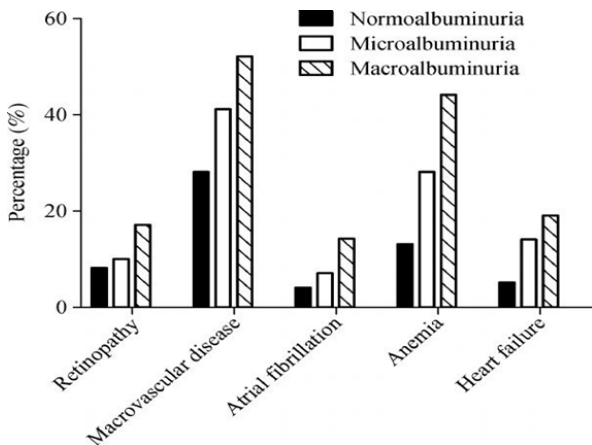
A study on Korean Type 2 diabetic patients with renal insufficiency identified shorter duration of diabetes and lower frequency of retinopathy among normoalbuminuric patients suggesting that renal insufficiency in these patients might be at an earlier stage compared to renal insufficiency in microalbuminuria and macroalbuminuria (An et al, 2009). This was supported by earlier finding of Rigalleau et al (2007) in normoalbuminric diabetic patients showing stable AER over 38 months and lower risk of progression to ESRD or death compared to microalbuminuric and macroalbuminric patients. Even Perkins et al (2007) reported rapid eGFR decline in proteinuric DKD patients compared to NADKD patients. A consistent data was obtained by Boronet et al (2016). All these findings suggest that normoalbminuria might lessen eGFR decline (Chao et al, 2017).

Even though Type 2 DM with microalbuminuriais an independent risk factor for CVD, the burden of CVD in NADKD is also high(Chao et al, 2017). In a cross-sectional study with normoalbuminuric patient group (UAER<10mg/24h) and low albuminuria (LA) (UAER 10-29 mg/24h) group, the intensity of acute CVD, coronary events, peripheral vascular events, and ulcers was considerably higher in LA group (Penno et al, 2012). This was validated by the study on Korean Type DM patients with renal insufficiency having higher incidence of CVD and coronary artery disease in NADKD patients (An et al, 2009). This finding signified that other atherosclerotic disease including renal vasculature might account for renal function decline in normoalb uminuric condition. Nevertheless, MacIsaac et al (2004) noticed analogous intrarenal vascular disease among normoalbuminuric and microalbimuniric DKD patients.Hence, mechanism of renal

insufficiency in normoalbuminuria still needs to be explained.

The role of hypertensive nephroangiosclerosis in NADKD development was studied as this is one of the leading causes of CKD in Type 2 diabetic patients. Nonetheless conflicting data were obtained about correlation of NADKD and hypertension. Ekinci and colleagues (2013) reported hypertension as the chief contributing factor for renal insufficiency in normoalbuminuric DM patients in contrast An et al (2009) found lesser hypertension among NADKD patients and Laranjinhi et al (2016) found equal occurrence of hypertension among albuminuric and nonalbuminuric patients. Therefore, more studies are required to confirm hypertension as a causative factor for NADKD.

Typically Type 2 DM is related to obesity. Accordingly Boronat et al (2016) found more BMI and waist circumference in NADKD patients than in patients with microalbuminuric and macroalbuminuric DKD. Higher levels of hemoglobin and low-density lipoprotein (LDL) were observed in NADKD patients than proteinuric DKD patients (Chao et al, 2017). Consequently, it is crucial to monitor regularly the kidney functions in NADKD patients.

Possible clinical predictors and risk factors for NADKD

Conventional screening approach for DKD is evaluation of UAER. However, presence of normoalbuminuric phenotype in DKD raises a doubt about considering UAER as the best biomarker for clinical diagnosis as NADKD might remain unnoticed if UAER is solely measured. In fact, according to a research on Japanese Type 2 DM patients, the UAER seemed to be an unreliable predictor for altered renal structure in CKD. A significant proportion of normoalbuminuric DKD patients reveal lower GFR; GFR<60ml/min/1.73m^2 in Type 2DM and GFR<90ml/min/1.73m^2 in Type I DM (Moriya et al, 2014). Moreover, albuminuria is a non-specific marker since it is higher in other kidney diseases (interstitial nephritis, nephrosclerosis) and also in congestive heart failure. Additionally, deterioration of renal function in DN is not always linear with albuminuria progression (Gentile and Remuzzi, 2016). GFR is one of the best measures for overall kidney function with respect to health and disease. The normal GFR level varies with age, sex, and body size. In young adults normal GFR is approximately 120 to 130 ml/min/1.73m^2 and the value decreases with age. In patients younger than 40 years, CKD should be defined as GFR<75ml/min/1.73m^2, and between 40 to 65 years defined by GFR<60ml/min/1.73m^2 (Silve et al, 2017). According to National Kidney Foundation, GFR<60ml/min/1.73m^2 indicates a loss of 50% of the renal function in adults and GFR<15 ml/min/1.73m^{2}points to renal failure (Levey et al, 2003). The progressive reduction of GFR makes it the current gold standard for detecting several renal diseases. KDOQI guidelines proposed that screening of DKD should be done on the bases of UAER (threshold: 30mg/24hr) as well as evaluation of GFR in adults (threshold: 60ml/min/1.73m^2) using Cockcroft-Gault equation and Modification of Diet in Renal Disease (MDRD) equation (Rigalleau et al, 2007; Levey et al, 2003; KDOI, 2007).

A plethora of studies disclosed that diverse pathogenic factors including age, female sex, lower duration of diabetes, obesity, high hemoglobin level, reduced occurrence of retinopathy, high lipidemia, intrarenal vascular disease, metabolic syndrome, smoking, and environmental factors play role in the NADKD development and progress. So, early diagnosis and constant surveillance of renal function in NADKD patients would facilitate better prognosis.

Novel biomarkers for NADKD

Numerous studies aimed to detect unique biomarkers for NADKD since GFR is not routinely available for its complexity and the expenses of measurement protocols. In order to overcome the limitations of conventional predictors for DN, few novel biomarker candidates are being assessed recently in preclinical and clinical models (Gentile and Remuzzi, 2016). Exceptional advancement in the field of genomics and proteomics aid the researchers to identify novel biomarkers for NADKD.

The urinary liver-type fatty acid-binding protein (L-FABP), expressed in the proximal tubules of the kidney has been found to be substantially higher in Type 2 DM patients with normoalbuminuria. This elevated L-FABP precisely displays the severity of DKD (Silva et al, 2017). Besides, heart-type fatty acid binding protein (H-FABP) indicates renal tubular injury. Hence, both L-FABP and H-FABP might be beneficial in NADKD diagnosis (Chao et al, 2017; Silva et al, 2017).

Narita et al (2006) observed an increased transferrin (a glomerular damage marker) level in the non-proteinuric patient and deliberated this as an indicator for development of proteinuria. Lim et al (2012) recommended the use of zinc-a (2)-glycoprotein as a urinary biomarker for NADKD. It is believed that tubular and glomerular injury occurs at the initial stage of DKD when renal tubule gets exposed to different hemodynamic and metabolic factors related to DM. Hence, increased tubulin markers such as neutrophil gelatinase-associated lipocalin (NGAL), a small protein of lipocal superfamily and urinary kidney injury molecule 1 (uKim1) showing progressive increase from ACR 10mg/g creatinine to 30mg/g creatinine in NADKD patients are considered as dependable predictors of highly sensitive and specific NADKD (De Carvalho et al, 2016). Moreover, enhanced levels of inflammatory cytokines of TNF pathway (TNFα, TNFR1, TNFR2, and Total TNFα), which mark the risk of ESRD in DKD patients are mostly associated with normoalbuminuria and are contemplated as NADKD diagnostic marker (Chao et al, 2017). Furthermore, fibroblast growth factor (FGF-21, FGF-23), and soluble TNF receptors are suggested biomarkers for prediction of DN at early stage but requires confirmation by larger study samples (Gentile and Remuzzi, 2016). Additionally, Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early DN in Type 2 diabetic patients with normoalbuminuria (PRIORITY) trial is assessing the benefits of urinary peptide-based classifier made up of 273 different urinary peptides (CKD 273) to identify individuals predisposed of developing DN (Gentile and Remuzzi, 2016). Further researches are required to discover more probable biomarkers for NADKD, so as to include them in DN diagnosis and monitoring guidelines and facilitating timely diagnosis of NADKD.

Treatment of NADKD

At present no effective treatment for NADKD is available. It is still dubious whether the treatment and preventive measures for classical DKD (with microalbuminuria or macroalbiminuria) would be equally effective for the new DKD phenotype. The use of RAAS inhibitors for NADKD treatment is not yet established due to contradictory findings. Recently, Dwayer et al (2012) stated about the protective role of RAAS inhibitors in NADKD progression by lowering proteinuria. In contrast, MacIsaac et al (2004) could not identify significant difference in result on using RAAS blockers for proteinuric and non-proteinuric patients (GFR<60ml/min/1.73m^2). Correspondingly, DEMAND study showed identical reduction in GFR measures in proteinuric and non-proteinuric patients, with or without ACE (Silva et al, 2017). To confirm this and also to identify new alternatives to boost the treatment for NADKD patients additional researches are essential.

Prognosis

There is ambiguity regarding the prognosis of NADKD with renal insufficiency. Few studies showed better prognosis with lesser mortality rates in normoalbuminric DKD in contrast to albuminuric DKD (Ekinci et al, 2013; Rigalleau et al, 2007). On the other hand no significant difference in terms of GFR decline following treatment was observed among proteinuric and non-proteinuric patients by MacIsaacc et al (2004). Also Ruggenetti and Remuzzi (2006) in a cohort study using gold standard method were unable to detect any major difference in eGFR reduction among proteinuric and non-proteinuric patients. Hence, it is vital to conduct more longitudinal studies to find out an approach to prevent lowering of GFR and renal dysfunction in NADKD patients.

CONCLUSION

Overall, it can be concluded that a significant proportion of diabetic patients with renal insufficiency (eGFR<60mL/min/1.73m^2) have normal albumin levels in urine (normoalbuminuric). These patients known as NADKD patients exhibit clinical features distinct from microalbuminuric and macroalbuminurc DKD patients. The presence of this new entity proposes the importance to review the conventional 5-stage classification of DN and to shift the classical DKD paradigm. A distinct pathological pathway is thought to account for NADKD. However, there is a scarcity of clinical and basic research on this patient group and thus majority of the medical community remains unaware of this phenotype due to which NADKD is an often missed entity. Identification of precise biomarkers may open up the avenue for timely diagnosis of NADKD. In order to establish a widespread recognition of NADKD, substantial multicentre and large-sample based clinical research and investigation on DM patients with normoalbuminuria and renal insufficiency is critical. This will elucidate its pathogenesis, risk factors, prevalence, and progression

and subsequently facilitates early diagnosis, provides prognostic information, and proposes accurate therapeutic regimen in clinical settings.

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