



KAPITEL 5 / CHAPTER 5⁵
THE NETWORK OF FUNCTIONAL AND METABOLIC DISORDERS IN
CHILDREN WITH DIABETIC NEPHROPATHY

DOI: 10.30890/2709-2313.2023-24-03-011

Introduction

Diabetic nephropathy (DN) has increased due to the global epidemic of type I diabetes (T1D). The primary biochemical abnormality associated with diabetes and DN is hyperglycemia, which can be harmful due to its stimulation of many intracellular enzymatic processes, oxidative stress, and inflammation. [1].

Patients with diabetes mellitus experience a persistent decrease of kidney function, which is referred to as diabetic nephropathy or diabetic kidney disease. After ruling out other potential causes of albuminuria, the diagnosis of diabetic nephropathy (DN) is made based on the detection of abnormally high urine albumin levels in these patients. The presence of DN is confirmed in two of the three samples that fall into the microalbuminuria (30 to 300 mg of albumin/24 h) or macroalbuminuria (greater than 300 mg of albumin/24 h) range. [2].

For individuals with diabetes, maintaining appropriate blood glucose control is a vital component of their treatment. Excessive levels of blood glucose can enzymatically attach to proteins like hemoglobin and produce glycosylation. The amount of glucose present in the surrounding environment determines the amount of glycosylated hemoglobin production. Glycated hemoglobin levels are higher in those with elevated blood glucose levels. Advanced glycation products (AGA) are produced by non-enzymatic glycation, which happens as a result of spontaneous contact between glucose and amino groups of proteins. In diabetic kidneys, AGA causes functional and morphological renal impairment concurrently with other metabolic diseases [3,4].

Research into endothelin-1 (ET-1)'s function in the kidney has been ongoing for a long time. Nevertheless, the intricate processes by which endothelin regulates the physiology and pathology of this organ remain incompletely understood. ET-1 is made,

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secreted, and bound by podocytes. Proteinuria, glomerulosclerosis, and the advancement of renal disease are caused by damage to podocytes, which are essential for maintaining the integrity of the glomerular filtration barrier [5]. Within the glomeruli, endothelial cells are thought to be the main source of ET-1 [6].

The significance of endothelium-podocyte communication in maintaining a functional filtration barrier, the cellular site of ET-1 generation and function in T1D and DN, and the overall impact of this process on the cardiovascular system remain unclear. Autocrine and/or paracrine ET-1 signaling is considered to be crucial in contributing towards the development of albuminuria.

Additionally, it has been demonstrated that ET-1 is involved in a variety of pathological processes in the glomeruli. Numerous chronic kidney diseases, including diabetic nephropathy [7], sickle nephropathy [8], and focal segmental glomerulosclerosis [6], have been linked to its involvement in their etiology. We evaluated ET-1 levels in T1D patients and patients with DN to answer the question of whether there was a shift in these levels in patients with early stages of T1D and DN.

It is interesting that, in the two to five years after the onset of T1DM, the frequency of microvascular complications is higher than anticipated, particularly in teenagers [4]. The International Society for Pediatric and Adolescent Diabetes (ISPAD) and the International Diabetes Foundation (IDF) released the most recent global consensus report in 2018. It suggested that annual screening for microvascular complications, such as albuminuria and glomerular filtration changes, should begin at age 11 and after two years of diabetes duration.

A crucial aspect of controlling type 1 diabetes is appropriate screening for complications in diabetic patients, as well as blood pressure control and glycemia levels [9, 10]. In the current study, we examined the relationships between the primary clinical indicators (disease course, blood pressure, HbA1c, albuminuria) and the levels of GFR, ET-1, cholesterol, and lipids oxidation ratio in children with T1D and DN. To assess the earliest pathological alterations associated with metabolic and functional problems, we incorporated individuals with T1D disease duration ≤ 1 year into the research analysis. The study's objective was to assess the most common metabolic and



functional abnormalities in kids with diabetes and kids with DN.

5.1. Clinical characteristics of children with DN

The study, which was developed as an analysis, included children with T1DM who were being monitored at the endocrinology section of Clinical Pediatric Hospital #6 (Kyiv, Ukraine) from 2014 to 2020. Patients involved in the trial following T1D onset up to 1 year of disease duration were referred to as the T1D group. Patients with DN in the group of children included those who had had the illness for at least a year. In this group, the average length of illness is 6.2 years.

Every patient received numerous adjustable dose intervals of insulin treatment and was examined every three months. Age chronologically, length of diabetes, height, weight, body mass index (BMI), blood pressure, Hb1Ac, and serum cholesterol are all noted at every hospital visit. Boys' and girls' WHO BMI-for-age z-scores were utilized to evaluate the BMI values of the study participants' children. Children whose weight-for-height Z scores fall between 0 and $\leq \pm 1$ are said to have a normal body weight. Every patient was grouped according to fundamental clinical facts (Table 1).

Table 2 - Clinical characteristics of patients

Parameter	Control group	T1D	DN group (T1D with diabetic nephropathy)
Age	12,4 \pm 1,3	11,59 \pm 3,46	11,85 \pm 4,09
Boys/girls	13/12	16/19	17/24
Body weight, kg	52,41 \pm 2,33	51,09 \pm 2,19	52 \pm 4,01
Hight, cm	151,3 \pm 2,03**	145,36 \pm 4,43	153,02 \pm 3,98**
BMI, kg/m ²	18,43 \pm 0,85	18,26 \pm 0,98	18,3 \pm 0,79
Serum creatinine, mcmol/mL	55,01 \pm 3,22**	41,6 \pm 2,89	57,33 \pm 1,82**
Albuminuria, mg/day	11 \pm 2,07	35 \pm 13,01	248,49 \pm 23,67**
Hb1cA, %	2,11 \pm 0,1	9,5 \pm 0,44**	9,35 \pm 0,32**
Systolic blood pressure, mmHg	111,34 \pm 0,88 **	96,66 \pm 3,84	127,51 \pm 1,79**
Diastolic blood pressure, mmHg	65,2 \pm 1,45	64,89 \pm 1,45	75 \pm 1,18**



Glomerular filtration rate (GFR) used to assess kidney function. Schwartz formula for children and adolescents 1 to 17 years old used [10]:

$eGFR = 0.413 \times (\text{height}/\text{Scr})$ if height is expressed in centimeters

OR $41.3 \times (\text{height}/\text{Scr})$ if height is expressed in meters

$eGFR$ (estimated glomerular filtration rate) = mL/min/1.73 m²

Scr (standardized serum creatinine) = mg/dL

5.2. Basic kidney function and compensation of T1D parameters in children with DN

Our results show that in control group GFR value was $103,94 \pm 4,95\%$ mL/min/1.73 m². In contrast, T1D group - $129,55 \pm 5,74\%$ mL/min/1.73 m² which is statistically different as compared to control group value ($p < 0,01$). GFR level in DN group was higher as compared to control group value ($113,74 \pm 3,52$ mL/min/1.73 m² vs. $103,94 \pm 4,95\%$ mL/min/1.73 m², $p < 0,01$) (Figure 1).

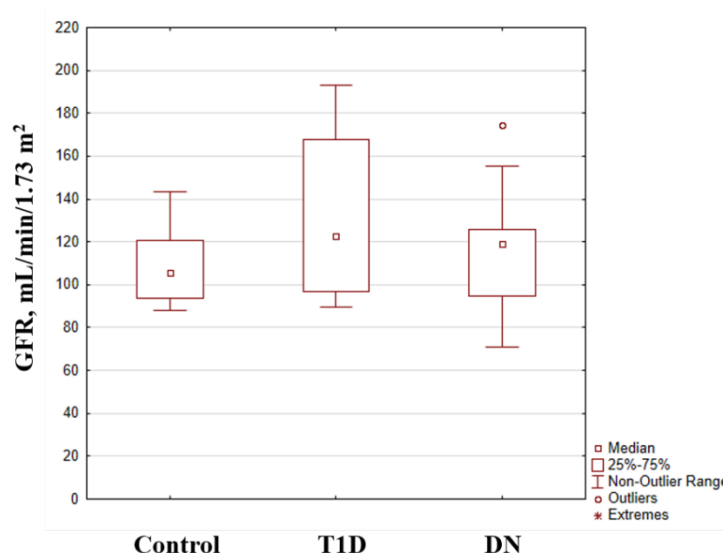


Figure 1. GFR levels in children with T1D and DN.

It's interesting to note that 63,2% of patients with T1D experience five or more episodes of decompensation, or diabetic ketoacidosis (DKA), year, which results in hospitalization. On the other hand, 41,9% of the DN group's patients had five or more



KDA events detected annually (Figure 2).

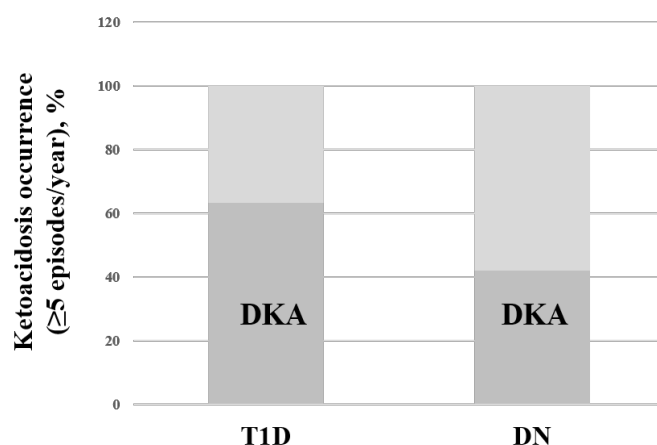


Figure 2. Ketoacidosis occurrence in children with T1D and DN.

5.3. Endothelin-1 and metabolic disorders in children with DN

Blood pressure changes in children with T1D as well as in group with DN evaluated.

Children with T1D have significantly lower systolic blood pressure ($p < 0,01$) than the control group ($96,66 \pm 3,84$ mmHg and $111,34 \pm 0,88$ mmHg, respectively). When comparing the DN group value to the control group and T1D patients, it is significantly higher ($p < 0,01$). The T1D patient group's and the control group's diastolic blood pressure readings were $64,89 \pm 1,45$ mmHg and $65,2 \pm 1,45$ mmHg, respectively. Nonetheless, the value of the DN group is larger ($p < 0,01$) than that of the T1D patient and control groups.

We examined the level of ET-1 in order to assess the potential effector in the development of microvascular diseases and variations in blood pressure. The ET-1 levels of every T1D patient included in the study are uniform. The group's variations and their reliance on the length of the illness have not been assessed. ET-1 levels in T1D patients are substantially higher ($p < 0,01$) than in the control group, at $1,71 \pm 0,1$ pg/mL and $3,4 \pm 0,1$ pg/mL, respectively. Figure 3A shows that patients in the DN group had greater levels of ET-1 ($4,38 \pm 0,2$ pg/mL) than those in the T1D group ($p < 0,001$). In children with DN, we discovered a positive direct connection ($r = 0,65$, $p < 0,05$) between ET-1 levels and the development of the disease (Figure 3, B).

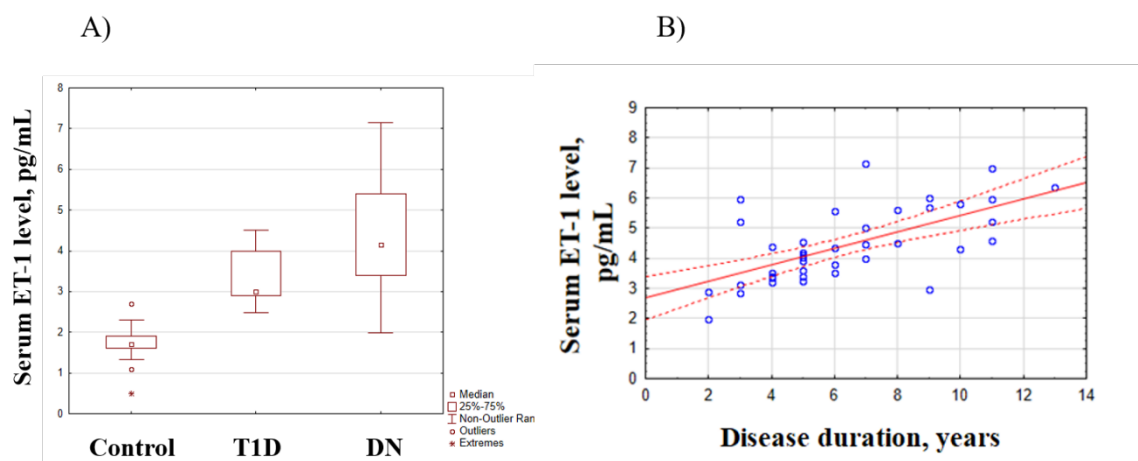


Figure 3. Serum ET-1 in patients with T1D and DN (A). Correlation between serum ET-1 and disease duration in patients with DN (B).

We assessed blood cholesterol levels in every patient who was a part of the trial. According to our findings, the T1D group's cholesterol value is $3,89 \pm 0,09$ mmol/L, while the control group's is $3,81 \pm 0,07$ mmol/L. Conversely, the T1D group and control groups have lower values ($p < 0,01$) than the DN group, which has a value of $5,11 \pm 0,27$ mmol/L (Figure 4A).

We also investigated the plasma level of lipid oxidation ratio in children. The ratio displays the non-oxidized/oxidized lipid fractions. The value in the control group is $1,27 \pm 0,08$. The children in the T1D group had a value of $1,03 \pm 0,06\%$, which is less than the value in the control group ($p < 0.05$). The oxidation level of DN lipids in children is $0.76 \pm 0.07\%$, which is less than the value in the control group ($p < 0.01$) (Figure 4B).

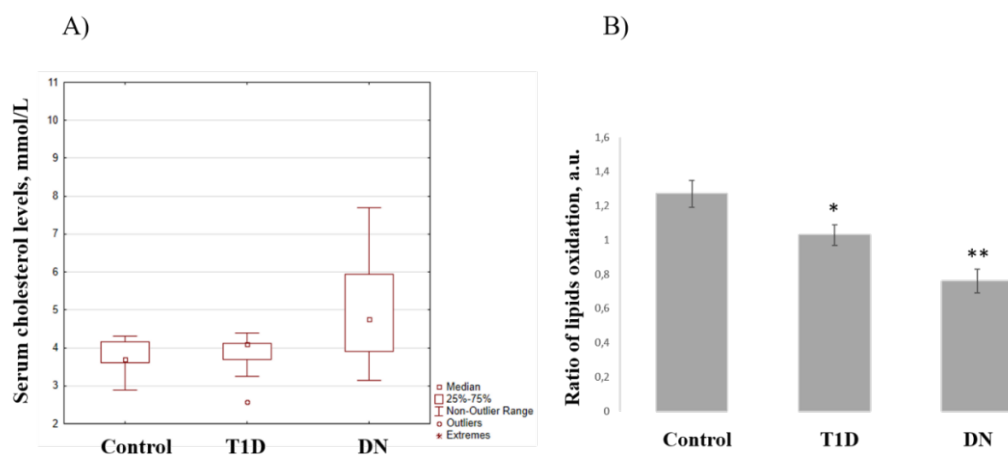


Figure 4. Blood cholesterol values (A) and lipids oxidation ratio (B) in patients with T1D and DN.



Discussion

The primary cause of death in people with type 1 diabetes is diabetic nephropathy [11, 12]. 25% of DN T1D patients go on to develop ESRD [13]. Furthermore, coronary artery disease (CAD) [14] and total mortality are significantly increased by DN. A protracted quiet phase without overt clinical nephropathy signs and symptoms is characteristic of the natural history of diabetic nephropathy (DN). Teens with T1D who receive inadequate therapy as a result experience early complications. According to a survey [15], ACE inhibitors (ACEi) and angiotensin-receptor blockers (ARB) were only prescribed to one-third of participants under the age of 20 who had a clinical diagnosis of microalbuminuria. These medications are meant to be a fundamental renoprotective treatment.

By the time $GFR < 60 \text{ mL/min/1.73 m}^2$, renal structural alterations are well-established and roughly 50% of renal function is lost. Earlier identification of GFR decline would allow interventions to decrease the rate of GFR loss and prolong the time to development of end stage renal disease (ESRD).

Despite the fact that there is substantial data supporting the benefits of blood pressure and glucose management in reducing microvascular problems in T1D [16,17]. One of the main causes of end-stage renal disease and death in T1D patients is vascular diseases, including hypertension [18, 19]. It is unclear, therefore, if hypertension plays a role in the progressive decline in renal function. Furthermore, it's unknown how the development and advancement of these ailments are related to one another.

Rapid GFR decline was observed to be associated with increased time-varying blood pressure by Vupputuri et al. The primary risk factor for chronic kidney disease (CKD), which strikes 70% of people over the age of 80, is the age-related decrease in glomerular filtration rate (GFR) [20]. Several observational studies have found an association between baseline blood pressure (BP) and subsequent GFR decline or incident CKD, but there are also studies that have shown no relationship or even a higher GFR [21].

Our research revealed that children with T1D who have had the disease for less



than a year have a 25% increase in GFR when compared to the value of the control group. These patients also had elevated serum ET-1 levels and normal blood pressure. According to our earlier research [22, 23], patients with T1D have elevated levels of apoptotic effectors and intracellular hypoxia (HIF-1 alfa, marker). We hypothesize that elevated GFR may be a reaction to metabolic abnormalities, such as hyperglycemia, hypoxia, apoptosis, and ET-1 activation, which may be present in T1D. Furthermore, our analysis of the patients' decompensation episodes (DKA) revealed that the T1D group had a high frequency of DKA (≥ 5 episodes/year), which could be a contributing factor to the metabolic effect on the kidney and "shock" GFR.

Individuals who have DN symptoms have higher GFR levels. In contrast to T1D patients, this group's GFR value was lower. When compared to the control group, the GFR of the children in the DN group increased by 12%. Every child diagnosed with DN has elevated systolic blood pressure, elevated serum ET-1 levels (in comparison to the T1D group), and elevated cholesterol levels. Considering all of these alterations, we think that the length of the disease influences the later onset of artery and vein damage. In children with DN, there is a clear and positive correlation between the serum ET-1 level and the progression of the disease.

Other factors besides vasoconstrictor markers might be involved in vascular changes, i.e. lipids peroxidation, apoptosis. In this study we found that children with DN have higher as compared to T1D group level of cholesterol and increased lipids oxidation level. This is an evidence that other metabolic disorders, including lipids oxidation, may have effect of vascular dysregulation on children and T1D and DN.

Unlike its well-known involvement in renal and vascular damage, ET-1 is typically thought to function in an autocrine rather than an endocrine manner. It is well known that neutral endopeptidases in the proximal tubule kill circulating ET-1 after it has been filtered by the glomerulus [24]. Therefore, we hypothesize that ET-1's autocrine activity has a direct impact on the renal vasculature in kids with T1D and DN. Blood pressure variations are caused by the ET-1 activity's endocrine manner. And the length of the sickness determines these impacts. In the past, we observed a progressive rise in the apoptotic marker caspase-3 in kids with DN and T1D.



Moreover, we found decreased level of Vitamin D in all diabetic patients. In group with T1D Vitamin D level was in insufficiency range, in patients with DN Vitamin D deficiency found [25]. Thus, we can't exclude fact that lack of Vitamin D protective functions on blood vessels, endothelium, kidney cells [25,26] may have a case in diabetic children and children with DN.

The American Diabetes Association (ADA) advises screening for nephropathy at the time of type 2 diabetes diagnosis and five years following type 1 diabetes diagnosis. Blood pressure should be constantly monitored by physicians because nephropathy and hypertension are connected. The current study adhered to the annual monitoring of BP, GFR, Hb1Ac, and ET-1. Regarding the annual assessment of functional and metabolic abnormalities and the continued prescription of appropriate medication, we think this is reasonable.

According to a retrospective study on pediatric type 1 diabetes, 2.9% of patients developed end-stage renal disease, and these conditions were strongly linked to poor glucose control ($A1C \geq 10\%$), increased LDL cholesterol (>100 mg/dL), and diagnostic age greater than 6 years [27]. Children with DN in our study had an average illness course of 6.2 years. Within five to six years of the onset of the condition, increased lipid oxidation ratios and cholesterol levels were found.

Studies on the natural course of type 1 diabetes in young people indicate that functional alterations frequently signify severe disease, and structural harm to the glomeruli, interstitium, and vasculature is visible even before overt albuminuria appears [28]. According to the Oxford Regional Prospective Study, microalbuminuria was linked to greater GFR at 5 years (22% increased risk per each 10 mL/min/1.73 m² rise in GFR) and poor glycemic control (30% increased risk per 1% increase in A1C) [29]. Currently, albuminuria is utilized as a marker to identify underlying renal illness, but it may not be perfect [29, 30].

Microvascular, vascular, and direct nephron damages in type 1 diabetes may be related to vitamin D3 deficiency and apoptotic activation, according to both in vitro and in vivo evidence. Children with T1D and DN had lower levels of vitamin D and apoptotic activation, according to our earlier research [30]. The entire web of



connections between the following conditions, however, is still unknown: hyperglycemia, apoptosis, vitamin D3, hypoxia, microvascular and vascular diseases, loss of kidney function, and DN progression.

Based on our findings, we draw the conclusion that children with T1D experience an increase in GFR during the first year of the disease, which represents the onset of early functional alterations. Albuminuria and BP fluctuations follow these early problems in their appearance. The latter is a sign of DN and results in additional renal damage caused by albumin. Our theory is that the metabolic abnormalities associated with type 1 diabetes, such as hyperglycemia, hypoxia, and decompensation episodes, are what cause the early functional deficits in children with the disease. In children with T1D, DN is linked to the metabolic abnormalities already stated, elevated ET-1 levels, and lipid disorders, all of which may have an impact on blood pressure and the kidney vasculature. And the length of the sickness determines these impacts.

Conclusions

Lastly, we concentrate on the fact that DN is distinguished by an extended clinically silent phase devoid of illness signs or symptoms. To further stop the onset and progression of DN, better ways of identifying early mediators of renal injury are desperately needed. We invest a great deal of money in researching these problems in kids with DN and T1D. Specifically, we hypothesize that early, intricate assessment and tracking of the aforementioned alterations may facilitate comprehension of the profound underlying mechanisms of T1D progression and the emergence of its consequences.