www.nephropathol.com DOI: 10.34172/jnp.2021.18 J Nephropathol. 2021;10(2):e18

Journal of Nephropathology

Vascular endothelial growth factor expression in diabetic nephropathy; a clinico-pathological study

Sagarika Sharma[1], Suchitha Satish[1*], Manjunath Sanjeev Shetty[2]

[1]Department of Pathology, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, India
[2]Department of Nephrology, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, India

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 5 April 2020
Accepted: 31 July 2020
Published online: 13 August 2020

Keywords:
End-stage renal disease
Renal biopsy
Diabetic nephropathy
Vascular endothelial growth factor
Glomerular filtration rate

ABSTRACT

Introduction: Diabetes is an illness of epidemic magnitude, and the figures are rising each year. Diabetic nephropathy (DN) is a dreaded long-term complication of diabetes and the most common reason for end-stage renal disease (ESRD). Microalbuminuria is considered as a non-invasive indicator of early onset of DN. Renal biopsy is vital to know the extent of renal damage. Vascular endothelial growth factor (VEGF) plays a key role in angiogenesis and has been implicated in the pathogenesis and development of the disease.

Objectives: To assess the expression of VEGF in different classes of DN and to evaluate its association with the known clinical and histopathological prognostic factors.

Patients and Methods: Fifty-five patients of DN undergoing a renal biopsy were studied and classified according to the "pathologic classification of DN" by Tervaert et al. Glomerular and tubular staining of VEGF was recorded. *P* values of less than 0.05 were considered statistically significant.

Results: Of 55 patients, eight patients belonged to class II, 24 to class III, and 23 to class IV. VEGF was positive in six (75%) of class II, 17 (70.83%) of class III and eight (34.7%) of class IV biopsies. A statistically significant correlation between classes of DN with estimated glomerular filtration rate (eGFR), serum creatinine, serum urea, diabetic retinopathy, hematuria, VEGF positivity and staining intensity was observed.

Conclusion: A precise assessment of renal damage in DN can be conducted by studying renal biopsies. VEGF expression is increased in the early stage of diabetes however; further studies could open up new avenues for early diagnosis and management.

Original Article

Implication for health policy/practice/research/medical education:
In a study of 55 patients with DN, VEGF expression was studied. A statistically significant correlation between the classes of DN with VEGF positivity and staining intensity was seen. The role of VEGF in diabetic retinopathy is well established as is the efficacy of anti-VEGF therapy in its treatment. A similar strategy can be envisaged for the potentially dangerous complication of DN. Larger longitudinal and cross-sectional studies would however be required to confirm the preeminent role of VEGF as a causative factor in DN, before treatment options can be explored.

Please cite this paper as: Sharma S, Satish S, Shetty S. Vascular endothelial growth factor expression in diabetic nephropathy; a clinico-pathological study. J Nephropathol. 2021;10(2):e18. DOI: 10.34172/jnp.2021.18.

Introduction

Diabetes is a disease assuming epidemic proportions, and the number of people developing the disease is growing every year (1). The kidney is affected in ~40% of diabetic patients, and diabetic nephropathy (DN) is the chief cause of end-stage renal disease (ESRD) (2). Projections from Indian Council of Medical Research–India Diabetes study have shown that India possibly has 62.4 million people with diabetes, making DN an important cause of renal failure (3). A study from India has shown that 31.3% of renal failure in India is caused by DN (4). These staggering numbers convey the magnitude of the problem the country is facing and the need to gear up for its diagnosis and treatment.

Microalbuminuria is a marker of DN, and it is regarded as the non-invasive indicator of early DN. Nevertheless, currently no technique can estimate who develops DN, before any damage ensues. Quantification of pathological

*Corresponding author: Suchitha Satish, Email: satishsuchitha@gmail.com,ssuchitha@jssuni.edu.in

kidney injury is difficult with the available clinical and laboratory investigations. A precise assessment of renal injury in DN can only be attained by the histopathological evaluation of tissue biopsies. Therefore, it is imperative to determine and distinguish the diverse pathologies at an initial stage to prevent progression and probable complications (5).

The metabolic and hemodynamic variations in diabetes disturb the glomerular filtration barrier, causing ultrastructural alterations of the glomeruli. Variations seen are fusion and detachment of podocyte foot processes, thickening of glomerular basement membrane (GBM), reduced endothelial cell glycocalyx, and accumulation of extracellular matrix in the mesangium and glomerulosclerosis, finally progressing to albuminuria and ESRD (6). The sequence of events is initiated by the activation of intracellular signaling molecules that lead to the dysregulation of vascular growth factors and cytokines, such as VEGF and angiopoietins. These factors play an important role in the functional and structural regulation of the glomerular filtration barrier (7).

Emerging evidence suggests that vascular endothelial growth factor (VEGF) is critical in glomerular physiology and in the pathogenesis of glomerular disease in diabetes. VEGF and angiopoietin systems are necessary for the preservation of glomerular physiology since DN is characterized by an imbalance in the expression of the above growth factors. Despite the latest breakthrough clinical trials indicating the advantageous effects of tight glycemic control and blood pressure control, the prevalence of DN continues to rise; therefore, there is a need to recognize novel targets for therapeutic intervention (7).

An improved understanding of the role of angiogenesis in DN is crucial for the discovery of effective inhibitors to this disease. Currently, the inhibition of the renin-angiotensin system is the only treatment available to delay the advancement of this grave disease. Recognizing and quantifying the pathological angiogenesis related to DN could be regarded as an effective tool to reduce or rather cease the morbidity and mortality associated with the disease (8).

Objectives

To assess the expression of VEGF in different classes of DN and to evaluate its association with the known clinical and histopathological prognostic factors.

Patients and Methods

Protocol of the study

This study was undertaken in the departments of pathology and nephrology, JSS medical college and hospital, Mysore. A total of 55 patients of biopsy proven DN were studied between March 2016 to March 2019.

Inclusion criteria

Diabetics with a rapid deterioration of renal functions or hematuria who underwent a renal biopsy to rule out acute precipitating factors or non-diabetic renal disease, with a histological diagnosis of DN, as defined by light and immunofluorescence microscopic studies were included in the study.

Exclusion criteria

Patients with acute precipitating factors for renal dysfunction, isolated micro-albuminuria, uncontrolled diabetes as evidenced by HbA1c levels, on anti-proteinuric drugs and smocking and patients with contraindications for renal biopsy were excluded from the study.

Data was collected as per case report form, which included detailed analysis with respect to history, duration, mode of treatment, renal symptoms and details of micro- and macro-vascular complications of diabetes. Preliminary investigations included routine hematological profile, blood sugar levels, urinalysis, blood urea and creatinine, serum electrolytes, and ultrasound imaging of the kidney was conducted prior to the biopsy procedure. Fundus examination was performed by an ophthalmologist. An informed consent prior to renal biopsy was taken from all patients. Renal biopsy was performed under local anesthesia using 2% lignocaine and an automated gun (Bard company) with disposable needles. Two cores were taken. The tissue was placed in 10% formalin for light microscopic (LM) examination and in saline for immunofluorescence (IF) studies.

For LM, multiple step serials were stained and studied using hematoxylin and eosin, periodic acid–Schiff (PAS), Masson's trichome (MTS) and Jones methenamine silver stains. Renal lesions in DN were classified according to "pathologic classification of DN" by Tervaert et al (9).

For IF, the biopsy specimens, one to two sections were placed on each slide and then stained with fluorescein iso thiocyanate labeled anti-human antibodies of IgG, IgA, IgM, C3, C1q, kappa and lambda light chains, and viewed under immunofluorescent microscope- Olympus BX 41.

Immunohistochemical staining

Immunohistochemistry was performed on four micrometer thick sections on poly-l-lysine coated slides. Antigen retrieval was conducted in citrate buffer at pH 9 in a pressure cooker for 20 minutes. The sections were then incubated for 60 minutes with VEGF (Daco, dilution 1/50). A labelled streptavidin-biotin-peroxidase method was used to visualize the immunoreactions with diaminobenzidine as chromogen. Sections from the placenta were used as positive control and sections treated with tris-buffer solution instead of primary antibody were used as negative control. Glomerular and tubular

staining of VEGF was recorded as percent positive area of the sampled tissue along with the intensity of staining. Brown cytoplasmic reactivity was taken as positive. The glomerular and tubular staining was graded as 1+ when less than 25% of glomeruli/tubules affected; 2+ when 25% to 50% of the glomeruli/tubules affected and 3+ when greater than 50% of glomeruli/tubules affected.

Intensity of staining quantified into three categories

Weak reactivity was graded as 1+ when staining was seen at high magnification (40X).

Moderate activity was graded as 2+ when staining was seen at medium magnification (20X). Accordingly, strong reactivity was graded as 3+ when staining was seen at low power magnification (10X).

Ethical Issues

The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of JSS Medical College and Hospital, JSS Academy of Higher Education and Research, India (Ethical code; JSSMC/PG/4700/2017-18). Consent of the patients was obtained before the study.

Statistical evaluation

The statistical analyses were performed using statistical package for social sciences IBM SPSS© version 23 (SPSS Inc, Chicago, IL, USA). The comparison of data from multiple groups was made by Pearson's chi-square test and between two groups by independent samples *t* test. *P* values of less than 0.05 were considered statistically significant. Percentage was used for categorical data. Graphs were generated using Microsoft excel.

Results

The demographic data, clinical parameters and laboratory findings in different classes of DN are given in Table 1.

Estimated glomerular filtration rate (eGFR) was calculated in adults using the abbreviated Modification of Diet in Renal Disease study equation: "eGFR (ml/min per 1.73 m2) = 186.3 x (serum creatinine in mg/dL-1.154) x (age-0.203) x (0.742 if female) x (1.21 if black)".

Grading was done as grade I (>90 mL/min/1.73 m^2), grade 2 (60-89 mL/min/1.73 m^2), grade 3 (30-59 mL/min/1.73 m^2), grade 4 (15-29 mL/min/1.73 m^2) and grade 5 (<15 mL/min/1.73 m^2). In 55 patients studied, the mean eGFR was found to be 25.872 ± 21.64 mL/min/1.73 m^2 with a range of 5 to 89 mL/min/1.73 m^2. It showed a significant correlation with classes of DN (P<0.001).

Renal function was categorized according to serum creatinine levels (mg/dL) as follows; normal-mild renal insufficiency (<1.5 mg/dL), moderate renal insufficiency (1.5-3.0 mg/dL) and advanced renal insufficiency (≥3.0 mg/dL). Eight (14.5%) patients had normal-mild renal insufficiency, 18 (32.7%) patients had moderate renal insufficiency and 29 (52.7%) had advanced renal insufficiency.

Histology

Among the classes of DN, class III was the most common class of DN with 24 (43.6%) patients, followed by class IV DN with 23 individuals (41.8%) and class II DN with eight (14.5%) patients (Figure 1, Table 2).

The percentage of viable glomeruli, interstitial fibrosis and tubular atrophy (IFTA) score, interstitial inflammation score, arteriosclerosis and arteriolosclerosis score are given in Tables 3 & 4 and Figure 2.

VEGF staining

Out of 55 cases, 31 (56.4%) biopsies showed glomerular

Table 1. Demographic data, clinical parameters and lab findings

Parameters	Findings
Age (mean)	55.13 ± 9.68 years
Male: Female ratio	2.93:1
Duration of Diabetes (mean)	96.34 ± 36.98 months
Hypertension (%)	50 (90.1%)
Diabetic retinopathy (%)	26 (62%)
Proteinuria by dipstick (%)	49 (96.1%)
24 Hour proteinuria (mean)	4.91 ± 2.75 g/d
Haematuria (%)	23 (56.1%)
Serum creatinine (mean)	4.455 ± 3.16 mg/dL
eGFR (mean)	25.872 ± 21.64 ml/min/1.73 m^2
Blood urea nitrogen (mean)	85.05 ± 47.51 mg/dL

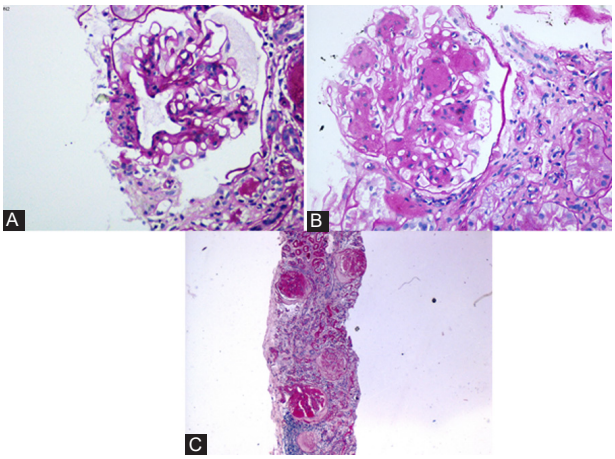

Figure 1. (A) Class II DN with mesangial expansion (PAS, X200). (B) Class III DN showing PAS positive KW nodules (PAS, X200). (C) Class IV DN with obsolete glomeruli, one glomerulus with KW nodules and IFTA (PAS, X40).

Table 2. Class wise distribution of various clinical parameters

Laboratory/clinical parameters	Class II	Class III	Class IV
Age (years)	56.25 ± 10.50	55.58 ± 8.98	54.26 ± 10.44
Serum creatinine (mg/dL)	2.13 ± 1.06	2.75 ± 1.95	7.04 ± 2.83
BUN (mg/dL)	45.23 ± 24.74	69.5 ± 51.24	109.65 ± 33.72
Urine albumin	2 ± 0.71	2.82 ± 0.91	2.73 ± 0.83
24 Hour urine protein (g/L/24 h)	8.74 ± 4.89	4.72 ± 2.09	4.17 ± 2.86
eGFR (mL/min/1.73 m^2)	39.25 ± 21.71	36 ± 22.21	10.65 ± 8.05

Table 3. Viable glomeruli percentage

	< 25%	26-50%	51-75%	>76%
Viable glomeruli	11 (20%)	12 (21.8%)	16 (29.1%)	16 (29.1%)

Table 4. IFTA score (0-3), Interstitial Inflammation (0-2), arteriosclerosis (0-2), arteriolosclerosis (0-2)

Parameter	Score 0	Score 1	Score 2	Score 3
IFTA score	8 (14.5%)	9 (16.4%)	12 (21.8%)	26 (47.3%)
Interstitial inflammation	7 (12.7%)	46 (83.6%)	2 (3.7%)	-
Arteriosclerosis	0 (0)	36 (65.5%)	19 (34.5%)	-
Arteriolosclerosis	26 (47.3%)	14 (25.5%)	15 (27.3%)	-

positivity for VEGF. In the eight cases of class II DN, VEGF positivity was seen in six (75%) cases. In 24 cases of class III DN, VEGF positivity was seen in 17 (70.8%) and of the 23 cases of class IV DN, VEGF positivity was seen in eight (34.8%) cases (Figure 3).

Tubular positivity was seen in 20 (64.5%) of the 31 VEGF positive cases. In class II, one (16.7%) case showed tubular positivity out of the six VEGF positive cases, having 1+ score. In class III, 12/17 (70.6%) cases showed tubular positivity with eight (66.7%) having 1+ and four (33.3%) having 2+ score. In class IV, seven (87.5%) cases showed tubular positivity out of the eight positive cases, with three (42.9%) having 1+ and four (57.1%) having 2+ score. The staining intensity of VEGF was divided into three grades, low, intermediate and strong. Out of the six positive class II cases, one (16.66%) was graded as low, three (50%) were graded as intermediate and two (33.33%) were graded as strong. Out of the 17 positive class III cases, eight (47.06%) were graded as low, six (35.29%) were graded as intermediate and three (17.65%) were graded as strong. Out of the 13 positive class IV cases,

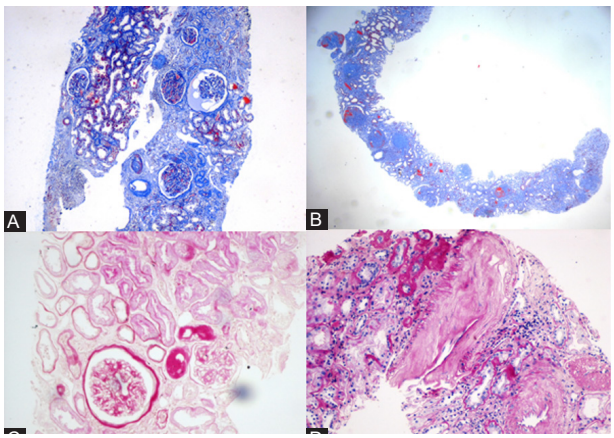

Figure 2. (A) IFTA score 2 (MTS, X100). (B) IFTA score 3 (MTS, X40). (C) Hyaline arteriolosclerosis (PAS, X100). (D) Intimal Fibroplasia and Tunica Media Hyperplasia (PAS, X200).

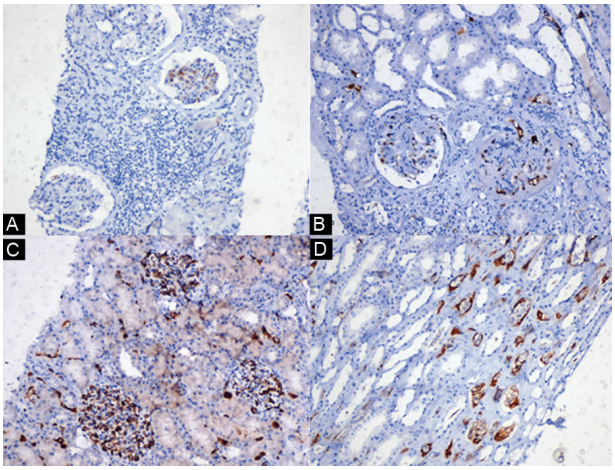

Figure 3. (A) Glomerular staining of VEGF – Score 1 (VEGF, X100). (B) Glomerular staining of VEGF – Score 2 (VEGF, X100). (C) Glomerular staining of VEGF – Score 3 (VEGF, X100). (D) Tubular staining of VEGF (VEGF, X100).

three (23.08%) were graded as low, seven (53.85%) were graded as intermediate and three (23.08%) were graded as strong.

Classes of DN showed significant correlation with eGFR (P = 0.000), serum creatinine (P = 0.000), serum urea (P = 0.001), diabetic retinopathy (P = 0.09), urine RBCs (P = 0.033), VEGF positivity (P = 0.010), and VEGF staining intensity (P = 0.042).

VEGF was positive in 31(56.4%) of 55 cases. It showed significant correlation with class of DN (P = 0.010).

Discussion

DN is defined as the presence of proteinuria with progressive deterioration of GFR and evolves over a course of 10-20 years. Presence of microalbuminuria gives a reliable diagnosis of clinical DN, however it may regress with aggressive treatment, but macroalbuminuria is irreversible. A proper assessment of renal injury in DN can only be accomplished by the histopathological examination of tissue specimens. Thus, kidney biopsy in DN could be an invaluable tool to identify the stage of the renal disease (5).

In the past few years, investigators have worked on the role of VEGF in the pathogenesis of microvascular complications of diabetes. VEGF has a crucial role in angiogenesis and is a powerful mitogen for endothelial cells, increases monocyte chemotaxis, tissue factor production and is also a vascular permeability factor (10,11).

Demographic data

Age

The mean age of our study was comparable with studies from Korea, China, Italy, India (10-17). There appears to be an accelerated rise in the incidence of DN in Asian countries due to change in lifestyle, which was reflected in the current study (18).

Gender

The present study showed a male predominance which was also seen in other studies (12,15,16,19) and the ratios varied from 1:1.12 to 3.5:1.

Duration of diabetes

The findings from the present study were found to be in agreement with previous studies (12-14,20). Patients with a longer duration of diabetes have a higher risk of developing nephropathy and a poorer prognosis (21). With the AusDiab complication study, longer duration of diabetes was a predictor for each of the complications associated with diabetes - retinopathy, neuropathy and peripheral vascular disease (18,22).

Clinical parameters

Hypertension

Findings from this study correspond with previous study done by Zhang et al (15), who found 106 (81.5%) patients with hypertension. Various factors are responsible for an elevation in blood pressure in diabetic patients and DN. The main reason for hypertension in both types of DM is volume expansion due to elevated renal sodium reabsorption and peripheral vasoconstriction because of deranged factors which control peripheral vascular resistance. Stimulation of renin-angiotensin-aldosterone-system, upregulation of endothelin-1 and reactive oxygen species, and downregulation of nitric oxide (NO) can also be responsible for hypertension. Additionally, hypertension in the presence of proteinuria can accelerate the decline of glomerular filtration rate and progress to ESRD (23).

Diabetic retinopathy

Identical molecular routes are responsible for the progression of both diabetic renal and retinal microvascular injury. Many cases have been reported with a coincidence of DN and diabetic retinopathy (DR), with DN patients already having DR and patients with DR having high probability of developing DN (24,25). In the present study and other studies, this finding has been substantiated (15).

Laboratory findings

Proteinuria

Proteinuria is characteristic of DN and is a distinct risk factor for both kidney and cardiovascular disease advancement (26). Proteinuria of more than 2 g/d is known to be linked to disease progression and adverse renal outcome (27). The degree of proteinuria perhaps reflects the severity of renal disease and proteinuria by itself is tubulotoxic (28). It is an indicator of disease severity and is also helpful for the clinician to plan the treatment. The diabetic environment affects all cell types in the kidney, which adds either primarily or secondarily to the occurrence of albuminuria/proteinuria and reduced GFR.

Within the glomerulus, both the hemodynamic consequences and damage to the different parts of the glomerular filtration barrier-podocyte, GBM, and glomerular endothelial cell primarily cause proteinuria. Besides this, tubulo-interstitial damage may reduce tubular protein reuptake. Secondarily, mesangial cell damage is probably responsible for proteinuria by (i) mesangial matrix expansion which leads to decreased glomerular filtration surface area producing glomerular hyperfiltration or (ii) by mesangiolysis causing structural changes in the capillary loops. Proteinuria by itself may

lead to a reduction in GFR by causing tubulo-interstitial damage (26).

Hematuria

Findings for hematuria correlated with other studies (29-32). Urine RBCs showed a significant correlation with class of DN with a *P* value of 0.033. Hematuria in the absence of urinary tract infection is one of the pointers of renal damage in DN as well as in non-diabetic renal disease (NDRD). However, if hematuria is seen with or without proteinuria in the initial course of the disease, it increases the possibility of NDRD (33).

Serum creatinine

In the present study the number of cases with class III and IV DN was much higher than class II cases. Lack of early screening measures and awareness about the disease, preventing an early diagnosis could also be factors contributing to this. Studies have shown that elevated serum creatinine levels during renal biopsy was associated with an increased rate of development of renal failure and acts as a very strong adverse clinical prognostic indicator, which is supported by our study, as the mean value of serum creatinine for class IV DN cases was much higher than those of classes II and III.

Blood urea nitrogen

A linear increase in the values of BUN with the class of DN was seen, with class IV having the highest mean. The values from the present study were found to be correlating with other studies (14,34). Levels of serum urea and creatinine can be regarded as important prognostic markers and predictor of renal damage among diabetics. Assessment of renal function test is simple, dependable, economical and sensitive that can be regarded as an adjunct in the management and long-term treatment of diabetes mellitus (16).

Estimated glomerular filtration rate

There was statistical significance between eGFR and the class of DN with a $P<0.001$. The findings in the present study were not corresponding with previous studies (12,15,20), because there were greater number of class III and class IV DN cases with a higher mean serum creatinine. Assessment of eGFR is the best method to study the overall renal function. Serum creatinine is the most utilized indirect endogenous filtration indicator of determining eGFR (17).

Histology

Class III was the predominant class with 24 (43.7%) patients and was found to be correlating with studies by An et al (12) and Wang et al (20).

The pathological hallmarks of DN are increased thickness of GBM and mesangial expansion. Mesangial expansion is considered more important than thickening of GBM, since expansion can eventually lead to renal insufficiency. Several studies have demonstrated a close relationship between the extent of glomerular mesangial expansion in DN and worsening of serum creatinine levels besides the severity of proteinuria (28).

Viable glomeruli

Findings from the present study were in agreement with the study done by An et al (12) which echoes that "as the percentage of global sclerosis increases by 10%, the risk of progression of DN to ESRD increased by a factor of 1.5 ($P<0.001$) and that glomerular lesions can considerably influence renal survival ($P< 0.001$)" (Table 3).

Interstitial fibrosis and tubular atrophy (IFTA) score

The findings of class II and class IV were corresponding with study by An et al (12), but this study had most class III cases with IFTA score 1, which was not correlating with the present study (Table 4).

It has also been shown that tubulo-interstitial lesions like tubular atrophy with or without inflammatory cells in the interstitium play an important role in the advancement of DN (35).

Interstitial Inflammation

The findings correlated with the study by An et al (12), where most cases of classes III and IV had an interstitial Inflammation score of 1 (Table 4).

IFTA and interstitial Inflammation are significantly associated with renal outcomes in a univariate Cox analysis ($P < 0.001$). In a "multivariate Cox regression analysis after adjusting for baseline proteinuria, mean arterial pressure (MAP) and eGFR, IFTA remained an independent risk factor for renal survival ($P = 0.028$)" (12).

Arteriosclerosis and arteriolosclerosis

These findings were not correlating with the study by An et al (12), where most cases of classes II, III and IV had arteriosclerosis and arteriolosclerosis score of 2.

VEGF staining

A significant statistical correlation of VEGF immunostaining with the class of DN ($P = 0.010$) and between VEGF intensity ($P = 0.042$) and class of DN was observed. Tubular staining of VEGF was seen in 20 (64.5%) of 31 positive cases. In class II DN, one (16.7%) biopsy of six, class III, 12 (70.6%) of 17 biopsies and in class IV DN, seven (87.5%) of eight biopsies showed tubular positivity.

It was found that VEGF expression was increased in early

stages of DN with 75% positive cases in class II, 70.83% in class III, and 56.52% in class IV which concurred with other studies (10,34,36-38).

DN does not develop in the absence of hyperglycemia. Hyperglycemia causes renal damage directly or through hemodynamic alterations. It initiates activation of protein kinase C, increased production of advanced glycosylation end products, and diacylglycerol synthesis. Moreover, it is responsible for hemodynamic changes such as glomerular hyperfiltration, shear stress and albuminuria (39).

Current experimental studies support a key role of "hyperglycemia-driven secretion" of VEGF in the course of DN (40). It was shown that VEGF could promote cell division in glomerular capillaries, increase vasoactive substances, dissolve and decrease the number of anions on the GBM and enhance vascular permeability, thus resulting in proteinuria; furthermore, VEGF could also cause an increased activity of mononuclear cells in the glomerulus, mesangial cell activation, and glomerular sclerosis, thus intensifying the progression of DN (14).

The ability of VEGF to cause vascular leakage or increased neovascularization can promote the pathological processes during the initial phases of DN (34).

It has been reported that in the kidney, the glomerulus, is a major source of VEGF production in humans. VEGF protein is primarily localized in the glomerular epithelial cells and to a smaller extent in the tubular epithelial cells of the normal kidney. One can hypothesize that VEGF produced by the podocyte, may play a role in regulating the glomerular permeability to proteins (11).

Renal cortical VEGF expression is elevated in the early stages of type 1 and type 2 diabetes which coincides with renal hypertrophy, suggesting that VEGF may be involved in stimulation of protein synthesis (36,37).

Biopsies of patients with early DN showed higher VEGF staining in the glomeruli as compared to biopsies of control specimens. Although this does not prove any association, the up-regulation of VEGF may contribute to the changes in early DN (11). However, the small number of patients with class II DN in the present study impedes any absolute conclusion about the role of VEGF in the early stage of disease, which was also observed in a study by Cha et al (11).

In advanced DN lesions, there is a deficiency of local VEGF action/ expression and this may be the cause for insufficient capillary repair and glomerulosclerosis with renal scarring (34).

Pathologic findings on biopsy differ from urinary VEGF levels. Although VEGF staining in the glomeruli is diminished and tubular staining is increased with deteriorating DN, the VEGF excretion in the urine increased with rising proteinuria. Patients with early DN having microalbuminuria might produce most of their VEGF from glomerular visceral epithelial cells. As compared to this, patients with advanced DN with global sclerosis and overt proteinuria may secrete most of their VEGF in the tubules, which would explain the up-regulation of VEGF staining in the tubules. It is possible that as DN progresses, increased tubular epithelial VEGF production counterbalances the diminished glomerular VEGF production (11,13).

Treatment with VEGF antibody was shown to improve both the early features of DN and late renal changes including reduction in total mesangial volume in type 2 experimental models (14,34). However, these effects in a model of type II diabetes failed to verify this (41). Contrary to this, many experimental studies of various glomerular diseases have established a favorable role of VEGF therapy through inhibition of capillary rarefaction, glomerulosclerosis, and renal fibrosis (42,43). VEGF regulation is very firmly controlled within the glomerulus and either local absence or excess of VEGF is harmful to the glomerulus (44). Specially in the setting of a tight VEGF regulation, these facts of local VEGF expression and activity needs to be interpreted together with the structural changes (34).

Correlating data from experimental diabetic models with human tissue biopsies is very challenging, as lesions in experimental models of DN are always very trivial and may share characteristic features of human pathologies that are so early, they are commonly not represented in a kidney biopsy (34).

Other important microvascular complication of diabetes - diabetic retinopathy (DR) remains the leading vascular-associated cause of blindness throughout the world. VEGF is implicated in the pathogenesis of DR and anti-VEGF therapy, which is less damaging and safer than laser treatments explored as primary therapy for the management of vision-threatening complications of DR (45). Studies like the present one provide information that could aid in understanding the role of VEGF in the pathogenesis and progression of DN and also exploring the possibilities of using anti-VEGF antibodies in DN.

Conclusion

An early diagnosis of DN is needed to prevent the progression of the disease into ESRD. Diagnostic tests are helpful in predicting onset, progression and response to therapy in DN. Renal biopsies are helpful in classifying renal diseases into three major categories: DN, NDRD, and a superimposed NDRD on an underlying DN and is also useful in estimating the extent of damage in DN and starting suitable therapy.

Many growth factors and cytokines have been implicated in the pathogenesis of glomerular changes in DN. Very few studies have studied the renal expression of

VEGF in DN.

DN is a substantial health problem because of its increasing incidence, morbidity, and mortality. Despite the huge challenges, novel treatment regimens targeting the cytokine imbalance in diabetics in an appropriate and precise way could go a long way in the prevention of development and progression of DN.

VEGF blocking therapies in DN have been found to have beneficial results in experimental studies. The results of these studies are indeed a ray of hope and could be a harbinger of an effective treatment option in DN. However, human trials are still sometime away. Anti-VEGF treatment thus remains an exciting possibility in the future.

Limitations of the study

The cases were not equally distributed in the different classes, with fewer number of class II cases, which may not be representative. No follow up data was available for all the cases. Serum and urinary VEGF was not estimated. A larger sample size would help confirm the findings of this study.

Authors' contribution

SSh and SSa; study design, data acquisition, analysis and interpretation. SSa; revising critically for important intellectual content. MS; consultant of the study. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

None.

References

1. Ossman SS. Diabetic Nephropathy: Diabetic Nephropathy: Where We Have Been and Where We Are Going. 2006;19(3):153-156. doi: 10.2337diaspect.19.3.153.
2. American Diabetes Association. Standards of medical care in diabetes--2014. Diabetes Care. 2014;37 Suppl 1:S14-80. doi: 10.2337/dc14-S014.
3. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of Diabetes and Prediabetes (Impaired Fasting Glucose and/or Impaired Glucose Tolerance) in Urban and Rural India: Phase I Results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) Study. Diabetologia. 2011;54(12):3022-7. doi: 10.1007/s00125-011-2291-5.
4. Ritz, X Zeng. Diabetic Nephropathy - Epidemiology in Asia and the Current State of Treatment. Indian J Nephrol. 2011;21(2):75-84. doi: 10.4103/0971-4065.82122.
5. Gonzalez Suarez ML, Thomas DB, Barisoni L, Fornoni A. Diabetic nephropathy: Is it time yet for routine kidney biopsy? World J Diabetes. 2013;4(6):245-55. doi: 10.4239/wjd.v4.i6.245.
6. Gnudi L. Angiopoietins and diabetic nephropathy. Diabetologia. 2016;59(8):1616-20. doi: 10.1007/s00125-016-3995-3.
7. Dei CA, Gnudi L. VEGF and angiopoietins in diabetic glomerulopathy: how far for a new treatment? Metabolism. 2012;61(12):1666-73. doi: 10.1016/j.metabol.2012.04.004.
8. Zent R, Pozzi A. Angiogenesis in diabetic nephropathy. Semin Nephrol. 2007;27(2):161-71. doi: 10.1016/j.semnephrol.2007.01.007.
9. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556-63. doi: 10.1681/ASN.2010010010.
10. Bortoloso E, Del Prete D, Gambaro G, Dalla Vestra M, Sailer A, Baggio B, et al. Vascular endothelial growth factor (VEGF) and VEGF receptors in diabetic nephropathy: expression studies in biopsies of type 2 diabetic patients. Ren Fail. 2001;23(3-4):483-93. doi: 10.1081/jdi-100104731.
11. Cha DR, Kim NH, Yoon JW, Jo SK, Cho WY, Kim HK, et al. Role of vascular endothelial growth factor in diabetic nephropathy. Kidney Int Suppl. 2000;77:S104-12. doi: 10.1046/j.1523-1755.2000.07717.x.
12. An Y, Xu F, Le W, Ge Y, Zhou M, Chen H, et al. Renal histologic changes and the outcome in patients with diabetic nephropathy. Nephrol Dial Transplant. 2015;30(2):257-66. doi: 10.1093/ndt/gfu250.
13. Kim NH, Oh JH, Seo JA, Lee KW, Kim SG, Choi KM, et al. Vascular endothelial growth factor (VEGF) and soluble VEGF receptor FLT-1 in diabetic nephropathy. Kidney Int. 2005;67(1):167-77. doi: 10.1111/j.1523-1755.2005.00067.x.
14. Li X, Wu TT, Chen J, Qiu W. Elevated expression levels of serum insulin-like growth factor-1, tumor necrosis factor-α and vascular endothelial growth factor 165 might exacerbate type 2 diabetic nephropathy. J Diabetes Investig. 2017;8(1):108-114. doi: 10.1111/jdi.12542.
15. Zhang J, Wang Y, Li L, Zhang R, Guo R, Li H, et al. Diabetic retinopathy may predict the renal outcomes of patients with diabetic nephropathy. Ren Fail. 2018;40(1):243-251. doi: 10.1080/0886022X.2018.1456453.
16. Bamanikar SA, Bamanikar AA, Arora A. Study of Serum urea and Creatinine in Diabetic and nondiabetic patients in a tertiary teaching hospital. J Med Res. 2016;2(1):12-15.
17. Babaliche P, Nadpara RA, Maldar A. Association Between

Estimated Glomerular Filtration Rate and Microvascular Complications in Type II Diabetes Mellitus Patients: A 1-Year Cross-Sectional Study. J Natl Med Assoc. 2019;111(1):83-87. doi: 10.1016/j.jnma.2018.06.003.
18. Tapp RJ, Shaw JE, Harper CA, de Courten MP, Balkau B, McCarty DJ, et al. The prevalence of and factors associated with diabetic retinopathy in the Australian population. Diabetes Care. 2003;26(6):1731-7. doi: 10.2337/diacare.26.6.1731.
19. Kanesaki Y, Suzuki D, Uehara G, Toyoda M, Katoh T, Sakai H, et al Vascular endothelial growth factor gene expression is correlated with glomerular neovascularization in human diabetic nephropathy. Am J Kidney Dis. 2005;45(2):288-94. doi: 10.1053/j.ajkd.2004.09.020.
20. Wang J, Zhao L, Zhang J, Wang Y, Wu Y, Han Q, et al. Wang J, Zhao L, Zhang J, et al. Clinicopathologic features and prognosis of type 2 diabetes mellitus and diabetic nephropathy in different age groups: more attention to younger patients. Endocr Pract. 2020;26(1):51-57. doi: 10.4158/EP-2019-0238
21. Van Buren PN, Toto R. Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis. 2011;18(1):28-41. doi: 10.1053/j.ackd.2010.10.003.
22. Tapp RJ, Shaw JE, de Courten MP, Dunstan DW, Welborn TA, Zimmet PZ, et al. Foot complications in Type 2 diabetes: an Australian population-based study. Diabet Med. 2003;20(2):105-13. doi: 10.1046/j.1464-5491.2003.00881.x.
23. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. N Eng J Med. 2002;346(15):1145-51.doi:10.1056/NEJMcp011773.
24. Klein R, Zinman B, Gardiner R, Suissa S, Donnelly SM, Sinaiko AR, et al. The relationship of diabetic retinopathy to preclinical diabetic glomerulopathy lesions in type 1 diabetic patients: the Renin-Angiotensin System Study. Diabetes. 2005;54(2):527-33. doi: 10.2337/diabetes.54.2.527.
25. Kofoed-Enevoldsen A, Jensen T, Borch-Johnsen K, Deckert T. Incidence of retinopathy in type I (insulin-dependent) diabetes: association with clinical nephropathy. J Diabetes Complications. 1987;1(3):96-9. doi: 10.1016/s0891-6632(87)80064-8.
26. Jefferson JA, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. Kidney Int. 2008;74(1):22-36. doi: 10.1038/ki.2008.128.
27. Mazzucco G, Bertani T, Fortunato M, Fop F, Monga G. The prognostic value of renal biopsy in type 2 diabetes mellitus patients affected by diabetic glomerulosclerosis. J Nephrol. 2005;18(6):696-702.
28. Arif M, Arif MK, Arif MS. An evaluation of renal biopsy in type-II diabetic patients. J Coll Physicians Surg Pak. 2009;19(10):627-31. doi: 10.2009/JCPSP.627631.
29. Tone A, Shikata K, Matsuda M, Usui H, Okada S, Ogawa D, et al. et al. Clinical features of non-diabetic renal diseases in patients with type 2 diabetes. Diabetes Res Clin Pract. 2005;69(3):237-42. doi: 10.1016/j.diabres.2005.02.009.
30. Akimoto T, Ito C, Saito O, Takahashi H, Takeda S, Ando Y, et al. Microscopic hematuria and diabetic glomerulosclerosis--clinicopathological analysis of type 2 diabetic patients associated with overt proteinuria. Nephron Clin Pract. 2008;109(3):c119-26. doi: 10.1159/000145454.
31. Chang TI, Park JT, Kim JK, Kim SJ, Oh HJ, Yoo DE, et al. Renal outcomes in patients with type 2 diabetes with or without coexisting non-diabetic renal disease. Diabetes Res Clin Pract. 2011;92(2):198-204. doi: 10.1016/j.diabres.2011.01.017.
32. Chong YB, Keng TC, Tan LP, Ng KP, Kong WY, Wong CM, et al. Clinical predictors of non-diabetic renal disease and role of renal biopsy in diabetic patients with renal involvement: a single centre review. Ren Fail. 2012;34(3):323-8. doi: 10.3109/0886022X.2011.647302.
33. Mak SK, Gwi E, Chan KW, Wong PN, Lo KY, Lee KF, et al. Clinical predictors of non-diabetic renal disease in patients with non-insulin dependent diabetes mellitus. Nephrol Dial Transplant. 1997;12(12):2588-91. doi: 10.1093/ndt/12.12.2588.
34. Hohenstein B, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CP. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int. 2006;69(9):1654-61. doi: 10.1038/sj.ki.5000294.
35. Bohle A, Wehrmaan M, Bogenschutz O, Batz C, Muller CA, Muller GA. The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. Pathol Res Pract. 1991;187(2-3):251-9. doi: 10.1016/s0344-0338(11)80780-6.
36. Hovind P, Tarnow L, Oestergaard PB, Parving HH. Elevated vascular endothelial growth factor in type 1 diabetic patients with diabetic nephropathy. Kidney Int Suppl. 2000;75:S56-61.
37. Senthil D, Choudhury GG, Mclaurin C, Kasinath BS. Vascular endothelial growth factor induces protein synthesis in renal epithelial cells: a potential role in diabetic nephropathy. Kidney Int. 2003;64(2):468-79. doi: 10.1046/j.1523-1755.2003.00135.x.
38. Bortoloso E, Del Prete D, Dalla Vestra M, Gambaro G, Saller A, Antonucci F, et al. Quantitave and qualitative changes in vascular endothelial growth factor gene expression in glomeruli of patients with type 2 diabetes. Eur. J. Endocrinol. 2004;150(6):799-807. doi: 10.1530/eje.0.1500799.
39. Schena FP, Gesualdo L. Pathogenetic mechanisms of diabetic nephropathy. J Am Soc Nephrol. 2005;16 Suppl 1:S30-3. doi: 10.1681/asn.2004110970.
40. Khamaisi M, Schrijvers BF, De Vriese AS, Raz I, Flyvbjerg A. The emerging role of VEGF in diabetic kidney disease. Nephrol Dial Transplant. 2003;18(8):1427-30. doi: 10.1093/ndt/gfg242.
41. Schrijvers BF, De Vriese AS, Tilton RG, Van de Voorde

J, Denner L, Lameire NH, et al. Inhibition of vascular endothelial growth factor (VEGF) does not affect early renal changes in a rat model of lean type 2 diabetes. Horm Metab Res. 2005;37(1):21-5. doi: 10.1055/s-2005-861027.
42. Ostendorf T, Kunter U, Eitner F, Loos A, Regele H, Kerjaschki D, et al. VEGF(165) mediates glomerular endothelial repair. J Clin Invest. 1999;104(7):913-23. doi: 10.1172/JCI6740.
43. Kim YG, Suga SI, Kang DH, Jefferson JA, Mazzali M, Gordon KL, et al. Vascular endothelial growth factor accelerates renal recovery in experimental thrombotic microangiopathy. Kidney Int. 2000;58(6):2390-9. doi: 10.1046/j.1523-1755.2000.00422.x.
44. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111(5):707-16. doi: 10.1172/JCI17423.
45. Gupta N, Mansoor S, Sharma A, Sapkal A, Sheth J, Falatoonzadeh P, et al. Diabetic retinopathy and VEGF. Open Ophthalmol J. 2013;7:4-10. doi: 10.2174/1874364101307010004.