Research Article

Assessment of the Renal Vascular Lesions in Diabetic Patients: A Single Center Study

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Abstract

Background: Diabetes mellitus is a metabolic disease distinguished by hyperglycemia secondary to insulin defects. Long term damage of hyperglycemia commonly occurs in different organs as eye, kidney, nerves, and blood vessels. Diagnosis of Diabetic kidney disease is based on a constellation of histological findings. Pathophysiology of DKD is a complex and dynamic process. One possible mechanism is due to high filtration of glucose and increased reabsorption of both glucose and sodium chloride in the proximal tubules. Thus, the delivery of sodium chloride to the distal tubules is reduced, leading to dilatation of afferent arteriole because of tubule-glomerular feedback. At the same time, vasoconstriction of efferent arteriole occurs due to high local level of angiotensin II, which causes changes in autoregulation and glomerular hypertension. **Aim:** To correlate the demographic data of the studied cases according to pathological diagnoses of kidney disease and to assess the vascular lesions in the renal biopsy from diabetic patients. **Material & Methods:** All specimens were formalin fixed and paraffin-embedded and tissue samples were stained by routine Haematoxylin and Eosin, PAS, silver, masson trichrome and congo red stains and were histopathologically examined to evaluate their diagnosis, Presence of nondiabetic renal disease and Score of both arteriolar hyalinosis and of arteriosclerosis. All cases were stained by: Immunoglobulin A, Immunoglobulin G, Complement 3, Anti-kappa light chain and Anti-lambda light chain antibodies. **Results:** Based on biopsy findings, cases were categorized into three groups: Isolated DKD, Isolated NDRD and Coexisting DKD and NDRD. There was a significant difference between different pathological diagnoses regarding parameters including HCV and hypertension. A significant difference was noted among the studied groups regarding different scores of arteriosclerosis and arteriolar hyalinosis. **Conclusion:** Marked arteriolar hyalinosis occurs mainly in DKD group and in coexisting DKD and NDRD group. However, more than half of NDRD group shows no arteriolar hyalinosis (score 0). DKD group shows arteriosclerosis score 1 in more than half of the studied cases.

Keywords: Diabetes mellitus, Renal vascular lesions, Periodic Acid Schiff (PAS), masson trichrome, congo red, Immunoglobulin A, Immunoglobulin G, Complement 3, Anti-kappa light chain antibody, Anti-lambda light chain antibody.

Abbreviations: Diabetes mellitus (DM), The International Diabetes Federation (IDF), American Diabetes Association (ADA), diabetic kidney disease (DKD), glomerular filtration rate (GFR), type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), renal pathology society (RPS).

Introduction

Diabetes mellitus (DM) is a metabolic disease distinguished by hyperglycemia secondary to defects in insulin secretion, action, or both. Long term damage of hyperglycemia of DM commonly occurs in different organs as eye, kidney, nerves and blood vessels. [1,2].

The International Diabetes Federation (IDF) informed that the incidence of diabetes in

Worldwide estimates were 9.3% and 463 million adults aged 20–80 years are suffering from diabetes [3].

Egypt was listed by IDF among the world top 10 countries in the number of patients with diabetes[4]. In 2017, Egypt was the eighth leading country regarding the prevalence of DM; it was estimated that more than 8 million adults living in Egypt with DM, which is a prevalence of almost 15% [3].

American Diabetes Association (ADA) defined diabetic kidney disease (DKD) as a clinical diagnosis based on presence of albuminuria (increased urinary albumin excretion is defined as ≥30 mg/g) and progressive decrease in glomerular filtration rate (GFR) in the background of a long duration of diabetes (>10 years' duration of type 1 diabetes mellitus (T1DM); may be present at diagnosis in type 2 diabetes mellitus (T2DM), and is typically associated with retinopathy [5].

Diagnosis of DKD is made based on a constellation of histological findings, with no single histological change being characteristic of DKD [6]. Structural changes in both types of diabetes are similar, but changes in T2DM are more heterogeneous and less predictably associated with clinical presentations [7]. Pathophysiology of DKD is a complex and dynamic process. One possible mechanism is due to high filtration of glucose and increased reabsorption of both glucose and sodium chloride in the proximal tubules. Thus, the transport of NaCl to distal tubules is reduced, which causes dilatation of afferent arteriole because of tubule-glomerular feedback. At the same time, vasoconstriction of efferent-arteriole occurs caused by high local intensity of angiotensin II, that causes changes in autoregulation and glomerular hypertension [8].

Aim of the work:
1- Correlate the demographic data of the studied cases according to pathological diagnoses of kidney disease.
2- Assess the vascular lesions in the renal biopsy from diabetic patients.

Material and methods
From 2014 to 2020, a total of all 895 biopsies were received at PathLab (Private Pathology Lab) from diabetic patients.

Clinical data of the investigated cases were obtained from patients' laboratory reports. These data include associated systemic diseases (comorbidities) such as hypertension, cardiovascular diseases, autoimmune diseases, chronic liver diseases, liver transplantation, solid tumors, hematological neoplasia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), hypothyroidism and gout. The method of obtaining all specimens were tru-cut needle biopsy.

Inclusion criteria:
1. Biopsies were confirmed diabetic patients (minimal sample were excluded if <6 glomeruli).
2. Enough residual tissue in the paraffin blocks.
3. Referral sheet with available clinical data.

Ethical Consideration:
The study was approved by the Institutional Ethics Research Committee (approval number 321:11-2019).

Histological and Immunohistochemical data
All specimens were formalin fixed and paraffin-embedded and tissue samples were stained by routine Haematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS), silver, masson trichrome and congo red stains and were histopathologically examined to evaluate their diagnosis.

The studied cases were histopathologically evaluated for the following parameters:
1. **Presence of nondiabetic renal disease.**
2. **Score of arteriolar hyalinosis.** Arteriolar hyalinosis is classified into: score 0: no hyalinosis, score 1: At least one area of arteriolar hyalinosis, score 2: More than one area of arteriolar hyalinosis [9].
3. **Score of arteriosclerosis (score worst artery):** Presence of large vessels and the arteriosclerosis is classified into; score 0: No intimal thickening, score 1: Intimal thickening less than thickness of media, score 2: Intimal thickening greater than thickness of media [9].

Scoring of vascular lesions were evaluated according to pathologic classification of diabetic kidney disease of renal pathology society (RPS) (table 1) [9].

Table (1): Renal vascular lesions of diabetic biopsy [9].

Lesion	Criteria	Score
Arteriolar hyalinosis	Absent	0
	At least one area of arteriolar hyalinosis	1
	More than one area of arteriolar hyalinosis	2
Presence of large vessels	--	Yes/No
Arteriosclerosis (score worst artery)	No intimal thickening	0
	Intimal thickening less than thickness of media	1
	Intimal thickening greater than thickness of media	2

All cases were stained by:
1- Immunoglobulin A (Polyclonal rabbit antibody, clone 267A-16, 1mL concentrate, incubated in a humidity chamber for 45 minutes at 4ºC (diluted at 1/200), Cell Marque, USA).
2- Immunoglobulin G (Polyclonal rabbit antibody, clone 269A-16, 1mL concentrate, incubated in a humidity chamber for 45 minutes at 4ºC (diluted at 1/200), Cell Marque, USA).
3- Complement 3 (Polyclonal rabbit antibody, clone, HPA020432, 100µl concentrate, incubated in a humidity chamber for 45 minutes at ºC (diluted at 1/300), Sigma Aldrich, USA).
4- Anti-kappa light chain antibody (Clone: CH15, 1ml concentrate, incubated in a humidity chamber for 45 minutes at 4ºC (diluted at 1/200), Leica, United Kingdom) was used with the product code (NCL-L-KAP-581).
5- Anti-lambda light chain antibody (Clone: SHL53, 1ml concentrate, incubated in a humidity chamber for 45 minutes at 4ºC (diluted at 1/200), Leica, United Kingdom) was used with the product code (NCL-L-LAM-578).

Immunostaining procedure: Leica IHC autostainer.
Positive control tissue
Positive controls are necessary to indicate correct tissue preparation and staining. Positive control tissue sections were processed in the same manner as the selected tissue samples and was included in each staining run. The known positive cases used as a positive control for IgA, IgG, C3, kappa and lambda antibodies

Negative control tissue
Negative control sections were processed for each run by omitting the specific primary antibody from the staining procedure and replaced with PBS. The negative control was used to indicate non-specific staining by the primary antibody. Absence of non-specific staining confirms the lack of cross reactivity with other non-target cellular component.

Identification:
Detect IgA, IgG C3, kappa and lambda staining along glomerular basement membrane and in mesangium.

Statistical analysis
Data was analyzed using SPSS version 22 (Statistical Software package version 22). Descriptive analysis was performed. Quantitative data was represented as mean, standard deviation and range. When the data was normally distributed one-way ANOVA test was used to compare between two groups. Qualitative Data were reported as frequencies and percentages and compared using either Chi-Square test. Graphs were produced by using Excel or SPSS version 22. P value was considered significant if it was ≤ 0.05.

Results
This retrospective study was conducted at Path Lab (private pathology lab) during the period from 2014 to 2020. Out of 895 cases were diagnosed as DM, 754 cases were included in the study according to the inclusion criteria.
Prevalence of DKD, NDRD and coexisting DKD and NDRD. Based on biopsy findings, cases were categorized into three groups (figure 1): Isolated DKD, Isolated NDRD and Coexisting DKD and NDRD.

Table (2): Demographic data of the studied cases according to pathological diagnoses of kidney disease

	Total clinical data 754 (%)	Pathological Diagnoses			P value
		DKD 203 (%)	NDRD 281(%)	Coexisting DKD and NDRD 270 (%)	
Presence of hypertension	511(67.8%)	138(68%)	173 (61.6%)	200 (74.1%)	**0.007***
HCV	123 (16.3%)	30(14.8%)	29(10.3%)	64 (23.7%)	**0.0001***
HBV	9 (1.2%)	2 (1%)	4 (1.4%)	3 (1.1%)	0.90
Cardiovascular diseases	34 (4.5%)	10 (4.9%)	13 (4.6%)	11 (4.1%)	0.9
Chronic liver diseases	12 (1.6%)	2 (1%)	6 (2.1%)	4 (1.5%)	0.6
Liver transplantation	4 (0.5%)	1 (0.5%)	0 (0%)	3 (1.1%)	0.20
Solid tumors	8 (1.1%)	2 (1%)	3 (1.1%)	3 (1.1%)	0.99
Autoimmune diseases	32 (4.2%)	4 (2%)	16 (5.7%)	12 (4.4%)	0.13
Hematological neoplasia	4 (0.5%)	1 (0.5%)	3 (1.1%)	0 (0%)	0.23
Hypothyroidism	7 (0.9 %)	2 (1 %)	2 (0.7%)	3 (1.1%)	0.88
Gout	3 (0.4%)	1 (0.5%)	0 (0%)	2 (0.7%)	0.37

Qualitative data represented by No (%). Chi-square test was used to compare number and percentages. Chi-square test used to compare every two groups with each other to detect any statistically significant differences. (*) statistically significant p-value (P <0.05).

There was a significant difference between different pathological diagnoses regarding parameters include HCV and hypertension (table 2).

Table (3): Vascular lesions according to histological diagnoses of kidney disease.

Lesion	Score	Pathological Diagnoses			Total 754 (%)	P-value
		DKD 203 (%)	NDRD 281 (%)	Coexisting NDRD and DKD 270 (%)		
Arteriosclerosis (score worst artery)	No arteries	27(13.3%)	90 (32 %)	37 (13.7%)	154 (20.4%)	**0.001***
	0	34(16.7%)	57 (20.3%)	74 (27.4%)	165 (21.9%)	**0.001***
	1	16(57.1%)	111 (39.5%)	125 (46.3%)	352 (46.7%)	**0.001***
	2	26(12.8%)	23 (8.2%)	34 (12.6%)	83 (11%)	**0.002***
Arteriolar hyalinosis	0	19 (9.4%)	181(64.4%)	48 (17.8%)	248 (32.9%)	**0.001***
	1	30(14.8%)	59 (21%)	65 (24.1%)	154 (20.4%)	**0.002***
	2	154(75.9%)	41 (14.6%)	157 (58.1%)	352 (46.7%)	**0.001***

Quantitative data represented by mean ± SD, qualitative data represented by No (%). Chi-square test was used to compare number and percentages, One-way ANOVA used to compare means. Chi-square test used to compare every two groups with each other to detect any statistically significant differences. (*) statistically significant p-value (P <0.05).

A significant difference was noted among the studied groups (DKD, NDRD and coexisting DKD and NDRD) regarding different scores of arteriosclerosis and arteriolar hyalinosis.

Figures (4-7) demonstrate the histopathological scores of vascular lesions including arteriosclerosis and arteriolar hyalinosis scores.
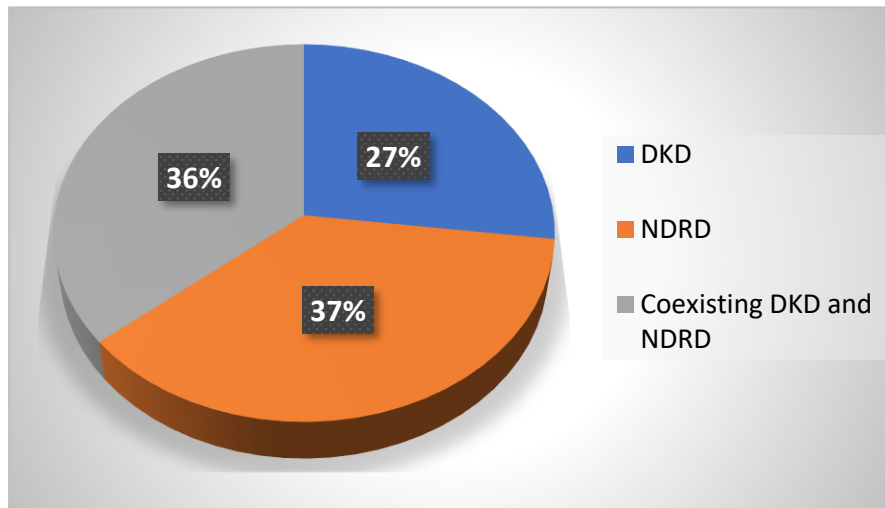
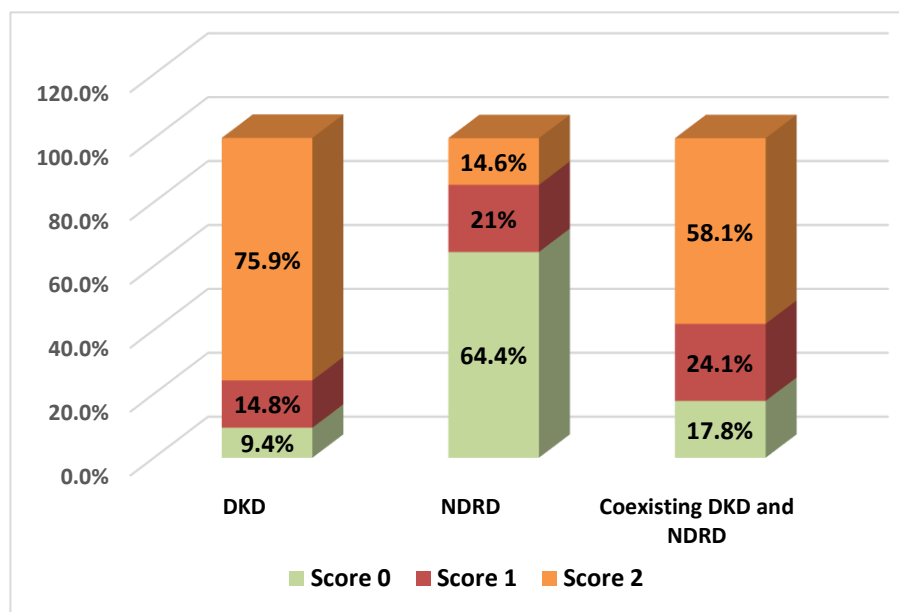**Figure (1): Percentage of the studied groups according to renal biopsy.**

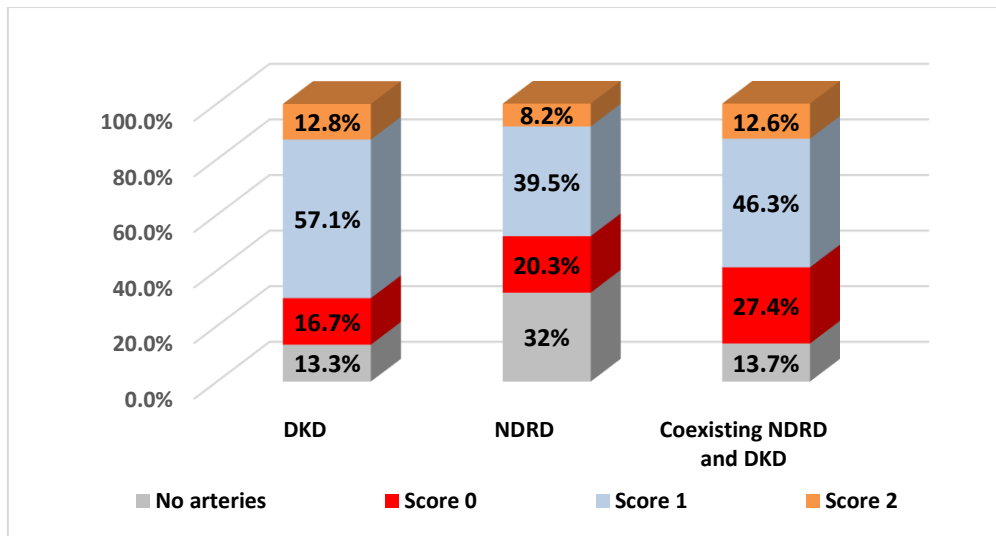**Figure (2): Arteriolar hyalinosis in the study groups.**

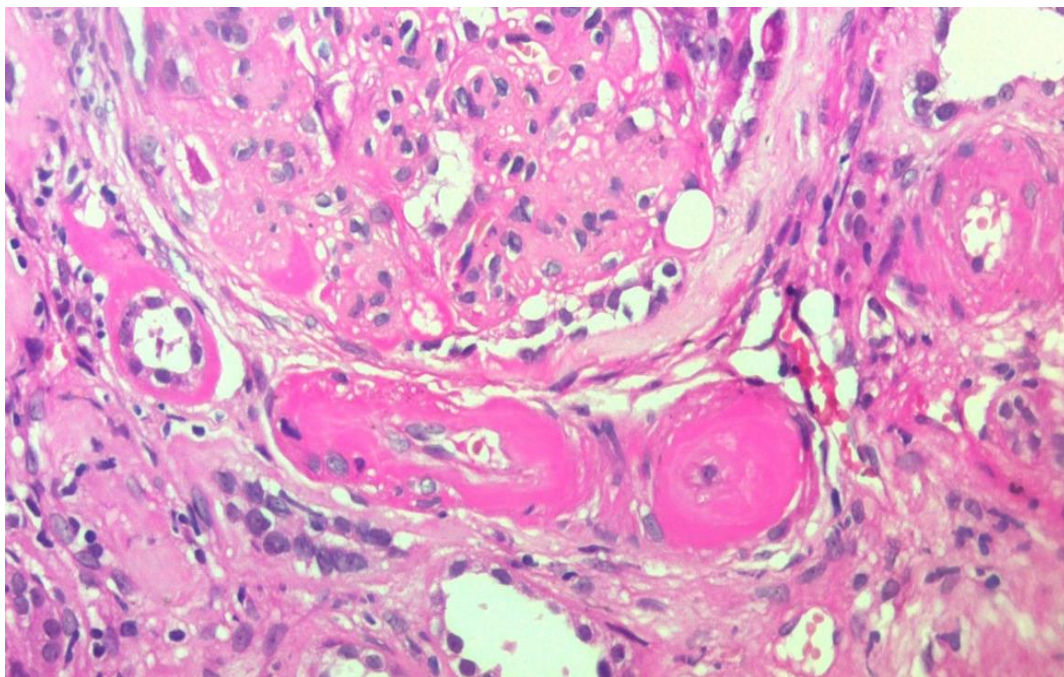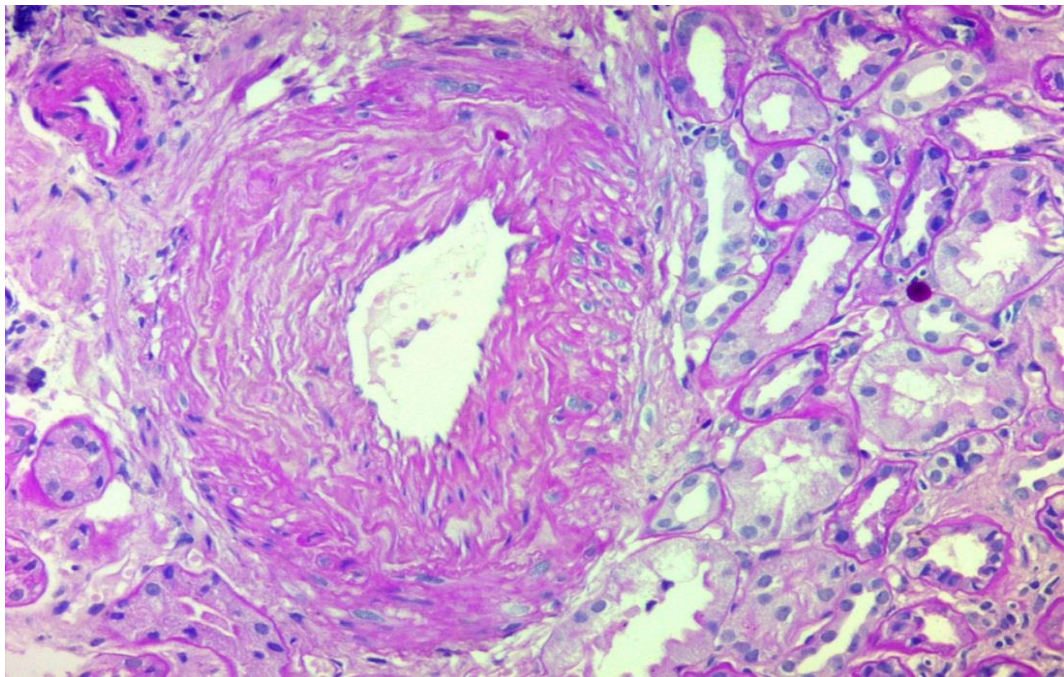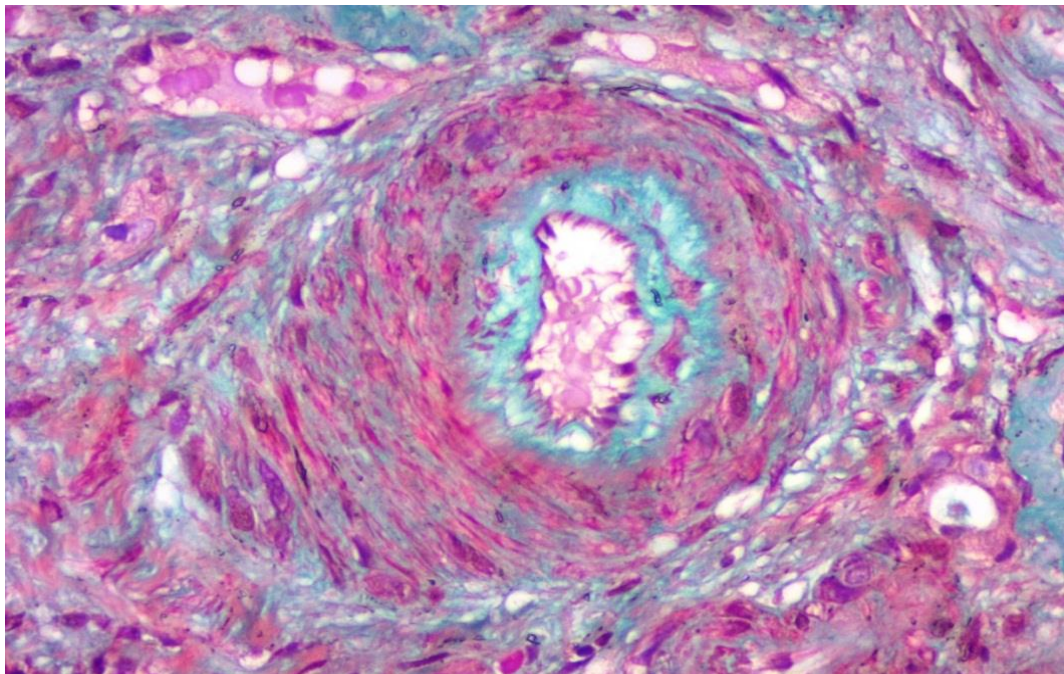**Figure (3): Arteriosclerosis in the study groups.**
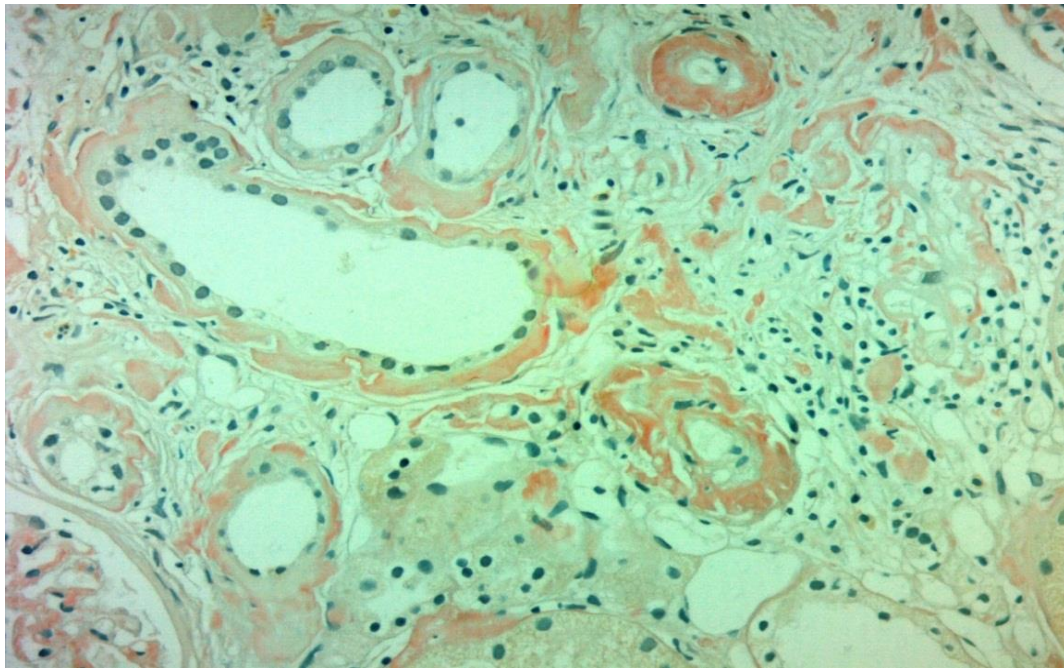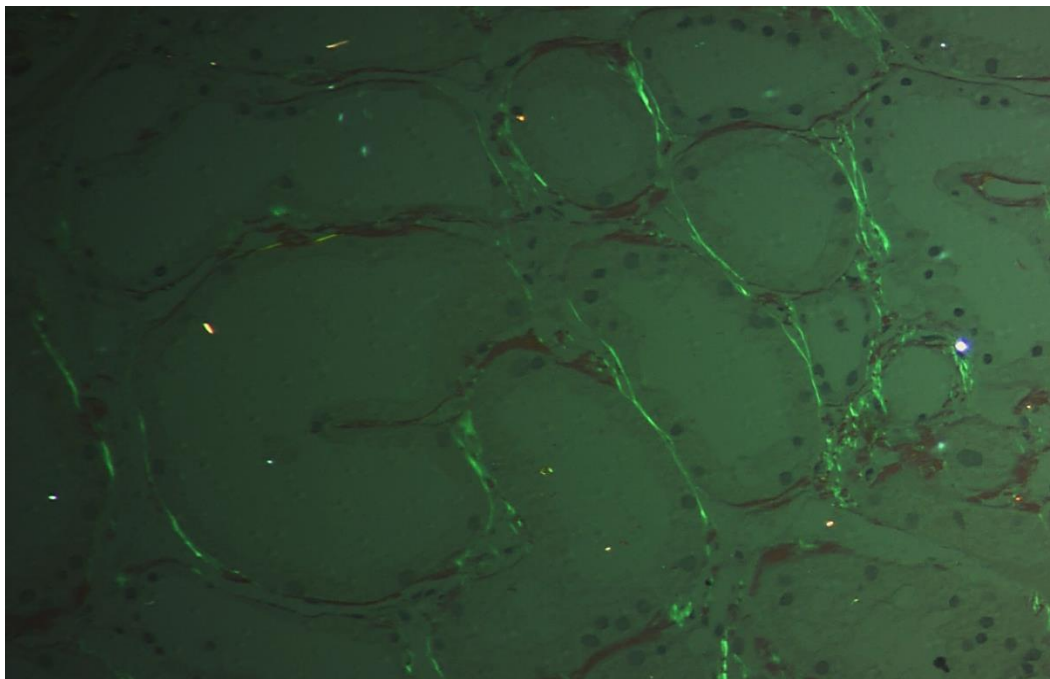

Figure (4): **Arteriolar hyalinosis, score 2** (H&E, ×400).

Figure (5): **Arteriosclerosis**, **score 1** (Masson trichrome, ×400).

Figure (6): **Arteriosclerosis**, **score 2** (PAS, ×٤00).
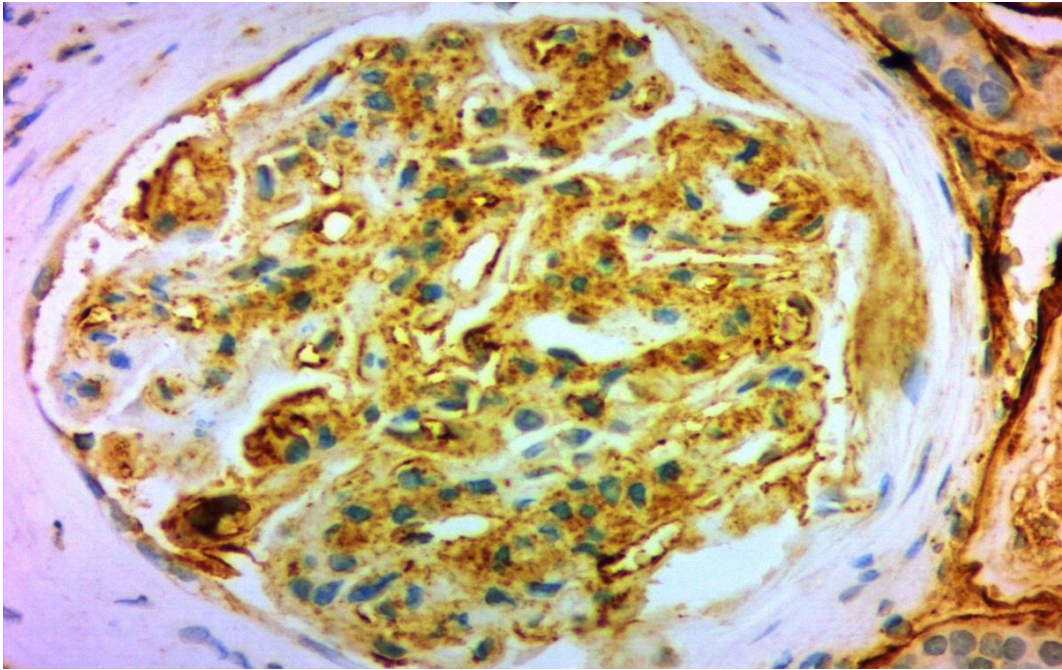
Figure (7): Amyloidosis as example of NDRD (orange colour of amyloid deposits under light microscopy in interstitum, tubular basment membranes and arteriole (congo red, ×200).
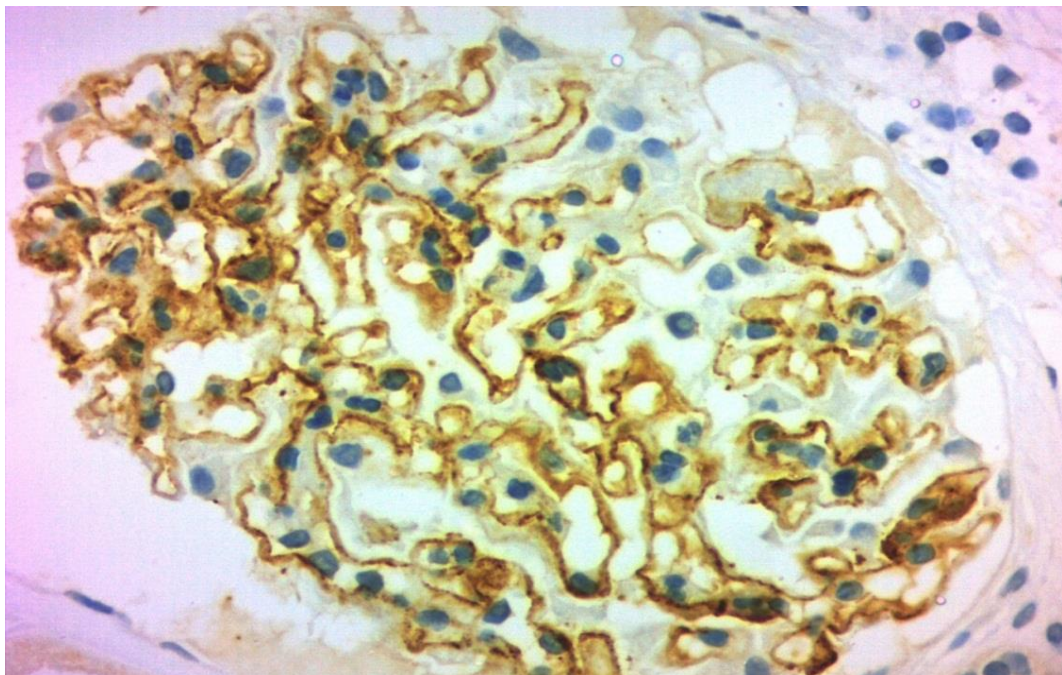

Figure (8): Amyloidosis (congo red stain under polarized light confirming the apple green birefringence of the deposits mainly in tubular basement membranes ×400).
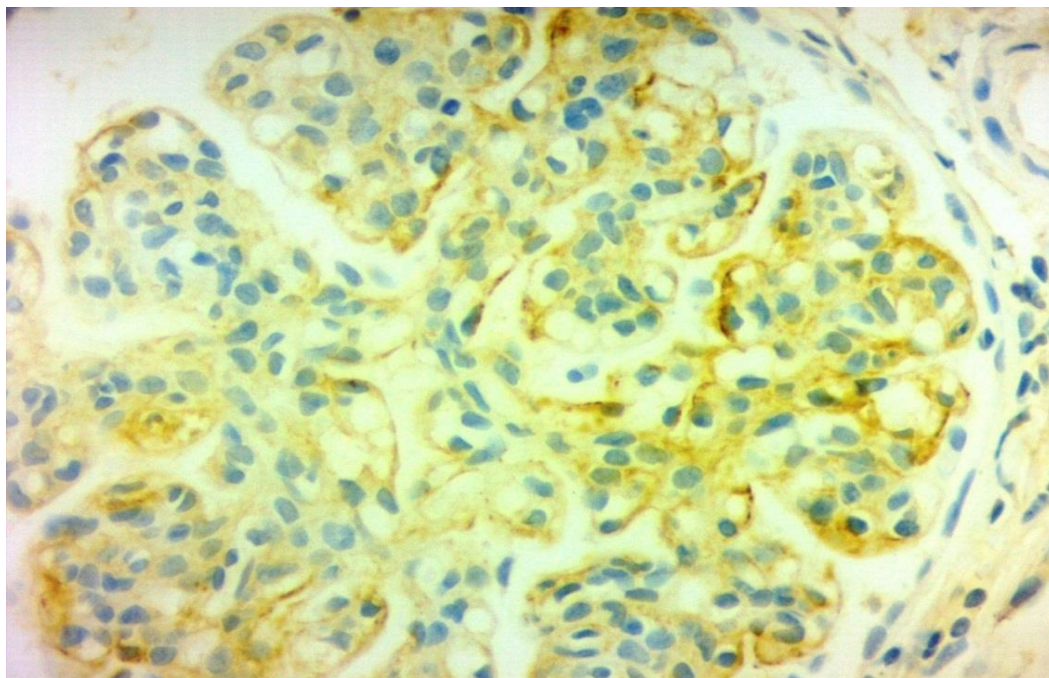
Figure (9): IgA nephropathy showed diffuse mesangial deposits of IgA (IHC, ×400).

Figure (10): Membranous glomerulonephritis showed diffuse glomerular basement membranes deposits of IgG (IHC, ×400).

Figure (11): membranoproliferative glomerulonephritis showed mesangial and glomerular basement membranes deposits of C3 (IHC, ×400).

Discussion

In this study we aim to study the vascular lesions in diabetic patients.

In our study, hypertension was the most common associated medical disease represented 67.8% of the study cases, 61.6% in isolated NDRD cases and 74.1% in coexisting DKD and NDRD cases. Hypert-ension was common also in a literature carried out by Horvatic et al., which represented 73.8% (59/80) of all studied cases, 69% (20/29) of isolated NDRD cases and 85.7% (12/14) of coexisting DKD and NDRD cases [10].

Hepatitis C virus infection is a common disease in Egypt. In our study it was 16.3% of studied cases, 10.3% of isolated NDRD cases and 23.7% of coexisting DKD and NDRD cases. The study reported by Sanghavi et al., who enrolled 399 patients found that hepatitis C virus infection represented 8.3% (33/399) of all studied cases and 8% (17/207) of isolated NDRD cases[11]. This difference may be attributed to different environmental factors.

In our study, arteriolar hyalinosis was in 67% of studied cases. Score 1 and score 2 were 20.4% and 46.7% respectively. Arteriolar hyalinosis score 2 was 75.9% of DKD group and 58.1% of coexisting DKD and NDRD group. Only 14.6% of isolated NDRD cases showed arteriolar hyalinosis score 2. No arteriolar hyalinosis was noted in 64.4% of isolated NDRD cases. In a study done by Comai et al., in 2019, arteriolar hyalinosis was in 77% of cases while score 1 and score 2 were 17% (6/35) and 60% (21/35) of cases respectively [12]. In another study conducted on 135 patients, arteriolar hyalinosis was 88% of cases, score 1 was 9% and score 2 was 89% of cases [13].

In our study, arteriosclerosis score 1 and score 2 were 46.7% and 11% respectively of studied cases, while score 0 was 21.9%. arterios-clerosis score 1 was 57.1% of DKD group and 46.3% of coexisting DKD and NDRD group. It differs from a study carried out by Zhang et al., in which arteriosclerosis score 2 was 53% (63/135) of the studied cases [13].

Conclusion

Marked arteriolar hyalinosis (score 2) occurs mainly in DKD group and in coexisting DKD and NDRD group, while more than half of NDRD group shows no arteriolar hyalinosis (score 0). DKD group shows arteriosclerosis score 1 in more than half of the studied cases.

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