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# PATHOLOGICAL STUDY OF DIABETIC NEPHROPATHY BASED ON RENAL BIOPSY: A PROSPECTIVE STUDY

**C. O. Alebiosu*, S. Kadiri#, E. U. Akang**
*Departments of Medicine, Ogun State University Teaching Hospital, Sagamu, #University College Hospital, Ibadan, and Department of Pathology, University College Hospital, Ibadan, Nigeria*

## ABSTRACT

**Background of the Study, Aims & Objectives:** There are few studies on the histological patterns of diabetic nephropathy in this environment on a prospective basis. This study is important because of the increasing prevalence of diabetes mellitus and diabetic nephropathy in this environment. The aim of this study was to show the renal histological features among Nigerian diabetics.

**Subjects, Method and Materials:** Fifty-five diabetics with proteinuria had their medical records reviewed. Tissues from renal biopsies were processed and examined for histological lesions. Quantitative data was analyzed with the student's t-test and $X^2$ analysis.

**Results:** Fifty-five patients, 35 males and 20 females, formed the study group out of the 138 diabetics screened (77 males and 61 females); majority 47 (85%) had type 2 while eight (15%) had type1 diabetes mellitus. The frequency of occurrence of clinical diabetic nephropathy was 39.9%. Twenty-five (45.5%) of the patients had biopsy specimens with varying degrees of glomerular obsolescence. The frequency of occurrence of nodular and diffuse glomerulosclerosis was 20% and 98.1% respectively. Armani-Ebstein lesion was rare among the patients studied.

**Conclusion:** There is a high prevalence of diabetic nephropathy in the study population. The frequency of occurrence of nodular and diffuse glomerulosclerosis among the diabetics studied was 20% and 98.1% respectively.

**KEYWORDS:** *Diabetes Nephropathy, Renal Biopsy, Pathological Study*

## INTRODUCTION

The commonest endocrine disease in Nigeria is Diabetes Mellitus[1] with a prevalence rate of 2.2%[2]. Nephropathy was recognized as a specific complication of diabetes mellitus about 40 years ago[3]. The commonest causes of chronic renal disease in Nigeria include glomerulonephritis, systemic hypertension, diabetes mellitus, and obstructive uropathy[4]. In the western countries however, diabetes mellitus is the leading cause of chronic renal disease[5].

The detection of proteinuria by standard dipstick techniques in a diabetic is generally taken as the definition of the onset of clinical diabetic nephropathy[6]. Lesions indicative of chronic diabetes but not necessarily indicative of clinically significant renal disease occur in advanced diabetes[6]. These include nodular and diffuse glomerulosclerosis, fibrin cap lesion and capsular drop lesions.

Information regarding features of diabetic nephropathy in Nigerian diabetics is scanty[8]. Few workers have studied the histological patterns of diabetic nephropathy in this environment. Greenwood and Taylor[7] gave 4.16% for nodular glomerulosclerosis among 48 patients autopsied in Ibadan with a corresponding figure of 22.9% for diffuse glomerulosclerosis. Smith and Adetuyibi[8] in their study of 88 autopsies reported diffuse glomerulosclerosis in 24.5% of cases and nodular glomerular lesions in 22.1%. Armani – Ebstein lesion represented 2.6% of the renal lesions. Thomas[9] reported glomerular histological manifestations including diffuse (84%) and nodular (21.9%) glomerulosclerosis respectively in her autopsy study of 32 diabetic kidneys in Ibadan. Tubular vacuolations was reported not to be a rare occurrence. Parson et al[10] gave 1.8% and 11.1% respectively among 54 autopsies among Ugandans in 1968.

The aim of this study was to show the renal histological features among Nigerian diabetics. The importance of the study lies in that there is an increasing prevalence of diabetes mellitus[11,12,13] and diabetic nephropathy[14,15,16] in this environment. Earlier studies were based on autopsy findings but this present study is unique in that it is based on percutaneous renal biopsy and we hope may aid better understanding of clinical diabetic nephropathy in Nigerians.

## METHODS

Fifty-five consecutive diabetic patients with proteinuria (positive 'albustix') presenting at the Medical Outpatient Unit of the University College Hospital, Ibadan without clinical or laboratory evidence of kidney or renal tract disease other than diabetic glomerulopathy, formed the study group. The study had local ethical committee approval. Fully informed written consent

*Correspondence: Dr. C. O. Alebiosu

was obtained from the subjects.

The inclusion criteria were prior clinical diagnosis and treatment for at least five years duration of diabetes mellitus and presence of persistent proteinuria. Persistent proteinuria was defined as dipstix 1+ proteinuria on early morning urine samples at least on 3 occasions about 4 weeks apart. The exclusion criteria include diabetes of less than five years duration, urinary tract infection or infestation, congestive cardiac failure and pregnancy. The subjects were divided into two groups type 1 and type 2 diabetes mellitus. Blood pressure was recorded. The blood pressure was measured with a standard clinical sphygmomanometer (cuff 25cm by 12cm) on the right arm with the subject in the supine or sitting position after resting for at least ten minutes It was measured thrice during the study while trying to establish persistent proteinuria. Pressure was recorded to the nearest 2mmHg; systolic and diastolic blood pressures were taken as the appearance and disappearance of the Korotkoff sounds respectively (phases I & V).

Packed cell volume, platelet count, prothrombin and bleeding times were obtained. Laboratory parameters to assess renal function, serum proteins, cholesterol, triglycerides, fasting and 2 hours postprandial blood sugars, (on two occasions) were also estimated. Urinalysis and urine microscopy were also done. Creatinine clearance was also done; direct questioning and urinary creatinine were used to assess completeness of 24 hours urine collections. Proteinuria was measured using the bromocresol green method.

Kidney biopsies were performed on all patients provided there was no contraindication. The exclusion criteria for kidney biopsy include:
(a) Lack of consent (b) Solitary kidney at ultrasonography (c) Deranged coagulation pattern (d) Thrombocytopaenia (e) Poor diabetic control (f) Uncontrolled hypertension and (g) Urinary tract infection (h) Bilaterally shrunken kidneys (< 9cm longitudinally)

Patients with unacceptable glycaemic states were controlled before biopsy. The procedure for the renal biopsy is as follows: premedication of intramuscularly 5mg diazepam was given 30 minutes before the procedure. Prior to the procedure, both kidneys were imaged by ultrasound and marked on the skin at full inspiration. Kidney sizes and the depth from the skin to the center of the kidneys were recorded. The patient was positioned prone with a firm pillow under the abdomen. Two- percent lignocaine was infiltrated from the skin down to the kidney and a 5mm incision made using a number 15 blade. The Trucut biopsy needle was advanced down to the lower pole (depth from the skin predetermined by ultrasonography) with the patient holding his/her breath in full inspiration. The trocar is advanced into the kidney and the cannula was flicked over the trocar to take a bite of the kidney tissue. The needle was removed and the Trucut is within the renal substance for few seconds. The patient was then asked to breathe normally. The patient was asked to take frequent liberal fluid thereafter and vital signs were recorded every 15 minutes for about 4 hours and thereafter every half-hour. The appearance of the urine passed hourly was observed during this period.

Tissues were fixed immediately in buffered formalin solutions. After fixation, the biopsy specimens were embedded in paraffin and sectioned at 2 – 4 um thickness smoothly. Tissues were stained with haematoxylin and eosin (to demonstrate infiltrates and cellularity), periodic acid silver methenamine stain (to demonstrate basement membrane) and trichome stain (to show the amount of interstitial fibrosis and vascular changes). Processing of the tissues was done at the Pathology Department of the University College Hospital, Ibadan. The kidney tissues were examined for glomeruli, tubules, interstitium and vessels. Table 1 shows the different histopathological lesions examined for in the slides. Sufficient tissue sample was indicated by the presence of at least 10 glomeruli. In examining the slides, each section was studied and graded on at least two separate occasions and the final grading given to any particular slide was the mean of the two independent assessments. Where necessary, new sections were prepared. Grading was done as documented by Kimmestiel and Wilson[17] and Thomas[9].

***Table 1:*** **Items for Histopathological Analysis of Kidney Tissue Studied.**

| Features | Lesions |
|---|---|
| Glomerular | Glomerular obsolescence<br>Nodular Glomerulosclerosis<br>Diffuse Glomerulosclerosis<br>Exudative lesions<br>Capsular Drop<br>Fibrin Cap |
| Vessels | Hyaline change<br>Intimal thickening<br>Diffuse capillary thickening |
| Tubules | Vacuolation<br>Cast<br>Atrophy/Necrosis |
| Interstitium | Fibrosis<br>Cellular Infiltrates |

In assessing nodular glomerulosclerosis, grades were allotted as follows: mild (+) refers to the presence of a single nodule in occasional glomeruli; (++) refers to many glomeruli containing nodules with many still spared; (+++) almost every glomerulus contained one or more nodules and were rendered almost completely ischemic.

In grading diffuse glomerulosclerosis, (+) mild refers to 'local' lesion within each glomerulus, and 'focal' within the kidney. With increasing severity, the thickening became 'diffuse' within the glomerulus and 'generalised' throughout the kidney (moderate ++). As the lesion progressed, the capillary lumina gradually became narrower with subsequent obliteration initially occurring 'locally' but eventually the whole glomerulus became almost ischemic and appeared hyalinised (severe +++). 'Local here implies lesions affecting one or more discrete areas in each glomerulus. 'Diffuse' implies lesions affecting the whole of each glomerulus. 'Focal' implies lesions either 'local' or 'diffuse'

affecting all or almost all glomeruli. In assessing exudative lesions, the presence (+) or absence (-) of these were noted.

Interstitial fibrosis and tubular atrophy and interstitial inflammation were graded as follows: mild (+) – focal areas involved less than 25% of the field; moderate (++) between 25 – 50% of the field was involved and severe (+++) extensive areas over 50% of the filed was involved. The grades represented only the ranking of the severity of the lesions.

Quantitative data was expressed as mean ± S. D. Student's t-test was used to assess the difference between the various subject groups. P-values < 0.05 were considered significant.

## RESULTS

Fifty-five patients, 35 males and 20 females, formed the study group out of the 138 diabetics screened (77 males and 61 females); majority 47 (85%) had type 1 while eight (15%) had type 2 diabetes mellitus. Thus the frequency of occurrence of clinical diabetic nephropathy is 13.9%.

Hypertension was present among thirty-two (52.2%) patients with twenty-six (47.3%) having normal diastolic blood pressure (<90mmHg) and twenty-four (43.6%) having normal systolic blood pressure (<140mm Hg). Among the hypertensives, sixteen (29.1%) had mild hypertension, 7(12.7%) had moderate hypertension while 6(10.9%) had severe hypertension. Twenty-three (41.8%) patients had systolic blood pressure between 140 – 160 mm Hg and 8(14.6%) had systolic blood pressure of ≥ 161mmHg.

## HISTOPATHOLOGICAL FINDINGS

The typical morphological changes observed in kidneys of the 55 patients are depicted in Table 2.

*Table 2:* **Frequency and Severity of Renal Histopathological Lesions**

| Lesion | Mild | Moderate | Severe | % of total |
|---|---|---|---|---|
| **Glomerular Lesions** | | | | |
| Nodular Glomerulosclerosis | 11 | 0 | 0 | 20 |
| Diffuse Glomerulosclerosis | 41 | 11 | 2 | 98.2 |
| Exudative lesions | 9 | 0 | 0 | 16.4 |
| **Vessels** | | | | |
| Hyaline change | 30 | 13 | 5 | 87.3 |
| Intimal thickening | 24 | 14 | 5 | 78.2 |
| **Tubules** | | | | |
| Vacuolation | 14 | 1 | 0 | 27.3 |
| Cast | 41 | 10 | 2 | 96.4 |
| Atrophy | 28 | 6 | 1 | 63.6 |
| **Interstitium** | | | | |
| Fibrosis | 15 | 17 | 1 | 60 |
| Cellular Infiltrates | 3 | 3 | 0 | 10.9 |

## GLOMERULAR LESIONS:

(a) Glomerular obsolescence (global sclerosis) – Fig 1. Twenty-five (45.5%) of the patients had biopsy specimens with varying degrees of glomerular obsolescence. Out of these, 18 (72%) patients had biopsy specimens with 80% of the glomeruli sclerosed and two (8%) had global sclerosis of all the glomeruli. Twenty-two out of the 25 (88%) patients showing varying degrees of globally sclerosed glomeruli belong to the subset of 32 patients that had systemic hypertension. The remaining 3 (2 males and a female) had moderate diffuse glomerulosclerosis without glomerular obsolescence.

(b) Diffuse glomerulosclerosis – Fig 2 Diffuse glomerulosclerosis was the most common form of glomerular lesion seen. It was present in 54 (98.2%) of the patients in varying degrees of severity: mild – 41 (74.6%), moderate 11 (20%) and severe 2 (3.6%).

(c) Nodular glomerulosclerosis – Fig 3: Nodular glomerulosclerosis was seen in 11 (20%) diabetics (8 males and 3 females).

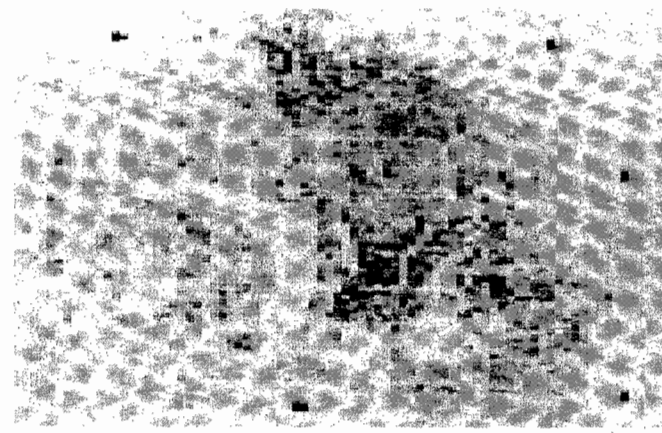

*Fig. 1:* **Renal Cortex Showing Glomerular Obsolescence And Mononuclear Infiltrates (H & E X 150)**

***Note That 5 Out Of 6 Glomeruli Show Glomerular Obsolescence (Arrow Heads). Mononuclear Inflammatory Cells Infiltrate The Interstitium.***

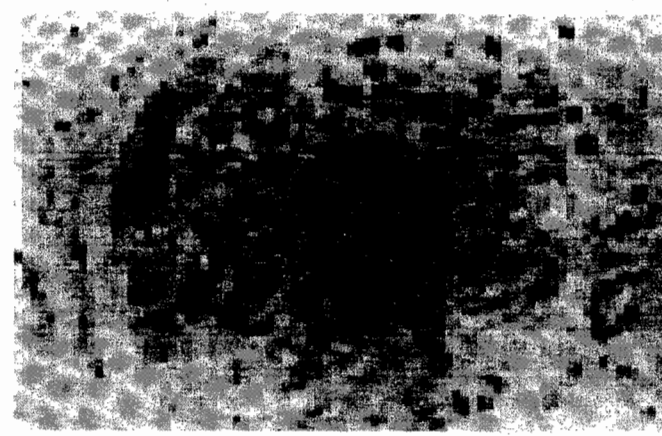

*Fig. 2:* **Renal Cortex Showing Diffuse Glomerulosclerosis (H & E X 200)**

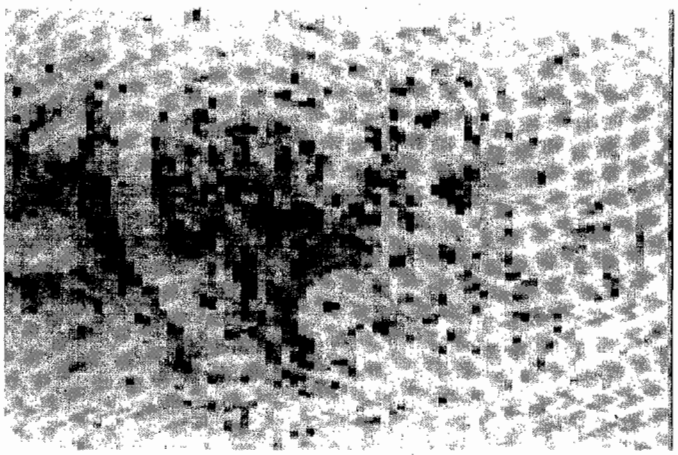

*Fig. 3:* **Glomerulus Showing Nodular Glomerulosclerosis (H & E X 200)**

***Note the oval shaped homogenous nodule (arrow head) with hyaline material mainly to the inner aspect, the outer aspect being spared (i.e. that facing Bowman's space).***

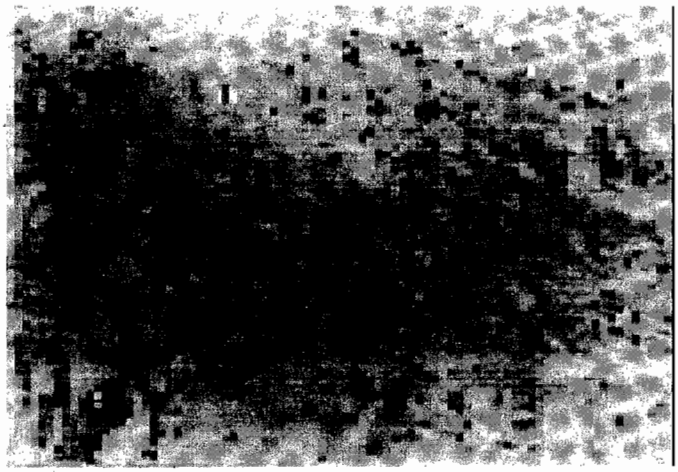

*Fig. 4:* **Renal Medulla Showing Tubulointerstitial Changes (H & E X 200)**

***Note hyaline-H and cellular-C casts, tubular atrophy-T and vacuolations-V)***

(d) Exudative lesions: Exudative lesions were seen in 9 diabetics (16.4%) – 6 males and 3 females (p = 0.86). They were found more commonly in the severe forms of diffuse and nodular glomerulosclerosis.

(e) Vascular Lesions: The arterial changes were characterised by intimal thickening and hyaline change. Hyaline change involved both afferent and efferent arterioles. There were 31 males and 17 females, (p=0.87). Thirty (54.6%) patients had mild hyaline vascular change, 13(23.6%) had moderate while 5(91%) had severe hyaline vascular change. Seven (12.7%) patients did not show any evidence of hyaline vascular change. Twenty-four (43.6%) patients had mild intimal thickening, 14(25.5%) had moderate and 5(9.1%) severe. Twelve (21.8%) did not show any evidence of intimal thickening. The sex distribution of those with thickening was 27 males, 16 females (p=0.86).

Tubular lesion – Fig 4: The epithelial cells of the proximal convoluted tubules showed varying degrees of vacuolations (Table 2). The typical Armani – Ebstein lesion was absent among the patients studied. Forty (72.7%) patients did not show any vacuolation, 14(25.5%) show mild and 1(1.8%) showed moderate vacuolation. The sex distribution was 12 males and 3 females (p=0.28). Varying degrees of hyaline and cellular casts were demonstrated in the tubules among the patients studied. Majority had mild casts 41 (74.5%) moderate in 10 (18.2%) and severe in 2(3.6%). No casts were seen in 2 (3.6%) of the patients. The sex distribution was 34 males and 19 females (p=0.29). Twenty (36%) of the patients did not show any evidence of tubular atrophy, 26 (47.3%) had mild atrophy, 8(14.5%) had moderate while 1(1.8%) had severe tubular atrophy. The sex distribution was 25 males and 10 females (p=0.37).

(f) Interstitial fibrosis– Fig 4: The interstitium was characterized by varying degrees of fibrosis and few cellular infiltrates, mainly mononuclear cells. No abscess was seen. Seventeen (30.9%) patients had mild fibrosis, 15(27.5%) had moderate fibrotic change and 1 (1.8%) had severe evidence of fibrosis. No fibrosis was seen in 22(40%) of the patients studied. Forty-nine (89.1%) patients did not have cellular infiltrates in the interstitium. Three (5.5%) patients had mild cellular infiltrates while another 3(5.5%) had moderate cellular infiltrates.

## DISCUSSION

Studies on clinical Diabetic Nephropathy (DN) among the same population have given figures ranging from 16% to 42.5%[10,11]. In this study, 39.9% of diabetic patients fulfilled the inclusion criteria for clinical DN. From these studies, it appears that there is a high prevalence of clinical DN in Ibadan, Nigeria. In other parts of Africa, studies have shown figures ranging from 6% to 46% [12,13]. The low rate of occurrence of type 1 diabetes in this study, (14.6%), is in keeping with reports from other parts of Africa.

This study is *unique* in that it is a prospective study, based on percutaneous renal biopsy. Thomsen[14] in his study of 102 renal biopsies demonstrated diffuse glomerulosclerosis (fig. 2) in 90% of cases and nodular glomerular lesions (fig 3) in 26%. Out of the 120 renal biopsy sections examined by Hirose[15] among Japanese diabetics, the frequency of diffuse and nodular glomerular lesions were 77.5% and 21.7% respectively while it was 84% and 21.9% respectively in Thomas[9] study. The findings in this study are comparable to those of earlier studies. Greenwood and Taylor[7] however recorded the occurrence of nodular glomerulosclerosis to be 2(4.7%) and diffuse lesions 11(26.6%), while Edington and Mainwaring[16] noted a rarity of nodular glomerulosclerosis in the same environment. The rarity of these lesions in the later two studies may be explained by the fact that not all patients who died having diabetic mellitus died as a result of diabetic nephropathy.

Vascular changes characteristic of hypertension is hyalinisation of afferent arteriole immediately[18] before it enters the glomerulus. Thirty-two (58.2%) patients had a history of systemic hypertension. In this study there is hyalinisation of both afferent and the efferent arterioles, a feature that is considered pathognomonic of diabetic nephropathy[18]. The good control of blood sugar may explain the rarity of the typical Armani-Ebstein lesions.

A patient with fairly long standing diabetes mellitus with proteinuria and associated retinopathy may be assumed not to require a renal biopsy, which will almost certainly show changes consistent with diabetic nephropathy. The indications for renal biopsy among diabetics are diagnostic and research purposes[19]. A thorough investigation including a renal biopsy[14,20], is indicated in the absence of diabetic retinopathy, the early onset and / or rapid progression of nephropathy, or the presence of signs and symptoms of other systemic diseases. The presence of haematuria and red cell casts will also suggest the possibility of other renal disease, although red cell casts have been noted in 4% of cases of biopsy-proven diabetic disease[21]. Furthermore, almost every form of glomerular disease has been shown to occur in diabetic patients[22], the diagnosis of a coincident glomerular disease by renal biopsy may be helpful from both the therapeutic and prognostic standpoints. Thus, renal biopsy is an important research tool and its use is entirely justifiable in careful constructed research protocols even among diabetics.

Finally, in diabetes nephropathy, the most advanced histological changes are not necessarily related to the degree of proteinuria or to the degree of deterioration of renal function[6,23]. This explains the resultant inclusion of some patients with seemingly significant degree of renal dysfunction *histologically* in this study. The correlation between renal function and the severity / type of the histological lesions is not very good[6,23].

In conclusion, the frequency of occurrence of nodular and diffuse glomerulosclerosis is 20% and 98.1% respectively. The typical Armani-Ebstein lesion is rare among the patients studied.

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