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CLINICAL RESEARCH

Renal Disease in Metabolic Syndrome: the Hidden Role of Intrarenal Ischemia

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Introduction: The pathogenesis of renal disease in obesity and metabolic syndrome (MS) is mostly unknown. This is in part because of the limited information about renal morphological changes in these conditions. We evaluated renal histology in subjects with MS and those without MS, who are participants in the European Nephrectomy Biobank (ENBiBA) project.

Methods: MS was defined with at least 3 of the following criteria: (i) body mass index (BMI) $\geq$27 kg/m^2; (ii) prediabetes: fasting glucose of 100–125 mg/dl or HbA1c >5.7%; (iii) systolic or diastolic blood pressure >140/90 mm Hg or the use of medications; and (iv) triglycerides >150 mg/dl or high-density lipoprotein cholesterol <40 (in men) or 50 mg/dl (in women). The absence of these criteria defined patients without MS. Exclusion criteria were diabetes or known causes of renal disease.

Results: A total of 157 cases were evaluated: 49 without and 108 with MS. Those with MS were older (54 $\pm$ 16 vs. 66 $\pm$ 11, $P < 0.0001$), had more prevalent chronic kidney disease (CKD, estimated glomerular filtration rate [eGFR] <60 ml/min): 24% (23%) versus 4% (8%) ($P = 0.02$), and had higher albumin-to-creatinine ratio (10 [4–68] vs. 4.45 [0–27], $P = 0.05$) than those without MS. Global sclerosis (3% [1–7] vs. 7% [3–13], $P < 0.0001$), nodular sclerosis, mesangial expansion, glomerulomegaly; moderate + severe hyalinosis, and arteriosclerosis were more frequent in those with MS than in those without (88 [82] vs. 29 [59]; 83 [77] vs. 30 [61]; $P < 0.05$). These vascular changes were independent of differences in age.

Conclusion: In MS, ischemic renal disease may play a role in renal disease. In addition, some patients may develop lesions compatible with diabetic nephropathy such as increased mesangial expansion and nodular sclerosis. Further analyses are needed to study the consequences of the pandemic of obesity on renal health.

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The pandemic of overweight and obesity affects about 2 billion people worldwide, and these numbers are expected to increase in the next decades.[1,2] The consequence of these figures in public health are severe. Obesity is a risk factor for major diseases such as diabetes, hypertension, dyslipidemia, chronic liver disease, infections, cardiovascular events, and some types of cancer and renal disease, among the most important.[3] Understanding the pathogenesis of obesity-related diseases is fundamental to treat and prevent the consequences of this pandemic.

In nephrology, the impact of obesity and being overweight are particularly relevant. The risk for CKD increases with weight and starts below the threshold of obesity. Hsu *et al.*,[4] in >300,000 subjects, observed a relative risk for end-stage renal disease of 1.87 with BMI of 25 to 30 kg/m^2, which increased to 3.57 with a BMI of 30 to 35 kg/m^2 or to >6 with a higher BMI. Others observed that obese or overweight subjects with MS are those particularly at risk for CKD.[5-7] MS indicates the coexistence of diseases such as obesity, dyslipidemia, hypertension, hyperglycemia, inflammation, obesity or overweight, etc. The common factor linking these conditions is supposed to be insulin resistance. Interestingly, the higher the number of MS traits present or the level of insulin resistance, the higher the risk for CKD.[8] Thus, MS could be considered a link between obesity and renal damage.

However, the pathogenesis of CKD in patients with overweight and obesity is not clear. In a seminar study, D'Agatti and colleagues observed that patients with severe obesity (mean BMI 42 kg/m^2: [range, 31–63) developed glomerulomegaly (100%) and a secondary form of focal segmental glomerulosclerosis. Of relevance, about half of the cases showed "diabetoid" changes, that is, mild mesangial expansion, nodular mesangial sclerosis, and/or mild focal thickening of glomerular and tubular basement membrane.[9,10] This finding is intriguing because the patients with these changes had no clinical diabetes. Therefore, features similar or close to those found in diabetic nephropathy may be observed in obese subjects without diabetes, suggesting a continuum between diabetes and obesity in renal disease. Whether these findings apply to patients with MS and less severe forms of obesity but without diabetes is unknown. On the other hand, a recent study of our group observed that vascular damage, that is, arteriolar hyalinosis and arteriosclerosis can be very frequent in patients with diabetes, irrespective of the levels of albuminuria or proteinuria or the stage of diabetic nephropathy.[11] Vascular damage is a common finding in diabetes, obesity, and MS. However, the role of intrarenal ischemia in obese patients without diabetes is unknown. This is mostly because patients with obesity and MS and mild signs of CKD do not undergo a renal biopsy. In any case, renal morphological changes in obesity and MS are mostly unknown. This issue represents a major gap in our understanding of the pathogenesis of renal damage in the context of obesity and MS.

In this study, we evaluated renal morphologic changes in subjects with and without MS included in the ENBiBA.

METHODS

Protocol–Study Design

This study is part of the ENBiBA. The design of the project has been published.[11] In most of the patients with obesity, diabetes, or MS, renal biopsies are seldom performed, mainly due to the lack of proteinuria. This is the cause of a major gap in our knowledge of the pathogenesis of renal disease in these conditions. ENBiBA was designed to solve this limitation by analyzing unaffected renal tissue of nephrectomy specimens as well as serum, urinary samples, and clinical data.[11] After surgery, a sample of renal parenchyma, including both cortical and medullary, is taken and embedded in paraffin. Most of the nephrectomies were due to cancer, and so, samples are taken from an area at least 5 cm from the tumor. Finally, ENBiBA is an initiative of DIABESITY, a working group of the European Renal Association-European Dialysis and Transplantation Association and includes 15 centers from 6 countries (see participants in the Appendix).[11] The protocol was approved by the ethics committees of all participants.

Patients

For this study, only patients with and without MS were included. Patients with type 1 or type 2 diabetes were excluded from the analysis. Inclusion criteria were as follows: >18 years, capacity to understand the informed consent, and presence or absence of MS. Exclusion criteria were diabetes; previous renal disease, that is, urinary tract obstruction, glomerulonephritis, reflux nephropathy, chronic pyelonephritis, polycystic disease, interstitial nephritis, nephrolithiasis, severe renal artery stenosis, acute kidney injury, etc.; previous radiotherapy or renal toxicity due to chemotherapy; and particularly, the extension of the tumor to the whole kidney limiting the availability of unaffected tissue.

Definition of MS

Patients were classified as with MS when at least 3 of the following criteria were met: (i) overweight or

obesity: BMI ≥27 kg/m^2, (ii) impaired fasting glucose: fasting glucose of 100 to 125 mg/dl or HbA1c >5.7%, (iii) hypertension: systolic or diastolic blood pressure >140/90 mm Hg or the use of medications; and (iv) dyslipidemia: triglycerides >150 mg/dl, or high-density lipoprotein cholesterol <40 or 50 mg/dl in males and females, respectively. These criteria were selected based on a combination of previous definitions and consensus on MS.[12,13] The criterion of BMI >27 kg/m^2 was chosen because the risk for CKD starts before the classic cut-off point of 30 kg/m^2.[4] Patients were classified as without MS when none of the 4 criteria described above were observed.

Clinical Data

We collected data on weight; height; smoking habits; dyslipidemia; hyperuricemia; gout; hypertension; impaired fasting glucose; and treatment for previous conditions, if any; cardiovascular events; laboratory analysis; HbA1c; albumin-to-creatinine ratio in urine spots, albuminuria, and total proteinuria in 24-hour urine in some cases. Renal function was estimated by means of the CKD-Epidemiology Collaboration creatinine-based formula unadjusted for body surface area. We did so to avoid the artificial reduction of eGFR in patients with overweigh and obesity.[14]

Sampling and Histological Variables

According to the protocol, in each subject, the pathologist took a sample of unaffected renal tissue (~3 × 2 × 0.5 cm) at least more than 5 cm from the tumor. In all cases, the pathologist of the coordinating center (Hospital Universtiario de Canarias - University of La Laguna) checked the absence of neoplastic lesions or infiltrates in the sample. Histological sections (3 micron) were processed for light microscopy and stained with periodic acid-Schiff and Sirius red. Histological evaluation was performed by the pathologist of the coordinating center (RR-R). This included the analysis of glomerular, tubular, interstitial, and vascular pathological specimens, following the BANFF classification of 1999 with minor changes, as published before.[11,15,16] The evaluation included (i) number of glomeruli; (ii) glomerular sclerosis (nodular, segmental, diffuse, and global); (iii) increased mesangial matrix: uniform increase in the matrix with the width of mesangial inter-space exceeds the length of 2 mesangial cell nuclei: (absence; <25%; 26%–50%; >50% of affected nonsclerotic glomeruli; mesangial proliferation: >3 nuclei in 1 mesangial area; and nodular mesangial expansion, i.e., round formations of matrix); (iv) tubular atrophy and interstitial fibrosis: <5%, 5%–10%, 10%–20%, >20%, etc.; (v) inflammation: mononuclear cell interstitial inflammation in total parenchyma (scarred and unscarred), <5%, 5%–10%, 10%–20%, >20%, etc.; (vi) arterial sclerosis (fibrointimal thickness); and (vii) arteriolar hyalinosis: in mild (1+ [deposits in 1 arteriole]), moderate (2+ [deposits in more than 1 arteriole]), or severe (3+ [circumferential deposits]). The thresholds for arteriosclerosis were mild (0%–25%), moderate (25%–50%), and severe (>50%); and refer to the percentage of occlusion of the vascular lumen. Vascular damage was analyzed in the most severely affected vessels.

The pathologist was blind to the clinical characteristics of patients. Then, patients were classified into those with and those without MS, according to the previous definition.[12,13] Immunofluorescence and electron microscopy were not performed.

Morphometric Analysis to Evaluate Mesangial Expansion and Glomerulomegaly

Histological sections (3 μm) were stained with periodic acid-Schiff to select all the glomeruli sectioned through the hilum per sample to evaluate glomerular area and mesangial expansion. The glomeruli with clear vascular poles were used for morphometric analysis using the ImageJ software (National Institute of Health). A minimum of 30 glomeruli from each kidney were analyzed. Glomerulomegaly was defined as a value greater than the 90th percentile of the tuft area in patients without MS. To determine the mesangial area, glomeruli were photographed and analyzed with ImageJ software by assessing the periodic acid-Schiff-positive and nuclei-free area in the mesangium.

Definitions of Albuminuria and Proteinuria

Urinary protein excretion is measured by using either isolated urine spots or 24-hour collections. Normoalbuminuria, microalbuminuria, or overt proteinuria were defined considering the following criteria: (i) urinary albumin excretion (spot urine): <30, 30 to 299, and >300 ug/mg creatinine; (24 h urine collection): <30, 30 to 299, and >300 mg; and (ii) proteinuria >500 mg in 24h urine collection.[6]

Statistical Analysis

Patients were grouped based on the presence or absence of MS. Continuous variables were compared using parametric or nonparametric tests when appropriate. Dichotomous variables were compared using chi-square test. For analysis, SPSS Statistics for Windows version 17.0 (Chicago, IL) was used.

Sensitivity Analyses

We evaluated renal morphology in the cases with eGFR above or below 60 ml/min. In addition, in multivariate logistic regression, we analyzed the impact of

Table 1. Characteristic of patients with and without metabolic syndrome

Characteristics	Total	MS-NO	MS-YES	
N and *n* (%)	157	49 (30)	108 (70)	*P* value
Age (yr) mean ± SD	63 ± 14	54 ± 16	66 ± 11	
Gender (male), *n* (%)	108 (69)	23 (47)	78 (72)	0.003
Smoking habits[a]				
Never	72 (48)	23 (48)	49 (48)	
Current	34 (22)	10 (21)	24 (23)	
Previous	45 (30)	15 (31)	30 (29)	
Metabolic syndrome				
BMI >27 kg/m^2 (yes), *n* (%)	108 (70)	———	108 (100)	
BMI <27 kg/m^2 (yes), *n* (%)	49 (30)	49 (100)	——	
Weight (kg)	81 ± 18	65 ± 10	88 ± 16	
BMI (kg/m^2)	28 ± 5	20.8 ± 2.5	30.6 ± 3.6	
Impaired fasting glucose (yes), *n* (%)	62 (57)	———	62 (69)	
Fasting glucose (mg/dl)	98 ± 16	89 ± 8	103 ± 17	<0.0001
HbA1c (%)	5.6 ± 0.4	5.2 ± 0.3	5.7 ± 0.4	<0.0001
Hypertension (yes), *n* (%)	98 (62)	——	98 (91)	
Blood pressure levels				
Systolic (mm Hg)	134 (17)	126 (13)	137 (18)	<0.0001
Diastolic (mm Hg)	77 (11)	77 (8)	77 (11)	——
ACE inhibitors, *n* (%)	77 (49)	——	42 (39)	
AR blockers, *n* (%)	35 (22)	——	35 (32)	
Calcium channels blockers	26 (17)	——	26 (24)	
Beta-blockers, *n* (%)	32 (20)	——	32 (30)	
Diuretic, *n* (%)	44 (28)	——	44 (41)	
Dyslipidemia (yes), *n* (%)	81(52)	——	81 (75)	
Total cholesterol	174 ± 45	168 ± 34	172 ± 49	ns
HDL-cholesterol	46 ± 16	55 ± 14	44 ± 15	0.001
LDL-cholesterol	104 ± 41	96 ± 32	105 ± 44	ns
Triglycerides	133 ± 68	90 ± 33	151 ± 71	<0.0001
Statins, *n* (%)	41 (26)	——	41 (38)	
Fibrates, *n* (%)	5 (3%)	———	5 (5%)	
Hyperuricemia (yes), *n* (%)	20 (13)	———	20 (18)	
Uric acid levels (mg/dl)	5.1 ± 2.4	3.8 ± 2	5.3 ± 2.3	<0.0001
Allopurinol, *n* (%)	10 (6)	———	10 (9)	
Gout, *n* (%)	4 (3)	———	4 (4)	
Cardiovascular events (yes), *n* (%)	30 (19)	3 (6)	27 (25)	0.03
eGFR: CKD-EPI (ml/min)	83 ± 26	87 ± 23	81 ± 27	Ns
eGFR <60 ml/min, *n* (%)	28 (18)	4 (8)	24 (23)	0.02
Albuminuria-to-creatinine (mg/g), mean (IQR)	8 (4–62)	4.45 (0–27)	10 (4–78)	0.05
24-h albuminuria (mg/24h), mean (IQR)	9 (0–76)	0.1 (0–13)	16 (0.6–16)	0.05

ACE, angiotensin-converting enzyme; AR, angiotensin receptor; BMI, body mass index; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration equation; MS, metabolic syndrome; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.
[a]Data available in 151 patients.

difference factors in the appearance of vascular damage, that is, arteriolar hyalinosis and arteriosclerosis. The covariates included were age, the presence of MS, the smoking status, and the use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Then, in the final model, we replaced MS by its single components, to evaluate the individual role of the MS traits in renal damage. The correlation between vascular lesions and histological and clinical variables were tested with the Kendall's Tau-b.

RESULTS

A total of 161 patients were included in the study. In all subjects, nephrectomy was due to cancer (kidney, ureter, or renal pelvis). Four patients were excluded due to insufficient material for histological analysis, that is, unaffected tissue unavailable. Thus, we evaluated 157 cases. In all samples, the pathologist (RR-R) ruled out neoplastic infiltration.

Patient Characteristics

Of the entire population, 108 (70) had MS and 49 (30) did not. Average age was 63 ± 14 years; and subjects with MS were older than those without (54 ± 16 vs. 66 ± 11, $P < 0.0001$). Male gender was more prevalent in subjects with MS (78 [72%] vs. 23 [48%], $P = 0.003$) than in those without. Smoking status was comparable between groups.

MS Traits

The average BMI in patients with MS was 30.6 ± 3.6 kg/m^2, 62% (69%) had impaired fasting glucose, 98% (90%) had hypertension, and 81% (75%) had dyslipidemia. Concerning treatments for these conditions, 77% of the cases with hypertension were on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and all of those with dyslipidemia were on statins or fibrates. Finally, patients with MS had higher levels of uric acid 5.3 ± 2.3 versus 3.8 ± 2.4 ($P < 0.0001$).

Parameters of Renal Function

eGFR was comparable between the groups. However, the number of cases of eGFR <60 ml/min was higher in subjects with MS than in those without MS: 24% (23%) versus 4% (8%), ($P = 0.03$). Urinary albumin excretion assessed by albumin-to-creatinine ratio or 24-hour albuminuria were numerically higher in patients with MS than in those without but of borderline significance ($P = 0.05$).

Renal Histology in Patients With and Without MS

Glomerular Lesions

The median number of glomeruli evaluated per case was 172 (116–222). No differences were observed between groups (Table 2). However, the cases with global sclerosis were 2 times higher in those with MS than in those without MS (10 [5–21] 7% vs. 5% [2–11] 3%, $P = 0.001$). Nodular sclerosis was found almost always in patients with MS (20 [17] vs. 1 [2], $P = 0.002$). Mesangial expansion and glomerulomegaly were more frequent in patients with MS (Figure 1).

Interstitial Lesions

Tubular atrophy, interstitial fibrosis, interstitial inflammation were mild and comparable between groups (Table 2).

Table 2. Morphological changes in renal tissue in patients with and without metabolic syndrome

	Total	MS- NO	MS- YES	
***N* and *n* (%)**	**157**	**49 (30)**	**108 (70)**	***P* value**
Glomerular lesions				
Glomeruli: *N*, median (IQR)	172 (116–222)	179 (107–247)	159 (117–218)	ns
Glomeruli with GS, median (IQR)	13 (7–27)	5 (2–11)	10 (5–21)	0.01
Percentage with GS of total Glomeruli	10 (4–17)	3 (1–7)	7 (3–13)	<0.0001
Glomeruli with NS				
Cases with NS (%)	21 ()	1 (2)	20 (18.5)	0.002
N, median (IQR) of glomeruli affected[a]	5 (2–19)	—	2 (1–4)	
Interstitium and tubuli				
Tubular atrophy				
<5%	77 (49)	29 (59)	48 (45)	ns
5%–10%	72 (46)	19 (39)	53 (49)	ns
10%–20%	2 (1.3)	1 (2)	1 (1)	ns
>20%	5 (3.2)	0	5 (5)	ns
Interstitial fibrosis				
<5%	77 (49)	28 (57)	49 (45)	ns
5%–10%	56 (37)	14 (29)	42 (39)	ns
10%–20%	20 (13)	6 (12)	14 (13)	ns
>20%	4 (2)	1 (2)	3 (3)	ns
Inflammation				
<5%	137 (87)	45 (91)	92 (85)	ns
5%–10%	5 (3)	0	5 (5)	ns
10%–20%	8 (5)	3 (6)	5 (5)	ns
>20%	7 (5)	1 (2)	6 (5)	ns
Vascular lesions				
Arteriolar hyalinosis				
Mild	40 (25)	20 (41)	20 (18)	
Moderate	114 (73)	29 (59)	85 (79)	
Severe	3 (2)	0	3 (3)	0.002[a]
Fibrointimal thickening				
Mild	44 (28)	19 (39)	25 (23)	
Moderate	105 (67)	28 (57)	77 (71)	
Severe	8 (5)	2 (4)	6 (6)	0.035[a]

GS, global sclerosis; IQR, interquartile range; MS, metabolic syndrome; ns, not significant; NS, nodular sclerosis.
[a]Moderate and severe lesions considered together.

Vascular Lesions

Arteriolar Hyalinosis. Moderate hyalinosis was highly frequent in both groups and severe hyalinosis was observed only in patients with MS (Table 2). Considering together moderate + severe hyalinosis, the lesions were more frequent in those with MS (88 [82] vs. 29 [59], $P = 0.002$) (Figure 2). In subjects with moderate + severe hyalinosis, moderate and severe fibrointimal thickening (arteriosclerosis) was observed in 86% of the cases (101 of 117).

Fibrointimal Thickening. Moderate arteriolar sclerosis was highly prevalent, affecting 60 to 76% of the cases (Table 2). Considering together moderate + severe lesions were more frequent in those with MS (83 [77] vs. 30 [61], $P = 0.035$) (Figure 2). In subjects with moderate and severe fibrointimal thickening (arteriosclerosis), moderate + severe hyalinosis was observed in 89% of the cases (101 of 113).

Sensitivity Analyses

Interstitial fibrosis (10% [45%] of 22 vs. 18% [14%] of 133; $P = 0.001$), inflammation (6% [43%] of 14 vs. 22% [16%] of 140; $P = 0.022$), and arteriosclerosis (4% [9%] of 44 vs. 24% (22%) of 110; $P = 0.049$) were more frequent in subjects with eGFR <60 ml/min and in those with higher levels. Tubular atrophy and arteriolar hyalinosis were comparable between groups.

In multivariate logistic regression, the variables associated with arteriolar hyalinosis were MS (odds ratio [OR]: 3.0, CI: 1.4–6.8, $P = 0.005$) and smoking (OR: 3.5, CI: 1.3–9.8, $P = 0.015$); age and angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers were not significant. When MS was replaced by its traits, hypertension (OR: 2.7, CI: 1.3–5.8, $P = 0.013$) and prediabetes (OR: 2.7, CI: 1.1–6.5, $P = 0.031$) were significantly associated with arteriolar hyalinosis, dyslipidemia reached borderline signification (OR: 2.2, CI: 0.9–5, $P = 0.056$), and obesity was not significant. The variables associated with arteriosclerosis were MS (OR: 3.65, CI: 1.04–13, $P = 0.043$), active smoking (OR: 3.2, CI: 1.1–48.4, $P = 0.02$), age (OR: 2.7, CI: 1.2–6, $P = 0.02$); the use of angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers was not significant. No single component of MS was significant when they replaced MS in the model.

Arteriolar hyalinosis was correlated with age, smoking, diverse MS traits (obesity, prediabetes, hypertension, and dyslipidemia), mesangial expansion, glomerulosclerosis, tubular atrophy, fibrosis, and inflammation (Supplementary Table S1). Arteriosclerosis was correlated with age, smoking, high-density lipoprotein cholesterol levels, glomerulosclerosis, tubular atrophy, fibrosis, and inflammation (Supplementary Table S1).

DISCUSSION

Our main finding was that patients with MS had an increased rate of vascular damage (arteriolar hyalinosis and arteriosclerosis) than those without MS. In addition, glomerular damage (glomerulosclerosis, glomerulomegaly, increased mesangial expansion, and nodular sclerosis) were more frequent in subjects with MS. These findings may indicate a relevant role of ischemic renal disease in MS, together with obesity-related glomerular damage and lesions comparable to those of diabetic nephropathy.

In clinical practice, patients with obesity and MS rarely undergo a renal biopsy. This is due to the clinical characteristics of renal disease in this population, that is, slow progression toward CKD and the absence

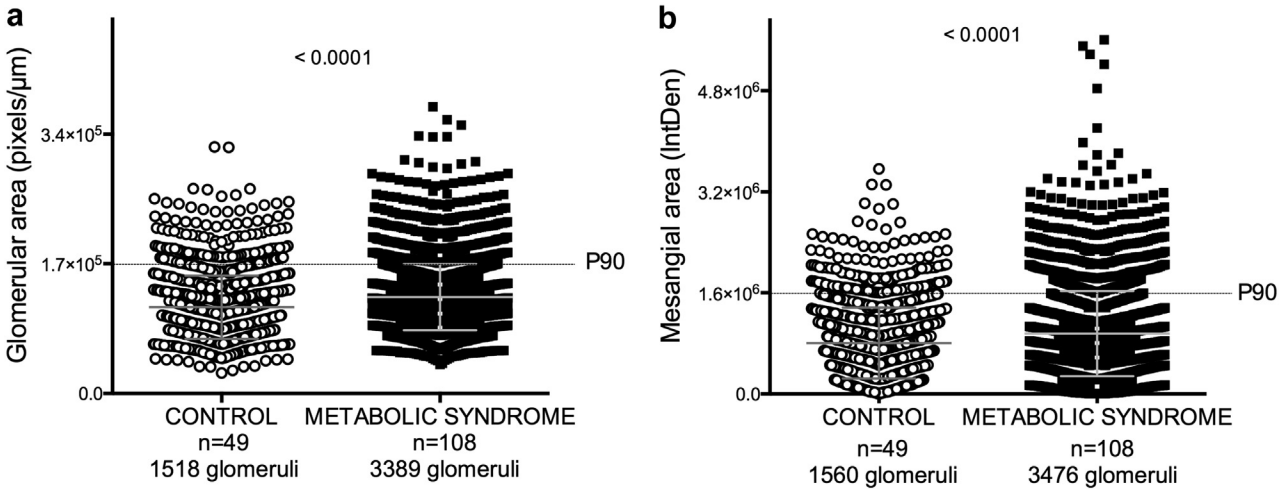

Figure 1. Glomerular area and mesangial expansion in patients with and without metabolic syndrome.

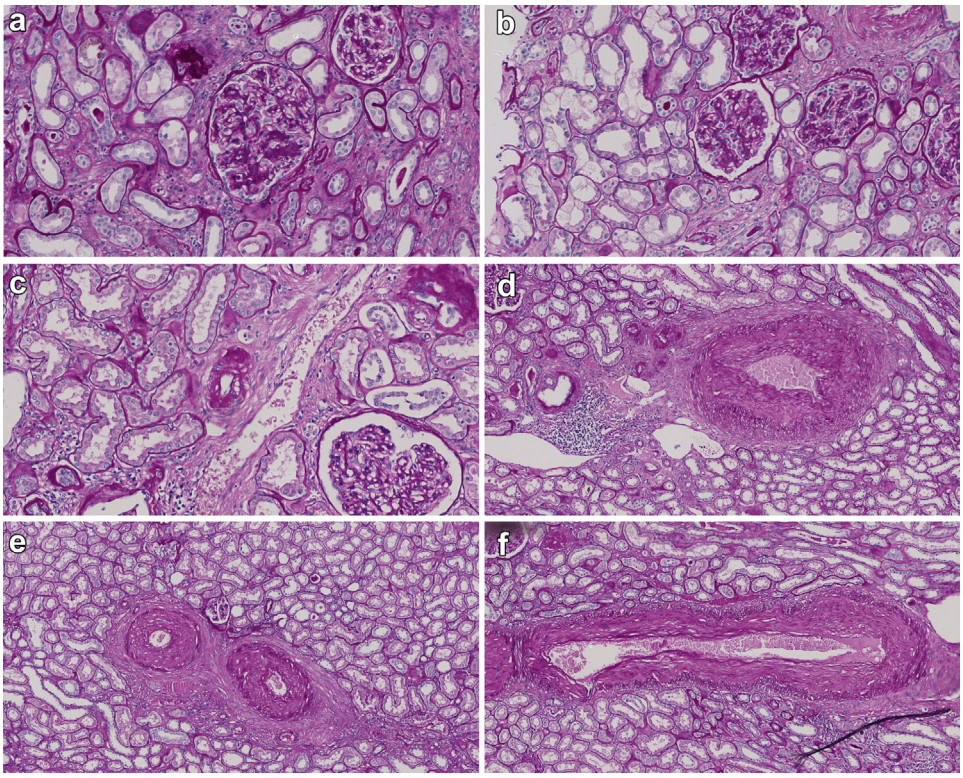

Figure 2. Representative cases of histological changes in patients with metabolic syndrome (a) mesangial expansion, (b) mesangial expansion, (c) arteriolar hyalinosis, (d) arteriolar hyalinosis and arteriosclerosis, (e) arteriosclerosis, and (f) arteriosclerosis.

of proteinuria. This has determined a limited and rather theoretical understanding of renal damage in obesity and MS. The ENBiBA project was developed to address this problem by studying the unaffected renal tissue of nephrectomy specimens, to ensure enough material for analysis.

The main finding of our study was that patients with MS had moderate and severe vascular disease. In fact, about 80% of the cases with MS had arteriolar hyalinosis and arteriosclerosis, indicating a role of ischemic disease in obese subjects with MS. This is an unexpected finding, and its interpretation is not simple. Cardiovascular disease is one of the most frequent causes of mortality and morbidity in patients with obesity and MS.[17] Obesity, hypertension, hyperglycemia, and dyslipidemia are known to induce endothelial and vascular damage. Thus, our data may indicate that these factors also affect renal vessels, namely arterioles, arteries, and peritubular vessels, a fact that may lead to intrarenal ischemia. In this line, a previous study of the ENBiBA group found that vascular damage was frequent and severe in patients with diabetes. In fact, in an analysis of 90 patients, 80% to 90% of the cases have arteriolar hyalinosis and arteriosclerosis.[11] The presence of the same vascular damage in patients with MS and obesity but without diabetes may indicate that vascular disease can start before the onset of diabetes in the context of obesity and MS. Therefore, ischemic renal disease may represent a common link between diabetes and MS in the development of renal damage. Several studies observed that renal hypoxia may be involved in the pathogenesis of CKD.[18,19] The kidneys represent less than 0.5% of total body weight but receive 20% to 25% of the cardiac output.[18,19] The majority of this amount of blood flow goes to the cortex. However, despite this "overflow," the capacity of oxygen extraction of kidney tissue is low, that is, 10% to 20%. Therefore, the kidney is extremely inefficient in oxygen extraction.[18-20] The causes of this aspect of renal physiology are not clear; however, the nature of the intrarenal vasculature, with multiple arteriovenous shunts may play a role in the limitation of kidney cells for oxygen extraction. These characteristics make the kidney particularly susceptible to hypoxia. According to our findings showing relevant vascular damage in subjects with MS, we may consider that renal ischemia and cellular hypoxia play a relevant role in the development of renal disease in this population. In addition, vascular changes (arteriolar hyalinosis and arteriosclerosis) were independent of age. This finding might indicate the role of accelerated senescence in intrarenal vascular damage in obesity and MS

changes.[19,21] To the best of our knowledge, this is one of the first reports that describe the hypothesis of vascular damage in the development of renal disease in MS. Clearly, this hypothesis must be tested in *ad hoc* designed studies.

Another finding of our study was that patients with MS had structural changes compatible with diabetic nephropathy, that is mesangial expansion + nodular sclerosis. These results are interesting but not necessarily unexpected. Previous studies observed that lesions compatible with diabetic nephropathy may be observed in obese patients without diabetes.[9,10,22] Moreover, D'Agati *et al*.[10] observed that about 50% of obese patients (mean BMI of 40 kg/m^2) who underwent a renal biopsy due to reduced GFR and/or proteinuria had changes compatible with diabetic nephropathy in the absence of diabetes.[9] The prevalence of similar changes in our group was about 20% (based on the cases with nodular lesions). The difference between both series may be that we included patients with less severe obesity (average BMI of 30 kg/m^2) or even overweight and without evidence of overt renal disease, that is, proteinuria. Therefore, our results may indicate that features of diabetic nephropathy may start at early stages of obesity in nondiabetic subjects with MS. Finally, patients with MS had glomerulomegaly, possibly as the consequence of obesity, as has been reported before in humans[9,10] and animal studies.[23,24] Mesangial expansion, nodular sclerosis, and glomerulomegaly reflect glomerular damage from diverse pathogenic factors observed both in MS and diabetes, such as hyperglycemia, dyslipidemia, hypertension, obesity, inflammation, and insulin resistance, among the most relevant. In fact, in animal models of obesity and insulin resistance, increased mesangial expansion and nodular sclerosis are not uncommon.[23,24] Another factor important to consider is smoking, which was significantly associated with vascular damage in multivariate models. Previous studies observed an association between intrarenal vascular damage and mesangial expansion and nodular sclerosis in the glomeruli.[25,26] Clearly, smoking may have played a role in vascular damage in our population with and without MS.

MS is a construct of diverse traits, all of them with a different association with renal disease. The presence of prediabetes and hypertension were independently associated with arteriolar hyalinosis in multivariate analysis. This may indicate that hyperglycemia and hypertension may play a stronger role in arteriolar damage in obese patients. However, no specific MS trait was associated with arteriosclerosis in the analysis. This finding is intriguing and may indicate that the sum of the components of MS may be more important than the individual role of the traits in renal disease. Finally, it is worth considering that establishing limits between diseases that represent *per se* a metabolic continuum, such as obesity, MS, and prediabetes-diabetes, may be misleading. We need practical definitions in clinical medicine. However, in human biology, practicality has to deal with process strongly interrelated. For example, the fact that hyperglycemia in the nondiabetic range—prediabetes—was independently associated with vascular damage and glomerular lesions, similar to that observed in patients with established diabetes, may indicate a continuum in renal disease between prediabetes and diabetes. Our results, considered together with our previous report on diabetes, may indicate a continuum for renal damage between obesity, MS, and diabetes. Nevertheless, this hypothesis is worth investigating in future studies.

Interestingly, eGFR was comparable between groups; however, the proportion of patients with eGFR <60 ml/min was lower in patients with MS than in those without MS. This may indicate the impact on renal function of the glomerular and vascular lesions observed in the context of MS. Previous studies identified MS, its components, and insulin resistance as risk factors for rapid GFR decline and CKD.[8] Our analysis confirms the relationship between MS and renal function loss and may indicate the histological background of this association. Another finding was that tubular damage was mild and comparable between groups. This is intriguing, particularly after observing that patients with MS have lower renal function. There is no simple explanation for this finding. We may argue that vascular and glomerular lesions are not severe and widespread enough to induce relevant tubular fibrosis. In addition, it is known that the kidney has an important capacity for compensation to damage, which may be linked to renal reserve. In any case, this lack of association between tubular damage, vascular disease, and renal function deserves detailed attention in future studies.

This study has limitations. The most important is that this is a cross-sectional study; therefore, the impact of vascular damage on renal function changes over time cannot be evaluated. This must be studied in *ad hoc* designed studies. In addition, the role of ischemia in renal disease must be tested with mechanistic analysis in models of disease. Finally, a limitation *per se* is the definition of MS. In clinical research, MS is used as a simple approach to evaluate insulin resistance because this condition is not simple to measure. Most of the definitions of MS combine diverse traits such as hypertension, obesity, dyslipidemia, and hyperglycemia. In general, the presence of at least 3 of them is required to diagnose MS.

However, the definitions of MS are not free of critique. The first is the lack of clear background of the cut-off points for MS traits. Thus, different combinations of traits may lead to diverse conditions with diverse impacts in major outcomes such as CKD. Finally, considering treated conditions such as hypertension or dyslipidemia as a positive trait may lead to variability. These may indicate that the population with MS is *per se* heterogeneous in reflecting insulin resistance, a fact that may limit the results of the study. However, this is to date a simple approach to evaluate insulin resistance that is particularly helpful in epidemiological studies. Therefore, future research is needed to confirm our hypothesis.

In conclusion, we found that vascular damage and lesions compatible with diabetic nephropathy may be relevant players in renal damage in the context of obese patients with MS but without diabetes. This information may help both for preventing and treating CKD in this population.

APPENDIX

List of the DIABESITY working group of the European Renal Association

Center Name	City	Country	Principal investigator
University Clinical Center	Maribor	Slovenia	Radovan Hojs, Sebastjan Bevc
Hospital Universitario Fundación Alcorcón	Madrid	Spain	Gema Fernández, Clara María Cases Corona
Hospital de Bellvitge	Barcelona	Spain	María Quero, Laia Pujol, Sergi Beato Montserrat Gomà and Josep Cruzado
Hospital Sant Joan Despí Moisès Broggi	Barcelona	Spain	Meritxell Ibernon
Hospital Universitario Vall d'Hebron	Barcelona	Spain	Francisco Moreso, Marina López-Martínez
Rigshospitalet	Copenhagen	Denmark	Mads Hornum, Bo Feldt-Rasmussen
IIS-Fundación Jiménez Díaz-UAM	Madrid	Spain	Alberto Ortíz, Beatriz Fernández-Fernandez, Elena Gomá-Garces, Teresa Stock da Cunha, Ana B Sanz; María Garranzo, Carmen Gonzalez-Enguita, Ana María Autrán-Gómez; Pablo Cannata
Galilee Medical Center	Galilee	Israel	Khalid Khazim, Fedaa Ghanem
Hospital Universitario de Canarias	Tenerife	Spain	Esteban Porrini, Rosa Rodríguez-Rodríguez, Natalia Negrín Mena, Tomás Concepción
Hospital de Santa Cruz	Lisboa	Portugal	Ivo Laranjinhia
Centro Hospitalar Lisboa Norte	Lisboa	Portugal	Luís Mendonça
Centro Hospitalar São João	Porto	Portugal	Miguel Bigotte Vieira
Ospedale San Raffaele	Milano	Italy	Trevisani Francesco, Arianna Bettiga, Federico Di Marco, Andrea Salonia, Francesco Montorsi, Dell'Antonia Giacomo
Hospital 12 de Octubre	Madrid	Spain	Enrique Morales, Manuel Praga

DISCLOSURE

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AUTHOR CONTRIBUTIONS

EP had the idea of the study and designed the protocol. RR-R did the histological analysis of all samples. AERR participated in data analysis, and in the morphometric tests. MH and EM discussed several aspects of the results. All authors collaborated in the design of the protocol. All authors contributed with samples and data of the subjects included in the study. All authors read the final manuscript of the study.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Correlations between vascular lesions, clinical variables, and other histological parameters (Kendall's Tau-b).

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