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Prevalence and causes of microscopic haematuria in Type 1 (insulin-dependent) diabetic patients with persistent proteinuria

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Summary. The prevalence and causes of microscopic haematuria were examined in all Type 1 (insulin-dependent) diabetic patients with persistent proteinuria (diabetes duration ≥ 5 years) attending the outpatient clinic at Hvidöre Hospital during 1985. One hundred eighty-four patients (69 F/115 M) out of 1024 Type 1 patients had persistent proteinuria (18%). Microscopic haematuria was defined as ≥ 3 erythrocytes per high power field in two or more sterile urine samples. Twenty-three Type 1 patients with persistent proteinuria (7 F/16 M, aged 35.4 ± 13 years) had microscopic haematuria (12.5%). No significant changes were found between the group with and without microscopic haematuria: blood pressure 148/89 ± 22/11 versus 145/91 ± 20/11 mmHg, duration of diabetes when persistent albuminuria occurred 17 ± 8 versus 20 ± 10 years, serum creatinine 99 ± 24 versus 98 ± 31 μmol/l, simplex retinopathy 61 versus 54%, proliferative retinopathy 39 versus 42%, and no signs of retinopathy 0 versus 4%. Kidney biopsy was performed in 13 out of the 23 patients with microscopic haematuria. Diabetic glomerulosclerosis was present in all 13 patients, but 9 patients had a non-diabetic renal disease superimposed (mesangioproliferative glomerulonephritis ($n = 5$), membranous glomerulonephritis ($n = 3$) and sarcoidosis ($n = 1$). Microscopic haematuria is a rare finding, frequently reflecting superimposed non-diabetic glomerulopathies, in Type 1 diabetic patients with diabetic nephropathy and well preserved kidney function.

Key words: Microscopic haematuria, persistent proteinuria, diabetic glomerulosclerosis, non-diabetic glomerulopathy, Type 1 diabetes.

Histological evidence of diabetic glomerulosclerosis is present in approximately 90% of Type 1 (insulin-dependent) diabetic patients with diabetes of more than 10 years' duration [1, 2]. Persistent proteinuria, a decline in glomerular filtration rate and elevated blood pressure, i.e. diabetic nephropathy, will develop in only half of these patients [3, 4]. Diabetic nephropathy is diagnosed clinically if the following criteria are fulfilled: persistent proteinuria, diabetic retinopathy, diabetes of more than 10 years' duration, and no clinical or laboratory evidence of kidney or renal-tract disease other than diabetic glomerulosclerosis [5, 6]. According to this definition, microscopic haematuria is regarded as a sign of a non-diabetic glomerulopathy. However, two recent studies have suggested that microscopic haematuria is a frequent and an early sign of diabetic nephropathy [7, 8].

In order to elucidate this diagnostic dilemma, we examined the prevalence and causes of microscopic haematuria in all Type 1 diabetic patients with persistent proteinuria attending the outpatient clinic at Hvidöre Hospital during 1985.

Subjects and methods

Patients

We examined the records of all Type 1 diabetic patients visiting the outpatient clinic at Hvidöre Hospital during 1985. All patients fulfilling the following criteria were included: age ≥ 18 years, onset of diabetes before the age of 41 years and diabetes duration ≥ 5 years. We identified 1024 patients. All were insulin-dependent from the time of diagnosis. Persistent albuminuria (> 300 mg/24 h in two out of three 24-h urine collections at home) was demonstrated in 69 females and 115 males (18%).

Methods

As part of our detection program for microvascular lesions, urine testing with Albustix was performed in all patients at each visit to the outpatient clinic (approximately 4 times a year). In case of repeatedly positive Albustix readings, determination of 24 h urinary albumin excretion was performed using a radial immunodiffusion technique [9] or a radioimmunoassay [10]. The latter method was routinely applied after 1984. Microscopic examination of the urinary sediment was performed in a midstream specimen in all patients with persistent albuminuria ($n = 184$). Eight ml of urine was centrifuged at 1500 rpm for 5 min. The supernatant was removed, and resuspended

Table 1. Clinical data in 184 Type 1 (insulin-dependent) diabetic patients with persistent proteinuria

Patients	Number and sex	Age (years)	Duration of diabetes at onset of persistent proteinuria (years)	Retinopathy (%)	Serum creatinine (μmol/l)	Arterial blood pressure (mmHg)	Frequency of arterial hypertension (%)
Normal urine microscopy	161 (62 F/99 M)	38 ± 14	20 ± 10	96	98 ± 31	145/91 ± 20/11	48
Significant haematuria	23 (7 F/16 M)	35 ± 13	17 ± 8	100	99 ± 24	148/89 ± 22/11	44

Data obtained at the onset of persistent proteinuria or at the time when a proteinuric patient was referred to our hospital

Table 2. Clinical data in 23 Type 1 (insulin-dependent) diabetic patients with persistent proteinuria and microscopic haematuria

Patient no[a]	Age/Sex	Duration of diabetes (years)	Retinopathy[b]	Arterial blood pressure (mmHg)	Serum creatinine (μmol/l)	Albuminuria (mg/24 h)	Urine microscopy		
							Red	White	Casts
1	57/F	28	P	190/105	90	451	2-4	0-1	0
2	35/M	14	P	125/85	82	375	2-5	3-10	0
3	27/F	16	P	135/85	65	350	5-8	0	0
4	37/M	10	S	125/90	115	936	2-5	0	0
5	60/F	29	S	130/70	113	1308	2-4	4-6	0
6	27/M	12	S	170/95	108	363	5-10	0	0
7	64/M	40	S	160/80	103	606	2-5	2-4	0
8	43/M	14	S	185/95	95	2970	5-10	2-5	0
9	49/M	27	P	150/80	123	677	2-5	1-3	0
10	47/M	12	S	130/80	64	1364	2-4	0-2	0
11	31/M	14	S	135/90	122	1889	3-5	0	0
12	25/M	18	S	150/100	118	4304	8-10	0	0
13	25/M	13	S	140/80	72	1099	3-5	0	0
14	23/M	7	S	110/80	133	500	3-7	0	0
15	32/M	19	P	180/110	96	1300	3-6	2-4	0
16	29/F	14	S	140/80	76	3894	4-6	0	0
17	23/F	11	P	140/85	56	2262	5-10	0	Granular
18	27/M	12	P	160/95	141	1191	5-15	6-20	Erythrocytes
19	20/M	15	P	180/100	88	3000	4-10	0	0
20	30/M	13	S	160/104	100	1001	5-15	0	Erythrocytes
21	39/F	19	S	120/80	119	821	2-4	2-4	0
22	16/F	12	S	130/80	75	1000	2-5	0	0
23	49/M	20	P	160/95	113	3224	10-12	0	Granular

[a] In patients nos. 11 to 23 a kidney biopsy was performed.
[b] P = proliferative; S = simplex

Table 3. Renal pathology in Type 1 (insulin-dependent) diabetic patients with persistent proteinuria and microscopic haematuria

Patient no.	Light microscopy		Immunofluorescense microscopy					
	Diabetic glomerulosclerosis	Non-diabetic glomerular lesions	Immunoglobulins	Complement fractions	Localisation in glomerulus			
					Mesangium	Capillary	Global	Segmental
11	Nodular	Membranous GN	IgM, IgA	C_{1q}, C_3	+	0	+	+
12	Diffuse	Sarcoidosis (Interstitial lesion)	IgM	C_{1q}, C_3	+	+	+	+
13	Diffuse	Mesangioproliferative GN	IgM, IgA	C_3	+	0	+	+
14	Diffuse	Membranous GN	IgM	C_3	+	+	+	+
15	Nodular	Absent	0	0	−	−	−	−
16	Diffuse	Mesangioproliferative GN	0	0	−	−	−	−
17	Diffuse	Absent		C_{1q}, C_4	+	0	0	0
18	Nodular	Mesangioproliferative GN			No Tissue			
19	Diffuse	Membranous GN	IgM, IgA	C_{1q}, C_3, C_4	+	+	+	+
20	Nodular	Mesangioproliferative GN	IgM	C_3	+	+	+	0
21	Diffuse	Mesangioproliferative GN	IgM, IgA	C_3	+	+	+	+
22	Diffuse	Absent	IgG, IgM	0	+	0	+	0
23	Nodular	Absent	IgM	0	+	0	+	0

GN = Glomerulonephritis

sediment (about 0.15 ml) was examined with phase-contrast microscopy. Significant haematuria is defined as 3 or more erythrocytes per high power field on urinalysis of two or more sterile urine samples. Serum creatinine concentration was measured yearly [11]. Blood pressure was measured at least once a year in the sitting position after 10 min rest with a standard clinical sphygmomanometer (cuff size 25 × 12 cm) on the right arm. Arterial hypertension was diagnosed according to WHO criteria [12] (≥ 160/95 mmHg) or if antihypertensive medication was applied. Ophthalmoscopy through dilated pupils was carried out at least yearly.

All 23 patients with persistent proteinuria and significant microscopic haematuria were referred for further examination to the department of nephrology. Two patients (nos. 5 and 8) did not attend. Kidney biopsy was not performed in eight patients because of minimal albuminuria (< 1 g/24 h) in five patients (nos. 1-4, 6), anticoagulant treatment in two patients (nos. 7, 9), and nephrolithiasis on intravenous pyelogram in one patient (no. 10). A renal biopsy was performed in the remaining 13 patients. Techniques of the examination of renal tissue samples are described in detail elsewhere [12], and are summarized as follows:

Light microscopy. The tissue was fixed in 4% formaldehyde buffered to pH 7.0 ("Lillie's fluid"), embedded in Paraplast® (Fisher Company, Fair Lawn, New Jersey, USA), cut into 4, 3 and 2 μm sections and stained with haematoxylin-eosin, periodic-acid Schiff, picric acid + sirius red, Masson's trichrome and silver methenamine + haematoxylin-eosin.

Immunofluorescent microscopy. Kidney specimens were frozen using dry ice, embedded in Tissue-Teck (Miles, Naperville, Illinois, USA)-gelatine, and sections of 2 μm were cut at −24 °C on a Leitz Histocryotome (Wetzlar, FRG). Direct immunofluorescent staining technique was applied, using FITC-conjugated rabbit or goat antisera specifically reactive to human IgG, IgM, IgA as well as complement C_{1q}, C_3, and C_4 [13].

Histological classification of the glomerular lesions was according to the World Health Organization standard [14]. Duration from onset of persistent proteinuria to renal biopsy was 2.5 years, range 0.5 to 5 years.

Statistical analysis

Data are given as mean ± SD. Statistical analysis was performed using Mann-Whitney's test for unpaired comparison. A p value of < 0.05 was considered statistically significant.

Results

The clinical characteristics and pertinent laboratory findings are summarized in Table 1. There were no significant differences in age, duration of diabetes at onset of persistent proteinuria, retinopathy, serum creatinine and arterial blood pressure between patients with and without haematuria. Our material showed a male predominance, particularly in those with haematuria. The prevalence of significant haematuria was 12.5% in our 184 Type 1 diabetic patients with persistent proteinuria.

Serum creatinine was < 150 μmol/l in all our patients with haematuria, while 13/161 patients without haematuria had a serum creatinine > 150 μmol/l.

The individual data in the 23 patients with haematuria are presented in Table 2. Diabetic glomerulosclerosis (nodular, $n = 5$; diffuse, $n = 8$) was demonstrated in all 13 patients having a kidney biopsy performed because of persistent proteinuria and significant haematuria, Table 3. However, superimposed non-diabetic glomerular lesions were demonstrated in 8 patients. Mesangioproliferative glomerulonephritis ($n = 5$) was defined by more than 9 mesangial cells in one or more of the glomerular segments. Membranous lesions ($n = 3$) were characterized by pink subepithelial deposits and/or black "spikes" in some of the capillary loops in all the glomeruli.

Data of the different immundeposits and their localization are shown in Table 3. The deposits have a brilliant staining and a fine or coarse granular pattern with a diffuse distribution to all the glomeruli represented in the biopsy. The distribution in the individual glomeruli was more or less global, but in some of the cases had a pronounced fluorescence in segmental areas. In all biopsies a dull applegreen fluorescence of IgG was found in many of the tubular and all the glomerular basement membranes.

Discussion

Our study has revealed a low prevalence (12.5%) of significant haematuria in a consecutive series of 184 Type 1 diabetic patients with persistent proteinuria. The urinalysis was performed at the onset of persistent proteinuria or at the time when a proteinuric patient was referred to our hospital. All our patients had a serum creatinine < 150 μmol/l, except 13 patients without haematuria. In contrast, O'Neill et al. [7] have demonstrated significant haematuria in 9 out of 30 insulin-treated Type 1 and Type 2 (non-insulin-dependent) diabetic patients with persistent proteinuria and a mean serum creatinine of 171 μmol/l. None of these patients underwent a renal biopsy or further nephrologic evaluation. Hermann et al. [8] have reported an even higher prevalence of significant haematuria (68%) in 19 Type 1 diabetic patients with persistent proteinuria and a serum creatinine ≥ 177 μmol/l. The discrepancy between our study and the two above-mentioned investigations cannot be explained by differences in the applied criteria of significant haematuria, since these criteria were practically identical. Even though O'Neill et al. [7] investigated a heterogenous group of insulin-treated diabetic patients, this cannot explain the controversy, since all the patients in the study of Hermann et al. [8] had Type 1 diabetes. Hermann et al. [8] suggested that the high prevalence of arterial hypertension in his material (100%) may contribute to the development of microscopic haematuria. This confounding variable can be ruled out in our study, since arterial blood pressure was nearly identical in patients with and without significant haematuria. The differences in kidney function may well explain the observed differences in prevalence of haematuria. Our patients were examined when serum creatinine was

normal or near normal, while serum creatinine was clearly elevated in the two other studies.

The causes of microscopic haematuria were elucidated by kidney biopsy in 13 out of our 23 patients. Diabetic glomerulosclerosis was demonstrated in all 13 patients, but superimposed non-diabetic glomerular lesions were demonstrated in 8 of these patients. Immunofluorescence microscopy revealed a generalized distribution of granular deposits of immunoglobulins together with complement C_3 in the glomeruli. This suggests an immune complex pathogenesis of the superimposed glomerular lesions not different from that in human glomerulonephritis [15]. Diabetic glomerulosclerosis per se is characterized either by no deposits at all [15] or a focal distribution of immunoglobulins or complements in the glomeruli [16]. The minor differences in immune pattern and localization of the glomerular deposits compared to human glomerulonephritis might be due to modification in the development of glomerulonephritis lesions in glomeruli already damaged by diabetic lesions. The occurrence of non-diabetic glomerulopathy in diabetic patients with persistent proteinuria is well documented, as reviewed by Kasinath et al. [17]. Furthermore, microscopic haematuria is frequently present in such cases [17–19].

We conclude that microscopic haematuria is a rare finding, frequently reflecting superimposed non-diabetic glomerulopathies, in Type 1 diabetic patients with diabetic nephropathy and well preserved kidney function. We recommend further nephrological examination including percutaneous kidney biopsy in proteinuric Type 1 diabetic patients with significant haematuria.

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