Retinal and renal complications in patients with a mutation of mitochondrial DNA at position 3,243 (maternally inherited diabetes and deafness). A case–control study

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Abstract

Aims/hypothesis We assessed the prevalence and determinants of retinal and renal complications in patients with maternally inherited diabetes and deafness (MIDD).

Methods This was a multicentre prospective study comparing the prevalence of retinopathy and renal disease in 74 patients with MIDD and 134 control patients matched for sex, age and clinical presentation at onset of diabetes, duration of diabetes and current treatment. Comparisons were adjusted for HbA_{1c} and hypertension.

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Results In MIDD patients, HbA_{1c} (7.6 ± 1.6 vs $8.5 \pm 2.0\%$, $p < 0.002$), systolic blood pressure (126.6 ± 16.2 vs 133.1 ± 17.3 mmHg, $p < 0.007$) and prevalence of hypertension (33.8 vs 64.2% , $p < 0.0001$) were lower than in control patients. Prevalence of diabetic retinopathy was 3.7-fold lower in MIDD patients (6/74, 8 vs 40/134, 29.6%, $p < 0.0001$). Differences between groups remained significant after adjustment for hypertension, systolic blood pressure and HbA_{1c}. In MIDD, urinary albumin excretion (314.8 vs 80.1 mg/24 h, $p = 0.035$) and creatinine plasma levels (103.5

vs 82.2 μ mol/l, $p = 0.0178$) were higher and GFR was lower. Impaired renal function (GFR < 60 ml/min) was four- to sixfold more frequent in MIDD. Differences between MIDD and control diabetic patients further increased when adjusted for HbA_{1c} and systolic blood pressure ($p < 0.0001$). Adjustment for treatment with an ACE inhibitor or angiotensin II receptor antagonist did not modify the results.

Conclusions/interpretation This study indicates that diabetic retinopathy is less prevalent in MIDD than in control diabetes. This suggests that retinal alterations due to mitochondrial disease may have a protective role. By contrast, nephropathy is far more frequent in MIDD, suggesting the presence of a specific renal disease independent of diabetic nephropathy.

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Keywords Diabetes mellitus · Diabetic nephropathy · Macular pattern dystrophy · Maternally inherited diabetes and deafness · Mitochondrial 3243 mtDNA mutation · Mitochondrial nephropathy · Prevalence · Renal failure · Retinopathy

Abbreviations

ARA II angiotensin II receptor antagonist
MDRD Modification of Diet in Renal Disease
MIDD maternally inherited diabetes and deafness

Introduction

Maternally inherited diabetes and deafness (MIDD) is the type of diabetes most frequently related to mitochondrial disease. It is associated with a point mutation of mitochondrial DNA, an A to G transition at position 3243 (m.3243 A>G) encoding for transfer RNA leucine (tRNA^{Leu[UUR]}) [1, 2]. We previously reported the MIDD phenotype in 54 patients from a multicentre study [3] and found a low 7.5% frequency of diabetic retinopathy, while kidney function was impaired in 75% of patients. We hypothesised that the retina was protected against microangiopathy/diabetic retinopathy by a reduction in oxygen requirements as a result of the specific pigmentary lesion, the so-called pattern dystrophy, which was present in 85% of these patients [3–5]. By contrast, in a series of Japanese patients with MIDD, a 13.4% prevalence of pattern dystrophy and a 55% prevalence of diabetic retinopathy were observed [6].

The aim of the present study was thus to ascertain, in a larger series of European patients with MIDD, the prevalence of diabetic retinopathy and renal function abnormalities compared with that in patients with 'common' forms of diabetes matched for sex, age at onset, type, treatment and duration of diabetes. We also investigated the potential role of the other determinants of diabetic complications, i.e. high blood pressure and hyperglycaemia [7–9].

Methods

Patients The French Mitochondrial Diabetes Study Group (GEDIAM) multicentre prospective study [3] was initiated in September 1995. At the time of the study, 74 patients with MIDD (from 57 families) were included. All participants were Euroid. The A3243G mutation was present in all of them. Diabetes was classified according to its clinical presentation at onset [10], i.e. insulin-dependent ($n=12$) and non-insulin-dependent ($n=62$). Non-insulin-dependent diabetes was sub-classified, according to treatment at the time of the study, into insulin-requiring ($n=32$) and non-insulin-treated diabetes ($n=30$). These patients were compared with 134 unrelated patients with the ‘common’ subtypes of diabetes (type 1 and 2) recruited from the Department of Clinical Immunology and Diabetology of Cochin Hospital (Paris, France) and referred to as ‘controls’ in the present report. These control diabetic patients were free of the m.3243 A>G mutation as well as of other monogenic diabetes, including MODY. Control diabetic patients were matched to patients with MIDD according to sex, age at onset of diabetes, clinical presentation at onset (i.e. insulin dependency or non-insulin dependency), duration of diabetes and current treatment (diet and/or glucose-lowering oral agents and/or insulin). Matching was performed with two controls for one patient with MIDD in 59 cases and with one control for one patient with MIDD in 16 cases (Table 1). The study conformed to the principles of the Declaration of Helsinki and participants gave informed consent.

Methods A structured interview and a standardised examination of the patients were used to ascertain family and personal history of diabetes, hearing loss, treatment, associated manifestations, and retinal and renal complications [3]. Diagnosis of diabetes was based on American Diabetes Association 1997 criteria [11]. HbA_{1c} was assayed by HPLC. Hypertension was defined as a blood pressure in excess of 140/85 mmHg on two occasions after 10 min in the resting position or the use of antihypertensive treatment [12]. Of the control participants, 54 (42%) versus 12 MIDD patients (18%; $p<0.001$) were being treated with an ACE inhibitor or angiotensin II receptor antagonist (ARA II). The 24 h urinary albumin excretion and plasma creatinine levels were determined. Glomerular filtration rate was calculated according to the Cockcroft and Gault formula [13], previously validated by our group in diabetic patients [14], and the Modification of Diet in Renal Disease (MDRD) equation [15]. Impaired renal function was defined as GFR <60 ml/min. A standardised ophthalmological examination was performed including colour fundus photographs in all cases and fluorescein angiography in some cases, with grading by the same investigator (P. Massin). The retinal epithelium alterations were graded according to published criteria [4, 5]. Diabetic retinopathy was considered to be

present if any characteristic lesion as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale [16] was present, i.e. microaneurysms, retinal haemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, or new vessels. Diabetic retinopathy was classified using four grades of severity as follows: (1) no diabetic retinopathy; (2) background diabetic retinopathy for any signs of diabetic retinopathy less severe than severe nonproliferative diabetic retinopathy; (3) severe nonproliferative diabetic retinopathy with numerous peripheral retinal haemorrhages and/or moderate intraretinal microvascular anomalies and/or definite venous beadings; and (4) proliferative diabetic retinopathy with new vessels on the disc or elsewhere on the retina. Macular oedema was diagnosed from the presence of hard exudates within one disc diameter of the foveola.

Molecular studies As previously described, the m.3243 A>G detection was based on an allele-specific PCR [17].

Statistical analysis Statistical analysis was performed using JMP (SAS Institute, Meylan, France). Data are expressed as means $\pm$ 1 SD for continuous variables. Odds ratios are given with 95% CI. Quantitative variables were compared using ANOVA in univariate analyses. The χ^2 test was used for the comparison of class variables. Determinants of diabetic microvascular complications, either for retinopathy or nephropathy, were analysed using a linear multivariate model with the group (MIDD or control) as the explanatory variable, and HbA_{1c} and hypertension as covariates. Hypertension was entered into the model in two ways: (1) as a class variable, i.e. presence or not of hypertension; and (2) as a continuous variable using systolic blood pressure. To proceed to the determinants of nephropathy, we also used a model including the variables already mentioned and the variable treated or not with an ACE inhibitor or ARA II.

Results

Data from the 74 patients with MIDD and the 134 diabetic controls are given in Tables 1 and 2. Patients with MIDD and controls were comparable for matching criteria. As previously reported [1, 3, 6, 10, 18], mean BMI in the MIDD group was lower than in controls (Table 1). In patients with MIDD, mean HbA_{1c}, mean systolic blood pressure (but not mean diastolic blood pressure) and prevalence of hypertension were lower than in controls (Table 2). Macular pattern dystrophy was present in 54/74 MIDD patients (73%) and in none of the controls ($p<0.001$). Deafness was present in 73 MIDD patients (98%).

Table 1 Characteristics of patients with MIDD and matched diabetic controls

Characteristic	MIDD	Diabetic controls	<i>p</i> value
<i>n</i>	74	134	
Women/men	43/31	81/53	0.74
Type of diabetes			0.96
Non-insulin-dependent	28	56	
Insulin-requiring	30	56	
Insulin-dependent	16	22	
Age at onset of diabetes (years)	37.5±9.1	38.7±10.0	0.38
Age at the time of the study (years)	48.6±10.0	49.6±9.6	0.48
Known duration of diabetes (years)	11.1±8.6	10.9±8.1	0.88
BMI (kg/m ²)	20.3±3.1	29.3±6.0	<0.0001

Results are expressed as *n* or mean±SD

Retinopathy Diabetic retinopathy was present in six patients with MIDD (8%). Of these, five had background retinopathy, including a case with severe macular oedema involving the centre of the macula, and one had proliferative retinopathy. In the control group, retinopathy was observed in 40/134 patients (29.8%), with a background form in 30 and a severe form in ten (five with severe pre-proliferative diabetic retinopathy and five with proliferative diabetic retinopathy). Nine of these ten cases had severe macular oedema involving the centre of the macula. Only two of the six MIDD patients with diabetic retinopathy had macular pattern dystrophy. Thus, diabetic retinopathy was nearly fourfold less frequent in patients with MIDD than in the control group (Table 3). In multivariate analysis, after adjustment for HbA_{1c} and systolic blood pressure or the presence or not of hypertension, differences between the two groups remained statistically significant (Table 3).

Nephropathy Data on urinary albumin excretion were not available in seven cases of MIDD and in one control case. Calculations were thus performed on 67 MIDD and 133 controls, whose clinical characteristics did not differ from

the whole groups (data not shown). Mean urinary albumin excretion was increased in MIDD compared with control diabetic patients (Table 4). The number of patients with microalbuminuria, macroalbuminuria or normal urinary albumin excretion was comparable between the two groups, i.e. 33, 11 and 88 in the control group subject, versus 20, 10 and 37 in the MIDD group, respectively (*p*=0.2). This result remained unchanged after adjustment for treatment with an ACE inhibitor or ARA II. In MIDD patients compared with controls (Table 4), creatinine plasma levels were higher and GFR (calculated according to either the Cockcroft and Gault formula or the MDRD equation) was lower. Impairment of renal function was 5- to 12-fold more frequent in MIDD than in controls, depending on the method used for GFR calculation. Differences were even greater when comparisons were adjusted for known determinants of diabetic nephropathy (hypertension and HbA_{1c}, or systolic blood pressure and HbA_{1c}), ranging from 14- to 20-fold in MIDD compared with controls. Adjustment for treatment with an ACE inhibitor or ARA II did not modify the results. Four patients from the MIDD group and none from the control group presented with end-stage renal failure, requiring either further renal transplantation or dialysis.

Discussion

The phenotype of MIDD is characterised by matrilineal transmission, early onset of diabetes (with either type 1 or type 2 presentation) and a low BMI. Hearing loss is observed in almost all cases, as well as macular pattern dystrophy, whose presence is almost specific to the disease at least in Europid patients. Cardiomyopathy, neuromuscular disorders and neuropsychiatric disturbances are also often associated with MIDD [3, 19]. Although we have previously reported a low frequency of diabetic retinopathy that contrasted with a high frequency of nephropathy [3], the prevalence of diabetic microvascular complications has not been systematically assessed in MIDD. In particular, the patients have not been compared with a control series of diabetic patients free of

Table 2 Determinants of diabetic microvascular complications in patients with MIDD and matched diabetic controls

Parameter	MIDD	Diabetic controls	<i>p</i> value
<i>n</i>	74	134	
HbA _{1c} (%)	7.6±1.6	8.5±2.0	0.002
Hypertension	25 (33.8)	86 (64.2)	0.0001
Systolic blood pressure (mmHg)	126.6±16.2	133.1±17.3	0.007
Diastolic blood pressure (mmHg)	77.0±10.4	76.0±8.9	0.46

Results are expressed as *n* (%) or mean±SD

Diabetic controls were matched for sex, age at onset of diabetes, clinical presentation at onset (i.e. insulin dependency or non-insulin dependency), duration of diabetes and current treatment

Table 3 Prevalence of diabetic retinopathy in patients with MIDD and matched diabetic controls

Parameter	MIDD (<i>n</i> =74)	Diabetic controls (<i>n</i> =134)	Unadjusted comparison	Model 1	<i>p</i> value	Model 2	<i>p</i> value
Diabetic retinopathy	6 (8)	40 (29.8)	<i>p</i> <0.0001	3.2 (1.3–9.0)	0.017	3.3 (1.3–9.7)	0.014

Results are expressed as *n* (%) or OR (95% CI)

Diabetic controls were matched for sex, age at onset of diabetes, clinical presentation at onset (i.e. insulin dependency or non-insulin dependency), duration of diabetes and current treatment

Multivariate comparisons were performed using two models. Model 1 compared the two groups (MIDD and diabetic controls) when adjusted for HbA_{1c} and the presence/absence of hypertension; model 2 compared the two groups after adjustment for HbA_{1c} and systolic blood pressure

MIDD and analysed according to major determinants of diabetic microangiopathy, i.e. glycaemic control and blood pressure.

The aim of the present study was thus to assess the prevalence of diabetic retinopathy and renal complications in patients with MIDD in comparison with that observed in patients with ‘common’ forms of diabetes matched for sex, age at onset, type and duration of diabetes. Comparisons between groups were adjusted for HbA_{1c} levels and hypertension. We found that the frequency of retinal complications due to diabetes was nearly fourfold lower, while renal complications were 6- to 14-fold more frequent in MIDD than in the ‘common’ forms of diabetes. Adjustment for treatment with an ACE inhibitor or ARA II did not modify the results.

Despite a mean duration of diabetes of 12 years, our patients exhibited a low frequency (8%) of diabetic retinopathy. Studies in patients with common forms of diabetes have shown that retinopathy develops in approximately 30 to 50% of the patients after 10 to 12 year duration, according to disease subtype [20–24]. The prevalence of diabetic retinopathy observed in MIDD patients in the present series supports previous findings made in smaller series [25–28]. It should be pointed out that most of the patients from the series reported by other investigators had macular pattern dystrophy. This may explain the apparent discrepancy between our results and those from a Japanese series [6], where diabetic retinopathy was frequent (55% of the cases), but macular pattern dystrophy was rare (13%).

Table 4 Renal values as indicated in patients with MIDD and matched diabetic controls

Parameter	Urinary albumin excretion (mg/24 h)	Plasma creatinine (μmol/l)	GFR Cockcroft (ml/min)	GFR MDRD (ml/min)	Impaired renal function Cockcroft ^a , <i>n</i> (%)	Impaired renal function MDRD ^a , <i>n</i> (%)
MIDD (<i>n</i> =67)	314.8±869	103.5±70.3	64.5±23.9	74.5±26.9	31 (46.3)	20 (29.8)
Diabetic controls (<i>n</i> =133)	80.1±29	82.2±19.4	102.4±32.1	82.4±18.1	9 (6.8)	10 (7.5)
Unadjusted comparison					11.86 (5.4–26.6)	5.23 (2.3–12.4)
<i>p</i> value	0.035	0.0178	<0.0005	0.032	<0.0001	<0.0001
Model 1					15.9 (6.1–41.3)	6.0 (2.5–15.5)
<i>p</i> value	0.005	0.002	<0.001	0.007	<0.001	<0.001
Model 2					14.7 (6.0–39.9)	7.4 (2.9–20.0)
<i>p</i> value					<0.0001	<0.001
Model 3					19.1 (7.6–54.7)	10.5 (4.0–30.9)
<i>p</i> value					<0.0001	<0.001
Model 4					15.9 (6.7–43.9)	6.5 (3.1–17.0)
<i>p</i> value					<0.001	<0.001
Model 5					19.2 (7.0–60.7)	8.6 (3.3–24.2)
<i>p</i> value					<0.001	<0.001

Results are expressed as *n* (%), mean±SD or OR (95% CI)

Diabetic controls were matched for sex, age at onset of diabetes, clinical presentation at onset (i.e. insulin dependency or non-insulin dependency), duration of diabetes and current treatment

Multivariate comparisons were performed using several models. Model 1 compared the two groups after adjustment for treatment with an ACE inhibitor or ARA II; Model 2 compared the groups after adjustment for HbA_{1c} and presence or not of hypertension; Model 3 compared the groups after adjustment for HbA_{1c} and systolic BP; Model 4 compared the groups after adjustment for HbA_{1c}, presence or not of hypertension and treatment with an ACE inhibitor or ARA II; Model 5 compared the groups after adjustment for HbA_{1c}, systolic BP, presence or not of hypertension and treatment with an ACE inhibitor or ARA II

^a<60 ml/min

The main known risk factors for diabetic retinopathy are high plasma glucose [7, 9, 21] and hypertension [8]. Systolic blood pressure and HbA_{1c} were lower in patients with MIDD than in controls, but differences in the prevalence of diabetic retinopathy between the two groups remained significant after adjustment for these risk factors. This suggests the presence of protective factors against diabetic retinopathy in MIDD. Some ocular factors, such as high myopia, amblyopia and retinitis pigmentosa, are known to provide a protective effect [29, 30], but they were absent in our patients. It is widely accepted that protection by such factors occurs through a reduction in retinal metabolism, a decrease in oxygen needs, the production of vasoproliferative factors and/or a reduced retinal blood flow [31]. The same mechanism(s) may underlie the low prevalence of retinopathy in MIDD patients, as alterations of pigment epithelium were present in 73% of the patients from our series. Several anatomical and electrophysiological studies have shown that retinal dysfunction extends beyond the alterations observed by fundus examination [26, 32, 33]. Such a generalised retinal dysfunction may result in reduced retinal metabolism and oxygen demand, and constitute a relative protection against diabetic retinopathy. Mitochondrial dysfunction in the retina may also result in decreased production of superoxide, which is considered to be a causal link between elevated glucose and the major biochemical pathways involved in the development of diabetic microvascular complications [34].

In contrast with the low prevalence of retinopathy, MIDD patients exhibited a high prevalence of kidney disease. Albumin excretion rate and creatinine plasma levels were higher, and GFR lower in MIDD patients than in control diabetic participants. In the latter, the prevalence of abnormal urinary albumin excretion was consistent with that described in common forms of diabetes with the same disease duration [7, 9]. Overall, renal dysfunction was observed in 53.7% of MIDD patients compared with 28.5% in diabetic controls. Moreover, differences between groups increased after adjustment for hypertension and glycaemic control. One may thus hypothesise that renal impairment in MIDD patients is more likely to be a result of specific mitochondrial defects than of hyperglycaemia and/or hypertension. Indeed, renal disease is commonly observed early in the course of mitochondrial cytopathies, generally occurring before the emergence of diabetes [35]. Specific clinical and pathological renal features [36–38] have been described in patients with mitochondrial cytopathies, with no histological change indicative of diabetic nephropathy, even though patients presented with diabetes [35, 38, 39].

In conclusion, our data indicate that diabetic retinopathy is less prevalent in MIDD patients than in a control group with ‘common’ forms diabetes. This better retinal prognosis in MIDD might result from protective specific retinal alterations due to mitochondrial dysfunction. By contrast,

nephropathy is much more frequent in MIDD than in ‘common’ forms of diabetes, suggesting the role of a specific mitochondrial kidney disease, leading to a more severe renal prognosis in MIDD patients. In these patients, early renal protection should be proposed.

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Duality of interest The authors declare that there is no duality of interest associated with this study.

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