A Screening Approach for Mitochondrial tRNA$^{Leu(UUR)}$ A3243G Mutation in a Hospital-Based Population with Diabetes

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To the Editor: Diabetes caused by mitochondrial tRNA$^{Leu(UUR)}$ A3243G mutation is one of the most common types of mitochondrial diabetes mellitus (MDM). Seventeen years ago, we reported that the prevalence of MDM was 0.4% in clinically diagnosed type 2 diabetes mellitus (T2DM) patients (n = 716).[1] Recently, we reviewed all the studies reporting MDM cases from grade three and first-class hospitals in China (unpublished) and found that the prevalence of MDM in a pooled randomly selected T2DM population was 0.64%. MDM patients are usually characterized by early age at diagnosis, low beta-cell function, and lack of obesity, insulin resistance, and autoantibodies associated with type 1 diabetes mellitus (T1DM). Owing to the mitochondrial DNA heteroplasmy, there is a great variation in clinical presentation and severity of affected organ impairment among MDM patients. Furthermore, because of a poor response to oral hypoglycemic agents and increased lactate levels, MDM patients usually need insulin therapy, but not metformin. However, according to previous reports, not all MDM patients present with typical clinical features, especially at an early stage, and sometimes they are misdiagnosed as T1DM or T2DM and receive incorrect treatment. Currently, the correct diagnosis of MDM depends on DNA sequencing. However, because of the low prevalence of MDM and the complexity of genetic testing, it is not practical for every patient with diabetes to be sequenced. In this study, based on a review of all the reports of MDM in China, we established a scoring system for screening MDM patients (MDM-score) based on multiple clinical indices and determined its effectiveness in identifying MDM patients from a hospitalized diabetes population.

From May 2011 to September 2017, 1094 hospitalized patients with diabetes were recruited, all of whom were diagnosed with diabetes in accordance with the 1999 World Health Organization criteria. This study was conducted in compliance with the 1975 *Declaration of Helsinki* as revised in 2000 and approved by the institutional ethics committee. Written informed consent was obtained from all the study participants.

Clinical information was collected at enrollment. Hemoglobin A1c (HbA1c), plasma glucose, serum insulin, urinary albumin/creatinine ratio (ACR), insulin autoantibody (IAA), islet cell autoantibody (ICA), and glutamic acid decarboxylase autoantibody (GADA) were measured. The reference range for fasting serum insulin was determined in 84 healthy participants (32 males and 52 females), who were recruited from the communities near the hospital and aged 25–50 years. They met the following criteria: (1) 18.2 kg/m^2< body mas index (BMI) <25 kg/m^2; (2) waist circumference <90 cm for males and <85 cm for females; (3) no history of hypertension, systolic pressure <140 mmHg (1 mmHg = 0.133 kPa), and diastolic pressure <90 mmHg at enrollment; (4) alanine aminotransferase <50 U/L; (5) serum creatinine <100 μmol/L; (6) total cholesterol <6.2 mmol/L, triglyceride <1.7 mmol/L, low-density lipoprotein cholesterol <4.1 mmol/L, high-density lipoprotein cholesterol ≥0.9 mmol/L for males and ≥1.0 mmol/L for females; (7) fasting plasma glucose <6.1 mmol/L, plasma glucose at 2 h during 75 g oral glucose tolerance test ranging from 4.0 to 7.8 mmol/L, HbA1c <6.0%; (8) serum uric acid <428 μmol/L; (9) ACR <26 mg/g for males and <32 mg/g for females; and (10) no history of smoking, alcohol ingestion, and other diseases such as cardiovascular disease or cancer. The 2.5% and 97.5% percentiles of serum insulin levels were 2.2 and 10.8 μU/ml, respectively. If the patients met one of the following criteria, they were diagnosed with T1DM: (1) severe insulin deficiency (fasting serum C-peptide <0.01 ng/ml); (2) if they had residual beta-cell function (fasting serum C-peptide ranging from 0.01 to 0.6 ng/ml), they depended on insulin treatment within 5 years of diabetes duration; and (3) persistently positive autoantibody associated with T1DM. When the patients had none of the abovementioned evidence of T1DM or clinical features for specific forms of pancreatic dysfunction such as pancreatitis, they were diagnosed with T2DM. DNA was extracted from peripheral blood leukocytes and polymerase chain reaction (PCR) was used to amplify the flanking sequence of mitochondrial DNA A3243G. PCR products were sequenced on an ABI 3730 sequencer (ABI, Foster City, CA, USA).

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Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.230729

Received: 22-12-2017 **Edited by:** Qiang Shi

How to cite this article: Tian LH, Han XY, Huang XT, Zhang SM, Gong SQ, Ma YM, Cai XL, Zhou LL, Luo YY, Li M, Liu W, Zhang XY, Ren Q, Zhu Y, Zhou XH, Zhang R, Chen L, Gao XY, Liu Y, Zhang F, Ji LN. A Screening Approach for Mitochondrial tRNA$^{Leu(UUR)}$ A3243G Mutation in a Hospital-Based Population with Diabetes. Chin Med J 2018;131:1117-9.

After reviewing the published papers on MDM in the Chinese population, of all MDM patients, we found that 92.6% had a young age at diagnosis of diabetes (≤45 years old), 94% had a BMI <24 kg/m^2, 85.4% had abnormal hearing, 61.4% received insulin injections, and 98% had a history of hyperglycemia or diabetes on their mother's side. They had detectable fasting serum C-peptide levels but not hyperinsulinemia. Thus, excluding T1DM patients based on clinical features, C-peptide levels, and autoantibody associated with T1DM, we calculated the MDM-score according to the following criteria: (1) age at diagnosis of diabetes ≤45 years (1 point); (2) family history of diabetes and/or abnormal hearing ability on their mother's side (1 point); (3) BMI <24.0 kg/m^2 (1 point); (4) abnormal hearing ability according to physical examination (1 point); and (5) impaired beta-cell function, there was endogenous insulin secretion, but <11 μU/ml, or on insulin treatment (1 point). After evaluating these five parameters for each patient, a total score was obtained (MDM-score).

Statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS for Windows, version 23.0; IBM SPSS, Chicago, IL, USA). We determined the ability of the MDM-score to discriminate between MDM and T2DM using the area under the curve (AUC) of the receiver operating characteristic curve (ROC). When the AUC of the ROC curve was >0.8, the clinical variable was considered useful for differentiating MDM from T2DM. The sensitivity and specificity of the MDM-score cutoff were also calculated.

A total of 57 patients were diagnosed with typical T1DM and latent autoimmune diabetes in adulthood. Among the 1037 T2DM patients, 59.3% were diagnosed at <45 years old, 33.3% had a family history of diabetes on their mother's side, 27.1% had a BMI <24.0 kg/m^2, 0.6% had impaired hearing ability according to physical examinations, and 51.9% received insulin treatment. In the patients using single oral hypoglycemia agents, 55.1% had serum insulin levels <11 μU/ml. In patients with insulin treatment, 100% had detectable C-peptide levels. No cases of MDM were found in the T1DM patients. In clinically diagnosed T2DM patients, five MDM patients were identified (0.5%). No hearing loss was found in any of the MDM patients through physical examination. Only one patient agreed to be followed and was diagnosed with hearing abnormality by electronic testing. As shown in the flow chart [Figure 1], in T2DM patients, 54 (5.2%) patients had a MDM-score of 4, 262 (25.3%) patients had a MDM-score of 3, and 721 (69.5%) patients had a MDM-score of <2. No case with an MDM-score of 5 was identified. In five MDM patients, one (20.0%) patient had a MDM-score of 4, and all patients had a score of >2. In ROC analysis, the AUC of ROC was 0.884 (95% confidence interval: 0.801–0.967, $P = 0.003$) to distinguish between MDM and T2DM. The cutoff of MDM-score ≥3 could discriminate between MDM and T2DM, with 100% sensitivity and 69.9% specificity; its positive predictive value was 1.6% and negative predictive value was 100.0%. Through this approach, only about one-third of the patients were sequenced and all MDM patients were identified from a total of 1037 T2DM patients. Thus, we successfully established a clinical screening approach to distinguish MDM patients from other types of diabetes using the MDM-score.

MDM patients who were described as having similar clinical features to T2DM, with or without hearing loss, were first reported in 1992.[2] In China, MDM pedigrees were first reported in 1995, and these patients were described as having special clinical presentations including matrilineal inheritance, early onset of diabetes, lack of obesity, dependence on insulin treatment, and hearing loss.[3] In 2005, the Chinese Diabetes Society recommended that patients with these combination of features should be screened for mitochondrial tRNA$^{Leu(UUR)}$ A3243G mutation.[4] However, as shown in our study, few patients with MDM had all these clinical features, it would miss most MDM patients if only such patients were screened for the tRNA$^{Leu(UUR)}$ A3243G mutation. In contrast, if we only screened the patients with an MDM-score ≥3, we would identify all MDM patients. Importantly, it would not be necessary to screen patients with a MDM-score ≤2 for the tRNA$^{Leu(UUR)}$ A3243G mutation. Of note, in our study, the hearing ability of all participants was evaluated by physical examination at enrollment, and only one MDM patient was followed up and his hearing ability was evaluated by electronic testing after hospital discharge. As we know, impaired hearing can occur in the MDM patients before or after the onset of diabetes, and most patients only have abnormal hearing at the electronic measurement level.[5] Therefore, it is usually not necessary to evaluate the hearing ability of each patient by electronic testing in a clinical setting; however, the electronic measurement of hearing loss would be useful for patients whose MDM-score was >2, which might reduce the cost of genetic testing.

Our MDM-score was based on a review of the reported MDM cases in China. The cutoff point for BMI, age at diagnosis of diabetes, abnormal hearing, and family history of diabetes on the mother's side were determined according to previous reports in which >85% of MDM cases had one of these features. In addition, we selected healthy subjects to determine the reference range of

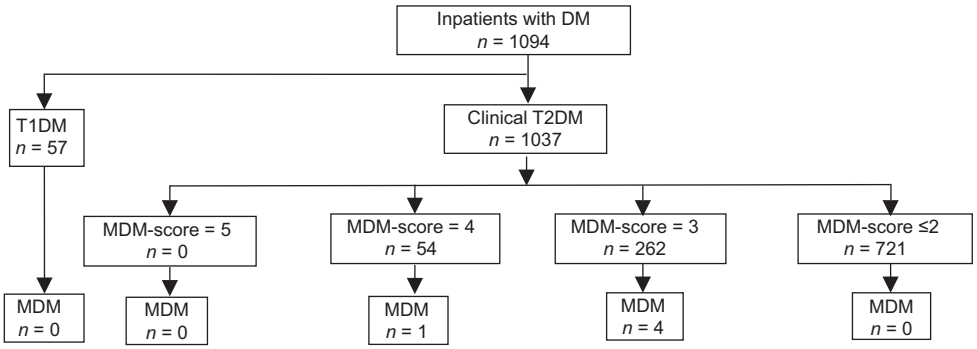

Figure 1: Application of the MDM-score in clinical screening for MDM patients. MDM-score: A summed score of the following parameters: (1) Age at diagnosis of diabetes ≤45 years old (1 point); (2) family history of diabetes and/or abnormal hearing on their mother's side (1 point); (3) BMI <24.0 kg/m^2 (1 point); (4) abnormal hearing (1 point); and (5) impaired beta-cell function, there was endogenous insulin secretion, but <11 μU/ml, or on insulin treatment (1 point). T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; MDM: Mitochondrial diabetes mellitus; BMI: Body mass index.

fasting serum insulin and used the 97.5% percentile as a cutoff point to assess the beta-cell function for patients without insulin injection to exclude as many as possible T2DM patients with insulin resistance, as MDM patients always present with reduced insulin secretion rather than insulin resistance. In fact, the first step in the clinical screening of MDM patients was to exclude patients with T1DM, which was not very difficult as T1DM patients often have severe insulin deficiency and autoantibodies associated with T1DM. Similarly, we observed that there were no MDM cases with severe loss of endogenous insulin secretion (fasting serum C-peptide <0.01 ng/ml) or positive autoantibodies (IAA, ICA, and GADA). In contrast to the previous studies conducted in China, in which PCR-RFLP was used to genotype the patients, we used Sanger sequencing, which is a more sensitive method. Thus, it was not surprising that the estimated prevalence of MDM in clinically diagnosed T2DM in this study was 0.5% which was slightly higher than that of our previous study.[1] Early insulin treatment was recommended for the MDM patients because of their impaired insulin secretion. Some studies have shown that coenzyme Q10 and vitamin B1 may also benefit MDM patients, but their effectiveness requires confirmation.

There were some limitations to our study. The sample size was not large enough to evaluate the effectiveness of the MDM-score, and only the inpatients from one center were included in the study. A further study with a larger sample size in multiple centers, including outpatients and inpatients, is needed to validate the current results.

In summary, the proportion of MDM patients of clinically diagnosed T2DM patients was 0.5%, and the MDM-score was helpful and economical to identify MDM patients from T2DM patients. Electronic hearing testing might improve the specificity of the MDM-score.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

This study was supported by grants from the Beijing Science and Technology Committee Fund (No. Z141100007414002), the National Key Research and Development Program (No. 2016YFC1304901), the National High-Technology Research and Development Program of China (No. 2012AA02A509), and the Beijing Science and Technology Committee Fund (No. D131100005313008).

Conflicts of interest

There are no conflicts of interest.

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