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Prevalence and Clinical Characteristics of Mitochondrial tRNA A3243G Mutation in Inherited Type 2 Diabetes Mellitus

Dr. Shashi Kant Verma[1], Dr. Kamlesh Palandurkar[2], Dr. Neeraj Kumar Agrawal[3], Dr. KiranRajendra Giri[4], Dr. S. P. Mishra[5]

[1]Senior Resident, Department of Biochemistry, AIIMS Patna, Bihar, India
[2]Associate Professor, Department of Biochemistry, IMS BHU, Uttar Pradesh, India
[3]Professor and HOD, Department of Endocrinology and Metabolism, IMS BHU, Uttar Pradesh, India
[4]Associate Professor, Department of Pharmacology, IMS BHU, Uttar Pradesh, India
[5]Professor & HOD, Department of Biochemistry, IMS BHU, Uttar Pradesh, India

Corresponding Author
Dr. S. P. Mishra
Professor & HOD, Department of Biochemistry, IMS BHU, Uttar Pradesh, India
Email ID:drsurendram2@gmail.com

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ABSTRACT

Introduction: Transfer RNA (tRNA) genes in the mitochondrial DNA genome are crucial for protein synthesis. Dysfunctional mitochondria, due to tRNA mutations, impair insulin secretion as they fail to operate optimally. These tRNA mutations may also contribute to insulin resistance. Moreover, the loss of tRNA modifications can lead to pancreatic β cell dysfunction. The limited understanding of the prevalence of the A3243G mitochondrial gene mutation in familial type 2 diabetes mellitus (T2DM) spurred this investigation. Our objective was to explore the prevalence and clinical characteristics of the mitochondrial tRNA A3243G mutation in inherited T2DM.

Methods: The study enrolled a total of 100 subjects, divided into two groups: 50 with paternal inheritance and 50 with maternal inheritance. Participants were recruited from the Department of Endocrinology and Metabolism, SS Hospital, IMS, BHU, and all were undergoing treatment for diabetes with a familial history of the disease. Comprehensive clinical, molecular, and biochemical evaluations were conducted on all patients. Mitochondrial DNA (mtDNA) genes were amplified using polymerase chain reaction (PCR) and subsequently sequenced as part of the molecular assessments.

Results: Participants in the maternal inheritance (MDI) group had a mean age of 43.24 ± 5.83 years, slightly higher than 41.99 ± 6.39 years in the

paternal inheritance (PDI) group. Baseline and biochemical parameters (Hb, FPG, and HbA1c) were similar between both groups. The A3243G mutation was found in 6% of MDI participants and 2% of PDI participants, indicating a higher prevalence in the maternal inheritance group. Most participants in both groups did not exhibit this mutation. Comorbidities such as polyneuropathy, visual impairment, and renal impairment were noted, although many reported no additional symptoms. Treatment strategies ranged from lifestyle modification alone to combinations of oral hypoglycemic agents, insulin, and lifestyle adjustments as necessary.

Conclusion: The mitochondrial A3243G mutation was identified in 4% of the diabetic patients. The occurrence of the A3243G mutation in the mitochondrial tRNALeu(UUR) gene, while low, is consistent with findings from previous studies. However, expanding the study to include a larger cohort and screening for additional mtDNA mutations beyond A3243G could provide deeper insights into the epidemiological dynamics and prevalence of MIDD or mitochondrial diabetes mellitus (mtDM).

Key Words: Assessments, OSPE, Practical Examination, Reliability, Validity.

INTRODUCTION

Diabetes mellitus (DM) ranks among the most critical chronic non-communicable diseases globally. In recent years, the rising incidence of DM and its related complications has placed a substantial strain on healthcare systems worldwide, emerging as a significant public health issue. According to the International Diabetes Federation (IDF), 8.8% of the global adult population is affected by diabetes. Current global data indicate that 463 million people are living with diabetes, with projections suggesting an increase to 700 million by 2045, representing a 51% rise from 2019. In India, the prevalence of diabetes has increased from 7.1% in 2009 to 8.9% in 2019. Often dubbed the diabetes ‘capital’ of the world, India ranks second after China in the global diabetes epidemic, with 77 million individuals currently affected, a figure expected to reach 134 million by 2045 [1-3].

Diabetes encompasses a range of disorders characterized by chronic hyperglycemia. The pathophysiological mechanisms leading to diabetes may involve improper insulin secretion, insulin resistance in the liver, muscle, and adipose tissues, or a combination of these defects. The risk of developing diabetes is influenced by a complex interplay of genetic and environmental factors [4]. Gene variants that contribute to the two predominant forms of diabetes, autoimmune type-1 diabetes mellitus (T1DM) and metabolic syndrome–associated type-2 diabetes mellitus (T2DM), are "low penetrance" variants that affect an individual's susceptibility to the disease [5,6]. Numerous gene mutations have been identified in recent years and are often referred to as "high penetrance" gene variants for diabetes. Carriers of these variants have an almost 100% likelihood of developing diabetes at some point in their lives. These monogenic forms of diabetes mellitus constitute a heterogeneous group of diabetes caused by single-gene defects. The incidence of monogenic diabetes has increased over the past few decades, partly due to greater public awareness and the broader availability of genetic testing [7-9].

The limited knowledge regarding the prevalence of the A3243G mitochondrial gene mutation in familial type-2 diabetes mellitus (T2DM) prompted this study. Our aim was to investigate the prevalence and clinical characteristics of the mitochondrial tRNA A3243G mutation in inherited T2DM.

MATERIAL AND METHODS

This cross-sectional was conducted at Department of Biochemistryand Department of Endocrinology and Metabolism, Sir SundarLal Hospital, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India, spanning a one-year period from November 2016 to October 2017. Approval for the study protocol was granted by the Institutional Ethics Committee (IEC).

Participants were provided with a detailed explanation of the study protocol, and written informed consent was obtained from each before enrollment. The study comprised a total of 100 subjects, divided into two groups: 50 patients having type-2 diabetes with maternal inheritance, and 50 with paternal inheritance. The study group consisted of patients undergoing diabetes treatment at the Department of Endocrinology and Metabolism, SS Hospital, IMS, BHU, who also had a familial history of diabetes mellitus (DM). Exclusion criteria encompassed individuals younger than 18 years, those lacking a family history of DM, individuals with chronic diseases or mental illnesses, and those unwilling to provide written informed consent.

Demographic details and clinical information of participants were gathered at the time of enrollment. This included data such as height, weight, age at diagnosis, glycosylated hemoglobin (HbA1C), fasting plasma glucose (FPG), and family history, sourced from office records, hospital charts, and direct interactions with participants. Familial cases were categorized into paternal diabetic inheritance (PDI) and maternal diabetic inheritance (MDI). All participants consented to both biochemical and molecular analysis of their blood samples.

Body mass index (BMI) was calculated using the formula weight (kg)/height (m)^2 during hospital admission for treatment. Overweight and obesity were defined with cutoffs set at 23.0 kg/m^2 and 25.0 kg/m^2 respectively [10].

Blood samples were obtained from subjects and were coded to prevent potential bias. Each subject provided 5 ml of venous blood following an overnight fast, which was then divided into two vials: 3 ml in clot-activated tubes and 2 ml in ethylene diamine tetra-acetic acid (EDTA) coated vials. The clot-activated tubes were centrifuged at 3,000 rpm for 10 minutes to separate the serum, which was then collected into micro centrifuge tubes. Remaining serum samples were stored at -80°C for future analysis. The 2 ml of whole blood in EDTA vials was used for genomic DNA extraction.

Genomic DNA isolation from fresh blood was conducted under cold conditions (4°C) throughout the experiment. Blood samples were homogenized in ice-cold sucrose, EDTA, and Tris-HCl buffer (composed of sucrose 10.8 g, EDTA 0.5 mol/l, Tris-HCl 1 mol/l, pH 8.0, final volume adjusted to 100 ml with nuclease-free water, autoclaved, and stored at 4°C). The homogenate was centrifuged at 4000 rpm for 10 minutes at 4°C to pellet the nuclei, followed by a wash with the same buffer. The resulting pure nuclear pellet was suspended in Tris-HCl, EDTA, and NaCl buffer (containing Tris-Cl 1 mol/l, EDTA 0.5 mol/l, NaCl 5 mol/l, pH 8.0, final volume adjusted to 100 ml with nuclease-free water, autoclaved, and stored at 4°C). To lyse the nuclei, 1% sodium dodecyl sulfate (SDS) was added and gently mixed. Proteinase-K was then added to a final concentration of 100 µg/ml, and the mixture was incubated overnight at 37°C. The lysate treated with proteinase-K was combined with an equal volume of Tris-saturated phenol (pH 8.0), followed by extractions with phenol:chloroform (1:1) and chloroform:isoamyl alcohol (24:1) mixtures, each followed by centrifugation at 10,000 rpm to separate phases. The final aqueous phase containing DNA was transferred to a fresh tube. To precipitate the DNA, 1/10th volume of 3 mol/l sodium acetate (pH 5.2) and two volumes of ice-cold absolute alcohol were added to the aqueous phase. The DNA precipitate was washed with 70% ethanol, air dried, and then dissolved in an appropriate volume of TE buffer (pH 8.0). The sample was incubated at 37°C until the DNA was completely dissolved.

The tRNALeu(UUR) gene of interest was PCR amplified using previously described primers with slight modifications in methodology [11]. The PCR primers used were 5’-TCTAGAGTCCATATCAACAA-3’ (nt 2953–2972) and 5’-TTTGGTGAAGAGTTTTATGG-3’ (nt 3480–3461), generating a 528-bp fragment encompassing the full mitochondrial

tRNALeu(UUR) gene. PCR was performed in a 96-well thermal cycler (Veriti, Applied Biosystems) with an annealing temperature of 53°C. The reaction mixture (20 µl) consisted of 1x PCR buffer (20 mMTris-HCl pH 8.4, 50 mMKCl), 1.5 mM MgCl2, 5 mMdNTP mix, 10 µM of each primer, 1.25 U Taq polymerase (New England Biolabs, MA, USA), and 2 µl DNA template. PCR conditions included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, elongation at 72°C for 60 sec, and final extension at 72°C for 7 min. The PCR product (23 µl) was subsequently digested with 10 U of ApaI restriction endonuclease (BioLabs), followed by separation on a 2% agarose gel containing ethidium bromide and visualization under UV transilluminator. In the absence of the A3243G mutation, the PCR product yielded a single 528 bp fragment (Figure 1a). However, presence of the A3243G mutation resulted in restriction at the ApaI site, producing two fragments of 234 bp and 294 bp (Figure 1b).

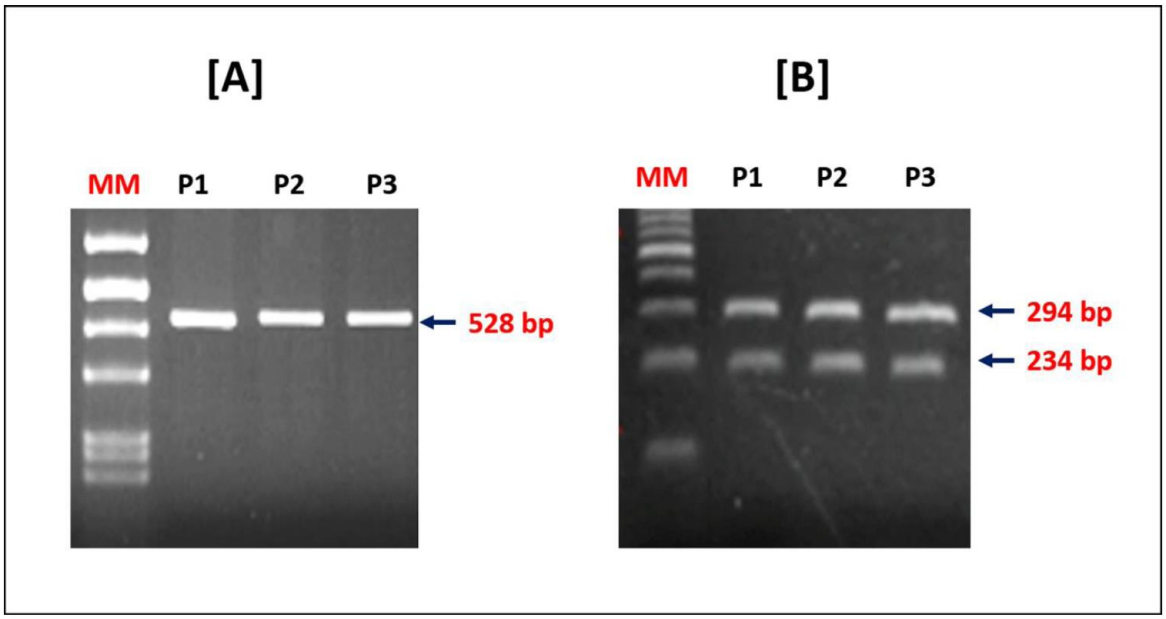

Figure 1: Screening of the A3243G mutation by PCR-RFLP: a 528bp fragment digested with restricted enzyme ApaI.
[A] A3243G mutation absent: PCR product showing undigested 528bp amplicon. MM: 100bp DNA ladder as molecular marker; P1, 2 and 3 are patient samples.
[B] A3243G mutation present: 528bp amplicon cleaved into two fragments of 234bp and 294bp. MM: 100bp DN ladder as molecular marker; P1, 2 and 3 are patient samples.

RESULTS

The baseline characteristics of the study participants are summarized in Table 1. The mean age of participants in the MDI group was 43.24 ± 5.83 years, compared to 41.99 ± 6.39 years in the PDI group, with no significant difference between the groups ($p = 0.65$). The mean Body Mass Index (BMI) was 23.78 ± 1.65 in the MDI group and 22.54 ± 1.47 in the PDI group, also showing no significant difference ($p = 0.81$). Gender distribution was similar across both groups, with the MDI group consisting of 24 males and 26 females, and the PDI group consisting of 26 males and 24 females ($p = 0.71$).

Table 1: Baseline characteristics of study participants

Variables	MDI Group (n=50)	PDI Group (n=50)	p value
Age (years)	43.24 ± 5.83	41.99 ± 6.39	0.65
BMI	23.78 ± 1.65	22.54 ± 1.47	0.81
Gender			
Male	24	26	0.71
Female	26	24	

Table 2 presents the biochemical variables of the study participants. The mean hemoglobin (Hb) levels were 12.17 ± 1.14 g/dl in the MDI group and 11.56 ± 1.08 g/dl in the PDI group, with no significant difference (p = 0.85). Fasting plasma glucose (FPG) levels were slightly higher in the PDI group (151.17 ± 23.48 mmol/L) compared to the MDI group (145.91 ± 24.72 mmol/L), but this difference was not statistically significant (p = 0.35). Hemoglobin A1c (HbA1c) levels were 7.35 ± 0.51% in the MDI group and 6.98 ± 0.48% in the PDI group, with no significant difference observed (p = 0.47).

Table 2: Biochemical variables of study participants

Variables	MDI Group (Mean ± SD)	PDI Group (Mean ± SD)	p value
Hb (g/dl)	12.17 ± 1.14	11.56 ± 1.08	0.85
FPG (mmol/L)	145.91 ± 24.72	151.17 ± 23.48	0.35
HbA1c (%)	7.35 ± 0.51	6.98 ± 0.48	0.47

The prevalence of the A3243G mutation among study participants is shown in Table 3. The mutation was present in 3 participants (6%) in the MDI group and in 1 participant (2%) in the PDI group, with no statistically significant difference between the groups (p = 0.307). The majority of participants in both groups did not have the mutation, with 47 participants (94%) in the MDI group and 49 participants (98%) in the PDI group being mutation-free.

Table 3: Prevalence of A3243G mutation in study participants

A3243G mutation	MDI Group (n=50)	PDI Group (n=50)	p value
Present; n (%)	3 (6)	1 (2)	0.307
Absent; n (%)	47 (94)	49 (98)	

Table 4 describes the clinical features observed among patients with inherited diabetes mellitus. Polyneuropathy was reported in 6% of patients with a diabetic mother and 4% of patients with a diabetic father (p = 0.864). Visual impairment (short-sightedness) was present in 12% of patients with a diabetic mother and 10% of patients with a diabetic father. Renal impairment was reported in 4% of patients with a diabetic mother and 2% of patients with a diabetic father. The majority of patients in both groups reported no additional symptoms, with 78% of patients with a diabetic mother and 84% of patients with a diabetic father being asymptomatic.

Table 4: Clinical features among patients with inherited DM

Clinical Features	Patients of Diabetic Mother		Patients of Diabetic Father		P Value
	n	%	n	%	

Polyneuropathy	3	6	2	4	0.864
Visual Impairment (Short-sightedness)	6	12	5	10	
Renal Impairment	2	4	1	2	
No Additional Symptoms	39	78	42	84	
Total	50	100	50	100	

The management strategies employed for patients with inherited diabetes mellitus are detailed in Table 5. Among patients with a diabetic mother, 10% were managed with lifestyle modifications (LM) only, 40% with LM and oral hypoglycemic agents (OHA), 28% with combined therapy (OHA + insulin), and 22% with insulin alone. For patients with a diabetic father, 16% were managed with LM only, 42% with LM and OHA, 24% with combined therapy, and 18% with insulin alone. There were no significant differences in the management strategies between the two groups ($p = 0.784$).

Table 5: Management of patients with inherited DM

Management	**Patients of Diabetic Mother**		**Patients of Diabetic Father**		**P Value**
	n	**%**	**n**	**%**	
Lifestyle Modification (LM) Only	5	10	8	16	0.784
LM and Oral Hypoglycemic Agents (OHA)	20	40	21	42	
Combined Therapy (OHA + Insulin)	14	28	12	24	
Insulin	11	22	9	18	
Total	50	100	50	100	

DISCUSSION

The current investigation involved 100 patients with type 2 diabetes mellitus (T2DM), divided into two groups: 50 with maternal inheritance and 50 with paternal inheritance. Among these patients, a strong prevalence was observed between diabetes and factors such as obesity, high BMI, and abdominal obesity, corroborating previous research findings [24-26]. Despite the lower BMI typically seen in the Indian population compared to Europeans, the risk of diabetes remains significant even at lower BMI levels. Physical activity is known to mitigate the risk of obesity, cardiovascular disease, and metabolic syndrome. Previous studies have highlighted that a sedentary lifestyle and inadequate physical activity are linked to the development of diabetes [12-14].

As anticipated, elevated levels of HbA1c and fasting plasma glucose (FPG) were observed among the T2DM patients, indicating excessive glycosylation of hemoglobin and poor diabetes management. These findings are in line with earlier studies by various researchers [15,16]. HbA1c serves as a crucial marker for long-term glycemic control, reflecting the cumulative glycemic history over the past two to three months. It reliably measures chronic hyperglycemia and correlates with the risk of long-term diabetes complications. High HbA1c levels are considered an independent risk factor for coronary heart disease, stroke, and mortality in diabetic patients [17]. An increase of 1% in HbA1c concentration is associated with approximately a 30%

rise in all-cause mortality and a 40% rise in cardiovascular or ischemic heart disease mortality among individuals with diabetes. Conversely, reducing the HbA1c level by 0.2% could decrease mortality by 10% [18]. Therefore, enhancing glycemic control in T2DM patients may be more critical than managing dyslipidemia for preventing both microvascular and macrovascular complications associated with diabetes [19].

Mitochondrial diabetes, characterized as a mitochondrial disorder with persistent hyperglycemia due to improper insulin secretion, insulin resistance, or a combination of defects, presents a considerable clinical challenge. This rare, monogenic form of diabetes occurs with a frequency of approximately 1%. Accumulating evidence indicates that deletions, insertions, or point mutations in mitochondrial DNA (mtDNA) are associated with mitochondrial diabetes [20]. The A-to-G transition at position 3243 of mtDNA is identified as the most prevalent mutation linked to mitochondrial diabetes globally, with prevalence rates ranging from 0.1% to 10% [4]. The phenotypic expression of this mutation varies widely, presenting with clinical manifestations from mild to severe. The A3243G mutation is notably a significant cause of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), as well as myoclonic epilepsy and ragged-red fiber disease (MERRF) [41]. Recently, this mutation has also been recognized as a cause of maternally inherited diabetes and deafness (MIDD) [21].

In this study, the mitochondrial A3243G mutation was detected in 4% of the diabetic patients, consistent with previous reports [22,23]. Generally, a 2.5% prevalence of the A3243G mutation is typical, with early-onset diabetes patients who have a maternal family history being more susceptible to this otherwise rare monogenic form of diabetes. Contrarily, other researchers have reported lower prevalence rates of the A3243G mutation, ranging from 0.13% to 1.0% in their studies [24,25]. The A3243G mutation has been identified in various clinical samples, including blood, hair follicles, buccal epithelial cells, and muscle biopsies. Among diabetic patients, the highest quantities of mutant mtDNA A3243G are found in muscle tissue, followed by hair follicles, with the lowest levels in blood cells [26]. This suggests that the use of blood samples as the source of mtDNA in the aforementioned studies [24,25] may account for the lower detection frequency. Moreover, as expected, diabetic patients with the mitochondrial A3243G mutation in this study were younger, leaner, had lower blood glucose levels, experienced neurosensorial deafness, and more frequently had a maternal history of T2DM compared to diabetic patients without any mitochondrial mutations.

In addition to its maternal inheritance and association with progressive neurosensorial deafness, maternally inherited diabetes and deafness (MIDD) exhibits several key characteristics: it typically manifests in middle age (25-40 years), occurs in non-obese individuals, often necessitates insulin therapy due to a progressive insulin secretory defect, is complicated by other mitochondrial disorders, and represents 1-2% of the diabetic population [27]. Despite its relatively low frequency, the pharmacotherapeutic and pharmacoeconomic implications, along with the associated morbidity, underscore the importance of early and accurate diagnosis of this rare monogenic mutation. Although the frequency of the A3243G mutation found in the present study aligns with that reported in most previous studies, other mtDNA mutations have also been documented [21, 28-31], which may contribute to the development of MIDD or mitochondrial diabetes mellitus (mtDM). Differential diagnosis for such patients is crucial, as they may present with severe symptoms that could be mistakenly attributed solely to diabetes mellitus, necessitating different treatment approaches. Comprehensive prospective studies are warranted to determine the prevalence of these conditions accurately.

CONCLUSION

In this study, the mitochondrial A3243G mutation was identified in 4% of the diabetic patients. This mutation, located in the mitochondrial tRNALeu(UUR) gene, although infrequent, aligns with the prevalence rates reported in previous research. The A3243G mutation is associated with mitochondrial diabetes and maternally inherited diabetes and deafness (MIDD), representing a notable, albeit rare, cause of diabetes.

The detection of the A3243G mutation at a comparable rate to earlier studies underscores the importance of recognizing its role in the clinical spectrum of diabetes. However, the limited prevalence data emphasizes the need for broader epidemiological studies. Screening larger cohorts for the A3243G mutation, as well as other potential mtDNA mutations, is crucial. Such comprehensive screening can enhance our understanding of the genetic underpinnings and epidemiological distribution of MIDD and mitochondrial diabetes mellitus (mtDM).

Furthermore, the identification of additional mtDNA mutations may reveal novel insights into the molecular mechanisms contributing to these mitochondrial disorders. Detailed genetic analysis and large-scale studies could elucidate the full spectrum of mtDNA variations involved, potentially uncovering mutations with significant clinical relevance. This expanded understanding could improve diagnostic accuracy, enabling tailored therapeutic interventions and better management strategies for affected patients.

Overall, this study highlights the necessity for extensive genetic screening in diverse populations to fully characterize the prevalence and impact of mitochondrial mutations, thereby advancing the field of mitochondrial genetics and its application in clinical practice.

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