Prevalence of A6224G Insulin Receptor Substrate-1 and Mitochondrial DNA tRNA)Leu(UUR) 3243 Polymorphisms in Diabetes Mellitus Patients in Saudi Arabia

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Abstract: The prevalence of diabetes is high among the Saudi population and 90% of diabetics suffer from Type II diabetes mellitus (T2DM). Genetic polymorphisms are integral to the development of genetic markers to identify individuals at risk of developing T2DM. These genetic markers could be used for early detection and molecular diagnosis of diabetes. The objective of the present study is to detect the prevalence of A6224G polymorphism within the Insulin receptor substrate-1 (IRS-1) gene and tRNA)Leu(UUR) 3243 polymorphism in mitochondrial DNA among T2DM. Saudi patients. One hundred and six patients were recruited. Genotyping was carried out via PCR-RFLP technique. No patients with tRNA)Leu(UUR) 3243 polymorphisms were found. Three genotypes of A6224G polymorphism were detected. The prevalence of the detected genotypes was (40.6 for AA, 58.5 for AG and 0.94 for GG and). The frequency of A allele was 0.7 and for G allele was 0.3.

Kea words: Diabetes · Insulin Receptor Substrate-1 gene · Mitochondrial DNA · PCR-RFLP

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by high blood glucose level [1]. Diabetes mellitus is a major public issue due to its high prevalence and long term complications. Worldwide, the number of people with diabetes is increasing due to population growth, aging, urbanization, life style, physical inactivity. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [2]. In Saudi Arabia diabetes represents a major clinical and public health problem. The prevalence of diabetes is high among the Saudi population and 90% of diabetics suffer from Type II (T2DM) [3,4]. Growing concern about diabetes worldwide and particularly the staggering impact the disease is having in the Gulf Region, where the picture is especially alarming. The Co-operation Council for the Arab States of the Gulf (GCC) has some of the highest rates of type II diabetes in the world. Qatar, Bahrain, United Arab Emirates, Saudi Arabia, Kuwait and Oman are of the International Diabetes Federation's 'top10' countries for diabetes prevalence [5]. The anticipated prevalences for diabetes 2010-2030 in the Gulf countries are: United Arab Emirates (UAE) 18.7-21.4%, Kingdom of Saudi Arabia (KSA) 16.8-18.9%, Bahrain 15.4-17.3%, Kuwait 14.6-16.9% and Oman 13.4-14.9% [5, 6].

It is well established that genetic factors play an important role in the development of diabetes mellitus [7]. Genetic polymorphisms are integral to the development of genetic markers to identify individuals at risk of developing such disease [8].

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In Saudi Arabia several studies have been conducted to evaluate the association between different diabetes types (Type 1, Type 2 and gestational) with different genes polymorphisms using different molecular genetic approaches [9-14].

Mitochondria play a key role in biogenesis, in glucose metabolism and in adenosine-5-triphosphate (ATP) production, which has been linked to insulin secretion in pancreatic beta cells [15]. Several line of evidences showed numerous mitochondrial DNA mutations were significantly associated with development of diabetes [16-19].

Insulin receptor substrate-1 (IRS-1) is the first substrate of the insulin receptor in the insulin signaling pathway [20]. Due to this central role, the IRS-1 function could be related to the development of T2DM. Almind *et al*. [21] found association between Insulin receptor substrate-1 IRS-1 gene polymorphisms with T2DM.

The aim of the present work is screening for the Insulin receptor substrate-1 (IRS-1) and mitochondrial tRNA)[Leu(UUR)] 3243 polymorphisms among T2DM Saudi patients.

MATERIALS AND METHODS

Patients and Blood Sample Collection: In the present study 106 type 2 diabetes mellitus Saudi patients were recruited and collected from the king Abdul-Aziz specialized hospital, Taif governorate. Whole blood samples of T2DM patients were drawn from a peripheral vein into ethylenediaminetetraacetic acid (EDTA) tubes and stored at-20°C until DNA extraction step.

DNA Extraction: Genomic DNA was extracted from blood samples according to instructions of Blood DNA Preparation Kit (Jena Bioscience; Germany).

The procedure of this kit is consists of three major steps. Blood samples were subjected to the first step in order to cell Lysis. Proteins were precipitated from the lysate with protein precipitation solution. Then, DNA was precipitated and hydrated via ethanol and DNA hydration solution respectively.

Genotyping: PCR amplification and genotyping of Insulin receptor substrate-1 (IRS-1) A6224G and transfer ribonucleic acid tRNA)[Leu(UUR)] 3243 polymorphisms were carried out via PCR-RFLP technique. Amplification of specific targeted DNA fragments was carried out with a total volume of 25 ul consists of 50-70 ng DNA template, 12.5 ul of 2X superhot PCR Master Mix (Bioron; Germany) and 10 Pmol of each used primer. The sequence of each used primer is listed in (Table 1). The PCR program for amplification of tRNA) Leu (UUR) was as follow; initial denaturation at 95°C for 3 min, followed by 35 cycles each at 95°C for 1 min, annealing temperature at 55 for 1 min, extension temperature at 72°C for 1.5 min and final extension at 72°C for 10 min. [7]. Amplification of Insulin receptor substrate-1 (IRS-1) gene was carried out with denaturation at 94°C for 3 min. and followed by 35 cycles of 94°C for 45 sec, 58°C for 45 secand 72°C for 1 min., final extention at 72°C for 5 min. [8]. The amplified DNA fragments were eletrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5%-agarose gel (Bioshop;Canada) for 30 min using Tris-borate-EDTA Buffer. The gels were stained with 0.5 µg/ml of ethidium bromide (Bioshop; Canada), visualized on a UV Transilluminator, photographed using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland,USA).

The amplified DNA fragments specific for A6224G and tRNA[Leu(UUR)] 3243polymorphisms within Insulin receptor substrate-1 (IRS-1) gene and mitochondrial gene transfer ribonucleic acid were digested with 2-4 units of

Table 1: Names of studied genes and sequences of used primers

Gene	Target polymorphism	Primers
Insulin receptor substrate-1 (IRS-1)	A6224G	5-CGGTCTTGTAAGGGTAAC-3
		5-GAATTCACATTCCCAAGACA-3
Mitochondrial gene transfer ribonucleic acid	tRNA[Leu(UUR)] 3243	5-AGGACAAGAAATAAGGCC-3
		5-CACGTTGGGCCTTTGCGTA-3

Table 2: Polymorphisms, restriction enzyme and expected digestion fragment size

Target polymorphism	Amplified fragment size	Restriction enzyme	Expected genotypes	Fragment size
A6224G	324bp	*NsiI*	AA	324
			AG	324+239+85
			GG	239+85
tRNA[Leu(UUR)] 3243	294bp	*ApaI*	AA	294
			AG	294+179+115
			GG	179+115

NsiI and *ApaI* restriction enzymes respectively. According to the manufacture instructions specific restriction enzyme 10X buffer in reaction volume of 20ul was used.For each resrection enzyme, incubation temperature and period as well as heat inactivation protocol were optimized and carried out according to manufactorinstructions. The digested DNA fragments were eletrophoresed on 2.5% agarose gel stained with ethidium bromide. The gels were visualized on a UV Transilluminator, photographed using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland,USA). The digital image files were analyzed using Gene Tools software from Syngene.

Expected amplified fragment size and genotypes for each studied polymorphisms were illustrated in Table (2). Two DNA fragments and three genotypes could be produced in cas of A to G mutation was found in the amplified fragments of studied mtDNA and IRS-1 genes Table (2).

RESULTS

Amplification of A6224G and tRNA[Leu(UUR)] 3243 polymorphisms within Insulin receptor substrate-1 (IRS-1) gene and Mitochondrial gene transfer ribonucleic acid was carried out. 324bp and 294bp DNA fragments specific for A6224G and tRNA[Leu(UUR)] 3243 polymorphisms respectively were detected in all used DNA samples Figures (1 and 2).

The resultant (324 and 294bp) DNA fragments were digested with of *NsiI* and *ApaI* respectively.

No patients with the mitochondrial tRNA[Leu(UUR)] gene mutation at position 3243 were found. Three genotyped of A6224G polymorphism within Insulin receptor substrate-1 (IRS-1) gene after digestion with *NsiI* were observed Figure (3).

The genotypic distribution and allelic frequency of studied polymorphisms were summarized in Table (4).

Fig. 1: PCR amplified DNA fragment (324bp) of A6224G within insulin receptor substrate-1 (lRS-1) gene among diabetic patients
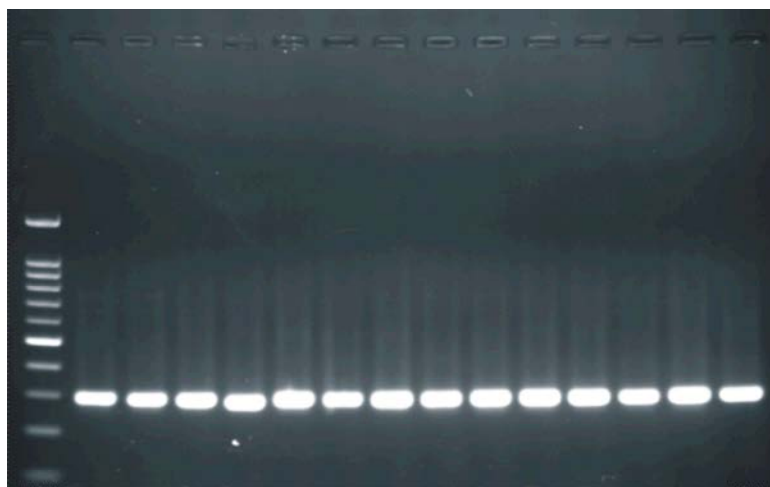

Fig. 2: PCR amplified DNA fragment (294bp) of A3243G polymorphism within mitochoundrial tRNA[Leu(UUR)] gene among diabetic patients
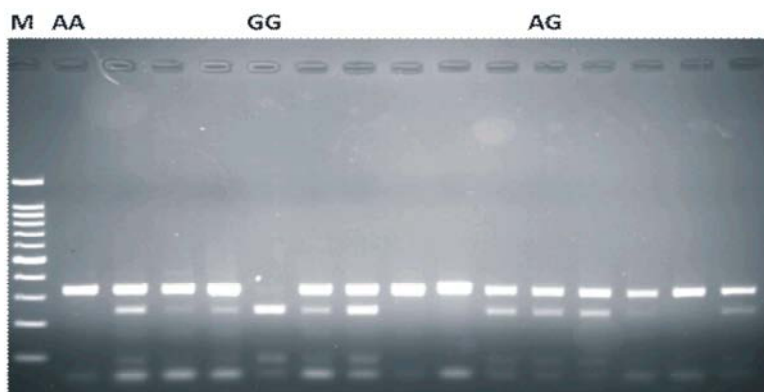
Fig. 3: Electrophoretic banding pattern of amplifired DNA fragment 324bp within lRS-1 gene after diagestion with *Nsil* restriction enzyme. Lane 1: slandered DNA marker (100bp); lane 2: AA genotype; lane 6: GG genotype; Lane 12: AG genotype

Table 4: Prevalence, genotypes and allele frequencies of A6224G polymorphism

polymorphism	No. of subject	Prevalence %	Allele frequency	
			A	G
GG	1	0.94		
AG	62	58.5	62	2
AA	43	40.6	86	62
Total	106		148 (0.7)	64 (0.3)

DISCUSSION

Insulin receptor substrate-1 (IRS1) gene encodes for substrate of the insulin receptor tyrosine kinase and involved in the signal transduction that characterizes insulin biological activity [8, 22]. So, different studies have been conducted to study the role of IRS1 as a candidate gene for type II diabetes. Several polymorphisms have been described in this gene, Laakso *et al.* [23] used single strand conformation polymorphism to analyze amino acid substation in noninsulin-dependent diabetes (NIDDM) among Finnish patients and identified three variants that predict amino acid substitution Gly818-Arg, Ser892Gly and Gly971Ar. The most common polymorphism was the Gly971Arg substitution which was found in 11 (9.8%) of 112 NIDDM patients. Almind *et al.* [24] reported that, mutation in codon 972 in IRS-1 impairs insulin-stimulated signaling, especially along the PI 3-kinase pathway and may contribute to insulin resistance in diabetic patients. Clausen and his co-workers [25] reported that, the combination of obesity and the codon-972 variant was associated with a 50% reduction in insulin sensitivity and suggested that, the codon-972 IRS-1 gene variant may interact with obesity in the pathogenesis of common insulin-resistant disorders. On the other hand there are different studies failed to confirm the association between IRS-1 polymorphisms and type 2 diabetes [8, 26]. The PCR

amplified DNA fragment (324bp) of A6224G within Insulin receptor substrate-1 (IRS-1) gene among Saudi type 2 diabetic patients and electrophoretic banding pattern of amplified DNA fragment 324bp after digestion with NsiI restriction enzyme were shown in Figures (1 and 3). The insulin receptor gene *NsiI* RFLP alleles and genotype distribution in the subjects were described in Table 4. One hundred and six type 2 diabetic patients were genotyped for A6224G polymorphism. Out of them, there were 43 patients with AA genotype with prevalence 40.6%, 62 were AG genotype with prevalence 58.5%. While GG genotype was recorded with only one patient with prevalence of 0.94% Table (4). The A allele frequency was 0.7 and G allele frequency was 0.3. On the other hand among 106 patients no presence of mitochondrial tRNALeu[(UUR)] A to G 3243 mutation was observed. The prevalence of AA genotype was 100%. The A allele frequency was1 and G allele frequency was 0. This finding is inconsistence with different studies among other Asian diabetic patients. Two different studies were conducted with Japanese diabetic patients to study the prevalence of mitochondrial tRNA[Leu(UUR)] A to G 3243 mutation. Otabe and his coworkers [27] reported that it was 0.9% and it was 0.01% by [28]. In addition among Korean diabetic patients [7] demonstrated that, the prevalence of mitochondrial tRNA[Leu(UUR)] A to G 3243 mutation was 0.02%.

CONCLUSION

In this screening of 106 type 2 diabetic Saudi patients, no patients with mitochondrial tRNALeu[(UUR)] A to G 3243 mutation were found. Among A6224G mutation within Insulin receptor substrate-1 (IRS-1) gene, one homozygote patient was observed. Further comprehensive studies would be needed to clarify the role of some polymorphisms in the etiology of type II diabetes. Thus, such polymorphisms could be employed as diagnostic marker of type II diabetes.

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