Single Nucleotide Polymorphism in Protamine 1 and Protamine 2 genes in fertile and infertile for men of Al-Najaf City

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Single Nucleotide Polymorphism in Protamine 1 and Protamine 2 genes in fertile and infertile for men of Al-Najaf City

MOHAMMAD Al ZEYADI* , ALAAULDEEN S.M. AL-SALLAMI* and MUSTAFA T. ALBALDAWY*,

* **Department of Biology, Faculty of Science, Kufa University**

Mohammed.mhawish@uokufa.edu.iq

ABSTRACT

Around 15% of the couples on the planet confront failure in the primary involvement in pregnancy. These issues in these couples can be explained as infertility. The objective of the study was to determination relationship among polymorphisms Protamin1 (G197T) and Protamine2 (C248T) were studied in three groups (Teratozoospermia n=37, infertile normozoospermia n=34 compare with fertile normozoospermia 17=0). **Methods:** Samples were collected at the Fertility Center Laboratories in Sadr Medical City. Analysis of SNPs was performed for PRM1 and PRM2 by using restriction fragment length polymorphism (PCR-RFLP). **Results:** The result showed Three types of polymorphism occur in both PRM1 (G197, G197T and G/T197) and PRM2 (C248, C248TandC/T248) genes found in all our patients.
In c**onclusion** the results are consistent with some previous studies and indicating that all our tested with SNPs was associated with teratospermia and normospermia as idiopatic male infertility in Iraqi population, which can find a genetically engineered solution in the future .

Keywords: Infertility ,Teratospermia ,Normospermia PRM1, PRM2, SNPs

1. INTRODUCTION

Infertility is a typical illness of the reproductive system, incapacity to have healthy birth following one year of effectively endeavors of unprotected free intercourses [1]. Around 15% of the couples on the planet confront failure in the primary involvement in pregnancy. These issues in these couples can be explained as infertility [2]. Some researchers classified reasons for infertility into four categories: male factors, female factors, congregated factors and idiopathic factors [3]. Additional factors that influence on male fertility involve weight of the body (body mass index), smoking and work [4]. Idiopathic is unknown causes of infertile males which is often associated with epigenetic and genetic abnormalities [5]. Large quantities of biomarker proteins[30], a large number of essential protein and specific proteins in tissue that have been found in the seminal plasma that represent precise indicator for pathologic status related with reproductive system[6]. For example, Protamines (PRMs) includes the biggest

amount of nucleoproteins in develop sperm of human. These proteins are translated in steps 1-4 of spermatids. While synthesis of the relating proteins begins, with temporal delay, in step 4 spermatids [7].Many of abnormal spermatogenesis and induce sperm chromatin damage or defect in imprinting and DNA breaks are have been reported as Mutation in protamine genes [8].The defected proteins of Protamine causes abnormal condensation of sperm chromatin ,these abnormality of increases break of DNA strand and immobility of spermatozoa that can led to male infertility.(9)

. Different studies reported that abnormal expressions of protamine gene in sperm of fertile men. Additionally, relationship of the changed PRM1/PRM2 ratio has been appeared with low count in sperms, reduced in motility of sperm and morphology, diminishes the fertilization capacity and increased sperm chromatin defect [9], [10]. Altered P1/P2 ratios in the sperm have additionally been accounted for to be one of the critical reasons for male infertility [9], [11], [12]. The purpose for this changed ratio might be an interrupted post-translation modification or mutation in the PRM/TNP genes [9], [12]. This study deals with nuclear protein (protamine) that has an important role in the spermatogenesis and detection part of problems that related with the male

The objective of this study was to test SNPs in (G197T) protamine1and protamine2 C248T in idiopathic infertile men(normospermia) with Teratospermia and with normal individuals(fertile normospermia) to determine and analyze their relationships with idiopathic male infertility in Al-Najaf City from Iraqi cases.

2. MATERIALS AND METHODS

DNA was extracted from blood samples for infertility patients after semen analysis[1] and divided into three categories : Teratospermia infertile patients (n=20) , Normospermia infertile patients(n=20) and compare with fertile normospemria (n=10) as healthy group .This procedure was measured according to the standards required by the manufacturer company (FAVORGEN). Amplifications of PCR were performed by using two primer pairs. for protamine1 amplification were: Primer sense was: (5´-cccctggcatctataacaggccgc-3´) and primer anti-sense: (5´- tcaagaacaaggagagaagagtgg-3´) for protamine 2 , the primer sense was: (5´- ctccagggcccactgcagcctcag- 3) and primer anti-sense: (5´-gaattgctatggcctcacttggtg-3´) The amplified fragments of PCR from PRM1 were 557 bp and from PRM2 were 599bp. At the beginning we set the thermo-cycle to 95°C degrees for five minutes. The condition of PCR of PRM1 was 32 cycles were , 95 °C 1 min, 66 °C for 1 min as annealing and 72 °C for 1 min as

extension in finally at 72 °C for 5 min. For PRM2, the PCR condition was 95°C degrees for five minutes, 32 cycles were , 95 °C for 45 sec as denaturation, 70 °C for 45 sec annealing and 72oC for 30 sec as extension . 72 °C for 5 min as finally step. PCR products were electrophoresed using 1.5% agarose gel with 2 µl ethidium bromide. Then PCR products of PRM1 were digested with BSeRI by adding 1µl of RE, 5µl of buffer and 5µl of DNA PCR product and incubate for 2.5 h at 37°C. And PCR products of PRM2 were digested with MScI by adding 1µl of RE, 5µl of buffer and 5µl of DNA PCR product and incubate for 2.5 h at 37°C and separated on a 1.5% agarose gel with 2 µl ethidium bromide.

3. Results and Dissection

Analysis of G197T SNP of PRM1

All PCR products of genomic fragments of PRM1 (557 bp) were used for digestion with BSeRI. After digestion of PCR products by restriction enzyme BSeRI (GAGGAG), In all 50 samples (40 infertile and 10 fertile individuals) after digestion with BSeRI both of fragments products were in 28 sample with two alleles carrying the GG in the PRM1 gene, detected in teratozoospermic patient 9 (45%), infertile normospermic patients 10 (50%) and a fertile control 9 (90%), which produces two fragments with different length (319 and 238 bp). and analysis of DNA of PRM1 gene with two alleles carrying the TT G197T SNP were 12, in teratozoospermic patients 6 (30 %), infertile normospermic patients 5 (25%) and fertile control 1 (10%) and produces one fragments (557 bp) (Table 1). And heterozygotes polymorphism G/T197 SNP were present in 10, 5 (25%) teratozoospermic patients, 5 (25%) infertile normospermic patients and control (0%) which produce all the three bands (557bp, 319bp, 238bp) (Figures 1,2).

Table 1 Frequencies and numbers of genotypes and alleles of PRM1 polymorphism G197T SNP in patients and control.

Type of Polymorphism	Total	Control N=10 (%)	Teratospermic Patients N=20 (%)	Infertile normospermic patients N=20 (%)
G197 SNP variant	28	9(90%)	9(45%)	10(50%)
G197T SNP variant	12	1(10%)	6(30%)	5(25%)

G/T197 SNP variant	10	0(0%)	5(25%)	5(25%)

Teratozoospermic patients.

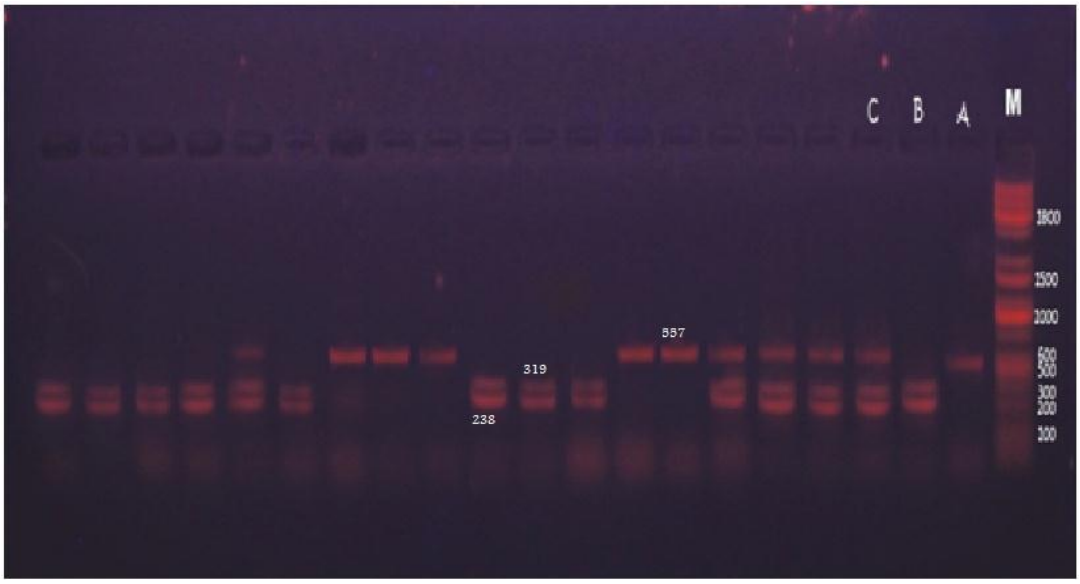

Figure (1) AnalysedG179T SNP by RFLP PCR of DNA from* Teratozoospermic *patients. M) Molecularmarker, A) PCR product that was undigested, B) completely digested, C) partially digested

Infertile normospermic patients.

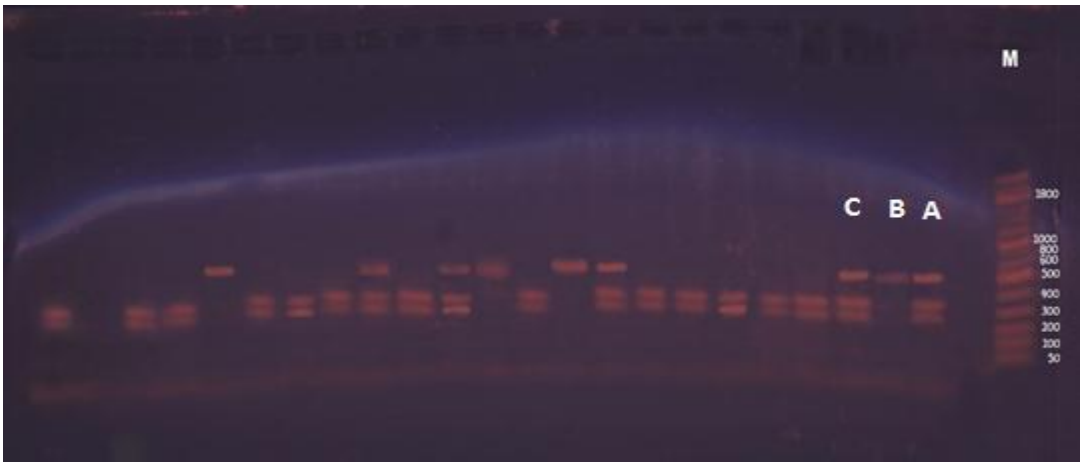

Figure (2) AnalysedG179T SNP by RFLP PCR of DNA from infertile patients. M) Molecularmarker, A) PCR product that was undigested, B) completely digested, C) partially digested,

Analysis of C248T SNP of PRM2

All PCR products of genomic fragment of PRM2 (599bp) were used for digestion with MscI. After digestion of PCR products by restriction enzyme MScI (TGGCCA), In all 50 samples (40 infertile and 10 fertile individuals) after digestion with MScI both of fragments products were in 33 sample with two alleles carrying the CC in the PRM2 gene, was detected in teratozoospermic patient 17 (85%), infertile normospermic patients 16 (80%) and a fertile control 9 (90%), which produces two fragments with different length (402 and 197 bp). and analysis of DNA of PRM2 gene with two alleles carrying the TT C248T SNP were 3, in teratozoospermic patients 1 (5 %), infertile normospermic patients 2 (10%) and fertile control 1 (10%) and produces one fragments (599 bp) (table 2). And heterozygotes polymorphism C/T 248 SNP were present 4, in teratozoospermic patients 2 (10%), in infertile normospermic patients 2 (10%) and control (0%) which produce all the three bands (599bp, 402bp, 197bp) (Figure 3,4)

Table 2 Frequencies and numbers of genotypes and alleles of PRM2 polymorphism C248T SNP in patients and control.

Type of Polymorphism	Total	Control N=10 (%)	Teratospermic Patients N=20 (%)	Infertile normospermic patients N=20 (%)
C248 SNP variant	42	9(90%)	17(85%)	16(80%)
C248T SNP variant	4	1(2%)	1(5%)	2(10%)
C/T248 SNP variant	4	0(0%)	2(10%)	2(10%)

A- Teratozoospermic patients.

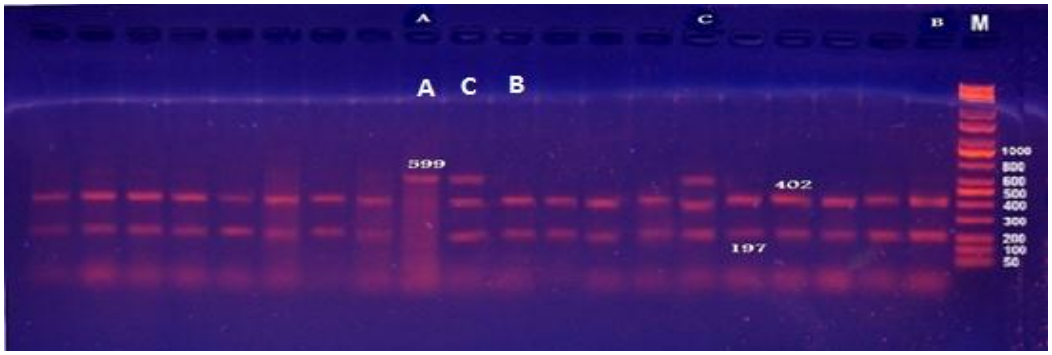

Figure (3) Analyses of C248T SNP by RFLP PCR of DNA from* Teratozoospermic *patients. M) Molecular marker, A) PCR product that was undigested, B) completely digested, C) partially digested .

B- infertile normospermic patients

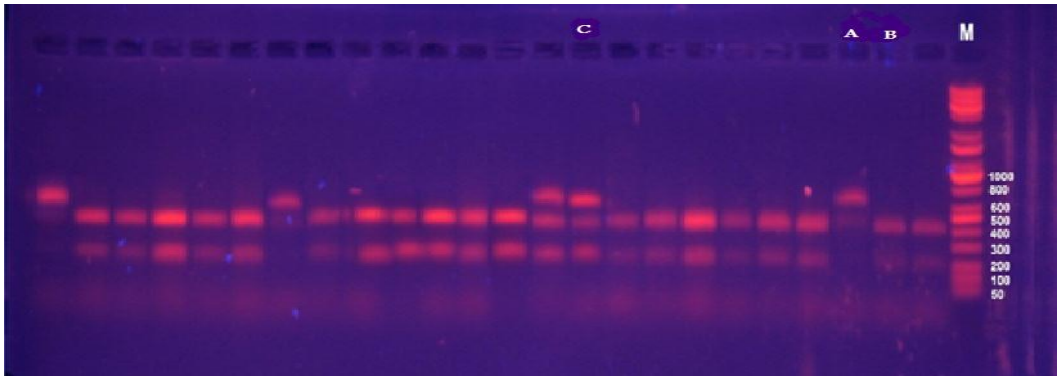

Figure (4) Analyses of C248T SNP by RFLP PCR of DNA from infertile patients. M) Molecular marker, A) PCR product that was undigested, B) completely digested, C) partially digested .

The present study has identified a common polymorphism in the PRM1 gene that is present at a significantly higher frequency, the results after digestion with BSeRI showed three type of polymorphism in PRM1 genes G197 (homozygote), G197T and G/T197 (heterozygote) in infertile patients compared with fertile men.

Our studies agreed with Iguchi et al, this study detect 1 SNP (G197T) resulting in change of arginine to serine in the PRM1 gene has been detected in 3 of 30 infertile patients [13].

Cristina et al., identified a common polymorphism in the promoter region of the Protamine1 gene that is found a higher significantly in infertile men with altered in spermatozoa morphology compared with control group [14].

Imken et al., detected some associated of SNPs in the Protamin1 gene to be with infertility, for example, detected point mutation G107C out of 281 in 135 infertile men. And point mutationG197T has been detected in 10% of 30 infertile men in USA population [15], [16].

The present study disagreed with Salamian et al., who reported that the absence of mentioned SNP of Protamine1 results in full enzymatic digestion of the amplified fragment [17].

Different former studies reported that aberrant PRM expression is related with men infertility de [18], Among these studies, small populations of infertile men with absence of PRM2 protein and deregulation of PRM1 expression have been reported [19], [20].

Our results also agreed with Iguchi et al., who discovered a novel SNP, G197T, in the Protamin1 gene in three from thirty infertile men, this SNP causes an amino acid to change from arginine to serine in a highly conserved arginine cluster [21].

The increase in the PRM1/PRM2 ratio may be due to an increase in the PRM1 gene expression or to an overall altered in PRM1 and PRM2 genes expression. Of potential relevance, both genes are located in the same chromatin loop and therefore could be subjected to a coordinate regulation [22], [23].

In the current study, we have identified a common polymorphism in the PRM2 gene that is present at a significantly higher frequency, our results after digestion with MScI showed three type of polymorphism in PRM2 genes C248 (homozygote), C248T (homozygote) and C/T248 (heterozygote) in infertile patients with compared with control.

Yang et al., work on the other region of PRM2 gene and found three genotypes were observed in the SNP locus (G398C) of PRM2 in infertile men and fertile controls [24].

Other previous studies reported the relationship between protamine variants and sperm parameters, such as sperm counts, motility, and morphology were assessed [25], [26]. It was reported that an elevated of sperm concentration and total sperm count was observed in the CC genotype of Protamine2, although the difference was not statistically significant [27].

The current study disagreed with Ahmad Salamian et al., who reported that the absence of mentioned SNP causes full enzymatic digestion of the amplified fragment [17].

In the spermatogenesis process, histones are replaced by protamines, promoting an 85–95 % association of sperm DNA with protamines [28]. Mutations in Protamine1, protamine2, and associated regulatory regions may have an effect upon expression of protamine. Altered PRM expression would induce inadequate chromatin condensation, DNA fragmentation, and men infertility [29]. In conclusion the results are consistent with some previous studies and indicating that all our tested with SNPs was associated with teratospermia and normospermia as idiopatic male infertility in Iraqi population, which can find a genetically engineered solution in the future .

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