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Single Nucleotide Polymorphisms Detection In *PRM1* Gene Of Infertile Men.

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ABSTRACT

At least 50% of married couples are infertility; it is the major concern among them. The male partner is the sole contributor to the problem. These men are diagnosed as unexplained cases and with no a etiology or idiopathic cases. The current study was concerted to estimate the role of single nucleotide polymorphisms (SNPs) in the protamine-1 gene PRM1 of 160 human male (130 infertile and 30 fertile individuals) by Restriction Fragment Length Polymorphism RFLP assay. We were detected 95 sample with alleles carrying Guanine (G) in sequence 197G of PRM1 gene fragment, fertile control 16 (53.3%) and an infertile patient79 (69.7 %), and 38 patients: fertile control 8 (26.6%) and infertile 30 (23 %) carrying a G SNP, while a heterozygotes polymorphism G/T197 SNP variant were present in 27 sample: control 6(20%) and infertile 21 (16.1%), suggesting that there are G197T SNP of PRM1 in the studied population Thus, the RFLP assay may be provides a rapid and a simple screening method to identification the polymorphism.

Keywords: infertile , RFLP_PCR , Polymorphism, PRM1.

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INTRODUCATION

About 10-15 % of married couples are known to experiment form of infertility problem, Approximately 50% of them related to male factors. weaken male fertility have been suggest for some causes[2] many of cases, which involve either inglorious sperm or unsuitable spermatogenesis [1,8]. The rise incidence of declining sperm count in European men and the low sperm counts reported in last decades proposed the tandem with lifestyle in the environment [8].

Actually, Some factors in lifestyle as diet ,caffeine , smoking, alcohol, drugs, and, circadian rhythm shifts may be induce reverse effects on male fertility [11,12]. The spermnucleus adopt rearrangement, which participate the removal of histone proteins and their alteration in different proteins in nucleus during spermatogenesis [20]. The head of sperm is highly condensed DNA with protamine1 PRM1 and protamine2 PRM2. When could identified the differences in protamine it was assume could be present the mutations in identical genes [21].

Some mutations in genes protamine have been identified to reason defect imprinting and in spermatogenesis and stimulate DNA breaks and damage of sperm chromatin [8,9,14]. The protamines have been proposed for several functions [15]. The generation of a condensed paternal genome with a more compact were the most obvious and hydrodynamic nucleus conserve the genetic message transfer by the spermatozoa during making it understand to nucleases or mutagens potentially present in the media. and devoid of epigenetic information [13]. And there are some addition function take part in reprogramming of it by the oocyte , and imprinting of the paterna l genome in spermatogenesis [13]. Also it has been suggested that protamines could be Contributes in checkpoint during spermatogenesis and have important role in fertilized of the ova [1,15].

MATERIALS AND METHODS

Study group included 160 infertile men with idiopathic infertility with a normal female partner after a gynaecological examination attending the infertility clinic at All Al Sader hospital. The control group included 50 healthy fertile donors who had fathered child in past 2-4 years. All participants were evaluated for complete medical history and physical examination.

Semen collection

By masturbation the semen fluid were collected after three days from asexual contact and after liquefaction at 37^{o}C for 40 minutes . According to WHO was performed the standard semen analysis [6].

Semen Analysis.

Appearance. Viscosity, Volume, pH, Motility, Sperm count, Sperm morphology.

Isolation of DNA from Sperm

After semen analysis, the remained semen sample was layered in Isolate sperm separation medium.

Isolation of sperm DNA

The sperm pellet was incubated at 55°C overnight with the sperm lysis medium (60mM DTT, 4% SDS and 350µg/ml protienase K (Bangalore Genei, India) made in lysis buffer). After complete digestion, sperm DNA was precipitated by adding equal amount of chilled isopropanol (Merck KGaA, Germany). And washed DNA pellet twice with 70% ethanol, than dried the ethanol at 37°C and by Tris-EDTA (TE) buffer dissolved it.

Detects the G/T SNP by PCR- RFLP assay (Restriction Fragment Length Polymorphism)

We analyses of PRM1 gene by used restriction enzyme BseRI which recognize GAGGAG and digested fragment of PRM1 gene (550 bp) to produces two fragments with different length (380 and 170 bp.) in G nucleotide polymorphism (SNP) at nucleotide 197 while in T polymorphism the GAGGAG changing to GAGTAG

and the BseRI enzyme can't recognize so produces one fragments (550 bp) in heterozygous G and T individual produces three fragments with different length (550, 380 and 170 bp.) so we digested the PCR products with BseRI by incubated it at for 1h and separated 2% of agarose gel, and visualized DNA by 5 µl of ethidium bromide staining .Primer pairs were used and PCR of 25µl reaction mixture was set up as described by Aoki et al (2006)[1] Forward(5'-cat agg cag ccc cta cac tc-3') Reverse (5'-ccc tct caa gaa caa gga gag aa-3'.) : programed 94°C /4 minutes than 35 cycles of 94°C /30s; annealing temperature for 30s 62.5°C, , followed by 72°C 60s for extension and a 5 minutes at 72°C in final hold .

RESULTS

Analysis of G197T SNP of *PRM1*

All PCR products of PRM1 genomic fragments (550 bp) were used for digestion with the restriction enzyme BseRI (GAGGAG). The polymorphism SNP was identified in the population screened in the present study. In all 160 samples (130 infertile and 30 fertile individuals) both of fragments which were products after BSeRI digestion were in 95 sample with two alleles carrying the GG in the PRM1 gene, was detected an infertile patient79 (69.7 %) and a fertile control 16 (53.3%) , which produces two fragments with different length (380 and 170 bp). Analysis of DNA of the PRM1 gene with two alleles carrying the TT G197T SNP were 38 patients a fertile control 8 (26.6%) and infertile 30 (23 %) produces one fragments (550 bp) (Figure 2). A heterozygotes polymorphism G/T197 SNP variant were present in 27 sample: control 6(20%) and an infertile 21 (16.1%) all the three bands (550bp, 380bp, 170bp). (Table 1). Which suggesting that there are G197T SNP of PRM1 in the studied population.

Table 1: Frequency and number of genotypes and alleles of PRM1 polymorphism G197 SNP in control and patients.

Polymorphism type	Total	Control N=30 (100%)	Patients N=130 (percentage)
G197 SNP variant	95	16 (53.3%)	79 (69.7 %)
G197T SNP variant	38	8 (26.6%)	30 (23 %)
G/T197 SNP variant	27	6 (20%)	21 (16.1%)

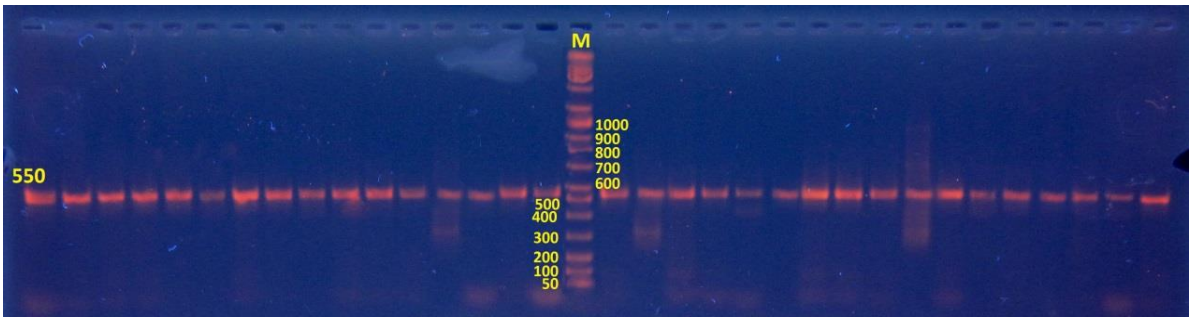

Figure 1: PCR product of PRM1 gene in fertile and infertile male produces one fragments (550 bp).

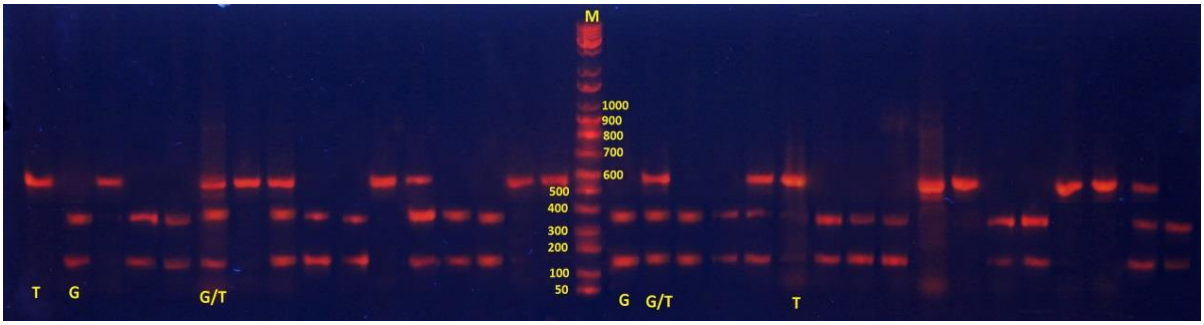

Figure 2: product of PCR- RFLP of *PRM1* gene in fertile and infertile male . T) Undigested PCR product for infertile male produce one fragments 550 bp (TT) , G) PCR- RFLP product of infertile male (GG) complete digested produce two fragments (380bp, 170bp), G/T) Partially digested PCR product for infertile male (GT) produce three fragments (555bp, 315bp, 105bp). M) Molecular marker (100 bp).

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DISCUSSION

Per haploid genome the human have one copy of the protamine 1 gene PRM1, it located on chromosome 16 [10]. The main DNA-binding proteins in sperm nucleus is Protamine1,which package DNA into sperm. Protein Protamine1 PRM1 is a single polypeptide have 50 amino acids, which have two different forms at least [13]. different speculations in Studies in different populations have indicated variations in human protamine genes [4, 7, 17]. Some investigations found some significant relationship between male infertility and transition protein 1 and reduced protamine in gene expressions [3]. Some single nucleated polymorphism several SNPs such as mutation in the PRM1 gene have detected in some populations these is interesting in male infertility [19]. We detected the Genotyping of PRM1 gene Polymorphism and analyzed it through PCR-RFLP (restriction fragment polymorphism length polymorphism) technique. All PCR products of PRM1 genomic fragments (550 bp) were used for digestion with BseRI(GAGGAG). Restriction enzyme. The absence of SNP results in full enzymatic digestion of the amplified fragment, which produces two fragments (380 and 170 bp). In all 160 samples (130 infertile and 30 fertile individuals) both of fragments which were products after BSeRI digestion were in 95 sample was detected with DNA from an infertile patient79 (69.7 %) and a fertile control 16 (53.3%) have two alleles GG in the PRM1 gene.

The DNA analysis from 38 patients: fertile control 8(26.6%) and infertile 30 (23 %) carrying a G197T SNP produces one fragments 550 bp. A heterozygotes polymorphism G/T197 SNP variant were present in 27 sample: control 6 (20%)and a infertile 21 (16.1%) . Imken et al 2009 were detected some associated of SNPs in the PRM1 gene to be with infertility, for instance , detected point mutation G107C out of 281 in 135 case infertile male [7]. and point mutationG197T has been detected in 10% of 30 infertile male in USA population[4] These results may in agreement with our results studies who represented in the sequence databases. Alternatively, rare SNPs may have been used for DNA sequencing . In previous study [4,19] they demonstrated the PRM1 gene variant Influence in DNA repair and in male infertility. There are correlation between polymorphism of patients according to studies in the world at this work. In addition some male infertility demonstrated there are some clinical and biological factors on the incidence of PRM1 gene mutations in male infertility and Several factors were included: age of patients , Binet stage at diagnosis, initial WBC count, previous treatment [1].

Island by Kichine et al 2008 detected some cases with G/C observed in 672 infertile men .and other other was reported C/A and demonstrated similar prevalence for fertile and infertile groupin Japanese [10]. The G197T SNP leading in Arginine change to Serine in protamine 1 according recently studies in some infertile patients.

CONCLUSION

In conclusion the happening of PRM1 gene difference or single nucleated polymorphism causing amino acid exchange in infertile men marked to the strong demand to keep and conserved the function of protamine during fertilization. Therefore, tested SNPs in this study may be could get us some indicator to affect spermatogenesis and some correlation between patents in different stage and age in idiopathic male infertility in Iraqi population. The chance is now available to look into the mechanisms of these associations of mutations and to choose if this PRM1 polymorphism might also have relation with the results of reproduction.

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