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The Impact of G1575A Matrix Metalloprotease-2 Gene Polymorphism on Male Fertility

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Background: Matrix metalloproteinases contain more than 20 enzymes that require zinc for their activities. Gelatinases are one of the subtypes of matrixmetalloproteinases, which degrade gelatin and collagen type 4, and are present in male reproductive tissues such as in prostate. G1575A Matrix Metalloproteinase-2 (MMP-2) gene polymorphism affects MMP-2 activity.
Objectives: The aim of this study was to investigate the prevalence of G1575A matrix metalloproteinase-2 gene polymorphism in fertile and infertile men.
Patients and Methods: In this study 200 fertile men as controls and 200 idiopathic infertile men as cases were investigated. For genotyping thefertile and infertile group the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) method was used.
Results: Genotype frequencies of G/A in fertile and infertile men were significantly different ($\chi^2 = 4.16$, df = 1, p = 0.041). Genotype frequencies of G/G and A/Ain fertile and infertile men were not significantly different ($\chi^2 = 3.32$, df = 1, P = 0.068 and $\chi^2 = 0.521$, df = 1, P = 0.47, respectively). The risk of infertility was 1.43 folds higher in individuals with the A/A genotype compared to those with the G/G genotype. In men with the A/A genotype the risk of infertility was 2.14 folds higher than individuals with the G/A genotype.
Conclusions: These finding suggests that genetic variation of MMP can affect male infertility.

Keywords: Matrix Metalloproteinase 2; Infertility, Male; Polymorphism, Genetic; Reproduction

1. Background

Infertility represents a major clinical problem that affects married couples both medically and psychologically. Nearly 15% of couples are infertile, 25% of which are related to male factors. Cause of infertility in 25% of infertile men with abnormal semen is unknown (1). Many environmental and genetic factors affect male infertility (2, 3). Major modifications in structural and functional properties of reproductive organs during adult life involve degradation of Extracellular Matrix (ECM) and remodeling of connective tissue. These modifications require enzymatic activities but the Matrix Metalloproteinases (MMPs) due to their features have important roles in this process (4). Mammals have 28 types of matrix metalloproteinases, 18 types of which have been found in testicles. One study suggested that CD 147 regulates expression of Matrix Metalloproteinase-2 (MMP-2) synthesis during spermatogenesis (5). It is believed that MMPs and their tissue inhibitors (TIMPs) have important roles in a number of physiological processes including ovulation and fertilization (6). The study showed that MMP-2 is basically limited to the main apical portion of acrosome of the sperm (7). Another study showed that the concentration of MMP-2 was significantly decreased in seminal plasma of patients with azoospermia compared with normospermia (8). Matrix metalloproteinases require zinc for their activities and are involved in the degradation of protein components of the extracellular matrix such as collagen, proteoglycans and elastin, and facilitate tissue remodeling and cell migration. Thus they can have a role in breaking the physical barrier between sperm and egg in fertilization (9, 10) and in fetal growth (11). Gelatinases are one of the subtypes of matrix metalloproteinases and includes matrix metalloproteinases 2 and 9 (collagenases A and B with molecular weight of 72 and 92 kD, respectively). These enzymes usually degrade gelatin and collagen type 4 (12-14). Matrix Metalloproteinase-2 is synthesized and secreted as a zymogen so the activation of MMP-2 is an important step in controlling its

activity. This function is supported by Membrane-Type 1 (MT1)-MMP and TIMP-2. Synthesis and activation of MMP-2 is regulated by local and hormonal pathways. It has been shown that G1575A MMP-2 gene (ID 4313) polymorphism affects MMP-2 activity (15). An allele of G1575A polymorphism has been shown to be associated with higher MMP-2 activity compared with the G allele (15). Since, it has been shown that azoospermic compared with normospermic men have low MMP-2 concentration in seminal plasma, the role of MMP-2 in normal spermatogenesis is important. To the best of our knowledge, the prevalence of G1575A matrix metalloprotease-2 gene polymorphisms has not been investigated in male fertility thus far.

2. Objectives

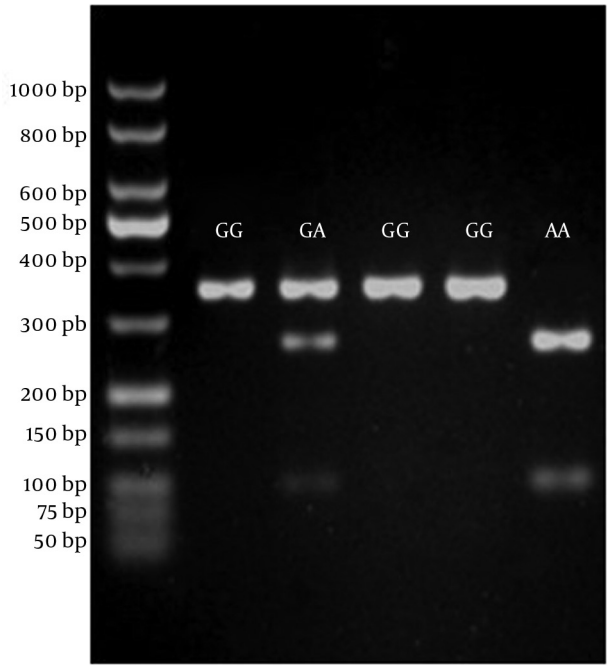
The aim of this study was to determine the prevalence of G1575A Matrix Metalloproteinase-2 gene polymorphism in fertile and infertile men residing in Hamadan, Iran. This case-control study consisted of 200 idiopathic infertile men and 200 fertile men. The fertile men had children and were staff members of the Hamadan University. Infertile men were selected from the Fatemieh Fertility Clinic of Hamadan University of Medical Sciences. Patients who had a specific reason for their infertility, such as varicoceles, were excluded. The two groups were matched according to age. The study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences. After collection and liquefaction of semen samples from infertile men, parameters such as sperm morphology, motility and concentration were determined (16).

3. Patients and Methods

3.1. DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes using the ethanol–chloroform method (17). Genotype analysis was performed by the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) method using forward, *5'-CACACCCACCAGACAAGCCT-3'* and reverse, *5'-TGGGGAATATGGGGAATGTT-3'* primers (15). For multiplication of each sample, a premix PCR kit (Bioneer, Korea) was used. For denaturation, annealing and extension processes temperatures were set at 94°C, 55°C and 72°C, respectively. The PCR product was subjected to electrophoresis on a 1.5% agarose gel, to confirm the 349 bp size of the PCR product. For genotype analysis, PCR products were digested using Rca1 (5 U) restriction endonuclease (Fermentas, USA). The final volume of RFLP was 30 μL. The homozygote GG genotype yielded a 349 bp product whereas two fragments of 260 bp and 89 bp were observed for AA homozygote subjects. Heterozygotes with the GA genotype on the other hand produced three 349 bp, 260 bp and 89 bp PCR products after Rca1 digestion (Figure 1).

Figure 1. Genotype Analysis of G1575A Matrix Metalloproteinase-2 Gene Polymorphism Using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Method



The PCR products were digested by Rca1 restriction endonuclease, run on a 1.5% agarose gel. The GG genotype produced a 349 bp,AA genotype 260 bp and 89 bp, and GA genotype 349 bp, 260 bp and 89 bpPCR product.

3.2.Statistical Analysis

Data were analyzed using the SPSS-16 software and represented as mean ± Standard Deviation (SD). Chi square analysis (χ^2 test) was applied to compare genotype frequencies between groups; odds ratios (OR) with 95% confidence intervals were calculated to determine the correlation between genotype, and fertility and infertility. The significance level was set at less than 0.05 for all tests.

4.Results

The homozygous GG genotype was detected in 53.5% of fertile men whereas 62.5% of infertile men showed the GG genotype. Moreover, homozygous AA genotype was detected in 1.5% and 2.5% of fertile and infertile men, respectively. In addition, the heterozygous GA genotype was detected in 45% of fertile males and 35% of infertile men. Analysis of genotype frequencies of G1575A Matrix Metalloproteinase-2 gene polymorphism showed that the G/A genotype was significantly different in fertile compared to infertile men ($\chi^2 = 4.16$, df = 1, P = 0.041), yet the frequencies of G/G and A/A genotypes did not show any significant differences between groups (P = 0.068 and P = 0.47, respectively). Allele frequencies of G1575A MMP-2 gene polymorphism are shown in Table 1. The allele and genotype odds ratio (OR) of G1575A MMP-2 gene

polymorphism in fertile and infertile men are shown in Table 2. Our results showed the risk of infertility in individuals with A/A genotype is 1.43 and 2.14 fold more than individuals carrying G/G and G/A genotypes respectively. The semen profiles of infertile subjects regarding MMP-2 genotypes are described in Table 3. According to the spermiogram, infertile men were divided to two asthenozoospermia (n = 97) and terato-asthenozoospermia (n = 103) groups. The genotype distribution of G1575A polymorphism in three groups of fertile, asthenozoospermia and terato-asthenozoospermia are shown in Table 4.

5. Discussion

The aim of the present study was to determine the prevalence of G1575A Matrix Metalloproteinase-2 gene polymorphism in fertile and infertile men in a western population of Iran. This polymorphism is located in the promoter region of MMP-2 gene, and due to the role of the promoter in regulation of gene expression, this polymorphism may affect gene expression and enzyme activity.We showed that frequencies of G/A in fertile and infertile men were significantly different, yet frequencies of G/G and A/A did not significantly differ in fertile and infertile men. According to our study, the risk of infertility in individuals with the A/A genotype is 1.43 folds more than individuals carrying the G/G genotype. Similarly, carriers of the A/A genotype were at a 2.14-fold greater risk of infertility than individuals with the G/A genotype. Thus, it can be concluded that the A/A genotype increases the risk of infertility compared with G/A or G/G genotypes. These differences may be due to the effect of G and A alleles on MMP-2 gene expression or its enzyme activity.

Table 1. Allele Frequencies of G1575A Matrix Metalloproteinase-2 (MMP-2) Gene Polymorphism in Fertile and Infertile Men

Allele	Infertile (n = 200)	Fertile (n = 200)
MMP-2 alleles	$\chi^2 = 1.86$; df = 1; P = 0.17	
G	320 (80)	304 (76)
A	80 (20)	96 (24)

Table 2. The Odds Ratio (OR) of Allele and Genotype of G1575A Matrix Metalloproteinase-2 (MMP-2) Gene Polymorphism Compared with the GG Genotype and G Alleles in Fertile and Infertile Men

Alleles	Infertile (n = 200)	Fertile (n = 200)
MMP-2 genotypes	OR = 1.45, CI (0.97-2.16)	Df = 1, P = 0.068
G/G	n =1 25	n = 107
G/A + A/A	n = 75	n = 93
MMP-2 alleles	OR = 1.26, CI (0.9-1.77)	Df = 1, P = 0.17
G	n = 320	n = 304
A	n = 80	n = 96

Table 3. Semen Profiles of Infertile Cases Regarding Genotype of G1575A Matrix Metalloproteinase-2 (MMP-2) Gene Polymorphism [a]

Genotype	Motility [b]				Sperm Count, million/mL	Normal Morphology
	Motility Grade A	Motility Grade B	Motility Grade C	Motility Grade D		
GG	3.83 ± 4.48	11.87 ± 9.13	18.05 ± 13.09	54.91 ± 26.02	44.57 ± 28.74	19.37 ± 10.95
GA	4.8 ± 6.22	10.4 ± 8.23	16.63 ± 12.32	55.11 ± 28.08	40.31 ± 27.96	20.21 ± 14.6
AA	2.75 ± 4.85	7.25 ± 6.07	12.5 ± 6.45	77.5 ± 15.54	33.25 ± 36.68	13.75 ± 13.76
P Value	0.439	0.384	0.581	0.25	0.522	0.588

[a] Data are presented as Mean ± SD (%).
[b] Grade of sperm movement according to the World Health Organization (WHO) criteria (16); A, rapid progressive; B, slow progressive; C, nonprogressive; D, immotile.

Table 4. The Genotype Distribution of G1575A Matrix Metalloproteinase-2 (MMP-2)Gene Polymorphism in Three Groups of Fertile, Asthenozoospermia and Terato-asthenozoospermia Infertile Men

Genotype	Fertile Men (n = 200)	Infertile Men (n = 200)		P Value	Total (n = 400)
		Terato-asthenozoospermia (n = 103)	Asthenozoospermia (n = 97)		
G/G	107 (53.5)	66 (64.08)	58 (59.79)	0.82 ($\chi^2 = 0.4$, df = 2)	249 (62.25)
G/A	90 (45)	33 (32.04)	38 (39.17)	0.57 ($\chi^2 = 1.13$, df = 2)	141 (35.25)
A/A	3 (1.5)	4 (3.88)	1 (1.04)	0.44 ($\chi^2 = 1.66$, df = 2)	10 (2.5)
Total	200 (100)	103 (100)	97 (100)		400 (100)

For instance, it has been shown that the existence of the A allele at the G1575A polymorphism leads to a higher enzyme activity compared with the presence ofthe G allele (15). Thus the increased risk of infertility in AA genotype carriers may be due to the higher enzyme activity of the A allele. Our results suggested that increasing enzymatic activity of MMP-2 might play a role in male infertility. Shimokawa et al. showed that there is an active-form and a pro-form of Matrix Metalloproteinase-2 and its degradation products are present in human seminal plasma (12). In vitro studies have shown that the Follicle Stimulating Hormone (FSH) increases expression of MMP-2 in testicular sertoli cells (11). An overexpression of MMP-2 has been found in the endometrium of patients with endometriosis (18) that proves the negative impact of MMP-2 overexpression on fertility. Therefore, despite the need for physiological activity of MMPs, overexpression or increased activity of these enzymes may inversely impact fertility. According to our results, semen profile analysis did not show any significant difference between infertile males regarding various genotypes of MMP-2. These findings suggest that genetic variation of MMP-2 does not have effects on semen profiles. Although, it has been reported that the activity of MMP-2 was significantly decreased in seminal plasma of cases with azoospermia compared with normospermia (8), yet the potential effect of MMP-2 genotype on semen parameters remains to be further studied in the subtype groups of infertile men. In conclusion, our findings showed that distribution of MMP-2 genotypes differed between fertile and infertile men suggesting a possible role of genetic variation of MMP in male infertility. However, more investigations are required to reveal molecular and genetic factors that underlie the male reproductive system.

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Authors' Contributions

Designing of the study and revision of the manuscript: Heidar Tavilani and Iraj Khodadadi. Writing of the manuscript and performance of experiments: Aboozar Mohagheghi. Statistical analysis and interpretation of data: Manoochehr Karami. Provision of patients: IrajAmiri.

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