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Research Article

Evaluation the polymorphism in metallothionine gene among infertile men in Mosul/Iraq

Mustafa Taha Al-Mashhadani[(1)] Owayes Muaffaq Hamed Al- Hassani[(2)]

Biology dep. / College of Sciences Mosul University, Iraq.

**e-mail : owsbio31@uomosul.edu.iq*

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Abstract-The present study aimed to show the relationship between the polymorphisms in metallothionine gene and frequency of infertility in men in Mosul city/Iraq for the first time. The included 150 subjects (130 infertile men and 20 healthy men), semen from all subjects were used to extraction of DNA followed by detection of polymorphisms in metallothionine gene using Amplification-Refractory-Mutation-System polymerase chain reaction using 4 specific primers. The results show a positive relationship between infertility and polymorphism in metallothionine gene as the results indicates presence of polymorphism of that gene with possible genotypes (AA, AG, and GG) and the genotype (AG) the highest among all other types of alleles in infertile men. The present study suggests a possible risk factor for the mutated allele genotype in undiagnosed infertile men in Mosul city/Iraq.

Keywords: Metallothionine, Polymerase Chain Reaction, Polymorphism, Mosul, Iraq.

I. INTRODUCTION

Infertility is defined by the World Health Organization (WHO) as the inability of a couple to conceive following a year of consistent, unprotected sexual activity [1]. Male infertility, defined as a man's incapacity to conceive a female, affects about 7% of all males and accounts for 40–50% of human infertility cases [2].

The risk factors for exposure to heavy metal in men considered as one of the most dangerous toxic effect, as the exposure to these heavy metals leads to irreversible damages in reproductive organ in male [3], as these heavy metals cause cellular weakness structurally and functionally in reproductive organ, and Lead, mercury, cadmium, chrome, and arsenic were effects on reproductive ability in male, as these can interact with gametocytes or Leydig cells or sperms directly and this leads to decrease in fertility as well as genetic disorders [4].

Previously known that the genetic mutations in MT2A A-5G for metallothionine leads to disfunction in mechanism of main encoded protein by this gene, as metallothionine have ability to bind all heavy metals through thiol group with cystine residues, which account to 30 % to its amine components for acids wastes [5] so these mutations leads to disfunction in encoded protein from that gene thus accumulation of heavy

metals in body organs. Because the metallothionine enrich with thiol, so it can bind many metallic materials as iron, magnesium, lithium, zinc, copper, chrome, nickel, arsenic, and selenium and others, so the main function of it is heavy material detoxification [6].

There are no studies on polymorphisms in metallothionine gene and their relationship with infertility in Mosul city/Iraq, so the present study aimed for the first time evaluate this relationship in Mosul/Iraq.

II. MATERIALS AND METHODS

Case Study

One hundred and fifty blood samples separated as (130 of infertile men) and (20 healthy control) included in this study.

Collection of Semen and Blood samples

Five ml of semen samples from all subjects in this study were withdrawn in EDTA tubes and used for DNA extraction.

DNA Extraction and determination of the purity and concentration of the extracted DNA

Extraction of DNA was done according to Iranpur and Esmailizadeh (2010) [7] followed by determining the concentration and purity of the extracted DNA using the Bio drop device, and the concentration at 25 ng/microliter was fixed at.

(ARMS-PCR) Amplification-Refractory-Mutation-System

The concentration of (25) ng/microliter was used for PCR reaction using TE buffer. 4 primers as shown in table (1) were used, the first two primers were used for the main gene band, while third primer used for normal allele, the last one used for mutated allele. PCR reaction was done using master mix (Biolaps/England) using special program for each reaction (table 2), then PCR amplicon followed by electrophoresis for 60 minutes at 50 volts, gel was visualized by UV trans-illumination device.

Table (1): Primers used for detection the polymorphism in metallothionein gene.

Primer	Sequence	tm	Band size
F1	5-CGCCTGGAGCCGCAAGTGAC		198 bp
R1	5-ATCCATGGCGAGCTGAAGA		
F2	5-ACTGCTTGCCGCGCTGCA,		135 bp
R2	5-TGGAGGAGGCGTGGTGGAGG,		100 bp

Table (3): PCR program used for detection the polymorphism in metallothionein gene.

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	45 sec.	35
3.	Annealing	67	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1
6.	Stop reaction	4	5 min.	1

Determination of genetic variation of the metallothionein gene in situ (rs28366003) using Mutiplex-ARMS-PCR technology.

The presence of the A⟶ G mutation in situ (rs28366003) was detected as 4 µl (100 nanogram) of template DNA and 1 µl (10 pmol) of each G20210A mutant primers supplied by the Korean company Macrogen were added to the contents of the master mix [8].

III. RESULTS AND DISCUSSION

Figure (1) showed relationship between infertility and genetic mutations in the (MT2A A-5G) as the results in the same figure show that the PCR reaction amplicon are 198 bp, 135 bp, and 100 bp and this indicates presence of polymorphism of that gene with possible genotypes (AA, AG, and GG) at different percentages as shown in figure (1). As the samples (7 and 10) represents healthy homozygotes (AA) with PCR amplicon 135 bp for normal allele and 198 bp for the main gene, while another samples (1,2,4,5, and 9) represents samples with carrier allele (AG) with PCR amplicon with 3 bands (198 bp) for the main gene, while (135 bp) band represents normal allele and (100 bp) represents mutated allele, last three samples (3,6, and 8) represents samples carry mutated homozygote genotype (GG) with 2 bands in PCR amplicon, first one with (198 bp) for the main gene, another with (100 bp) for mutated allele.

Table (3) show the allelic and genotypes frequency for (MT2A A-5G), and the genotypes frequency results for that the undiagnosed infertile men show that the mutated genotype (GG) was the highest between all homozygotes alleles (30%), while normal genotype (AA) was the lowest (20%), in contrast, heterozygotes alleles as (AG) was the highest among all types of alleles (50%) compared to the control group, which has a homozygote (GG) at (24%) represent the lowest percentage, while normal genotype (AA) appear at (33%), the heterozygote (AG) appeared to be the highest genotype among control group. While the allelic frequency results showed that the mutated allele in infertile was the highest (56%) and normal allele at (44%) compared which show that the mutated allele was (46%) and normal allele was (54%). Finally, the present study shows a possible risk factor for the mutated allele genotype in undiagnosed infertile men as the OR was (2.1467).

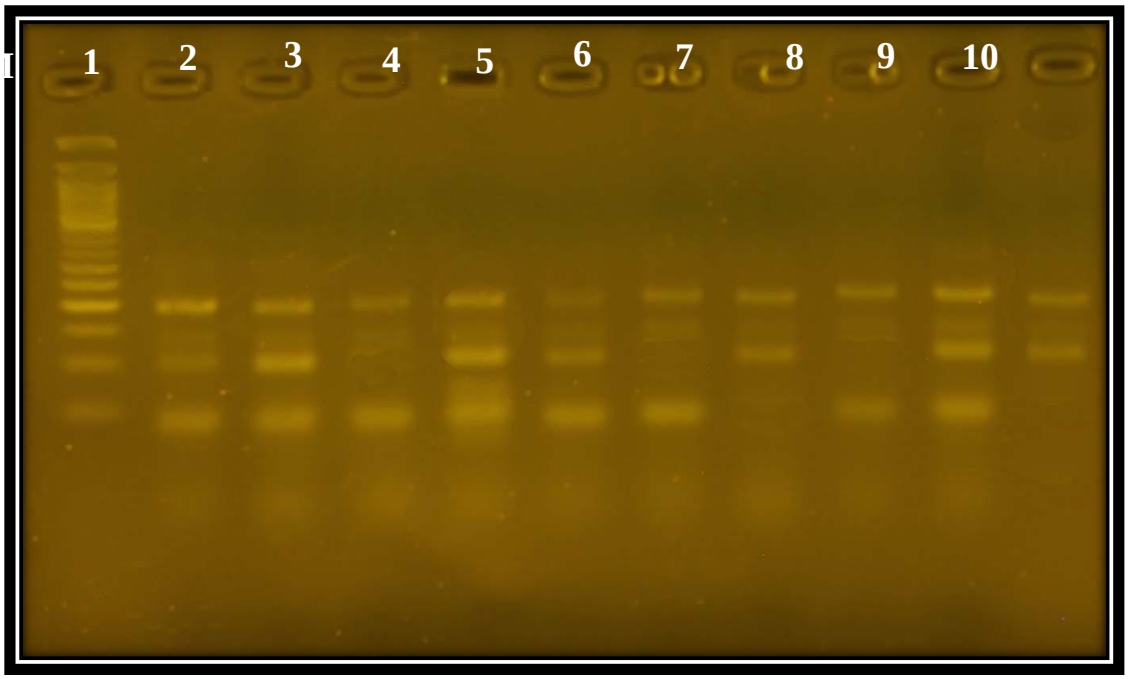

Figure (1): PCR reaction amplicon for polymorphism in (MT2A A-5G).

M= DNA marker

Table (3): Frequency of genotypes and alleles for MT2A A-5G in infertile and healthy men.

Genotypes	Patients NO.(20)		Control NO.(20)		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
AA	15	20	7	33	P = 0.2564	2.1467	0.5739 to 8.0293
AG	37	50	9	43			
GG	23	30	5	24			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
A	67	44	23	54	P = 0.2481	1.4996	0.7540 to 2.9827
G	83	56	19	46			

A= normal allele, G= mutated allele.

As previously known, the occurrence of the genetic mutation in the MT2A A-5G gene leads to a defect in the mechanism of action of the main protein encoded by the gene, as MTs have the ability to bind heavy metals such as zinc, copper and selenium and heavy metals such as cadmium, mercury, silver and

arsenic through a thiol group of cysteine residues, which represent approximately 30% of its amino components of acid waste [9,10]. These mutations will lead to dysfunction of the protein encoded by the gene, thereby increasing the accumulation of heavy metals in vital organs in the body [11]. Since metallothionine is rich in thiol, it binds many metallic minerals such as iron, magnesium, zinc, copper, nickel, cobalt, cadmium, arsenic and other metals, thereby attaining its role in the detoxification of heavy metals. The biogenesis of metallothionine appears to have increased several folds during oxidative stress to protect cells from cytotoxicity and DNA damage, so the biological efficacy of metallothionein will decrease, and the toxicity of heavy metals will increase [12,13].

IV. CONCLUSIONS

This study suggests that there is a positive relationship between polymorphism in metallothionine gene and frequency in infertility among men in Mosul/Iraq.

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