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Evaluation Level Hormones and GSTM1, GSTT1 Polymorphism in Infertility among Iraqi Men's

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Research article

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Abstract

Aims of Study: Detection of deletions Polymorphism of Glutathione S-transferases GSTM1 and GSTT1 genes in Iraqi patients with Oligozoospermia , Asthenozoospermia and fertile. Several studies refer to higher levels of oxidative stress are associated with infertility. The present study included 100 Iraqi men who were diagnosed as prostate cancer patients. The age of the patients ranged from 20 to 40 years beside 50 who were apparently healthy men. Participated in this study. Certain semen parameters were investigated according to World Health Organization (WHO) guidelines. Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin hormone and testosterone levels were also determined by enzyme -linked immune sorbent assay (ELISA). The purpose of this study to know the frequency of GSTM1 and GSTT1 genes in infertility patients and asset the relationship between the genes and some level hormones in infertility patients. Genotype (GSTM1 and GSTT1) genes were determined by polymerase chain reaction (PCR). The results showed that 36 samples of patients (oligozoospermia) have deletion in one genes or both (Null genotype), whereas 14 samples only were normal. Where, results showed that 34 samples of patients (asthenozoospermia) have deletion in one genes or both (Null genotype), whereas 16 samples only were normal. For control group, 50 samples were collected from apparently healthy men from different areas in dewania. Only eight samples showed genetic deletion while 42 samples were normal genotype. Hormones level. There is no significant different between oligozoospermia and asthenozoospermia cases & control group in regard to FSH, LH, Prolactin, and testosterone level.

Keywords: Hormones; Infertility; GSTM1; GSTT1; Polymorphism

Abbreviations: ANOVA: Analysis of Variation; CASA: Computer Assisted Semen Analysis; ELISA: Enzyme Linked Immune Sorbent Assay; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; LSD: Least Significant Difference; PCR: Polymerase Chain Reaction; SAS: Statistical Analysis System; WHO: World Health Organization.

Introduction

Infertility is a widespread problem worldwide. Infertility is the absence of pregnancy in the couple after a year to marry with an unplanned [1]. It is a heavy burden on countless families, and affecting people both medically

and psychosocially [2]. In addition, with important including psychological distress, social stigmatization, economic constraints, may lead to family separation [3]. According to the World Health Organization 72.4 million couples suffer from infertility worldwide [4]. A male partner factor contributes to 40% of the cases of infertility [5]. The cause may be related to a problem with the man, woman or both [6]. Despite medical advances in the treatment of infertility, the problem could not be satisfactorily tackled so far for several reasons [5], in particular genetic causes [7]. Often this type of infertility is associated with a defect in some enzymes that play a key role in phase-II cellular detoxification and bio activation reactions, and are generally considered to be ‘‘antioxidant’’ enzymes therefore, protection from oxidative stress is an important way to boost fertility [8,9].

Material and Methods

Seminal Fluid Analysis

Semen samples from infertile men and fertile controls were collected masturbation in a room adjacent to the laboratory and ejaculation into sterile plastic containers after 3-5 days of abstinence. Seminal fluid liquefaction for 30 min at 37°c, seminal fluid samples were evaluated according by computer assisted semen analysis (CASA). The variables taken into consideration were count, volume, pH, count, activity, motility, acrosome, normal sperm and abnormal sperm of Iraqi patients with Oligoaszoospermia, asthenoszoospermia and controls.

Blood Sampling

Venous blood samples (6ml) were collected from each for infertile men and fertile controls. The serum obtained by putting the blood samples in tubes centrifuged at 5000 rpm for five minutes the resulted serum also stored in the freezer (20°C) until used to measure hormones and other parameters.

Hormonal Assay

Serum testosterone, prolactin, LH & FSH for infertile men patients and control subjects was measured by using the Automated, Multiparametric Immunoanalyzer method by mini Vidas system.

Genetic Analysis

The GSTM1 and GSTT1 genotypes were analyzed by multiplex PCR according to the protocol [10]. Using a specific primer designed by NCBI. The primer was custom synthesized at Add Bio\Korea Company as a lyophilized product. The following primers were used

- GSTM1: F- (5-GAA CTC CCT GAA AAG CTA AAGC-3) R- (5-GTT GGG CTC AAA TAT ACG GTG G-3).
- GSTT1: F- (5-TTC CTT ACT GGT CCT CAC ATC TC-3) R- (5-TCA CCG GAT CAT GGC CAG CA-3).
- Albumin: F- (5-GCC CTC TGC TAA CAA GTC CTAC-3) R- (5-GCC CTA AAA AGA AAA TCG CCA ATC-3).

The amplification reactions were carried out in a volume of 50 µl containing (25mg) DNA; 10 mM Tris HCl; 50 mM KCl; 1.5 mM $MgCl_2$; 200 µM (each) dATP, dCTP,dGTP and dTTP (Geneaid); each primer was at 20 pM and 2.5 unit of Taq polymerase (Geneaid). The amplification was carried out as: Initial denaturation at 95□C for 3 mins, 30 cycles in thermo cycler (PCR Biorad T-100 Thermal cycler, Biolinx/India) as follow: 94□C for 1 min; 61□C for 1 min; 72□C for 1 min and 5 min final extension for last cycle. The PCR products were analyzed on 2% Agarose gel electrophoresis to detect the absence or presences of these genes. Albumin gene used as internal control.

Statistical Analysis

The Statistical Analysis System (SAS) program was used to detect the effect of difference factors in study parameters. Least significant difference (LSD) test, Analysis of Variation (ANOVA) was used to significant compare between means in Chi-square test was used to significant compare between percentages (0.01 probabilities) this study.

Results and Discussion

The internal control amplified Albumin fragment was 350 bp in length, whereas presence of the GSTM 1 and GSTT1 genes were identified by 215 and 480 bp fragments, respectively. Although these assays did not distinguish between heterozygote and homozygote positive genotypes, they conclusively identify the null genotypes (Figure 1).

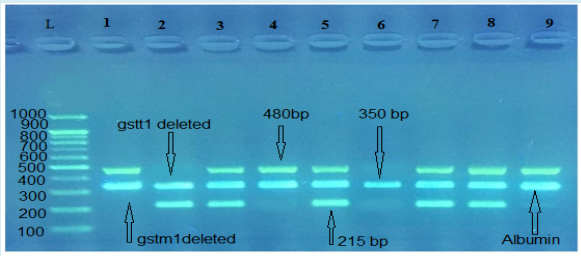

Figure 1: Electrophoresis of PCR products on agarose gel 2 % (100 volt / 1 hr) Lane 1,4,9 : gstm1 deletion, Lane 2 : GSTT1 deletion , Lane 3,5,7,8: Normal genotype, Lane-6 : Null genotype, Lane L : DNA ladder (100 -1000 bp).

The results covered 100 cases of patients diagnosed as infertility patients (oligozoospermia, asthenozoospermia)

and a 50 samples of controls. In the present study, observe significant different crude rates of the GSTM1, GSTT1 and null genotype in infertility patients. Data of patients were distributed according to selected characteristics as major risk factors for infertility while control samples subjected to some of these criteria except for affected side as they were apparently healthy men. The results showed that 36 samples of patients (oligozoospermia) have deletion in one genes or both (Null genotype), whereas 14 samples only were normal. Where, results showed that 34 samples of patients (asthenozoospermia) have deletion in one genes or both (Null genotype), whereas 16 samples only were normal. For control group, 50 samples were collected from apparently healthy men from different areas in dewania. Only eight samples showed genetic deletion while 42 samples were normal genotype. The descriptive parameters for both affected and healthy group are summarized in table (Table 1).

Group	Total	GSTM1 deletion	GSTT1 deletion	Null genotype	Normal
Oligozoospermia	50	19 (38%)	8 (16%)	9 (18%)	14 (28%)
Total & %	50	NO 36 & 72 %			NO 14 & 28%
Asthenozoospermia	50	17 (34%)	7(14%)	10(20%)	16 (32%)
Total & %		NO 34 & 68%			NO 16 & 32%
Control	50	4(8%)	3(6%)	1 (2%)	42 (84%)
Total & %		NO 8 & 16%			NO 42 & 84%

Table 1: Distribution of GSTM1 and GSTT1 genotypes among control subjects with infertility.

Hormones Level of Serum

The mean & SD of FSH concentrations in serum of oligozoospermia and asthenozoospermia cases and control individuals was (6.99 ± 0.09, 7.04 ± 0.06, 6.92 ± 0.08, respectively). While, the mean & SD of LH concentration in serum of oligozoospermia and asthenozoospermia cases and control individuals was (7.07 ± 0.08, 6.99 ± 0.06,7.33 ± 009, respectively). There is no significant different between oligozoospermia and asthenozoospermia cases & control group in regard to Prolactin concentrations in blood serum, so was (9.62 ± 0.15, 9.60 ± 0.14, 9.64 ± 0.17, respectively). The mean & SD values of serum testosterone in oligozoospermia and asthenozoospermia cases and control (6.60 ± 0.08, 6.59 ± 007, 6.85 ± 0.13, respectively) (Table 2).

Group	Mean ± SE			
	FSH (mlU/ml)	LH (mlU/ml)	Prolactin (Ng/ml)	Testosterone (Ng/ml)
Control	6.92 ± 0.08 a	7.33 ± 009 a	9.64 ± 0.17 a	6.85 ± 0.13 a
Oligozoospermia	6.99 ± 0.09 a	7.07 ± 0.08 a	9.62 ± 0.15 a	6.60 ± 0.08 a
Asthenozoospermia	7.04 ± 0.06 a	6.99 ± 0.06 a	9.60 ± 0.14 a	6.59 ± 007 a
LSD value	0.237	0.216	0.442	0.26
P-value	0.0001	0.0001	0.0001	0.0001
Means having with the different letters in same column differed significantly.** (P≤0.01).				

Table 2: Comparison between difference groups in Hormones level.
Means having with the different letters in same column differed significantly ** (P≤0.01).

Discussion

A similar study was conducted by Safarinejad, et al. [11] which showed an association between GSTT1 and GSTM1 with infertility. Observation about the relation between GSTT1 and GSTM1 with a risk of infertility matches to that reported in Indian, China and Russian population [12-14]. Some studies have reported also a significant relationship between deletion genes and infertility [15-17]. Results of the study proved that the null genotype of GSTT1 and GSTM1 increase the risk of infertility [11]. WHO found that GSTs concentrations were found to significantly decrease in the serum of patients with infertility which supports the hypothesis that GSTs are a protective factor against the development of infertility? Others conclude that infertility is a multi-factorial disease influenced by complex genetic as well as environmental factors, as noted by Pizzorno, et al. [18]. The current results found that FSH, LH, Prolactin, and testosterone are a non-reliable indicator for oligozoospermia and asthenozoospermia cases since no significant

differences was detected in the hormones levels between oligozoospermia and asthenozoospermia and control which indicate that the oligozoospermia and asthenozoospermia cases could be due to genes defect. These results are with agreement of that obtained by other studies Jaiswal, et al. [12], Samplaski, et al. [19]. It is likely that the impairment is not in the secretion functioning of the glands responsible for the production of sperm, but rather due to other factors related to the safety and preservation of sperm during spermatogenesis post-spermatogenesis [20].

Conclusion

The deletion of GSTM1, GSTT1 and null genotype (deletion of GSTM1 and GSTT1) were most common in infertility patients (oligozoospermia , asthenozoospermia) when compared with control group. No significant differences were detected in the hormones levels between oligozoospermia and asthenozoospermia and control which indicate that the oligozoospermia and asthenozoospermia cases could be due to genes defect.

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