ARTICLE / INVESTIGACIÓN

Men's ND1 gene genetic makeup Toxoplasmosis and Oligospermia affecting couples' infertility

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Abstract: *Toxoplasma* infection was higher in infertile couples than fertile couples, probably due to anti-sperm antibodies that were higher in couples with Toxoplasmosis. Investigations of *T. gondii* infections in men with infertility showed that among 100 cases of men's infertility, 36% were serologically positive for *Toxoplasma*-IgG and IgM. It has been concluded that *T. gondii* can affect men's fertility and result in infertility. Materials and Methods: Selective infertile males were asked about days of sexual abstinence. Seminal fluid samples were collected following a minimum of 2 days and a maximum of 7 days from abstinence. Every patient was given a clean, wide mouth, sterile, dry, graduated plastic and warm disposable container. The samples were obtained by masturbation in a private room near the semen analysis lab to reduce seminal exposure to temperature fluctuations and control the time from collection to analysis. Results: For the ND1 gene, samples of 8 different fertility groups have been sequenced. These sequences have been compared to reference sequences taken from the NCBI database. Several mutations in various nucleotide positions of the ND1 regions have been detected in samples from multiple groups. The base substitution has been positioned on the nucleotides (nts) 3480, 3567, 3591, 3693, and 4216. The T to C evolution was notorious at nt 3480 in ND1 genes. The SNP was detected in an asthenospermia human (Sample code: 010480).

Key words: Sequence, ND1 gen, Oligospermia Toxoplasmosis, Couples infertility.

Introduction

Toxoplasmosis is an essential global parasitic disease related to infertility and certain psychiatric disorders[1]. Toxoplasmosis has been suggested to have some unfavorable impacts on the reproductive capacity of both men and women[2]. Congenital infection caused by transplacental transmission of parasites can cause a wide variety of features in fetuses and infants, such as spontaneous abortions, still-births, newborns with classical congenital toxoplasmosis signs e.g., microcephalus or hydrocephalus, cerebral calcification and retinochoroiditis[3]. *Toxoplasma* infection was higher in infertile couples than fertile couples, probably due to the presence of anti-sperm antibodies that were higher in couples with Toxoplasmosis[4] Investigations of *T. gondii* infections in men with infertility showed that among 100 cases of men's infertility, 36% were serologically positive for *Toxoplasma*-IgG and IgM. It has been concluded that *T. gondii* can affect men's fertility and result in infertility[5]. It is known that the morphology of infected individuals can be affected by latent Toxoplasmosis, and the possibility of male offspring birth in both mice & humans is elevated, and all of those traits may be associated with observed variations in testosterone concentration between *Toxoplasma* infected & *Toxoplasma* free people[6].

Materials and methods

The study was conducted at Kamal AL-Samaraee Hospital in Baghdad province from January 1 to October 1 2019.

Blood collection

Using syringes with gauge 23 needles, 5 ml blood was collected by vein puncture, transported to non-heparinized tubes and left to clot at room temperature; then blood was centrifuged to obtain serum samples at 1500g for 5 minutes, then stored and frozen at (– 20°C).

Semen collection

Semen samples were collected according to WHO standard procedure / 2010 as follows:

First, selective infertile males were asked about days of sexual abstinence. Following a minimum of 2 days and a maximum of 7 days from abstinence, collection of semen samples was performed, then every patient was given a clean, wide mouth, sterile, dry, graduated plastic and warm disposable container.

Semen containers were labeled with the individual's name, identification number, and the time and date of sample collection. The samples were obtained by masturbation in a private room near the semen analysis lab to reduce seminal exposure to temperature fluctuations and control the time from display to analysis.

After masturbation, the semen fluid samples were immediately incubated at 37°C, and waited for complete liquefaction.

PCR reaction

To amplify ND1 and ND1 mitochondrial gene: Of the PCR primers, 2 sets have been designed and located on the flanking region of each gene.

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PCR application

To amplify the ND1 gene, for each primer pair, PCR reactions have been optimized with various annealing temperatures. About 160 ngs of the total sperm's DNA have been used in 50 µL of the reactions mixtures. The reaction mixture of PCR contains the followings:

1.5 µM Mg^{+2}

200 µM of deoxy nucleotide triphosphate (dATP, dCTP, dTTP and dGTP)

1 × Taq buffer, 0.6 µM of each primer and

1 unit of Taq DNA polymerase (Roche).

The standard PCR cycle has used initial denaturing temp. for 2 minutes at 94ºC, followed by 35 denaturing cycles for 30 seconds at 94ºC, then 58ºC of annealing temperature for 45 seconds & the primer extensions for 2 minutes at 72ºC. A final extension was for 5 minutes at 72ºC, and the reaction products were stored at 4ºC temp.

DNA sequencing

The automated DNA sequencing has been applied using a kit from "Applied BioSystem Big DyeTM termination V. 3.1 cycle sequencing". Sequencing for ND1 gene was done for mtDNA specimens of various fertility groups.

Evaluation of DNA sequencing

The computer-based sequencer (TM) was used to edit all the data obtained from automated sequences. The sequences have been compared with reference sequences of (NCBI database accession No.: NC-00187) to determine the mutation nature.

Codon usage analysis for the silent mutation: Synonymous mutations have been analyzed for codon usage. Results of the present study as well as data of (12) have been investigated for amino acid change prediction using a computer program MEGA Version (2.1)[7].

Mutation analysis for Nonsilent mutation: For determination of the altered amino acid effects on the protein properties, the proteins have been analyzed for polarity & hydrophobicity by the computer-based program (http://www.roselab.jhu.edu/ ~raj/ MISC/ hphobh.html) and ProtScal (http://download.invitrogen.com/evergreen/support_files/a_protscale_expasy.htm) respectively.

Statistical analysis

For statistical analysis of our data, version 16-SPSS was used. The numeric data have been expressed as mean ± SEM (standard error of the mean). The paired t-test was applied to compare the numeric data of patients and the healthy control groups. P-value <0.05 has been considered significant.

Ethical Clearance

This study was approved research ethics committee's faculty of Kamal AL-Samaraee Hospital No. 10.1186/s13104-019-4314-0:2019.

Results

The relationship between the age and the positive infertile patients is demonstrated in table (1). This table shows that the number and percentage of males were zero in the age groups (<20) and (20-24) years, and between (25-29) years was 2(4.34%). In this age group, it was lower than other age groups, while it was 14(30.35%) in the age groups (35-39) years, which was higher than other age groups. Females recorded 1(1.17%) in the age group (45-49) years, which was lower than females in all other age groups, and was zero in the age group (≥50) years, while it was 23 (27.06%) in the age group (35-39) years, and was higher than females in other age groups. The comparison of significance (CS) was high between all age groups (HS) (P-value = 0.000). The first row in each age group represented the number of patients, and the second row showed the percentage (%) of males to females in each age group. In contrast, the third row demonstrated the distribution percentage (%) of gender (males or females) among all age groups. The odds ratio of males to females in (<35:35≥) years group was approximately 1:6 (1:5.963). According to this ratio, every male corresponded 6 females ages fewer than 35 years; thus, females were higher in younger generations than males. The mean value of the age in males was 40, and in females was 31.18, while in all patients (couples) was 34.28.

Table 2 demonstrates marriage duration in positive infertile patients. The mean value was (8.35) and the standard deviation was (4.09). The infertile patients (males and females) were 70(53.44%) in marriage duration group (5-9) years, which was higher in this group, while it was 3 (2.29%) in the marriage duration group (20≥) years.

Family history of infertility, contact with cats in all patients, and disturbance in the menstrual cycle in females are shown in table 3.

It was found that only 10(7.64%) of positive infertile patients were with infertility family history, while other positive infertile patients, 121(92.36), were without a family history. According to this table, 77(58.77%) infertile patients had a history of contact with cats in their houses, while 54(41.23%) had never been in contact. Table (4-4) demonstrated that 48(56.47%) of females were with a regular cycle, while 37(43.53%) of them were with an irregular cycle.

DNA sequence analysis (ND1 genes)

The applied Bio System Big Dye (TM) termination Version 3.1 cycle sequencing kit was used to perform the automated DNA sequencing after the PCR product purification. Of the various fertility groups, 8 samples have been sequenced for ND1 genes. The sequences have been compared to the reference sequences taken from NCBI databases. The computer software program Sequencer (TM) has been applied for the sequence edition and determination of the mutation nature. The base substitutions have been located on the nucleotides (nts) 3480, 3567, 3591, 3693, and 4216 Table 4.

SNP T3480C

The (T to C) evolution was notorious at nt 3480 in the ND1 genes. This SNP was detected in an asthenospermia man (sample code 010480). This SNP is an equivalent change over on the 3rd position of the tyrosine codon, changing it from TAT to TAC Fig 1, Table 4.

Discussion

The odds ratio of males to females in (<35:35≥) years was (1:5.963); this result demonstrated that females were higher at younger ages than males, and it agreed with the results of (8) in Baghdad province showed that the percentages of women at (26-34) years age group was higher than other age groups, and the percentage of women at (17-25)

Age groups in the positive males & females (years)	No. & (%)	Gender		Total	CS. P-value CC Test
		Male	Female		
< 20	No.	0	2	2	C.C.=0.477
	% Age Groups	0.0	100	100	P=0.000
	% Gender	0.0	2.35	1.52	HS
20 - 24	No.	0	16	16	
	% Age Groups	0.0	100	100	Odds Ratio
	% Gender	0.0	18.83	12.22	(<35:35≥)
25 - 29	No.	2	18	20	(M:F)
	% Age Groups	10	90	100	(1:5.963)
	% Gender	4.34	21.17	15.37	
30 - 34	No.	8	17	25	
	% Age Groups	32	68	100	
	% Gender	17.39	20.00	19.08	
35 - 39	No.	14	23	37	
	% Age Groups	37.83	62.17	100	
	% Gender	30.35	27.06	28.24	
40 - 44	No.	9	8	17	
	% Age Groups	52.94	47.06	100	
	% Gender	19.56	9.42	12.97	
45 - 49	No.	6	1	7	
	% Age Groups	85.71	14.29	100	
	% Gender	13.04	1.17	5.35	
50 ≥	No.	7	0	7	
	% Age Groups	100	0.0	100	
	% Gender	15.22	0.0	5.35	
Mean ± SD		40.00 ± 7.94	31.18 ± 6.61	34.28 8.24	

Table 1. Distribution of positive infertile couples according to the age groups.

year group was high in apparently healthy unmarried women. It was found that the rate of positive infertile women with a history of one abortion was 17.64%, and those with past frequent abortions were 18.83%. These results approximately agreed with a study done by (9) who revealed that the percentage of infected women who suffered from one abortion was 23.5%, two abortions 17.6% and three or more abortions was 5.9%. The interpretation of these results is that chronic inflammation causes functional intrauterine abnormality and an endometrial reduction receptivity, which hurts the embryo implantation process and its early development[10]. They are mainly cases of chronic toxoplasmosis, brucellosis, listeriosis, rubella, cytomegalovirus and herpes infections[11]. 9.42% of positive infertile women had one successful pregnancy, and 2.35% had frequent (twice or more) previous pregnancies. The remaining women (88.23%) suffered from sterility without past pregnancy. These results can be explained in the following way: if the infection occurs in the late pregnancy stages, the strong bias of Th-2 and the diminished natural killer cells (NK) cells, macrophages & CD8+ T-cells functions can cause the parasite's survival facilitation, which it is unlikely to induce abortion. Conversely, the response of Th1 of T. gondii infections may trigger abortions early during pregnancies[12]. The genetic mutation in the base substitution was situated in nucleotides 3396, 3480, 3594, 3693, 3992 and 4216, and there was a change

Marriage duration in positive patients (years)	No.	%
< 5	15	11.45
5 - 9	70	53.44
10 - 14	37	28.24
15 - 19	6	4.58
20 ≥	3	2.29
Mean ± SD	8.35± 4.09	

Table 2. Distribution of the positive infertile patients according to the marriage duration.

Variables	Status	No.	%
History of infertility in the family	No	121	92.36
	Yes	10	7.64
Contact with cats	No	54	41.23
	Yes	77	58.77
Menstrual cycle	Regular	48	56.47
	Irregular	37	43.53

Table 3. Distribution of the observed frequencies of some demographical characteristics and related variables.

Figure 1. A shows the control sample chromatogram at nt 4216 in the Asthenospermia sample.

Case	Genes	Mutations	Codon changes	Amino Acid changes	Codon frequency Changes
Fertile	ND1	T3396C*	TAT- TAC	Silent	1.6-1.63
	ND1	G3693A*	CTG- CTA	Silent	0.38-1.55
	ND1	A4216G*	ATA- ACA	Silent	1.66- 0.22
Oligospermia	ND1	A3480G*	AAA- AAG	Silent	1.58- 0.26

* indicates mutations obtained from various semen sample groups in the study

Table 4. Analysis of DNA sequence & codon usage of the synonymous mutation in different semen sample groups of the mitochondrial DNA

in SNP T3396C position. At nt 3396, the T to C transition has been detected in the NDI genes. This SNP was seen in asthenospermia men (sample code: 010573)[13]. This SNP is a synonymous substitution in the 3rd position of tyrosine codons, changing it from TAT to TAC, and in SNP C3450T position, C to T transition has been observed in nt 3450 at ND1 regions. This transition was detected in an Oligozoospermic (sample code, 6445). This SNP synonymous substitution took place in the 3rd position of the proline codons, causing a change to the codons by using codon usage analysis in the synonymous mutant of mitochondrial mt DNA[13]. Other studies demonstrated the obtaining of genetic mutations which were determined by the present study. At the same time, our results agreed with (20), who found mutations from exo & endogenous origins such as DNA replication errors or environmental insult such as sunlight or smoking[14]. In addition, it was close to the results of (15), who showed that among 589,306 genomes, the wide screen of 874 genes resulted in the diagnosis of 13 adults who harbored mutation for (8) mendelian severe cases without reporting any clinical feature of an indicated disorder. Our results showed the promise of expanding genetic research, which looks for good persons who buffer the impacts of rare, deleterious and highly penetrant mutations[15]. Thus, our study was designed for the determination of genetic mutations in the ND1 genes, which is situated in the mitochondria of sperms, particularly in those suffering from sterility, and depending upon the past studies, our study showed that such mutation happened because of toxoplasmosis[16,17].

Conclusions

The present study showed that several mutations in various nucleotide positions of the ND1 regions were detected in samples from various groups. The base substitutions have been positioned on the nucleotides (nts) 3480, 3567, 3591, 3693, and 4216. The T to C evolution was notorious at nt 3480 in ND1 genes.

Recommendation

We recommended detecting the nucleotide positions of ND2 region from infertile males and determining the mutation that occurs in these positions.

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