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Biology Department

Microdeletion of the CATSPER1 ion channel gene with male infertility in IRAQ: a genetic disease screening study

A Graduate Research Project

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By

Aya Hussein Ali Al-Maamouri

Supervisors

Prof. Dr.

Ali Hussein Al-Marzoqi

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بسم الله الرحمن الرحيم

وآخر دعواهم أن الحمد لله رب العالمين

صدق الله العلي العظيم

سـوره يونس

الإهداء

اهدي هذا العمل المتواضع

إلى الوالدين الكريمين حفظهما الله

وإلى كل أفراد أسرتي

والى روح جدي وجدتي رحمهما الله

والى كل من ساهم في تلقيني ولو بحرف في حياتي

الدراسية

الباحثة

اية حسين المعموري

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In the Name of Allah, the Most Merciful, the Most Compassionate all praise be to Allah, the Lord of the worlds; and prayers and peace be upon Mohamed His servant and messenger.

First and foremost, we must acknowledge my limitless thanks to Allah, the Ever-Magnificent; the Ever-Thankful, for His help and bless. We are very sure that this work would have never become truth, without His guidance.

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Aya

Microdeletion of the CATSPER1 ion channel gene with male infertility in IRAQ: a genetic disease screening study

Abstract

Background

CATSPER1 gene is located on the long (q) arm of chromosome 11 at position 13.1 (11q13.1). Clinical studies have shown that the decreased sperm motility in infertile men was associated with CATSPER1 deletions or abnormal CATSPER1 expression.

Experimental Procedures: Subjects and semen analysis: In this study, one hundred and fifty- one (151) Subjects aged 17 to 61 years were analyzed consist of 126 Iraqi infertile men (patients), addition to 25 fertile men (controls) in Baghdad & Hillah city, Iraq, from September 2019 to January 2020. The DNA was extracted using DNA extraction kits (Invitrogen, USA). Then DNA amplification by multiplex PCR was performed using primer pair for the CATSPER1 gene.

Outcome: This investigation is the first, according to our knowledge, that revealing the relationship between the deletions of CATSPER1 gene and male infertility. In this study CATSPER1 gene was found in 66 (48.3%) case from 151 samples that detected by polymerase chain reaction "PCR'' technique. We revealed that microdeletions involving CATSPER1 is significantly related to oligo-asthenozoospermia. The high frequency of CATSPER1 deletion 25.76% (17/66) among oligo-asthenozoospermic patients and the significant difference between oligozoospermic, asthenozoospermic and azoospermic groups in the deletion of CATSPER1 may indicate that, CATSPER1gene deletions will increase the likelihood risk of male infertility due to it contribute to spermatogenesis and fertilization processes and to have a close association with sperm motility.

Conclusion: CATSPER1 gene deletion was found in higher percentage among Oligo–asthenozoospermia than other cases involved in this study.

Keywords:

CATSPER1, Male infertility, PCR, Cation channel of sperm1, oligospermia, Azoospermia, asthenozoospermia.

I. INTRODUCTION

The etiology of male infertility is complex and multidimensional; it affects a large percentage of couples globally and has several causes, including genetics, the environment, and lifestyle choices. The molecular basis of male infertility has been largely uncovered in recent years by genetic investigations, which have illuminated particular genes and pathways involved in spermatogenesis and sperm function (1). This study investigates the relationship between male infertility and the CATSPER1 ion channel gene in Iraqi population, a place where reproductive health issues are common (2). Fertilization and sperm motility are dependent on the Catsper ion channel complex, which is encoded by the CATSPER1 gene (3-4). This complex is expressed solely in spermatozoa. Infertility in males and decreased sperm function have been associated in certain studies with CATSPER1 mutations or microdeletions. It is still mostly unknown, however, how common and important CATSPER1 mutations are in Iraqi males. This study intends to fill that informational void by performing a thorough genetic disease screening in Iraq to determine the frequency of CATSPER1 microdeletions and their correlation with male infertility (5-6).

In order to understand the genetic basis of male infertility, population-specific research are crucial (7). This is because different groups have different genetic makeups and different environmental influences. Studying the function of CATSPER1 in male infertility is of utmost importance in Iraq, a country with significant challenges in reproductive health (8). The goal of this research is to help impacted individuals and couples who are having trouble conceiving find more specific diagnostic and treatment options (9). Finding and characterizing CATSPER1 microdeletions in Iraqi men with infertility diagnoses is the goal of this study, which employs cutting-edge genetic screening approaches and procedures (10-11). The results could help us learn more about the genetics of male infertility, which could lead to better genetic counseling and individualized reproductive treatments. In addition, by providing data from a location with distinct genetic and environmental factors that could impact the frequency and presentation of infertility related to CATSPER1, our study adds to the worldwide conversation about male reproductive health (12-13).

In order to put our results in context, we combed through and synthesized data from a wide range of sources, such as genetic studies on male infertility, studies of CATSPER1 function, and Iraqi population-specific studies on reproductive health (14). This all-encompassing method highlights the significance of population-specific factors while situating our study within the larger context of male infertility genetics (15).

II. Materials & Methods:

Subjects and semen analysis: Semen & blood samples were collected from **126** infertile men who attended infertility units at Al-Nahrain University /High Institute for Infertility Diagnosis Assisted Reproductive Technologies as well as private clinics in Baghdad and Hillah City for 3 months from (10/9/2019) to (11/12/ 2019).They were diagnosed by a specialist doctor as infertile males due to abnormal Seminal Fluid Analysis (SFA) according to the World Health Organization (WHO) guidelines (16) and inability to achieve pregnancy in the period of last 12 months or more despite regular unprotected intercourse. Also, **25** healthy fertile men, who have normal SFA according to WHO criteria and achieved one or more than one child in the last 24 months, were selected as control group for the study. Physical examinations, tests of the patients' sperm, and CATSPER1 deletion analyses were performed on each and every patient. After three to five days of not engaging in sexual activity, samples of sperm were collected by masturbating into a container that was sterile. At room temperature, specimens were transported to the laboratory, where they were examined for sperm count, sperm volume, pH, motility, and morphology. Additionally, fructose concentration was determined in accordance with the guidelines established by the World Health Organization (WHO). A minimum of two separate analyses of the subjects' sperm were performed. For the purpose of DNA analysis, blood samples were collected from all of the participants, including those with oligo-asthenozoospermia, asthenozoospermia, normozoospermia, oligozoospermia, azoospermia, astheno-teratozoospermia, and oligo-astheno-teratozoospermia. The DNA was extracted with the help of DNA extraction kits manufactured by Invitrogen in the United States. Following that, primers for the CATSPER1 gene were utilized in order to carry out a multiplex PCR amplification of the DNA. Those samples that were found

to have deletions after the initial screening were validated using further multiplex PCR amplification for an additional two times. The deleted loci were then validated through simplex PCR research. The comprehensive description of primers that were utilized for the detection of CATSPER1 deletion and the amplification settings condition was optimized as follows: the initial denaturation was performed at 95 ◦C for thirty seconds, followed by forty cycles of 95 ◦C for thirty seconds, sixty ◦C for ten seconds, and 72 ◦C for fifteen seconds; and finally, the extension was performed at 72 ◦C for five minutes. After carrying out a number of different optimizations, the electrophoresis conditions were brought to their optimal state. A total of 29 milliliters of milli-Q water, 2.5 milliliters of Tris-aceta-EDTA 10%, 5 milliliters of glycerol, and 12.5 milliliters of 40% acrylamide/bis-acrylamide, 300 microliters of 10% ammonium persulfate, and 32 microliters of tetramethyl ethylenediamine were necessary for the gel to be utilized at a concentration of 10%. Following that, the gel was dyed with red safe and imaged.

Specimens

Semen, blood and serum samples were obtained from 126 infertile men and 25 fertile men aged 17 to 61 years who attended infertility clinics at Al-Nahrain University infertility clinics as well as private clinics in Baghdad and private clinics in Hillah City from 10 September/2019 to 11 December/2019. The infertile men and fertile men were sub-grouped by the standard of semen mentioned in tabulations (1).

Table (1): Frequencies of Infertile men Groups and Control.

Specimens	Group	No.	Total
Patients	Oligo - asthenozoospermia	57	126
	Asthenozoospermia	24	
	Oligozoospermia	23	
	Azoospermia	11	
	Oligo-astheno- teratozoospermia	10	
	Astheno - teratozoospermia	1	
Control	Normozoospermia	25	25
Total		151	151

The overall procedure of the study summarized in the (Figure 3.1).

Materials

Instruments and Equipment

Table (2) shows the laboratory instruments and equipment that were used in this study and their sources.

Chemicals

The chemical materials and reagents which used in present study together with their producing companies were listed in table (3)

(Fig. 3.1) Experiment design of the study

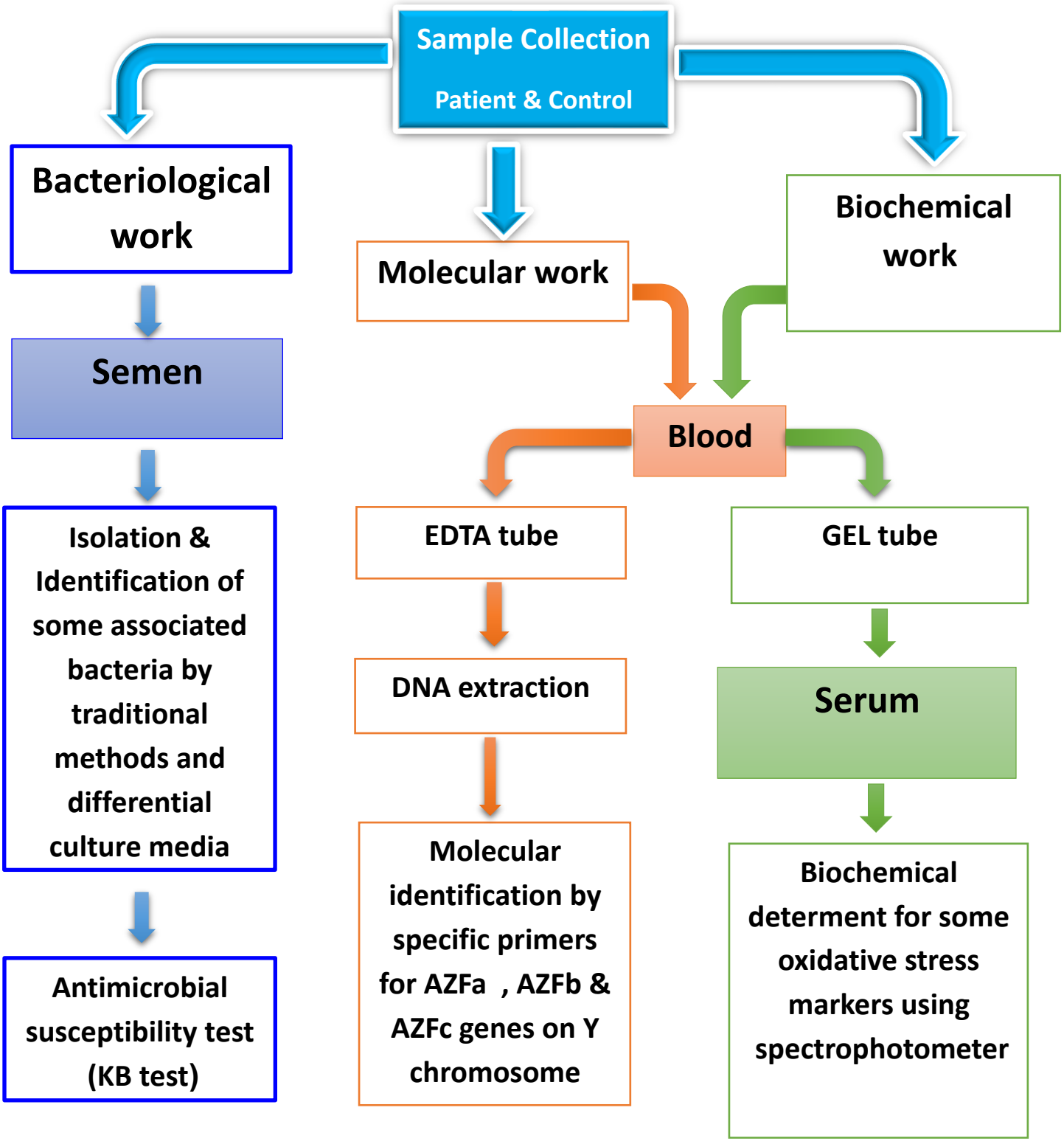

Table (2): Equipment & instruments

NO.	Equipment & instruments	Company / Country
1.	1.5 ml Eppndrof tubes	(Promeg/USA)
2.	Autoclave	(HIRAYAMA / USR)
3.	Beakers, Flasks & Cylinders (Different sizes)	(Superior/ Germany)
4.	Burner	(Memmert/ Germany)
5.	Cabinet hood	(BioLAB/ Korea)
6.	Centrifuge	(Hettich/Germany)
7.	Deep freeze(-20Cº)	(Forma Scientific Inc/ USA)
8.	Disposable plastic semen collection cups	(Bio-Hit/ Finland)
9.	)isposable Swabs &Transport media Swab	(AFCOSWAB /Jordan)
10.	Disposable syringes (5ml)	(Mediplast /U.A.E)
11.	EDTA Tube & Gel Tube	(Afcovac/Jordan)
12.	Electrophoresis power supply	(Pelex/ France)
13.	Eppendorf centrifuge	(MIKRO 120 / Germany)
14.	Horizontal Electrophoresis unit	(Pelex/ France)
15.	Incubator	(Memmert GmbH/ Germany)
16.	Light Microscope	(Olympus/ Japan)
17.	Micropipettes (Different sizes)	(Eppendroff/ Germany)
18.	Microwave oven	(Samsung /Korea)
19.	Nano drop2000c device	Thermo Scientific /USA
20.	Parafilm	ParafilmM/USA)
21.	PCR device	(Applied biosystem/USA)
22.	PCR Tubes(100 µl)	(Eppendorf /Germany)
23.	Petri dish	(Sterial/Jordan)
24.	PH meter	(Hanna instruments/Mauritius)
25.	Plane tubes(10ml) & Glass test tubes(10ml)	(DMD-DISPO/ Jordan)
26.	Platinum wire loop	(Himedia/India)
27.	Refrigerator	(Ishtar/ Iraq)
28.	Sensitive balance	(Sartorius /Germany)
29.	Slides & Coverslips	(OEM/ China)
30.	Spectrophotometer	(Optizen/Korea)

NO.	Equipment & instruments	Company / Country
31.	Test tube, Eppndroff &PCR (12 ×8) Racks	(Barcopharma/ China)
32.	Tips (Different sizes)	(Jippo / Japan)
33.	Ultraviolet light transiluminator	(Ultra violet products Inc./ UK)
34.	Vortex	(Griffin / England)
35.	Water bath	(Techne DRI-Block /UK)
36.	Water Distillator	(GFL/ Germany)
37.	Woody sticks	(RSD/China)

Table (3): Chemical Materials

NO.	Chemicals Materials	Company / Country
10.	5,5-Dithiobis (2-nitrobenzoic acid) DTNB	(Munchen/ Germany)
14.	Disodium hydrogen Orthophosphate (Na2HPO4)	(Riedei-de Haen/ Germany)
7.	Disodium-Ethylene Diamine tetra acetic acid (Na_2-EDTA)	(Himedia, India)
2.	Ethanol Absolute (96-99) %	(Fluka/ Switzerland)
17.	Ethylene Diamine tetra acetic acid (EDTA)	(Bio Basic Inc./Canada)
9.	Hydrochloric acid (HCl)	(BDH /England)
15.	Hydrogen peroxide(H2O2)	(BDH /England)
16.	Kovac's Reagent	(Himedia, India)
11.	Methanol	(Riedei-de Haen/Germany)
3.	Nuclease-Free Water	(Promega/ USA)
13.	Potassium dihydrogen orthophosphate (KH2PO4)	(Scharlau/European Union)
1.	Redsafe nucleic acid staining solution 1ml	(Intron / Korea)
12.	Reduced Glutathione(C10H17N3O6S)	(BDH /England)
6.	Thiobarbituricacid (TBA)	(Fluka / Switzerland)
5.	Trichloroacetic acid (TCA) (CCl_3.COOH)	(Poison/Australia)
8.	Tris (hydroxy methyl) aminomethane (C4H11NO3)	(Himedia, India)
4.	(Tris Borate EDTA buffer(10 x)	(Intron / Korea)

Table (4): Biological Materials & Kits

NO.	Chemicals Materials	Company / Country
10.	5,5-Dithiobis (2-nitrobenzoic acid) DTNB	(Munchen/ Germany)
14.	Disodium hydrogen Orthophosphate (Na2HPO4)	(Riedei-de Haen/ Germany)
7.	Disodium-Ethylene Diamine tetra acetic acid (Na_2-EDTA)	(Himedia, India)
2.	Ethanol Absolute (96-99) %	(Fluka/ Switzerland)
17.	Ethylene Diamine tetra acetic acid (EDTA)	(Bio Basic Inc./Canada)
9.	Hydrochloric acid (HCl)	(BDH /England)
15.	Hydrogen peroxide(H2O2)	(BDH /England)
16.	Kovac's Reagent	(Himedia, India)
11.	Methanol	(Riedei-de Haen/Germany)
3.	Nuclease-Free Water	(Promega/ USA)
13.	Potassium dihydrogen orthophosphate (KH2PO4)	(Scharlau/European Union)
1.	Redsafe nucleic acid staining solution 1ml	(Intron / Korea)
12.	Reduced Glutathione(C10H17N3O6S)	(BDH /England)
6.	Thiobarbituricacid (TBA)	(Fluka / Switzerland)
5.	Trichloroacetic acid (TCA) (CCl_3.COOH)	(Poison/Australia)
8.	Tris (hydroxy methyl) aminomethane (C4H11NO3)	(Himedia, India)
4.	(Tris Borate EDTA buffer(10 x)	(Intron / Korea)

Genomic DNA Extraction

Genomic DNA was isolated and prepared for molecular analysis from both patients and blood control specimens obtained in anticoagulant EDTA tubes and frozen for usage. The method of extraction was carried out using the PureLink ® Genomic DNA purification mini package (Invitrogen / USA).

Genomic DNA purification kit components & storage conditions

Table (3.10) identified the components for 250 DNA isolations from around 200µl of whole blood samples. In addition to some materials provided by the user,

which were a water path (55°C), (1.5mL) sterile, DNase free microcentrifuge tubes and (96-100%) ethanol%). All kit components were kept at room temperature (22-25 ° C), and proteinase K solution and RNase were kept at 4 ° C for long-term storage (> 1year).

Until DNA separation, PureLink ® Genomic Wash Buffer 1 and PureLink ® Genomic Wash Buffer 2 were diluted by adding 75 ml of ethanol and 87.5 ml of ethanol (96-100%) respectively. Mixed well and labelled the addition of ethanol on the labels, then both ethanol with wash buffers were kept at room temperature.

Table (5): Isolation Kit components

Component	Quantity
Genomic Lysis /Binding Buffer	50 ml
Genomic Wash Buffer 1	50 ml
Genomic Wash Buffer 2	37.5 ml
Genomic Elution Buffer	50 ml
RNase	5 ml
Proteinase K	5 ml
Spin Columns with Collection Tubes	5× 50 each
Collection Tubes (2.0 mL)	5 × 100

Procedure for genomic DNA purification

The procedure for purifying genomic DNA from EDTA blood samples using Invitrogen Kit was consist from several steps, which can be summarized in appendix (5 & 6).

Estimation the Concentration and Purity of DNA

The samples DNA concentration was estimated by using the Nano drop 2000c spectrophotometer by procedure described by (Desjardins & Conklin ,2010) as following:

1. Nano Drop software was opened and selected the nucleic acid application. The blank measurement was performed by pipetting (2-3µl) of nuclease-free water onto the lower optical surface of the device, then the lever arm was closed and selected "Blank" from the application. After that, the optical lower and upper surfaces were cleaned by clean and dry lens paper.

2. (2.5µl) of the extracted DNA was dispensed in the machine, the lever arm closed and the "Measure" was chosen to automatically calculate the DNA concentration in (ng /µL) and the purity detected by noticing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The values of concentration and purity of some DNA samples extracted during the current study are seen in Appendix (7).

Genes Detection by Polymerase Chain Reaction (PCR) Technique

In the present study, conventional PCR technique was used ,that based on thermal cycling "alternately heating and cooling the PCR sample through a defined series of temperature steps" (Kadri, 2019), in order to detect the presence or the absence of 7 specific genes on AZF regions of the human Y chromosome long arm(Yq AZF),in addition to other 3 genes that associated with male infertility which were CRISP2 on 6th chromosome short arm,CATSPER1 and PATE1on the long arm of chromosome 11(Heidary *et al*.,2019) by using ten primer pairs .Most of the primers sequences were selected according to the European Academy of Andrology (EAA)and European Molecular Genetics Quality Network(EMQN) guidelines(Krausz *et al*.,2014).The sequence of the primers and an description of a PCR protocol have been recorded in appendix(8).

Primers were suspended by dissolving the lyophilized product with the scheduled amount of nuclease free water according to manufacture company instruction (Macrogen / Korea) that shown in appendix (9) ,to give a final concentration of 100 picomoles (pmol)/ul and kept as a stock suspension in -20°C.

PCR Reaction Mixture

The following components formed the reaction mixture:

1. Master Mix package (Intron / Korea), with table parts (6)

2. Forward primer & Reverse primer

3. DNA template

Table (6) Master Mix components

Components	Concentration
DNA Taq polymerase	**1 U/ µl**
dNTP (dATP, dCTP, dGTP, dTTP)	**250 Mm for each one**
Tris-HCl (pH 9.0)	**10 mM**
KCl	**30 mM**
MgCl2	**1.5 mM**

To prepare the PCR combination, the dilution of the working primary solution must first be accomplished by incorporating DNase–Rnase-free water to reach a final concentration of 10 pmol/µl from the concentration primary stock of 100 pmol/µl, this was achieved using the formula N1×V1 = N2×V2 . Where, N1was the stock primer initial concentration = 100 pmol and V1 (the working primer volume we want to know), while N2 represented the primer concentration we wanted = 10 pmol and V2 was the volume we required that consist from multiplying (No. of specimens × volume for each).

For pipetting mistake additional hypothetical specimens were often taken (2-5), for example 10 samples were considered to be 15 samples. Thus, the formula became = 100 pmol × V_1 µl = 10 pmol × (No. of specimens (15) × volume for each 5) µl, therefore V_1=7.5 µl that was pipetting in sterile Eppendorf tube, inserting 67.5 µl of nuclease-free water to complete the 75 µl amount. Amplification of DNA was carried out by adding 5µl template DNA, 5µl master mix, 5µl of each 10 picomole/microliter forward and reverse primer in 100 µl PCR tube to get final reaction mixture volume of 20 µl, and mixing well by vortexing for 5–10 seconds.

PCR Program Conditions

Throughout our research, the system conditions for the conventional PCR thermal cycler were optimized before PCR mixing tubes were within for efficient amplification of the DNA and priming sets of interest. The PCR method is subdivided into three phases, the first is performed at a temperature of 94 ° C, called denaturation, and the second is hybridisation. It is usually performed at a temperature between 40 and 70° C, defined as the primary hybridization temperature. The third cycle is done at 72 ° C, defined as the elongation temperature (Kadri, 2019). The suggested optimization requirements included the temperature of annealing which was probably the most important in PCR programming (Kadri, 2019). According to instructions from supplier of primers (macrogen-Korea). The temperature of annealing for each was determined as:

C= (A+B)/2, where C= Final annealing, A= Annealing As primer sheet and B= Annealing As { (Forward primer + Reverse primer) /2 }. Reaction conditions for all primers were listed below:

CRISP2

As a first step, PCR optimization for the CRISP2 gene was achieved by using a temperature gradient as seen in table (7). This is really important to establish the optimal temperature for the annealing.

Table (7) CRISP2 gradient condition

Step	Temperature °C	Time/min.	Cycles
Initial denaturation	**95**	**3 Min.**	**1**
Denaturation	**95**	**30 Sec.**	**40**
Annealing	**56-58-60-62-64-66**	**10 Sec.**	
Extension	**72**	**15 Sec.**	
Final extension	**72**	**5 Min.**	**1**
Storage	**4**	**∞**	

After dissection of the PCR sample with 2% agarose gel recolored with 5 µg / ml Redsafe nucleic acid staining solution according to the method of (Lee et al., 2012), which will be mentioned in detail later, we determined the optimum annealing temperature for CRIAP2 by selecting the clearest band which was 60 ° C and PCR conditions as shown in table (8)

Table (8) CRISP2 PCR condition

Step	Temperature °C	Time/min.	Cycles
Initial denaturation	**95**	**3 Min.**	**1**
Denaturation	**95**	**30 Sec.**	**40**
Annealing	**66**	**10 Sec.**	
Extension	**72**	**15 Sec.**	
Final extension	**72**	**5 Min.**	**1**
Storage	**4**	**∞**	

CATSPER1

The requirements for CATSPER1gene gradient are similar to those seen in the table below (9).

Table (9) Gradient conditions for CATSPER1

Step	Temperature °C	Time/min.	Cycles
Initial denaturation	95	3 Min.	1
Denaturation	95	30 Sec.	40
Annealing	61-62-63-64-65-66	55 Sec.	
Extension	72	15 Sec.	
Final extension	72	5 Min.	1
Storage	4	∞	

The optimal temperature of annealing for CATSPER1 was 66 ° C dependent on the modification of PCR conditions for the CATSPER1 gene as in the following table (10).

Table (10) PCR condition for CATSPER1

Step	Temperature °C	Time/min.	Cycles
Initial denaturation	**95**	**3 Min.**	**1**
Denaturation	**95**	**30 Sec.**	**40**
Annealing	**66**	**55 Sec.**	
Extension	**72**	**15 Sec.**	
Final extension	**72**	**5 Min.**	**1**
Storage	**4**	∞	

III. Results & Discussion:

CATSPER1 is a novel gene located on the long (q) arm of chromosome 11 at position 13.1 (11q13.1) (figure 1), that has recently been identified to express specifically in sperms and some sperm-related organs such as testis, epididymis and prostate .It found this gene is contribute to spermatogenesis and fertilization processes, also its have a close association with sperm motility (17,18) Because no study has yet identified the deletion effect of *CATSPER1* gene on male infertility, it would therefore be important in this research to reveal the relationship between deletions of this gene and failure to conceive (infertility) using PCR to aggrandize the primer pair of it.

Figure 1:
Schematic representation of the human 11 chromosome and the CATSPER1 gene location marked red (NCBI)

In this study the cohort of subjects consisted of 43.71 % (66) men with deletion of *CATSPER1* (table 11), this too high prevalence of *CATSPER1* deletions in our outcomes can be positively correlated with male infertility. PCR amplification product of *CATSPER1* is illustration in the (figure 2).

Table 11:

The occurrence of *CATSPER1* gene deletions with PCR

CATSPER1 gene existence	NO.	%
Present	66	43.71%
Absent	85	56.29%
Total	151	100

It seems clearly according to (figure 3) that patients with primary infertility suffered from the highest deletion proportion from the gene under attention, who recorded 46.79% (31/66) of *CATSPER1* deletion, while secondary infertile men presented a deletions ratio about 34.85%(23/66) . This may be due to the fact that the number of patients with primary infertility is already greater than those with second infertility in our study. This result was in accordance with those of Zainab (2010) (19) in Baghdad; Al-Fahham *et al*. (2014) (20) in Al-Najaf Al-Ashraf; and AL-Hassani *et al*. (2019) (21) in Thi-Qar, who found that the primary infertility percentage was higher than secondary type. Primary infertility prevalence among population groups in Iraq is probably because the causes of the secondary type are correctable or patients with secondary infertility are in less urge for seeking medical help since they already got children.

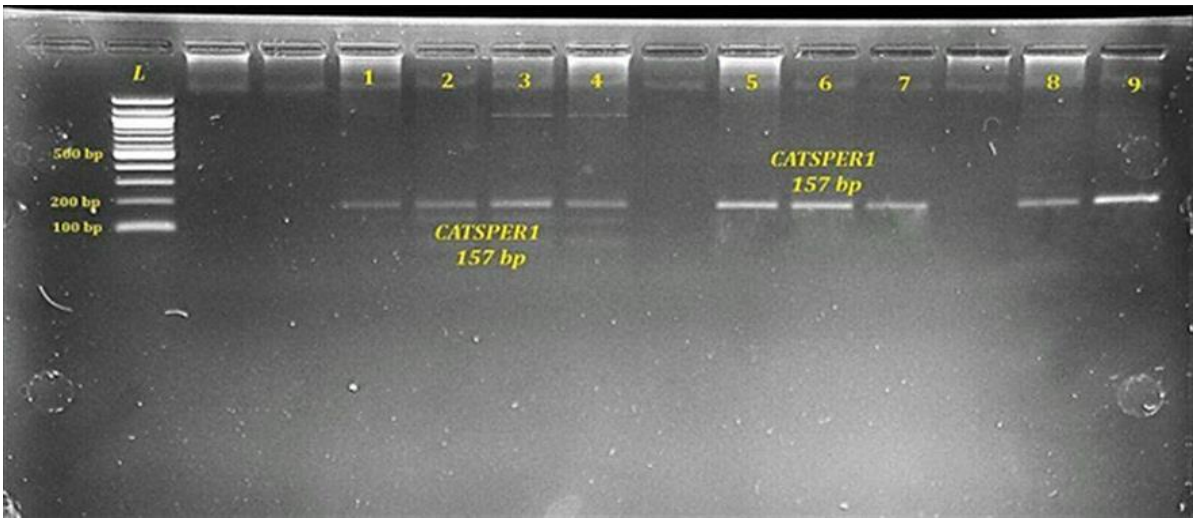

Figure 2:

Gel electrophoresis photograph of *CATSPER1* (157 bp) PCR products using a UV transilluminator (320 nm). L lane contain the 100 bp DNA Ladder, 5 % NuSieve® 3:1 agarose gel in 1X TBE buffer containing 5µl Red safe; (1-4),(5,6&7) and (8 & 9) positive results (deletion absent).

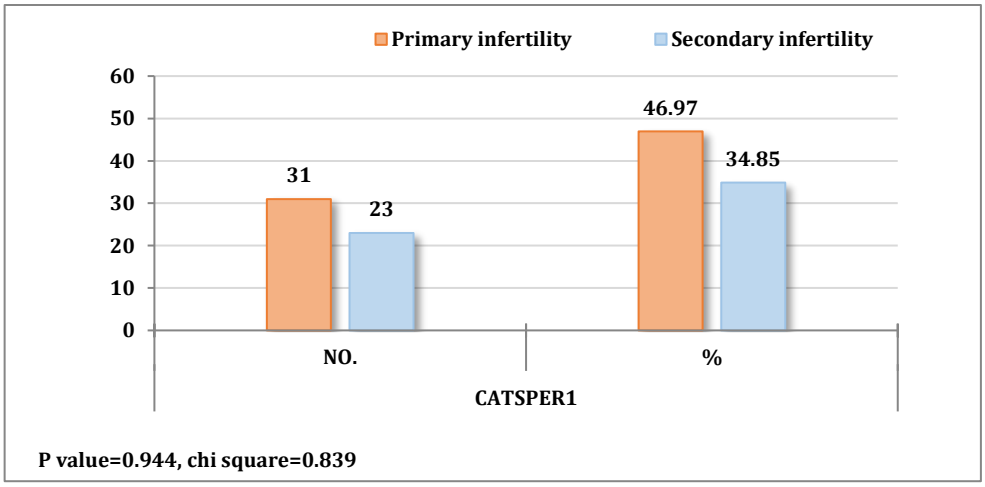

Figure (3):

CATSPER1 deletions associated with male infertility types. No significant differences (P value=0.944) between primary and secondary infertility in CATSPER1 gene deletions and this indicates that deleting this gene somewhat affects primary and secondary infertile men equally.

The highest deletion percentage of *CATSPER1*gene was found among (30-39) year-old participants in percent (34.85%)relative to other participating age groups, as included in (figure 4).

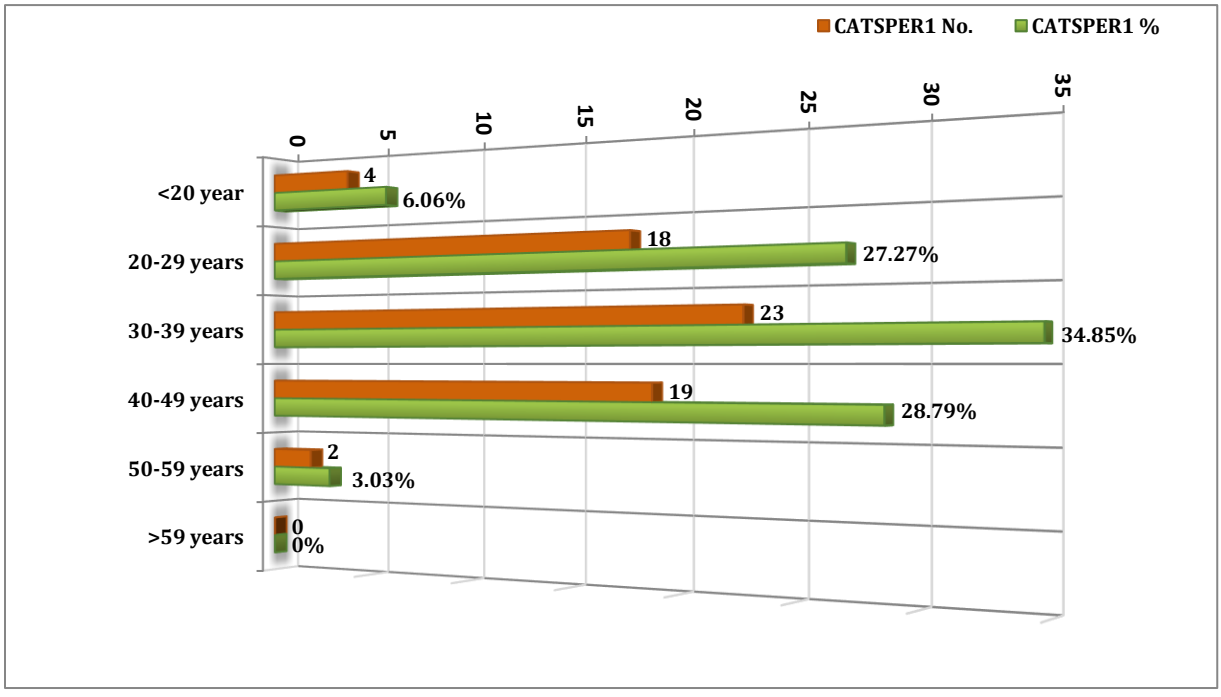

Figure (4):

The distribution of CATSPER1 gene deletions among age categories.

As men age, they are more likely to suffer from the decreasing of the sperm number and the muscles that are responsible for male ejaculation also get weaker over time, which could effect on motility of sperm and man's ability to effectively send his sperm to the woman's egg, therefor can significantly affect male fertility (22).

However, we noted the vast majority of *CATSPER1* deletions were among younger infertile men, because most of our patients were young and it could their concern regarding a child which made them consult the fertility specialist at earlier age. Our results were in good agreement with those of Al hadrawi *et al*. (2015) (23) who observed that among infertile men the most common age group was (30-39) years which constituted 44%. Also, we were in line with the findings of Al-Faisal (2010) and Mohammed (2020) (24, 25) reported the age group (30-34) years were the major age group in infertile men.

Table (12) show *CATSPER1* deletion percentage associated with male infertility cases of this study (azoospermia; oligo - asthenozoospermia; asthenozoospermia; oligozoospermia oligo - astheno- teratozoospermia; astheno - teratozoospermia and normozoospermia)

Table 12: The apportionment of *CATSPER1* gene deletions among male infertility cases & controls

Male Infertility Cases	*CATSPER1*	
	No.	%
Oligo - asthenozoospermia	17	25.76%
Asthenozoospermia	12	18.18%
Oligozoospermia	12	18.18%
Azoospermia	4	6.06%
Oligo-astheno- teratozoospermia	9	13.64%
Astheno - teratozoospermia	0	0.00%
Normozoospermia	12	18.18%
Total	66	100.00%

P value=0.695, chi square=9.087

Results showed that from 66 *CATSPER1* deletion, oligo- asthenozoospermic men formed the largest proportion of deletion 17 (25.76%), followed by asthenozoospermia and oligozoospermia who got the same ratio of deletion 18.18% (12/66) for each one of them, then oligo –astheno – teratozoospermia 13.64% (9/66) and azoospermia 6.06% (4/66) and actually no deletions for *CATSPER1* gene were discovered with astheno - teratozoospermia. Although the control group or individuals with normozoospermia had standard semen parameters but during this analysis, interestingly; they exhibited an incidence 18.18% (12/66) of *CATSPER1* deletions. The best explanation for that is these microdeletions in our fertile males are not inherited from the paternal germ line but occur recently as de novo events, which leads to a gradual decrease in semen parameters and thus infertility and such men even when helped successfully by ICSI to have child there is high risk of *CATSPER1* deletions transmission from fathers to their male offspring. Our results did not show significant changes in *CATSPER1* ($p=0.695$) among male infertility cases compared to the controls (normozoospermic), so, we can suggest deletion of this gene cannot be considered an actual a diagnostic marker of male infertility, but it is not plausible to generalize this suggestion due to the high presence of *CATSPER1* deletion in infertile males.

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Conflict of interest

The authors had no conflicts of interest to declare in relation to this article.

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