Screening of CD8 Gene in Females with Idiopathic Infertility: A Cross Sectional Study in Thi-Qar

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History:

- Received: March 19, 2020
- Accepted: June 25, 2020
- Published: Sept 1, 2020

DOI: https://doi.org/10.31838/ejmcm.07.02.05

ABSTRACT

Background: Infertility affects a major proportion of the individuals in the reproductive age group. About 40-50% of infertile cases are contributed by the female factors. Several cases of female infertility remain unexplained. There are several genetic factors associated with female infertility. Among these genetic factors, the most important factors are gene mutations, chromosomal abnormalities, and epigenetic factors. There are several single gene mutations reported in female infertility. The present study was conducted to screen the CD8 gene in unexplained cases of female infertility in the Thi-Qar province.

Material and methods: In the cross sectional, comparative study conducted over the year of 2018, 42 females were recruited. Among the 42 females, 11 were diagnosed to have primary infertility, 11 had secondary infertility, and 20 were healthy fertile controls. Venous blood was collected in EDTA coated vials by venipuncture. DNA was extracted and the CD8 gene was PCR amplified and sequenced to screen for any mutations.

Result: Only 1 out of 22 patients (4.55%) showed a sequence change in the CD8 gene. This patient showed one transition mutation (C418T) and one transversion mutation (T419G). These mutations were absent in the controls.

Conclusion: Our study did not confirm the role of CD8 gene mutations in idiopathic female infertility. However, further extensive studies should be conducted to understand the role of CD8 gene mutations in idiopathic female infertility.

Keywords: Female infertility, CD8, mutation, Thi Qar.

INTRODUCTION

According to the World Health Organization (WHO) infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse." About 1 in every 6 couples falls into this category worldwide (Estevez, 2013; Massart et al., 2012). Infertility is a complex disease with the involvement of several factors that include both genetic and environmental factors.

Genetic causes contribute to approximately 5–10% of all the infertile women. These causes include chromosome abnormalities, gene mutations and polymorphisms. Besides genetic causes other non-genetic or epigenetic factors such as the exposure to environmental factors, endocrine problems that include hormonal imbalances, infections in the reproductive tract, immunological factors, psychological factors, stress, and obesity may be completely or partially associated with a major proportion of infertility Tarin, 2015; Shah et al., 2003).

Iatrogenic factors, previous interventions, and surgery can also be associated with infertility (Shah et al., 2003). Advanced age of women is also another risk factor for female infertility (ACOG 2014).

Recent studies have revealed the role of immunity on the fertility status of women. Several studies have shown the role of natural killer cell (NK) mediated immunological mechanisms in infertility that involves the T lymphocytes. Elevated levels of peripheral blood NK cells have been reported in spontaneous abortions (Ramhorst et al., 2000; Yamada et al., 2001). Elevated levels of CD56_/CD16_/CD3 cells have been reported in women with IVF failures and in pregnancy losses (Fukui et al., 1999)

A study in recurrent spontaneous abortion and IVF failed cases showed elevated levels of CD8+ T-cells in the luteal phase and periglandular aggregation of these cells indicated focal endometriosis (Russell et al.,2013). In the premise of this background, the present study was aimed to screen the CD8 gene for mutations in the infertile women in Thi-Qar.

MATERIALS AND METHODS

Study population

The present study is a cross sectional analytical study done in Al Nasiriya City - fertility centre of Al-Hussein teaching hospital in the year 2018. Twenty-two females diagnosed with primary or secondary infertility were finally included in the study after applying the inclusion and exclusion criteria. Twenty healthy fertile females were included as controls in the study.

The patients were carefully examined for all the anatomical, endocrinal, and male partner factors before including them in the study by a specialist infertility doctor. All the participants provided signed informed consent after explaining the details of the experimental procedure. Patients who had obvious clinical causes of infertility were excluded from the study.

The participants were divided into 3 groups. First group included 20 healthy fertile individuals as a control. The second group included 11 patients with primary un-explained infertility and the third group included 11 patients with secondary un-explained infertility.

DNA extraction from blood

DNA was extracted by a commercial DNA extraction kit (G-spin dna extraction kit , intron biotechnology). Figure 1 shows the quality of the extracted DNA on an agarose gel.

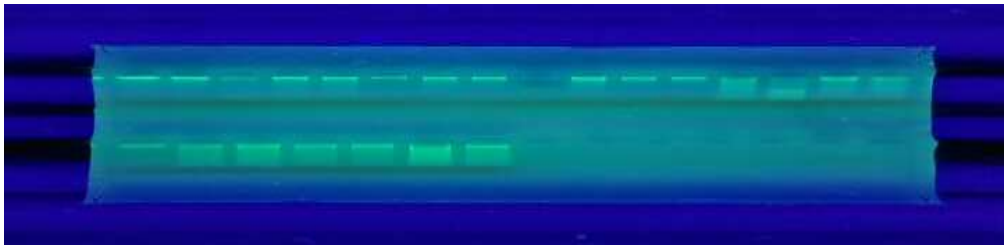

Figure 1: Gel electrophoresis of genomic DNA extracted from blood. The extracted DNA was run in a 0.8% Agarose gel at 5 volts/cm for 1:15 min. The DNA bands were visualized under UV after staining with Red Safe Nucleic acid staining solution.

Polymerase chain reaction

The whole CD8 gene was amplified and sequenced by the primer sets purchased from Integrated DNA Technologies Company, Canada. The amplicon size was 600 bps (Table 1).

Table 1: Details of the primers used to amplify CD8 gene

Primer	Sequence	Tm(°C)	GC (%)	Product size
Forward	5'-AGCGACCATCATTGTAGCCA- 3'	56.6	50.0	600 bps
Reverse	5'-GAGAGTGCAGACA TGACGCT- 3'	57.2	55.0	

Tm=melting temperature.

The composition of the reaction mixture and conditions for amplification of CD8 gene are listed in Tables 2 and 3 respectively.

Table 2: The composition of the PCR reaction mixture for CD8 gene amplification

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl
Reverse primer	10 picomols/µl
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table 3: The optimum conditions for PCR to amplify the CD8 gene

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	
2-	Denaturation -2	95°C	45sec	
3-	Annealing	52°C	45sec	40 cycle
4-	Extension-1	72°C	50sec	
5-	Extension -2	72°C	10 min.	

Purification of PCR products
Gene sequencing

The amplified PCR products were sent for sequence analysis (Sanger sequencing) after purification. Ten µl of purified PCR product along with 25 µl (10 pmol) of the forward primer was sent for sequencing. The sequencing was done using AB13730XL APPLIED BIOSYSTEMS machine in the national instrumentation center for environmental management NICM/USA.

Statistical Analysis
Data collecting and analysis

Demographic and clinical data of all the participants were collected in a predesigned pro-forma. The data were analyzed by the Statistical Package for Social Science (SPSS) version 25. Results were represented as mean ± SD for continuous variables and n (%) for categorical variables.

RESULTS

The PCR products were checked on a 1.5% Agarose gel to check for the amplification along a nucleotide ladder (Figure 2).

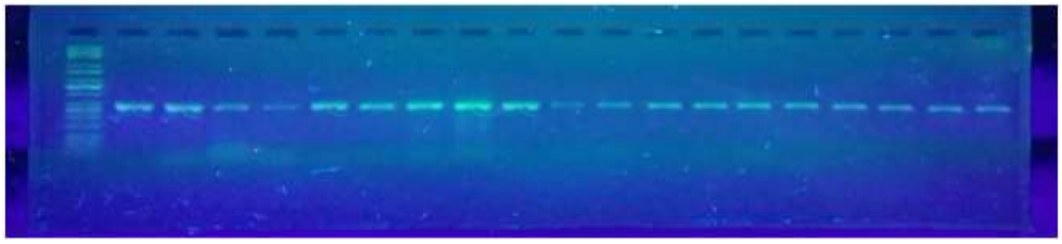

Figure 2: PCR products (600 bp) showed in all lanes when compared with ladder. The PCR products were electrophoresis on 1.5% agarose gel at 5 volt/cm^2 for 1.5 hours in 1x TBE buffer. A 100 bp DNA ladder was used to check the amplicon size.

Sequence analysis

Table 4 describes the details of the sequence analysis of the patients. Only one patient out of the total studied population showed a sequence change in the CD8 gene (4.55%). This patient showed one transition mutation (C418T) and one transversion mutation (T419G).

The point prevalence for each gene transition (C418T) and Transversion (T419G) solely was 2.38% (1/42/100 = 2.38 %). The overall prevalence of both the mutations were 4.76% (2/42/100= 4.76%).

Table 4: Full description of genetic analysis in studied samples.

No. Of sample		Location	Nucleotide	Range of nucleotide	Sequence ID	core	Expected	Identities	SOURCE
1-S		----------		135 to 527	ID: NG_044961.1	393	0.0	00%	H.SCD8
3-S		----------		109 to 514	ID: NG_044961.1	406	0.0	100%	H.SCD8
4-P	Transition Transversion	418 419	C>T T>G	112 to 527	ID: NG_044961.1	410	0.0	9%	H.SCD8
5-P		--------		106 to 515	ID: NG_044961.1	410	0.0	00%	H.SCD8
12-P	Error								
13-P		--------		109 to 526	ID: NG_044961.1	418	0.0	00%	H.SCD8
15-P		----------		112 to 527	ID: NG_044961.1	416	0.0	00%	H.SCD8
22-S		---------		110 to 527	ID: NG_044961.1	418	0.0	00%	H.SCD8
23-S		------		112 to 527	ID: NG_044961.1	416	0.0	00%	H.SCD8
24-S		--------		112 to 527	ID: NG_044961.1	413	0.0	00%	H.SCD8
25-S		---------		108 to 516	ID: NG_044961.1	409	0.0	00%	H.SCD8
33-CO		---------		108 to 516	ID: NG_044961.1	409	0.0	00%	H.SCD8
34-CO		----------		109 to 527	ID: NG_044961.1	419	0.0	00%	H.SCD8
36-CO		-------		89 to 527	ID: NG_044961.1	439	0.0	00%	H.SCD8
37-CO		Error							

DISCUSSION

In the present study we screened the CD8 gene in the females with idiopathic infertility. Mutations in CD8 gene was seen in only one of the patients (4.55%). The overall prevalence of the mutations in the CD8 gene was 4.7% in the study population.

It has been reported that female factor related infertility accounts for about 40-50% of infertility (Ganguly and Unisa, 2010; Mascarenhas et al., 2013). Several factors are associated with infertility. The factors like hormonal dysregulation, advanced maternal age, life style factors such as smoking or alcohol consumption, obesity, infection of the reproductive tract, immunological causes, surgery, anatomical causes, psychological stress, and abnormal gametogenesis are associated with the infertility. Most of these factors may be related to genetic abnormalities or genetic mutations.

Several studies have shown the association of single gene mutations with female infertility and infertility in general. Mostly these single gene mutations play at the hormonal levels and affect the fertility status of the women. Mutations in $FSH\beta$ gene have been seen to result in abnormal breast development or absence of breasts. These mutations also cause decrease in FSH and oestradiol levels and increase in the LH which together cause sterility in females (Layman et al., 1997). Deletions in the *Xp11* gene have been reported to cause menstrual dysfunction and ovarian failure in women (Layman, 2002). Fragile X syndrome is a genetic disorder associated with developmental problems and cognitive impairment. The causative gene for Fragile X syndrome is the *FMR1* gene. In this disorder the CGG triplet is expanded in the *FMR1* gene (Coffee et al., 2009). Another important gene associated with female infertility is the *GALT* gene. Mutations in this gene result in galactosemia which results in hypergonadotropic hypogonadism, secondary amenorrhea, and premature ovarian failure. Mutation in *GALT* gene is also associated with endometriosis (Fridovich-Keil et al., 2011). Leiomyomas otherwise called fibroids are the benign tumorous growth in the smooth muscle layers of the uterus. Mutations in the *MED12* gene have been identified in the females with leiomyoma (Perot et al., 2012). A study reported that about 60% patients with leiomyoma had mutations in the *MED12* gene (Perot et alk., 2012).

Another study reported 7q deletions in about 20% leiomyoma patients (Nilbert et al., 2012).

Endometriosis is a very common problem associated with inflammation and bleeding of the endometrium. Endometriosis causes severe pelvic pain and is associated with female infertility. Genome wide association studies have identified the locus 1p36 having the *WNT4* gene to be associated with endometriosis. *WNT4* gene plays an important role in the cell proliferation and embryogenesis (Zorrilla et al., 2013)

Primary ovarian failure (POF) is the partial or complete failure of the ovaries. Several genes have been associated with the pathogenesis of POF. X-linked genes reported to have mutations in the POF patients are *FMR1* and *BMP15* genes (Folsom and Fuqua, 2015; Barber and Franks,2013) Autosomal genes such as *AR*, *CDKN1B*, *CYP19A1*, *GDF9*, *FIGLA*, *FOXL2*, *FOXO1a*, *FOXO3a*, *INHA*, *LHX8*, *NOBOX*, *NANOS3*, *FSHR* and *SALL4* are reported to have mutations in the POF patients (Cordts et al., 2011).

Advancements in the genomic technology have enabled the discovery of several novel genes that are associated with infertility and reproductive disorders. However, since infertility is complex disease, it involves the interaction of several genes and epigenetic factors as well. Therefore, it is difficult to say that a single gene mutation can lead to infertility. Future studies will add to the present list of gene mutations reported to be associated with female infertility and this will help in the elucidation of molecular pathways and genetic associations in the idiopathic cases of female infertility.

CONCLUSION

Only one patient in the present study harbored mutation in the CD8 gene. The findings of the present study do not confirm the role of CD8 gene mutation in female infertility in out cohort of infertile females. However, larger genetic studies combined with functional assessment of the mutations can provide insights about the role of CD8 gene mutations in idiopathic female infertility.

ACKNOWLEDGEMENT

We; the authors; would like to thank the department of Fertility and Reproduction in Al-Hussein teaching hospital in ThiQar province, Iraq for their support and cooperation during our work especially the phase of data collection.

CONFLICTING INTERESTING

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

SOURCE (S) OF SUPPORT

There are no sources of support in the form of grants, equipment, or drugs. No funding was received for this work, apart from authors' participation.

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Cite this article: Enaas S. Jawad. 2020. Screening of CD 8 Gene in Females with Idiopathic Infertility: A Cross Sectional Study in Thi – Qar. European Journal of Molecular & Clinical Medicine, 7(2), pp. 38 – 42, DOI: https://doi.org/10.31838/ejmcm.07.02.05