Immunogenic Detection of IL-6 in Male Patients Suffering from Infertility Disorders

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ABSTRACT
Background; Infertility in men is one of the health and psychological problems around the world that is widespread and related to many physiological and medical causes. Many hormonal differences or viral or bacterial infections in general can lead to infertility in men. Aims of the study; The effect of interleukin 6 and its role in the development of male infertility. Methodology; This study was conducted on patients at Al-Sadr Medical City / Infertility Center / Al-Najaf Al-Ashraf and Dr. Ali Al-Ibrahimi's private center for embryology and infertility. Since October 2023, the control group (fertile men) included 40 healthy 25–45-year-old men. There were 60 infertile men aged 22-46 in the study. An enzyme-linked immunosorbent assay measured interleukin-6. DNA extraction was followed by PCR analysis of the interleukin 6 gene polymorphism. Result; The results showed that there was no statistical significance in age between the two groups. The results also showed an increase in interleukin 6 levels in general and by age groups in the patient group compared to the control group. In addition there is a statistical significance in the polymorphism gene tests between the two groups. Conclusions; The case group had higher interleukin 6 levels, and the gene polymorphism was significant. This suggests that inflammatory factors like viral or bacterial infections or autoimmunity caused the difference in interleukin 6 levels and the mutation in IL-6 gene could play a role in this increase.

1. Introduction

Infertility was defined as the failure to achieve pregnancy despite attempting to do so through the practice of full and regular sexual intercourse without the use of contraceptive methods for pregnancy for a period of one year or more (Leslie et al.,2020). According to the World Health Organization, approximately15% of couples can be classified within this particular group, where in 40% of infertility cases are attributed to male factors and an equivalent 40% are associated with female factors. Both sexes are implicated in the remaining 20% of instances (Aziziaram Z.,2021). Male infertility is a widely seen illness that exerts a significant psychological burden on affected individuals, particularly within the cultural context of Eastern societies (Yazdi et al., 2020). The etiology of this condition encompasses an microbiological factors, most notably bacterial, fungal, and viral infections, with other contributing factors. Given the limited attention given to viral causes in research and the predominant focus on bacterial factors, this study aimed to investigate the involvement of a specific viral strain (HHV6 and CMV) in the development of this infection (Brugh & Lipshultz., 2004). Male infertility is a widespread clinical concern observed among men of reproductive age throughout various societies. The condition of infertility with an unknown cause, sometimes referred to as idiopathic infertility, is a matter of significant concern due to the lack of understanding its underlying pathophysiology (Krauszet al., 2011). The occurrence of infection and inflammation in the reproductive system is considered a potential cause of male infertility. Sexually transmitted bacteria or urinary tract infection are the primary causative agents of epididymitis orchitis, with viral dissemination in the circulation also playing a contributory role (Oghbaei et al., 2020). IL-6 is a protein that aids the host in its self-defense. It exhibits pleiotropic activity, which implies that its effects vary depending on the target cells. IL-6 has the ability to stimulate, One example of a cytokine known for its pleiotropic function is interleukin-6 (IL-6). Numerous biological processes, including cellular proliferation, differentiation, and death (Hunter & Jones., 2015). exert regulatory influence on it. IL-6, apart from its principal role in inflammation, exerts influence on several physiological processes such as neuronal and endocrine cell function, bone and skeletal metabolism, and muscular activity, It promotes terminal differentiation and development on diverse

B cells, resulting in immunoglobulin secretion in B cells (Khan et al., 2023). Cytokines influence human sperm motility, increase the synthesis of human-produced reactive oxygen species (ROS), and decrease the number of ova-penetrating spermatozoa. Sperm's ability to reproduce Some of these still have debatable consequences. Furthermore, it has been claimed that an increase in cytokine expression may result in a faster rate of absorption.onto sperm cells, which was followed by a surge in legislative action (Tawfik et al., 2004). Although urogenital infections are the most common cause of male infertility, there are other probable causes, which are frequently complicated and/or unexplainable. As a result, if male infertility is present, the seminal cytokine concentration may provide additional information (Tvrdá et al., 2016). IL-6 is a physiological mediator that has a regulatory role in the acute phase response to inflammation or infection ,It has been proposed that an increase in cytokine expression may result in higher absorption onto sperm cells and, as a result, an increase in regulatory activity (Slaats et al., 2016).

2. Methodology

A hundred male specimens were collected for this case-control study. This study comprised patients from Al Sader Medical City/Infertility Center/Al-Najaf Al-Ashraf and the private Dr. Ali Al-Ibrahimi Center for Embryos and Infertility in Najaf. Starting in October 2023, the Control group consisted of 40 healthy fertile males aged 25–45 with at least 12 months of primary and secondary infertility. The Patients Cases group included 60 infertile men aged 22–46. Based on their infertility, forty-six men were classed as major infertile, and fourteen as secondary. To assure ethics and privacy, individuals masturbated in a separate laboratory to obtain their recent semen sample. This was done with a wide-opening sterile container. Participants were told the sample must be complete because each container holds their name, age, and sample collection time. Sperm was taken from each study participant's ejaculated samples after 72 hours (three days) of abstinence from sexual activity. For 30 minutes at 37 degrees Celsius, the samples liquefied. The physical properties of all samples were assessed after liquefaction. This involved monitoring sperm concentration, motility, grading activity, and normal sperm morphology using light and microscopy.This project was finished in October–January 2023. Before semen analysis, patient demographic and medical history data was obtained. A pre-configured data form received the data. Patients must provide name, sample number, age (in years), residence, and education level. Vertical dimension, mass, and job classification Long-term illness, smoking, Surgery history, primary and secondary infertility, sperm motility, count, and morphology are covered. An enzyme-linked immunosorbent test evaluated interleukin-6.

Molecular detection:

The Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) is a method used to identify a specific Single Nucleotide Polymorphism (SNP). It involves two separate reactions: one with a primer that specifically targets the normal DNA sequence and cannot amplify the mutant DNA at a specific location, and another reaction with a primer that specifically targets the mutant DNA and does not amplify the normal DNA **(Ali and Settin., 2013)**.

DNA Extraction from seminal fluid:

Spin column technology, specifically the PrestoTM Sperm DNA Extraction Kit, retrieved DNA from semen fluid. This kit effectively purifies DNA from human semen. Proteinase K and a sperm-cell-degrading reagent break down proteins and sperm cells. DNA binding to the spin column glass fiber matrix is more efficient with this method. A low-salinity elution solution extracts pure DNA after a wash buffer removes contaminants.

Sperm DNA Purification Procedure:

Use a 1.5-milliliter microcentrifuge tube to inject 900 microliters of Sperm Lysis Buffer. Following a gentle pipetting technique, 20 µl of Proteinase K and 80 µl of 1M DTT solution were mixed. Mix thoroughly. To make the solution, dissolve 1.5 grams dithiothreitol in 8 mL deionized ddH2O. Set volume to 10 ml. Dispense and store 1 ml aliquots at -20oC. Thaw the DTT solution to room

temperature before use. Combine 100 µl of semen with 100 µl of newly prepared Sperm Lysis Buffer (proteinase K and DTT) in a 1.5 ml microcentrifuge tube. Vortexing the mixture increases pathogenicity. One hour at 60 degrees Celsius lyses sperm cells. Prepare 200 µl of Elution Buffer per sample and transfer to a 1.5 ml microcentrifuge tube. Incubate at 60ºC for DNA elution (Step 5). The liquid was violently stirred while 200 microliters of GSB buffer was added. Amazing RNA removal To remove RNA from genomic DNA (gDNA), add 5 µl of RNase to GSB Buffer and vigorously mix. Incubating RNA at room temperature for five minutes ensures destruction. Add 200 µl of 100% ethanol to the sample lysate and vigorously shake for 10 seconds. Use a pipette to crush solids into powder. Gas Chromatography (GS) Columns were placed in 2 ml collecting containers. The mixture was centrifuged at 14-16,000 times gravity after being transferred to the GS column. After discarding the flow-through, move the GS column to a new 2 ml collection tube. The GS Column received 400 microliters of W1 buffer. The flow-through was eliminated after 30 seconds of 14-16,000 times gravity centrifugation. Returning the GS Column to the 2 ml collection vial. A 600 µl wash buffer was applied to the GS column. Discard the liquid after 30 seconds of centrifugation at 14-16,000 times the force of gravity. Returning the GS Column to the 2 ml collection vial. To dry the column matrix, centrifuge it for three minutes at 14-16,000 xg.

Estimation of DNA Purity and concentration:

A UV/Visible spectrophotometer measured human DNA purity. Elution buffer was used to blank a DNA sample and quantify its absorbance. DNA purity was measured by 260/280 nm absorbance. According to the extraction kit specifications, the ideal absorbance ratio for pure DNA is 1.8, with a concentration range of 4-10 µg/ml.

Primers used to amplify DNA of IL-6: All the primers (forward and reverse) have been prepared according to the manufacturer's recommendation, by dissolving the lyophilized short single stranded DNA in appropriate deionized water to get a final concentration 100 pmol/ml of each primer (Stock Solution). Working Solution was prepared by diluting 10 micro liter of stock solution with 90 micro liter of deionized water to yield a final concentration of 5 or 10 pmol/ml. one primers were used to detected (IL-6 gen) from human sminal fluid which are cox1 gene.

Table 1- Amp PCR Kit PCR primers used to amplify DNA gen of (IL-6)

The Gene	primer	Sequences of primer (5`→ 3`)	Size (bp)
IL-6 gene (rs 1800795 C/G)	Outer (F)	GACTTC AGCTTTACTCTTTGTCAAGACA	326 bp
	Outer (R)	GAATGAGCCTCAGACATCTCCAGTCCTA	
	F inner (Gallele)	GCACTT TTCCCC CTAGTTGTGTCTTCCG	205 bp
	R inner (C llele)	ATTGTGCAATGTGACGTCCTTTAGCTTG	184 bp

Thermocycling Condition:

IL-6 gene PCR thermocycler conditions were done using a standard instrument. The PCR program was developed to detect IL-6 SNPs. Tables (2) include IL-6 identification methodologies, temperatures, durations, and repetitions.

Table (2): The program IL-6 (rs 1800795 C/G)) Gene polymorphism

Target gene	Steps	Temperature (co)	Number of cycles	Time (second)
IL-6 -(rs 1800795 C/G) gene	Initial denaturation	95	1	10 min
	Denaturation	95	40	30 sec
	Annealing	55		30 sec
	Extension	72		30 sec
	Final Extension	72	1	10 min

Agarose gel electrophoresis:

PCR or genomic DNA extraction was followed by agarose gel electrophoresis. As detailed by Sambrook et al. (1989), extracted DNA was electrophoresed on agarose gel. 0.8% agarose gel to

validate DNA extraction. Pouring a Standard 1% Agarose Gel and Loading Samples and Running an Agarose Gel demonstrate electrophoresis' key principles.

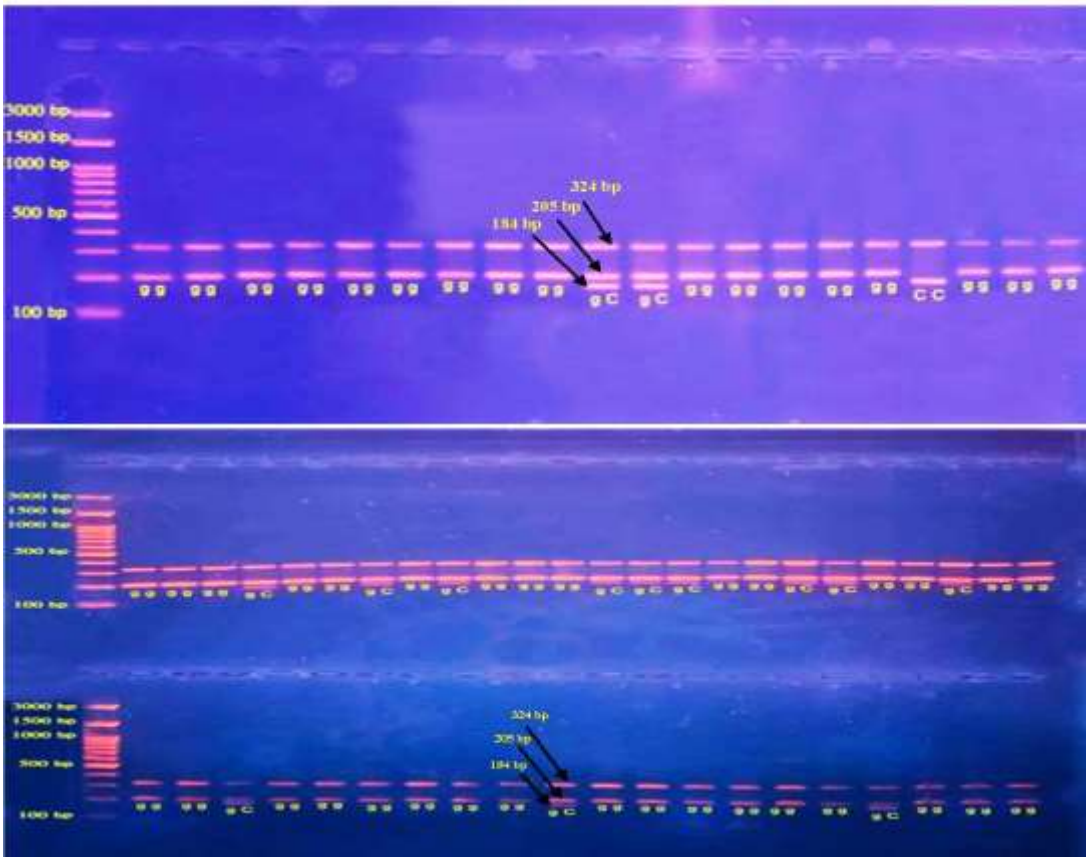

Figure (1): **):** Agarose gel electrophoresis image that show the PCR product analysis of IL6 -(rs 1800795 G/C) gene in infertility seminal fluid samples

Statistical analysis:

Statistical analysis is often used to analyze quantitative data, and provides methods for data description, simple inference for continuous and categorical data. The procedure involves the collection of data leading to test of the relationship between two statistical data sets. In this study all data are presented as mean ± standard deviation. The statistical analyses were performed using SPSS (version 26) and using using dependent t-test (two-tailed) and independent t-test (two-tailed) for normally distribution variables, whereas the Mann-Whitney *U* and Wilcoxon test used for those variables that were not normally distributed . $P < 0.05$ was considered statistically significant. The single nucleotide polymorphism (SNP analysis) test were chosen to search for specific genetic differences that may affect gene expression or protein functions.

3. Results and discussion

Socio-demographic and anthropometric characteristics

In a comparative study between a patient group consisting of 61 individuals and a control group of 28 individuals, it was noted that the average age was 35.28 years with a standard deviation of 5.45 for the patient group, and 33.68 years with a standard deviation of 5.76 for the control group, and there were no differences in age between the two groups. Statistically significant (P=0.20). While the average body mass index (BMI) was 27.97 ± 2.86 for the patient group, and 25.72 ± 1.37 for the control group, with strong statistical significance (P<0.001) indicating an increase in BMI in patients. It was found that 40 of the patients in the first group were smokers compared to 9 in the control group, indicating a statistically significant (P<0.01) association between obesity and smoking. Primary infertility was present in 46 patients in the patient group versus none in the control group,

showing statistical significance (P<0.001). 23 of the patient group had a history of surgery compared to only one individual in the control group, indicating a statistically significant relationship between a history of surgery and infertility (P<0.01). Regarding the duration of infertility, the results showed that 36 patients from the patient group had been suffering from infertility for less than 5 years, while 25 for more than 5 years, with significant statistical significance (P<0.001), indicating a link between the duration of infertility and the health status of the patient group.

Table 1 : Socio-demographic and anthropometric characteristics among the study groups

Characteristic		**Patients group (n=61)**	**Control group (n=28)**	**P. value**
Age (year) (Mean ± SD)		35.28 ± 5.45	33.68 ± 5.76	0.20 ^{NS}
BMI (kg/m^2) (Mean ± SD)		27.97 ± 2.86	25.72 ± 1.37	<0.001
Smoking	Yes	40	9	<0.01
	NO	21	19	
Fertility Type	**Primary**	46	0	<0.001
	Secondary	15	0	
	Non	0	0	
History of surgery	**Yes**	23	1	<0.01
	No	38	27	
Duration of infertility	**<5 years**	36	0	<0.001
	≥5 years	25	0	

SD: Standard deviation, NS: Non-significant

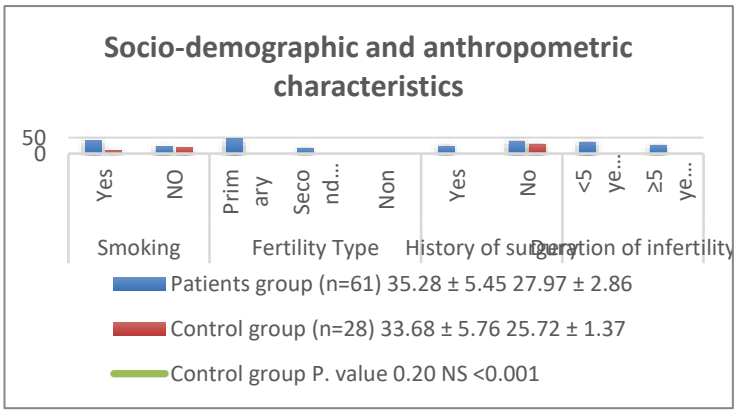

Fig 1: Socio-demographic and anthropometric characteristics

Comparison between sperm motility, sperm morphology and sperm count in patients and control group.

The disparities between the 61 individuals in the sick group and the 28 individuals in the control group are statistically significant. Regarding sperm motility, the sick group exhibited no motility, while the control group had a motility rate of 0.55 ± 0.08. The observed changes were extremely statistically significant (P<0.001). The mean morphology of sperm in the sick group was 16.47 ± 6.21, whereas it was 54.64 ± 6.51 in the control group. This demonstrates a substantial disparity and a notable level of statistical significance (P<0.001). Finally, when looking at sperm count, the patient group recorded a value of 9.77±3.36 compared to 56.71±8.37 for the control group, with clear statistical significance (P<0.001). These data confirm the existence of significant differences between patients and healthy individuals in parameters related to semen characteristics.

Table (2) : Comparison between sperm motility, sperm morphology and sperm count in patients and control group.

Parameters	Patients group (n=61)	Control group (n=28)	P. value
Sperm motility	0.068 ± 0.03	0.55 ± 0.08	<0.001
Sperm morphology	16.47 ± 6.21	54.64 ± 6.51	<0.001
Sperm count	9.77 ± 3.36	56.71 ± 8.37	<0.001

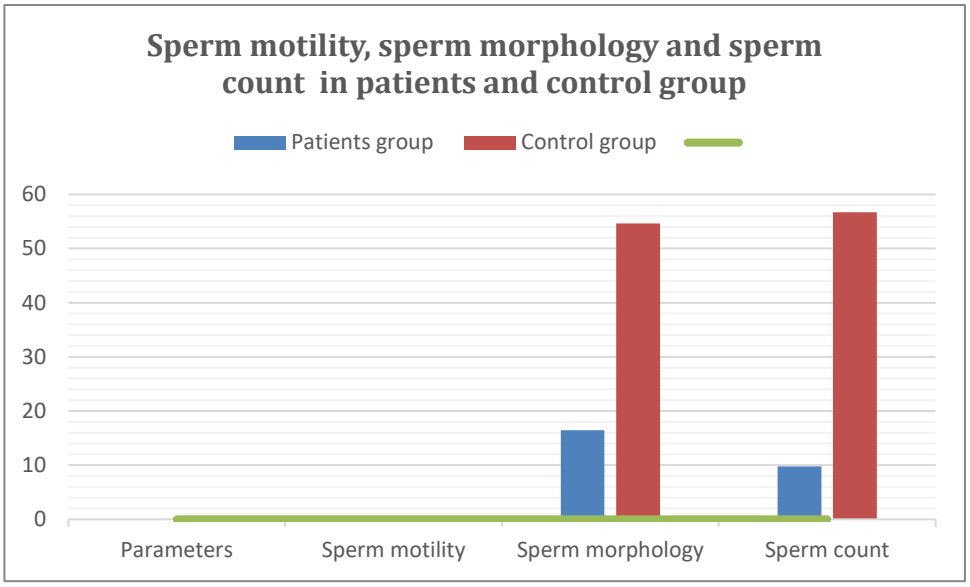

Fig 2: Sperm motility, sperm morphology and sperm count in patients and control group.

Comparison between sperm motility, sperm morphology and sperm count in patients and control group according to age.

The statistical results of the comparative study that included two groups, one for patients (61 individuals) and the other for controls (28 individuals), reveal significant differences between the two groups in all three measures, regardless of age. In the sperm motility scale, values of 0.066 ± 0.030 and 0.070 ± 0.032 were recorded for the patient group at ages 20-44 and ≥44, respectively, compared to 0.576 ± 0.084 and 0.534 ± 0.077 for the control group for the same ages, with significant statistical significance (P< 0.001) in both categories. For sperm morphology, the study showed values of 16.50 ± 6.31 and 16.45 ± 6.21 for the patient group compared to 55.33 ± 6.39 and 53.84 ± 6.81 for the control group for the age groups 20-44 and ≥45, respectively, with statistical significance (P<0.001). . In conclusion, the results showed differences in the sperm count measure, as the patients' values were 10.43 ± 3.74 for the age group 20-44 and 9.13 ± 2.86 for the ≥45 group, compared to 54.20 ± 7.85 and 59.62 ± 8.28, respectively, for the control group, confirming the large statistical differences. (P<0.001) between the two groups.

Table (3) : Comparison between sperm motility, sperm morphology and sperm count in patients and control group according to age.

Parameters			Patients group (n=61)	Control group (n=28)	P. value
Sperm motility	**Age**	**20-44**	0.066 ± 0.030	0.576 ± 0.084	<0.001
		≥ 44	0.070 ± 0.032	0.534 ± 0.077	<0.001
Sperm morphology	**Age**	**20-44**	16.50 ± 6.31	55.33 ± 6.39	<0.001
		≥ 45	16.45 ± 6.21	53.84 ± 6.81	<0.001
Sperm count	**Age**	**20-44**	10.43 ± 3.74	54.20 ± 7.85	<0.001

		≥ 45	9.13 ± 2.86	59.62 ± 8.28	<0.001

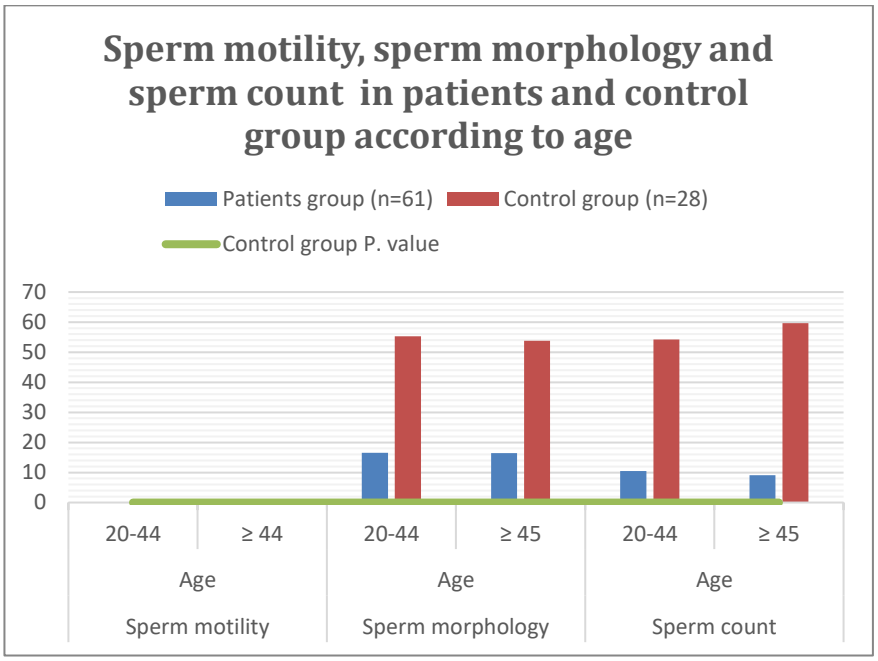

Fig 3: Sperm motility, sperm morphology and sperm count in patients and control group according to age

Comparison of IL-6 in patients and control group.

In the comparative study between a control group of 28 individuals and a patient group of 61 individuals, statistically significant differences in levels of the inflammatory marker IL-6 were recorded. The average IL-6 levels in the control group were 5.91, with a standard deviation of 1.28, while the levels in the patient group were higher, estimated at 7.86, with a standard deviation of 1.95, which indicates a noticeable increase in IL-6 levels among patients compared to the control group. The results were supported by a P value of less than 0.001, which indicates significant statistical significance and emphasizes the existence of a possible relationship between high levels of IL-6 and the disease status of the group.

Table (4) : Comparison between IL-6 in patients and control group.

Parameters	**Control group (n=28)**	**Patients group (n=61)**	**P. value**
IL-6	5.91 ± 1.28	7.86 ± 1.95	<0.001

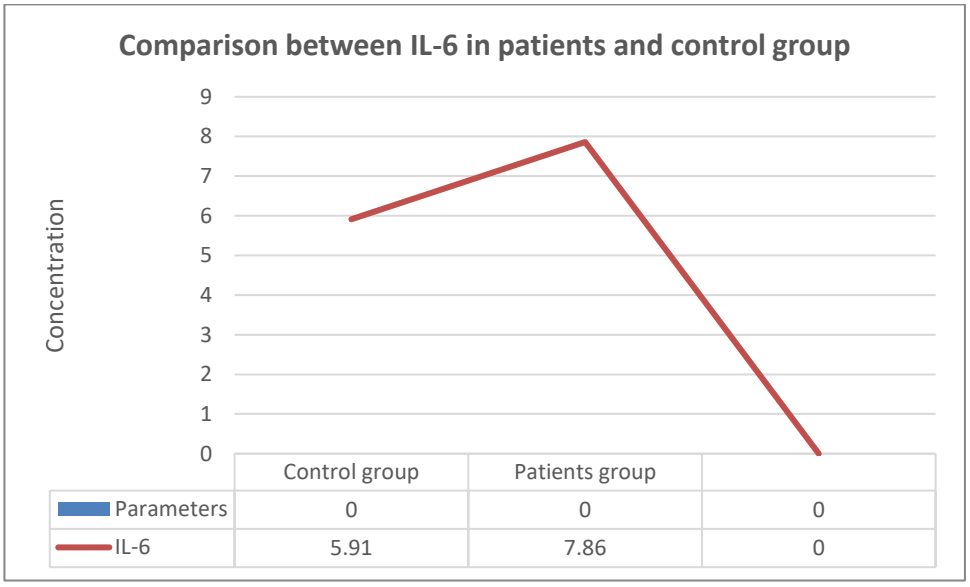

Fig 4: Comparison between IL-6 in patients and control group

Comparison of IL-6 in patients and control group according to age.

According to the data presented, the comparison between the control group and the patient group shows changes in the levels of the inflammatory protein IL-6 with respect to age. For the age group from 20 to 44 years, the average IL-6 levels in the control group were 6.05 with a standard deviation of 1.29, compared to 7.27 with a standard deviation of 0.83 for the same age group in the disease group. This difference was statistically significant (P<0.01). As for the age group 45 years and older, the mean IL-6 for the control group was relatively lower, 5.77 with a standard deviation of 1.29, while that mean increased in the patient group to reach 8.54 with a standard deviation of 2.61, with great statistical significance (P<0.001).

Table (5): Comparison of IL-6 in patients and control group according to age.

Parameters			**Control group (n=28)**	**Patients group (n=61)**	**P. value**
IL-6	**Age**	**20-44**	6.05 ± 1.29	7.27 ± 0.83	<0.01
		≥ 45	5.77 ± 1.29	8.54 ± 2.61	<0.001

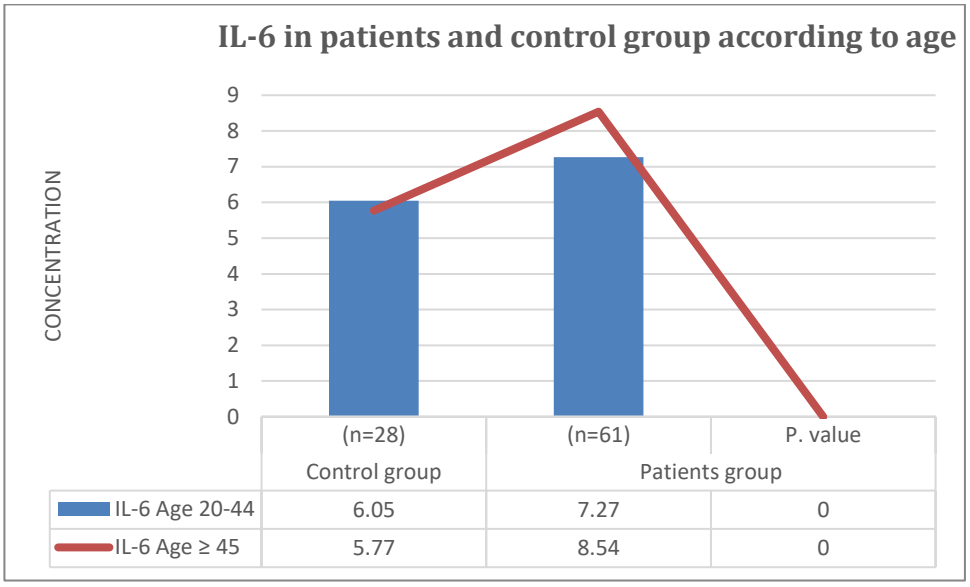

Fig 5: IL-6 in patients and control group according to age

Associations of genotype with category of the study individuals.

These results show the distribution of genotypes (CC, GC, and GG) between two different groups: a control group and a patient group. In the control group (29 people): there is one person with the CC genotype, two people with the GC genotype, and 26 people with the GG genotype. In the patient group (71 people): there was also 1 person with type CC, 20 people with type GC, and 50 people with type GG. In total (100 people): 2 people with type CC, 22 people with type GC, and 76 people with type GG. The P value presented is 0.03*, Demonstrating a statistically significant disparity in the distribution of genotypes between the two groups.

Table (6) : Associations of genotype with category of the study individuals

Parameters		**Genotype**			**Total**	**P. value**
		CC	**GC**	**GG**		
Group	**Control**	1	2	26	29	0.03*
	Patients	1	20	50	71	
Total		2	22	76	100	

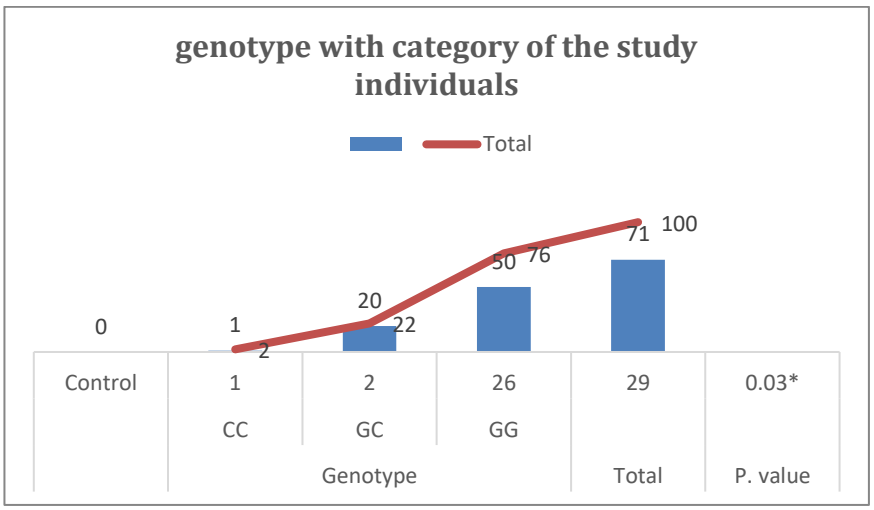

Fig 6: genotype with category of the study individuals

Type of infertility according to genotype.

Based on the table provided, the distribution of genotypes or genotypes (CC, GC, GG) for the two types of infertility, primary and secondary, is shown. In cases of primary infertility (53 cases): there is one case carrying the CC genotype, 15 cases carrying the GC genotype, and 37 cases carrying the GG genotype. In cases of secondary infertility (18 cases): There are no cases carrying the CC type, 5 cases carrying the GC type, and 13 cases carrying the GG type. In total in the sample (71 cases): there is one case with type CC, 20 cases with type GC, and 50 cases with type GG. The P value is 1.00, and this number indicates that there are no statistically significant differences between cases of primary infertility and secondary infertility regarding the distribution of the mentioned genotypes.

Table (7): Type of infertility according to genotype**.**

Parameters		**Genotype**			**Total**	**P. value**
		CC	**GC**	**GG**		
Type of infertility	**Primary**	1	15	37	53	1.00*
	Secondary	0	5	13	18	
Total		1	20	50	71	

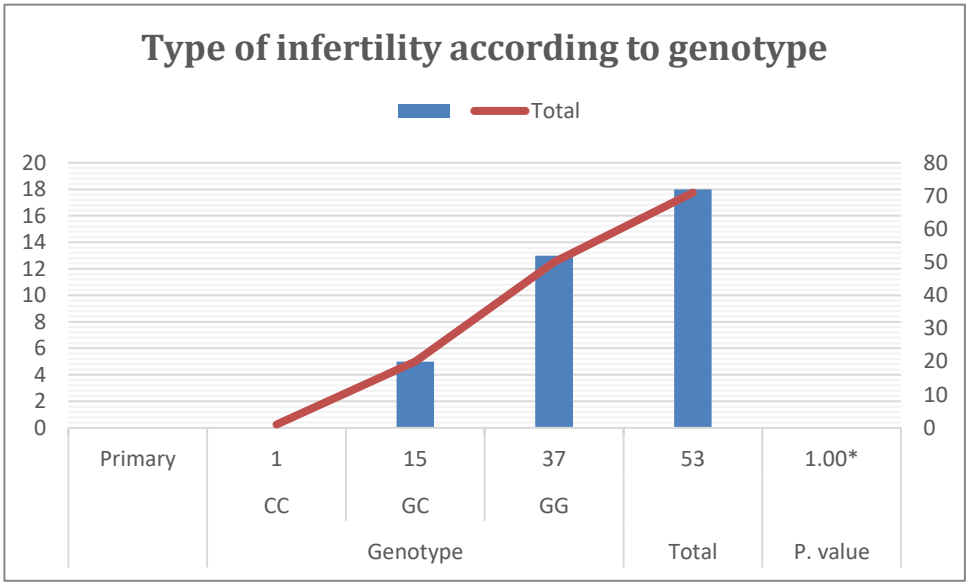

Fig 6: Type of infertility according to genotype

The distribution of genotypes (CC, GC, GG) for duration of infertility.

According to the presented data, the distribution of genotypes (CC, GC, GG) is evaluated based on the duration of infertility. For a period of infertility less than 5 years (43 cases): there is one case with the CC genotype, 15 cases with the GC genotype, and 27 cases with the GG genotype. For infertility lasting 5 years or more (28 cases): There were no cases with the CC genotype, 5 cases with the GC genotype, and 23 cases with the GG genotype. In total (71 cases): there is one case with type CC, 20 cases with type GC, and 50 cases with type GG. The P value is 0.17, which means that there are no statistically significant differences between the different infertility duration groups regarding the distribution of genotypes.

Table (8): the distribution of genotypes (CC, GC, GG) for duration of infertility

Parameters	**Genotype**	**Total**	**P. value**

		CC	GC	GG		
Duration of infertility	**< 5 years**	1	15	27	43	0.17
	≥ 5 years	0	5	23	28	
Total		1	20	50	71	

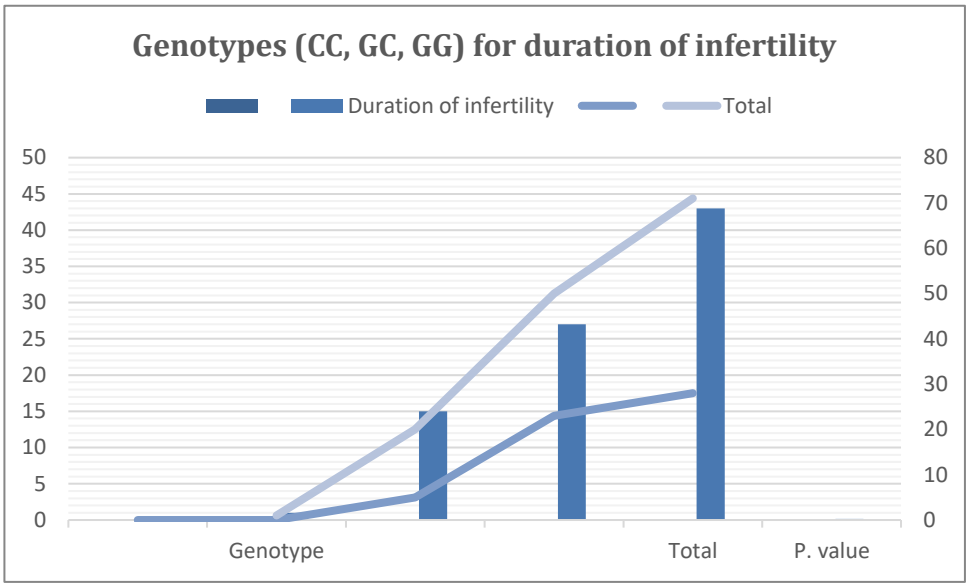

Fig 7: Genotypes (CC, GC, GG) for duration of infertility

The genotype distribution among different age groups.

The table shows the distribution of genotypes (CC, GC, and GG) divided by age. For the age group 20-44 years (36 people): There is one person with the CC genotype, 12 people with the GC genotype, and 23 people with the GG genotype. For the age group 45 years and over (35 people): There are no people in the CC type, 8 people in the GC type, and 27 people in the GG type. In total (71 people): 1 person with type CC, 20 people with type GC, and 50 people with type GG. The P value is 0.36, indicating that there are no significant differences between the two age groups with regard to the distribution of genotypes.

Table (9): the genotype distribution among different age groups:

Parameters		**Genotype**			**Total**	**P. value**
		CC	**GC**	**GG**		
Age (year)	**20-44**	1	12	23	36	0.36*
	≥ 45	0	8	27	35	
Total		1	20	50	71	

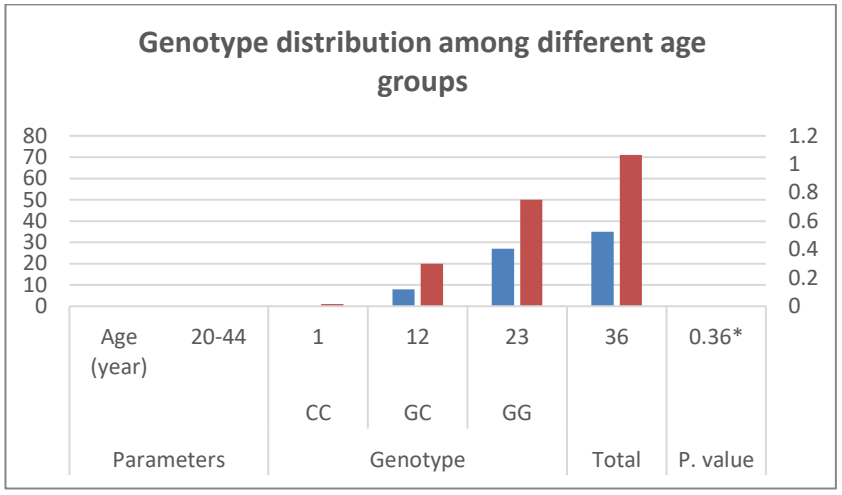

Fig 8: Genotype distribution among different age groups

Discussion:

There is a condition known as infertility, which is characterized by the inability to conceive a child while engaging in regular sexual activity and without taking any form of birth control for a period of one year or more. About fifteen percent of married couples are affected by this disorder, which is quite common. The problem of male infertility is rather common and has a considerable impact on the mental health of the individual who is affected by it, particularly in our eastern culture. There are a number of factors that contribute to the development of this illness. These factors include physiological factors as well as microbiological agents, including bacteria and viruses **(Brugh and Lipshultz., 2004; Leslie et al., 2020).** The study revealed significant disparities between the patients and the comparison group in relation to BMI, smoking habits, fertility type, surgical history, and duration of infertility. However, there was not a significant disparity in age between the groups. These findings are consistent with certain prior research, A study conducted by **(Muhsin et al., 2019)** shown that there is no statistically significant disparity in age between males who are healthy and those who are infertile. A separate study conducted by **(Eisenberg et al., 2014)** and published in "The Journal of Urology" found that obesity and high BMI could potentially heighten the likelihood of infertility in males. A meta-analysis conducted by **(Sharma et al., 2016)** examined the relationship between smoking and semen quality. The study uncovered evidence that smoking has a detrimental effect on sperm quality, increasing the risk of male infertility. The present investigation revealed a substantial and statistically significant disparity in sperm motility, sperm morphology, and sperm count, as demonstrated in tables (2) and (3). The findings are consistent with the research conducted by **Tilahun et al. (2022) and Jain et al. (2016)**. Sperm quality, encompassing factors such as count, motility, and morphology, plays a crucial role in male infertility. Regarding sperm count, An average male normally possesses a sperm count of 15 million sperm per milliliter or more. Nevertheless, males who are unable to conceive frequently display a diminished number of sperm, a condition referred to as oligozoospermia. The World Health Organization (WHO) has defined oligozoospermia as having a sperm count of less than 15 million sperm per milliliter. Research has indicated that various variables, including lifestyle choices, environmental conditions, and certain medical disorders, can contribute to a reduction in sperm count among individuals experiencing infertility **(Maurya et al., 2022)**. Sperm motility refers to the sperm's capacity to move rapidly and effortlessly, a crucial factor in the process of fertilization. Healthy sperm should exhibit a minimum motility rate of 40%, with at least 32% of that movement being forward. In contrast, males who are unable to conceive children commonly suffer from asthenozoospermia, a condition characterized by a total motility of less than 40% and a rising motility of less than 32%. This disease arises from metabolic dysregulation and is associated with genetic predisposition **(Maurya et al., 2022).** Normal sperm morphology is another crucial element in male conception. Healthy sperm possess an elliptical

head and an elongated tail, facilitating the process of fertilization with the egg. Teratozoospermia refers to a condition in which a significant proportion of sperm in men who are unable to conceive are abnormally shaped. This can occur due to DNA damage or genetic abnormalities, resulting in increased difficulty for sperm to successfully fertilize an egg **(Huang et al., 2023)**. The discrepancy in sperm count, morphology, and motility between infertile and fertile males is a complex issue that is influenced by a wide range of circumstances. In order to address this difficulty, it is essential to develop a complete plan that includes making changes to one's lifestyle, implementing medical interventions, and conducting further inquiry to determine the underlying factors and create effective remedies. Given the increasing global problem of male infertility, it is crucial to prioritize male reproductive health in order to improve fertility results. Compared to the healthy group, the current study discovered that infertile males had considerably elevated levels of IL-6 in their sperm. The results align with the findings of **(Camejo et al., 2001)**, who observed that infertile men had notably elevated IL-6 levels in their seminal plasma compared to fertile men. Moreover, these levels demonstrated a significant negative association with the number of sperm in the ejaculate, rates of penetration, and specific parameters related to sperm movement. According to the findings of A. Hachim et al. (2015), the samples from the normospermia group have lower levels of interleukin-6 measurement when compared to the infertile groups. This conclusion is compatible with the information presented in the previous sentence. Due to the fact that cytokines have a role in reproductive physiology and gonadal function, the relationship between cytokines and human reproduction has been the subject of a great deal of research. It is noticed that a wide variety of cells in the male reproductive system are responsible for the production of cytokines, and that these cytokines exercise their effects mostly in the immediate area. According to **(Wang et al., 2018; Alzaidi et al., 2021)**, sperm can be produced by the testis, the epididymis, and/or released by immune-competent cells that are present, even in the absence of inflammation. There are a number of cytokines that have been shown to have an impact on the movement, viability, and ability of sperm to effectively fertilize eggs. Research indicates that IL-6 can impact fertility through various pathways, including its potential to impede the normal motility of sperm and limit their capacity to survive and fertilize. Elevated levels of IL-6 can induce inflammation in the reproductive system, resulting in tissue damage and disruption of spermogenesis. Additionally, it may have an impact on the levels of hormones, such as testosterone, that are essential for the production of sperm **(Loveland et al., 2017; Chen et al., 2023).** Endocrine disorders, such as hormonal imbalances in the regulation of spermatogenesis, involving hormones like FSH, LH, and Testosterone, along with ILs like IL-6, can disrupt the process of spermatogenesis. This disruption is characterized by changes in the levels of these ILs, specifically a decrease in LH and Testosterone levels combined with an increase in FSH levels. These changes in IL levels affect the secretion of interleukin-6 by sertoli cells during spermatogenesis, ultimately leading to infertility **(Kumanov et al., 2006)**. Furthermore, inflammation can occur in any area of the reproductive system, particularly in the case of prostatitis **(Alshahrani et al., 2013)**. The study conducted by **(AL-saimary et al., 2014)** investigates the role of IL-6 in regulating spermatogenesis and its impact on pro-inflammation resulting from the failure of sperm production process. Additionally, the study examines the relationship between IL-6 and immune response. Existing research indicates a strong correlation between elevated levels of IL-6 and IL-10 and male infertility. Nevertheless, further investigation is required to comprehend the precise pathways and formulate efficacious treatment techniques. The findings of our study demonstrated a notable disparity in the IL-6 (rs-1800795 C/G)polymorphism between infertile guys and the healthy control group.

4. Conclusion and future scope

The human semen contains a repertoire of cytokines various interleukins (ILs) and some of their soluble receptors). Including IL-6 ,Seminal concentrations of cytokines might not only provide a measure of their release but also reflect their different interactions IL-6 with spermatozoa. The results of this study detected that the IL-6 most probably has a role in male infertility that is

associated with infection, immuno- infertility and varicocele. Moreover, the simultaneous determination of IL-6 in SP may provide a sensitive and useful marker of silent infection / inflammation of the male genital tract. A better understanding of these mediators in seminal plasma of normal men and patients with infertility may contribute to the management of male infertility in clinical practice

Ethical approval:

Verbal consent was taken from all participants in the research and they signed the ethical approvals form issued by the Ethics Department of Jabir Ibin Hayyan University for Medical and Pharmaceutical Sciences College of Medicine Department of Microbiology and important facilitation was obtained from the Thi Qar Health Diroctorate and Al-Habbobi Teaching Hospital for the purpose of conducting the research and collecting samples.

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