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Detection and Quantification of Human Herpes Viruses Types-6 and Cytomegaly Viruses in Sperm Samples of Patients with Fertility Disorders

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Abstract: Background; Viral infections are common around the world and cause many health problems. Infertility in men is a widespread health problem that can affect lifestyle and psychological state. Aims of the study; Study of the effect of herpes virus type 6 and cytomegalovirus on infertility disorders in men. Methodology; A case control study was done for a 100 specimens collected from men. This study has been conducted in Al Sader Medical City / Infertility Center / Al-Najaf Al-Ashraf and privet Center Dr. Ali Al-Ibrahimi for Embryos and Infertility in najaf . During the period from 10/10/ 2023 to 10/4/2024. The current study consisted of a sample size of 100 males, which was subsequently separated into two groups. The control group comprised 40 fertile men, aged 25 to 45 years, who had experienced both primary and secondary fecundity for at least twelve months. The Patients Cases group, consisting of sixty infertile males aged between 22 and 46 years, was categorized into two subgroups based on the type of infertility. There were forty-six guys who were classified as main infertility. There were a total of fourteen men who experienced secondary infertility. Male participants who are reproductive age, normal seminal analysis which is healthy and have a child as a Control group (Fertile men). Name of patient, sample number, Age (years), Education, housing area, Type of work, height, weight, Smoking, chronic diseases, History of surgery, Primary infertility, Secondary infertility, Sperm count, Sperm motility and Sperm morphology. Detection of CMV in semen plasma by CMV ELISA kit and detection of HHV 6 in semen plasma by HHV 6 ELISA kit. Result; The results showed that there was no statistical significance in age between the two groups. They also showed that there was statistical significance in smoking, type of infertility, and duration of infertility. The results showed statistical significance in the results for herpes and cytomegalovirus. There is also no statistical significance regarding the number of cases of cytomegalovirus infection according to age. On the contrary, there was statistical significance regarding age in herpes. The results also indicated that there was statistical evidence in the sperm analysis between the two groups. Conclusions; The statistical evidence between the two groups in the number of infection cases for all groups or by age indicates the role of viral infections in causing infertility in men.

Keywords: cytomegaly viruses, fertility disorders, herpes viruses-6

Introduction

Infertility is a medical condition affecting the reproductive system of either males or females, characterized by the inability to conceive a pregnancy despite performing frequent unprotected sexual

intercourse for duration of 12 months or longer (Amadi. 2020). Infertility exerts a profound influence on a substantial number of individuals, hence extending its repercussions to their familial and communal spheres. According to estimates, over 16.7% of individuals in the reproductive age group globally encounter infertility at some point in their lives (Thoma, M et al., 2021). Infertility in the male reproductive system is predominantly attributed to issues of the production of semen, insufficient or absent sperm count, as well as aberrant morphology and low motility. (Vander Borght, M., & Wyns, C. 2018). Semen analysis is a basic method to investigate the cause of infertility in the male. Six versions of a semen assessment methods guideline have been created by WHO (Mongkolchaipak, S., 2022). The volume of the substance is 1.5 mL, with a confidence interval ranging from 1.4 mL to 1.7 mL. The concentration of sperm is reported to be 15 million spermatozoa per milliliter, with a confidence interval ranging from 12 to 16 million spermatozoa per milliliter. The total quantity of sperm is reported to be 39 million spermatozoa per ejaculate, with a confidence interval ranging from 33 to 46 million (Levine H et al., 2017). Infertility can be classified into two distinct categories. Primary infertility; refers to the condition in which an individual who has never experienced a successful pregnancy encounters challenges in conceiving a child. Secondary infertility; refers to the condition wherein an individual has already experienced one or more successful pregnancies, yet encounters challenges in subsequent pregnancies (Hazlina et al., 2022).

Based on the assessed semen parameters, males infertility can be classified into the following major categories, Oligozoospermia (decreased sperm count); Asthenozoospermia (abnormal motility); Teratozoospermia (abnormal morphology); or"oligoasthenoteratozoospermia" combinations of these three conditions (Cooper et al., 2010). The results of a semen analysis test are of paramount importance as they have the potential to be linked to fertility; nevertheless, it is crucial to note that they do not provide an absolute determination of viability (Nallella., 2006). Abnormalities in sperm motility are an additional manifestation of male infertility. The successful passage of spermatozoa through the cervical mucus relies on the presence of rapid progressive motility, with a minimum velocity of 25 m/s (Barratt et al., 2010). Human herpesviruses 6A and 6B, commonly referred to as HHV-6A/B, are ancient viral pathogens of humans that were just discovered approximately three decades ago (Salahuddin SZ et al., 1986). The International Committee on Virus Taxonomy (ICTV) officially identified HHV-6A and HHV-6B as distinct viruses in 2012. Researchers cultured peripheral blood mononuclear cells from persons with lymphoproliferative disorders and AIDS. During their investigation, they found distinct, short-lived, and rather large cells that included inclusion bodies, which were located either within the nucleus or the cytoplasm. Using electron microscopy, researchers discovered a new virus and named it Human B-Lymphotropic Virus (HBLV) (Salahuddin et al., 1986). Human herpes virus 6 (HHV-6) is classified as a beta herpes virus and exhibits a significant genetic association with human cytomegalovirus (Godet et al., 2015). The reactivation of herpes simplex in the trigeminal nerve ganglia leads to the occurrence of recurring infections. Various factors such as age, exposure to sunlight or cold, trauma, physical or emotional stress, fatigue, pregnancy, immunosuppressive state, fever, respiratory illness, menstruation, systemic illness, or malignancy can contribute to the reactivation of the virus and subsequent recurrence of herpes simplex infections. The usual duration of incubation ranges from 3 to 9 days.(Stoopler ET, Greenberg MS . 2003). A characteristic juvenile exanthema, exanthema subatomic (roseola), has been related to HHV-6 as the causative agent. HHV-6 infections are most common during the first two years of life. Despite the fact that people with overt clinical disease are infrequent, HHV-6 can reactivate when the immune system is suppressed. HHV-6 has been linked to multiple sclerosis (MS) and other human diseases.(Dhanvijay M. M., Patil S. C. 2019). Human herpesvirus-5 (HHV-5), often

known as cytomegalovirus (CMV), is a member of the herpesvirus family that The Herpesviridae family, a group of DNA viruses, encompasses the cytomegalovirus (CMV) and exhibits both structural resemblances and biological characteristics such as latency and reactivation. (Whitley et al., 1998). CMV envelope glycoproteins play a vital role in virus entry and cell fusion. CMV is transmitted through direct contact with infectious bodily fluids , such as tears, saliva, nasal secretions, vaginal secretions, urine, or breast milk, can transmit CMV (Mack I et al., 2017). Human CMV infection is normally unrecognized in healthy people, but it can be lethal in people with compromised immune systems, such as HIV patients, organ transplant recipients, or infants. Because the viral genome encodes a number of proteins that impede immune response and antigen presentation (La Rosa C& Diamond DJ., 2012). CMV is distinguished by cell enlargement and intranuclear inclusions, which inspired the early use of the term "cytomegalic inclusion disease." Infected cells were initially described as "protozoon-like" or resembled owl eyes (Eggert-Kruse et al., 2009). The cytomegalovirus (CMV) persists within the host following an initial infection. Recent studies have provided evidence of its substantial role in male infertility, and the utilization of nested polymerase chain reaction (PCR) technology for early detection can facilitate the administration of efficient antiviral medicine, thereby enhancing the prospects of restoring fertility (Eggert-Kruse Wet al., 2018),

A wide range of cell types are susceptible to infection, and viral presence has been observed across various organ systems (Laib Sampaio et al., 2016). Viral replication and dissemination necessitate the inactivation of cellular and organismal defense mechanisms. HCMV tegument proteins target intrinsic, innate, and adaptive immune defense mechanisms, among others, that are antiviral in nature (Lee et al., 2016).

Methods

One hundred male specimens were gathered for the purpose of this case-control investigation. A group of patients from Al Sader Medical City/Infertility clinic/Al-Najaf Al-Ashraf and the private clinic Dr. Ali Al-Ibrahimi for Embryos and Infertility in Najaf were involved in this study starting in October 2023. After submitting the research proposal, the Medical Ethics Committee of The University Jabir Ibn Hayyan College of Medicine approved the study. The current study included a total of 100 male participants, who were separated into two groups: the Control group, which consisted of 40 healthy fertile guys aged 25 to 45 years, who had a minimum of 12 months of main and secondary fertility experience. The Patients Cases group, consisting of sixty infertile males aged 22 to 46 years, was separated into two subgroups based on the type of infertility. There were forty-six guys who were classified as main infertility. There were a total of fourteen men who experienced secondary infertility. The control group for the normal seminal analysis consists of healthy, fertile males of reproductive age who have fathered a child. The exclusion criteria encompass persons falling within the following age groups: individuals in the pre-reproductive stage of extreme youth, individuals with excessive obesity, unmarried cancer patients, and individuals with male infertility resulting from bad health. The recent semen sample was collected from the participants via masturbation in a specially designated laboratory room, which was isolated to ensure ethical and privacy standards. A sterile container with a wide opening was used for this purpose. Since each container holds participant identity information such as name, age, and time of sample collection, the participants were informed that the sample must be complete. After abstaining from sexual activity for at least 72 hours (three days), sperm was collected from the ejaculated samples of each individual involved in the study. The samples underwent liquefaction at a temperature of 37 degrees Celsius for

a duration of at least 30 minutes. After liquefaction, the physical characteristics of all samples were determined. This included measuring the sperm concentration, assessing sperm motility, evaluating sperm grading activity, and examining normal sperm morphology using light microscopy.This undertaking was completed between 10/10/ 2023 to 10/4/2024. Prior to the initiation of the semen analysis evaluation, data regarding the patients' demographic and medical history was collected. The data was inputted into a pre-configured data template. Information required: Patient's name and sample number (in years), regions of residence, educational background, occupational category, height, weight, tobacco usage, chronic medical conditions, primary and secondary infertility, surgical history, sperm count, sperm motility, and sperm morphology. The presence of HHV 6 in semen plasma can be identified using an HHV 6 ELISA kit, while the presence of CMV in semen plasma can be identified using a CMV ELISA kit.

Statistical analysis

The statistical analysis of the data was done by the Statistical Package for the Social Sciences (SPSS) version 26 (IBM, SPSS Inc, USA). For parametric variables, an independent sample t test was used, and a Mann–Whitney U test was used for nonparametric variables. The correlation between dependent variables was evaluated using Pearson's correlation coefficient analysis. The result was statistically significant when $P \leq 0.05$.

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 The University Jabir Ibn Hayyan College of Medicine approved the study, and important facilitation for the purpose of conducting the research and collecting samples.

Results and Discussion

Results

Socio-demographic and anthropometric characteristics among the study groups

Table (4.1) shows a non-significant difference in age between patients and control group (35.28 ± 5.45 vs. 33.68 ± 5.76, P=0.2) respectively, While there is a high significant difference in BMI between the two groups (27.97 ± 2.86 vs. 25.72 ± 1.37, P<0.001).

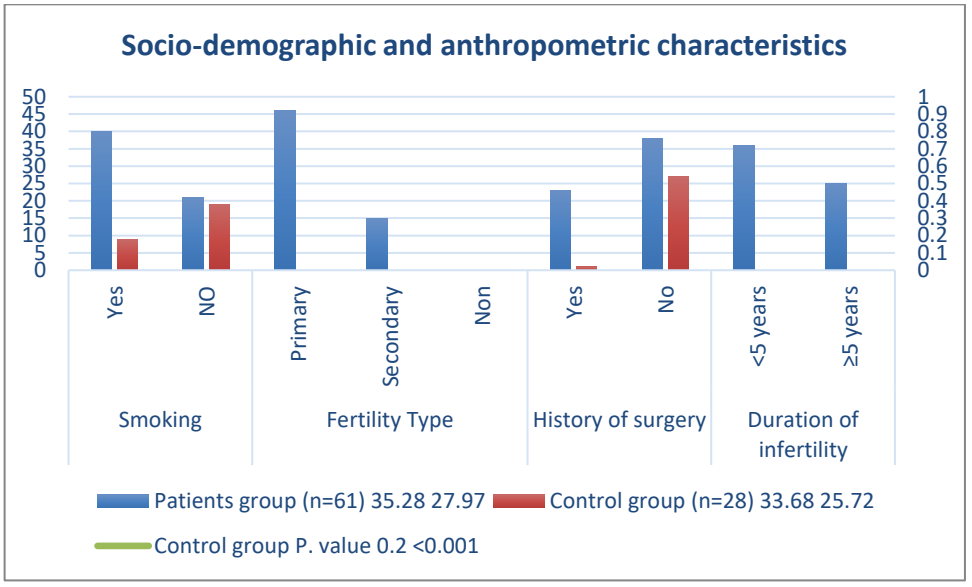
Also there is a significant difference between patients and control group in each of smoking (P<0.01), fertility type (P<0.001), history of surgery (P<0.01), and duration of infertility (P<0.001).

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

Characteristic	Patients group (n=61)	Control group (n=28)	P. value
Age (year)	35.28 ± 5.45	33.68 ± 5.76	0.20

BMI (kg/m^2)		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	

Figure 1. Socio-demographic and anthropometric characteristics among the study groups



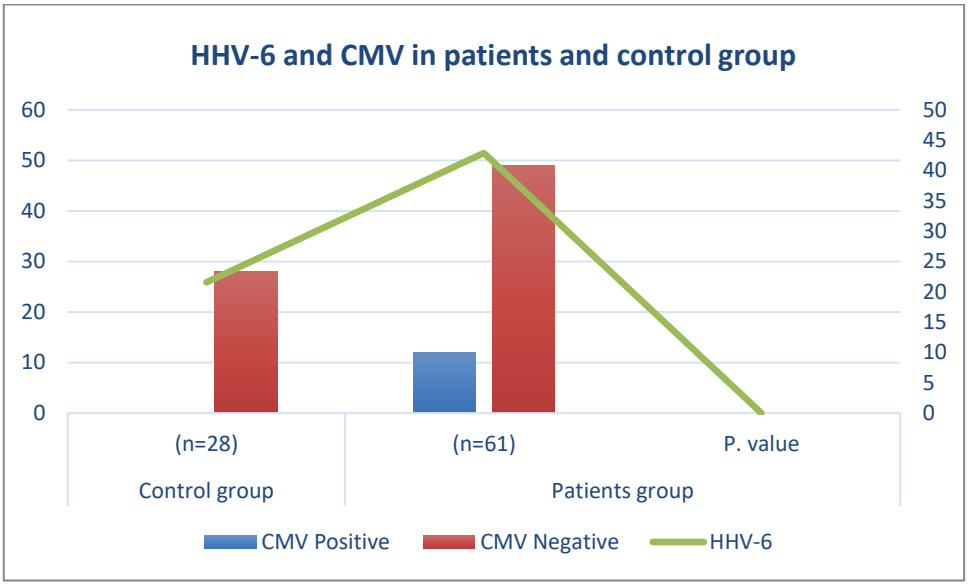
Comparison between HHV-6 in patients and control group.

When comparing levels of human herpes virus type 6 (HHV-6) between the control group and the patient group, statistically significant differences were observed. In the control group (n=28), the results of tests for the presence of cytomegalovirus (CMV) were negative in all cases, while in the patient group (n=61), the tests showed positive in 12 cases, with a P value of less than 0.05 indicating statistical significance. . The data also show a significant increase in the mean concentrations of HHV-6 in the serum of the patient group (42.89 ± 17.43) compared to the control group (21.57 ± 11.61), The P value obtained, which is less than 0.001, indicates strong evidence supporting the presence of a statistically significant difference between the two groups. This finding also supports the premise that HHV-6 infection is connected with the illness state of the group being studied.

Table 2 : Comparison between HHV-6 and CMV in patients and control group

Parameters		Control group (n=28)	Patients group (n=61)	P. value
CMV	Positive	0	12	<0.05*
	Negative	28	49	
HHV-6		21.57 ± 11.61	42.89 ± 17.43	<0.001

Fig 2: HHV-6 and CMV in patients and control group



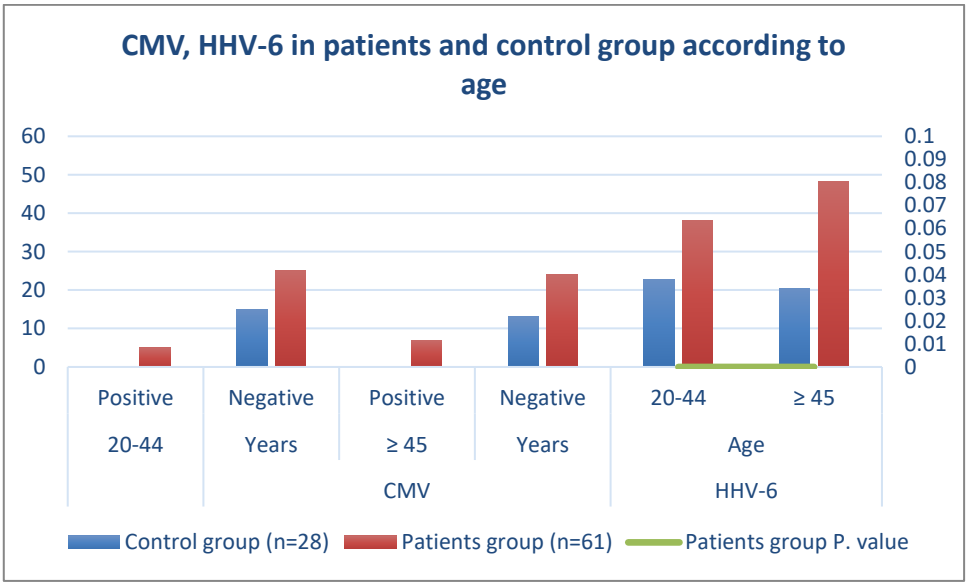
Comparison between CMV, HHV-6 in patients and control group according to age

Distinct results were obtained while analyzing the occurrence of human herpesvirus type 6 (HHV-6) and cytomegalovirus (CMV) in both the control group and the patient group, with categorization based on age. For CMV infection, in the age group from 20 to 44 years, no positive cases were recorded in the control group (0 out of 15), while there were 5 positive cases in the patient group (out of 30) with a P value of 0.09, indicating There was no statistically significant difference between the two groups. In the age group of 45 years and above, the results are similar, as no positive cases were recorded among members of the control group (0 out of 13) compared to 7 positive cases in the patient group (out of 31) with a P value of 0.08, which is a value close to statistical significance, but it is Don't reach it. Moving on to HHV-6, there was statistically significant evidence indicating increased levels of HHV-6 in the patient group compared to the control group, in both age groups, where values (22.81 ± 10.24) were recorded in the group from 20 to 44 years in the control group. And (38.23 ± 19.63) in the group of patients with P less than 0.01, and in the age group 45 years and above, the values were (20.37 ± 12.85) for the control group versus (48.27 ± 13.24) for the group of patients with P less than 0.001, which confirms the existence of differences. Significant between the two groups in both age groups in terms of HHV-6 levels.

Table 3 : Comparison between CMV, HHV-6 in patients and control group according to age

Parameters			Control group (n=28)	Patients group (n=61)	P. value
CMV	20-44 Years	Positive	0	5	0.09
		Negative	15	25	
	≥ 45 Years	Positive	0	7	0.08
		Negative	13	24	
HHV-6	Age	20-44	22.81 ± 10.24	38.23 ± 19.63	<0.01
		≥ 45	20.37 ± 12.85	48.27 ± 13.24	<0.001

Fig 3: CMV, HHV-6 in patients and control group according to age



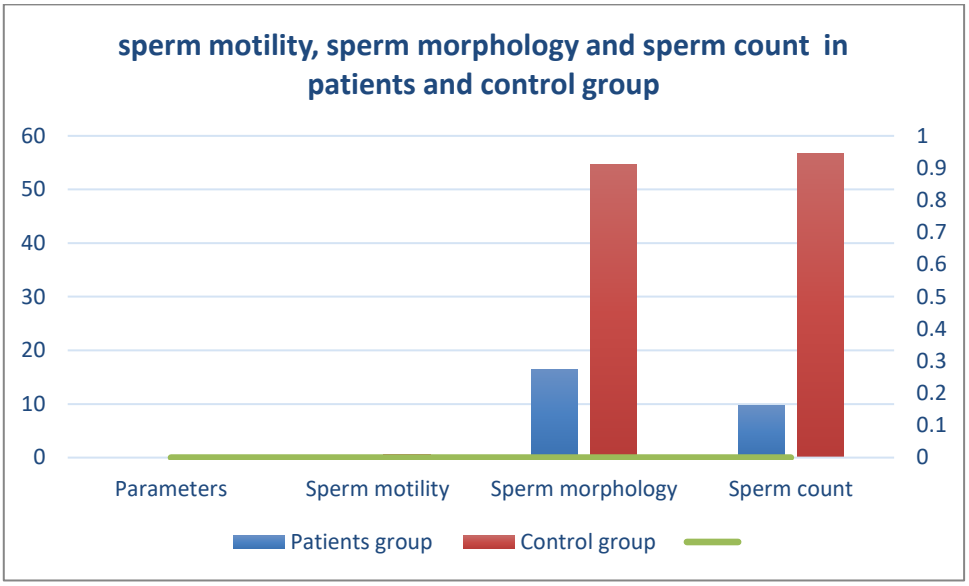
Comparison between sperm motility, sperm morphology and sperm count in patients and control group.

In a comparative study between a group of patients that included 61 people and a control group that included 28 people, the results were as follows: A decrease in sperm motility was observed for the patient group, as the average reached 0.068 with a standard deviation of 0.03 compared to 0.55 with a standard deviation of 0.08 for the control group, and this difference was statistically significant. The P value was less than 0.001. For sperm formation, the mean in the patient group was 16.47 with a standard deviation of 6.21 compared to the mean of 54.64 with a standard deviation of 6.51 in the control group, and these results were also statistically significant (P<0.001). As for sperm count, the average in the patient group was 9.77, with a standard deviation of 3.36, compared to 56.71, with a standard deviation of 8.37, for the control group, with a P value of less than 0.001, which indicates a significant statistical difference between the two groups.

Table 4: 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 4: sperm motility, sperm morphology and sperm count in patients and control group



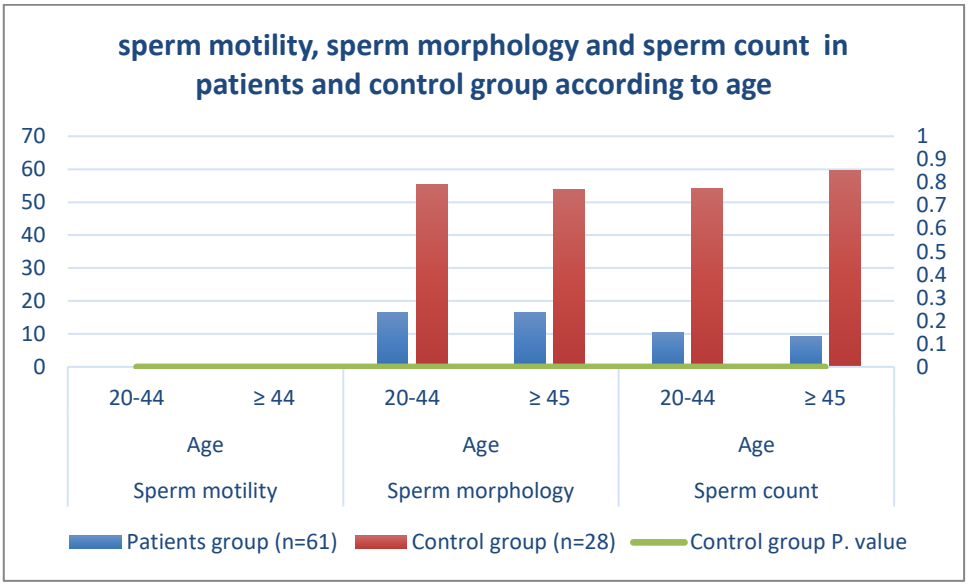
Comparison between sperm motility, sperm morphology and sperm count in patients and control group according to age

The comparative results of semen quality indicators, represented by motility, shape, and number in the patient group consisting of 61 people and the control group consisting of 28 people according to age group, show significant statistical differences. Regarding sperm motility, men in the age group from 20 to 44 years in the control group showed an average of (0.576 ± 0.084), while the patient group recorded (0.066 ± 0.030), and for the age group 44 years and older an average of (0.534 ± 0.077) versus (0.070 ± 0.032) for the patient group, with P values less than 0.001 for both groups, indicating a significant decrease in sperm motility in patients regardless of age. Regarding the normal shape of sperm, the results in the control group were much higher at (55.33 ± 6.39) for the age group 20-44 years, and (53.84 ± 6.81) for the age group 45 years and above, while the patient group recorded (16.50 ± 6.31) and (16.45 ± 6.21) respectively, with P values less than 0.001. Finally, when looking at the total sperm count, there was a significant decrease in the patient group in both age groups, as they averaged (10.43 ± 3.74) and (9.13 ± 2.86) versus (54.20 ± 7.85) and (59.62 ± 8.28) in the group. Control for each age group respectively, with a P value of less than 0.001, indicating a significant effect of the medical condition on sperm count at all ages.

Table 5: 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 5: sperm motility, Analysis of sperm morphology and sperm count in both patients and control group is conducted based on age.



Discussion

Infertility is the condition where couples are unable to achieve pregnancy, despite engaging in regular and unprotected sexual intercourse for a year or longer. It is a prevalent condition, affecting approximately 15% of couples. Male infertility is a prevalent condition that exerts a significant psychological toll on individuals, particularly within our eastern society. The causes of this condition are multifactorial, encompassing physiological factors, as well as microbial agents, particularly bacteria and viruses (Brugh and Lipshultz., 2004; Leslie et al., 2020). The study cohort's sociodemographic characteristics are outlined in Table 1. The research reveals substantial disparities in BMI, smoking status, fertility type, surgery history, and duration of infertility between the patients who were enrolled and the control group. However, the age disparity between the two groups is insignificant. These findings are consistent with the outcomes of other prior investigations. 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 a high body mass index (BMI) may elevate the likelihood of male infertility. A meta-analysis conducted by Sharma et al. (2016) examined the relationship between smoking and semen quality. The study provided compelling evidence that smoking has a detrimental impact on sperm quality, hence increasing the risk of male infertility (Eisenberg et al., 2014; Sharma et al., 2016). The present investigation demonstrated a substantial elevation in HHV-6 levels among patients in comparison to healthy individuals. These results are consistent with what was presented previously (Jones et al., 2018; Brown et al., 2021). Viral infections have a direct or indirect effect on infertility in men and occur through several different mechanisms (Eggert-Kruse et al., 2009; (Naumenko et al., 2011). The human herpes virus is also transmitted through the fetus, and it can directly affect the activity of the sexual organs in the human body, or it can lead to infections that cause infertility (Kaspersen & Höllsberg, 2013; Pagano, 2007). As for the CMV virus, there was a difference in many researches about its effect on infertility in men (Habibi et al., 2014; Klimova et al., 2010). The results indicated a relationship between infection with the virus and infertility, but it is recommended that more research be conducted to determine the pathophysiology of the virus (Bezold et al., 2007; Mohseni et al., 2018). These studies should also compare the semen parameters before and after therapy. Male infertility is a complex and difficult issue that is a significant concern and clinical challenge worldwide. Around 8-12 percent of couples globally are affected. Males contribute to over half of the infertility instances. Around 2% of these cases exhibit sperm with atypical characteristics, such as reduced sperm count, changes in semen acidity, impaired sperm movement, irregular shape, reduced viability, or a combination of these factors. On uncommon occasions, individuals may experience both erectile dysfunction and complete absence of sperm production (Kumar & Singh, 2015). Furthermore, it is important to mention that the incidence of infertility is significantly higher in underdeveloped nations. A considerable proportion of these incidents can probably be attributed to infectious diseases. Additionally, male infertility is often caused by acute or chronic infections that disrupt the seminal tract, making up the majority of such instances. In addition, it was noted that a percentage of the persons who were unable to conceive had no symptoms of illness, and around 50% of cases of infertility have an unknown cause. (Naumenko et al., 2014). The present investigation demonstrated a statistically significant disparity in sperm motility, sperm morphology, and sperm count, as indicated in table (5). The study cohort's sociodemographic characteristics are shown in Table 1. The research found notable disparities between the patients who were enrolled and the control group in terms of BMI, smoking status, fertility type, surgery history, and length of infertility. However, the age difference between the two groups is insignificant. The conclusions derived from these research are consistent with those acquired from prior investigations. Concerning sperm count, An average male normally possesses a sperm count of 15 million sperm per milliliter or above, indicating good health. Oligozoospermia is diagnosed when a semen analysis shows a consistently low sperm count in two separate tests. Historically, sperm concentrations that fell below 20 million sperm per milliliter were considered to be poor or oligospermic. Recently, the World Health Organization (WHO) has reassessed the standards for sperm and established a new benchmark of less than 15 million sperm/ml, which corresponds to the fifth percentile for males who are able to conceive. The concentration of sperm might fluctuate daily, and oligozoospermia can occur either temporarily or permanently (Maurya et al., 2022). Oligospermia, oligozoospermia, or low sperm count are terms used to describe semen with a low concentration of sperm. This is a common phenomenon in male infertility. Oligoasthenoteratozoospermia, a technical word, refers to the

condition where sperm has a decreased concentration and often shows significant abnormalities in both shape and movement. There has been a significant desire to replace the descriptive phrases used in semen analysis with more numerical information (Maurya et al., 2022). The human breast cancer susceptibility gene 2 (BRCA2) employs homologous recombination to mend DNA damage that arises during meiosis. There is a strong association between severe oligospermia and a common genetic variation known as a single-nucleotide polymorphism of the BRCA2 gene. A study was conducted to investigate the relationship between lifestyle factors and sperm DNA damage in men with a moderate decrease in sperm count (15–20 million sperm/ml sperm concentration). A clear association was found between sperm DNA damage and factors such as age, obesity, and occupational stress (Huang et al., 2023).

Conclusion

Our hypothesis is that male sterility may be caused by chronic IUTDs associated with CMV and HHV-6. This is based on the high concentrations of these viruses found in sperm, their prevalence among infertile men or those with chronic IUTDs, and the significant negative effects they have on the motility, sperm count, and morphology of infected patient samples.

Ethical approval:

Prior to the sample collection process, all patients participating in this study were duly informed and provided verbal consent. The study received approval from the Committee on Publication Ethics at the Thi-Qar Health Directorate, Al Habbobi Teaching Hospital-spasi-

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