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Study the Correlations Between HSV Viral Load and Semen Parameter in Infertile and Fertile Iraqi Male

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ABSTRACT

Background & Objective: The viruses are considered as one of the most prevalent factors that cause infertility because they represent a significant role in sexual transmission disease. The purpose of this study was to evaluate the correlations between viruses (HSV) viral load and semen parameters in groups of fertile and infertile Iraqi men.

Materials & Methods: This study comprised fifty infertile males and fifty fertile healthy males. Seminal fluid analysis was conducted on all participants to detect the presence of HSV using the HSV1, 2 quantification kits.

Results: There was a high significant correlation between sperm concentration and percentage of rapid linear progression motility (r= 0.478**) of infertile men. A strong meaningful connection was seen between the percentage of sperm concentration and both the percentage of sluggish linear or nonlinear motility (r = 0.681**) and the percentage of non-progressive motility (r = 0.604**). There was a strong negative connection (r = -0.725**) between the concentration of sperm and the number of immotile spermatozoa. On the others hand, there was a high significant correlation between rapid linear progression and slow linear or nonlinear (r =0 .413**) non-progressive motility (r = 0.704**) respectively, and high negative significant between rapid linear progression and immotile spermatozoa. The correlation between slow linear or nonlinear and non-progressive motility was highly significant (r= 0.555**) and negative significant between slow linear or nonlinear and immotile spermatozoa. Finally, A strong negative correlation (-0.813**) was observed between non-progressive motility and immotile spermatozoa.

Conclusion: The study found that HSV infection had an effect on semen parameters and will cause male infertility.

Keywords: Herpes Simplex Virus (HSV), Male Infertility, Motility, Semen Parameters, Immotile (IM)

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Introduction

Male infertility is characterized by the incapacity of a male to impregnate a fertile female, even after engaging in unprotected sexual intercourse for a minimum duration of one year **(1, 2)**. The man has exclusive responsibility for approximately 20% of infertility cases. In general, the male component significantly contributes to approximately 50% of all infertility cases. Given the frequent coexistence of male and female factors, it is crucial to conduct investigations for infertility in both partners and manage their conditions collectively **(3, 4)**.

Male infertility sometimes arises from inadequacies in the semen, with semen quality serving as an indicator of male fertility. Males whose sperm parameters fall below the normal values established by the World Health Organization (WHO) are classified as having male factor infertility **(5-7)**. The most notable among these are reduced sperm count (oligospermia), impaired sperm movement (asthenospermia), and atypical sperm shape (teratospermia). Additional characteristics that have a weaker correlation with infertility include the volume of semen and various indicators of epididymal, prostatic, and seminal vesicle function **(8)**.

Up to 90% of cases of male infertility are attributed to low sperm count, and there is a clear correlation between aberrant semen characteristics and sperm count. The issue of sperm count, motility, and morphology arises from a disruption in the regulatory mechanisms, encompassing elements before, during, and after the testes **(9)**.

Male infertility can arise from acquired, congenital, or occasionally idiopathic factors. These factors have a detrimental impact on the process of spermatogenesis and pose significant risks to male reproductive organs, ultimately leading to infertility.

Several factors contribute to this condition, such as smoking, alcohol use, drug usage, obesity, previous or current testicular infections, exposure to environmental contaminants, excessive heat exposure to the testicles, hormonal imbalances, testicular injuries, and ejaculatory/erectile problems, among others. Male infertility is often associated with viral infections. Specifically, the Herpesviridae family includes viruses including herpes simplex virus (HSV), cytomegalovirus, and human herpes virus **(10, 11)**.

Human herpes viruses (HHVs) are prevalent sexually transmitted viruses that might potentially impact male fertility. This can occur either through direct viral toxicity on cells in the genital tract or indirectly through local or systemic infectious or immunological reactions. Sexual transmission is possible for both HSV-1 and HSV-2. HSV, particularly HSV-2, has been identified in semen and has the potential to be absorbed into sperm cells. HSV invades the penile mucosa and initiates a persistent infection. The virus undergoes replication within the keratinocytes and subsequently disseminates to the nerve cells, where it enters a state of latency. Upon reactivation, the herpes simplex virus returns to the epithelial surface, causing cell death and vascular lesions. While the seminiferous tubules appear to be unaffected thanks to the safeguard provided by the blood-testicular barrier, HSV-2 infects nearly all tissues and organs of the male genital tract. The virus's capacity to invade nearly all organs of the male reproductive system suggests that it is likely to directly harm the spermatozoa and modify the quality of the sperm (such as by decreasing sperm count, motility, and the presence of sperm cells with normal morphology) **(12)**.

Methods

Study design and patient selection

The patient cohort comprises infertile males with suboptimal seminal fluid quality and no kids. They were picked from Al- Harthia International Lab and Kamal Al- Samara'ay IVF Hospital -Baghdad, Iraq. Samples included in this study were fertile and infertile men with different ages and from different areas of Iraq. Of the selected 100 men (50 patient and 50 healthy fertile men), the personal information for each patient and control was collected by a questionnaire administered by the consultant, following the necessary approval for sampling the patients and controls.

Collection of samples

Human semen samples were taken immediately after ejaculation by masturbation from men and directly placed into a clean, dry, and sterile disposable plastic Petri dish in a dedicated room specifically designed for this purpose. All participants had abstained from sexual activity for a period of 3-5 days, the collected samples were promptly transferred for semen testing.

Semen analysis

Macroscopic examination for seminal fluid analysis (SFA)

The seminal fluid analysis was carried out for the semen samples of patient and control groups according to the following procedure Appearance, volume, color, PH, viscosity, liquefaction time).

Microscopic Examination

The microscopic examination included the following parameters: Sperm Concentration, Sperm Motility (Progressive motility (PR), Non-progressive motility (NP), Immotile (IM)), Sperm Morphology.

HSV1-2 Quantification

Following ejaculation by masturbation, human semen samples were instantly transmitted for semen testing within a time frame of 30-60 minutes. The process of converting each semen sample from a solid or gel-like state to a liquid one was accomplished by following the specific methods described in the WHO recommendations published in 1999 and 2010. The semen was extensively analyzed after complete liquefaction, using both macroscopic and microscopic investigations according to the WHO 1999 and 2010 standards protocols. The Bosphore HSV 1-2 Genotyping Kit v1 utilizes multiplex PCR to simultaneously amplify HSV1 DNA and HSV2 DNA in a single reaction, employing primers and probes that are specific to each sequence. The fluorescence emitted by the HSV1 amplification is detected using a probe labeled with FAM at its 3' end, specifically in the FAM channel. The fluorescence emitted by the HSV2 amplification is identified using a second probe called JOE. Programming the Qiagen Rotor gene Q Real-Time PCR Instrument The thermal methodology for the Bosphore HSV 1-2 quantification Kit v1 consists of an initial denaturation to activate the HotStarTaq DNA Polymerase, followed by a two-step amplification cycle, and finally a terminal hold. Data is captured in real-time throughout the second stage of the amplification cycle.

Statistical analysis

Data were statistically analyzed by utilizing SPSS for Windows, version 26 (SPSS Inc. Chicago, IL, United States). Data were appeared as mean ± standard deviation. The association degrees between variables were analyzed by Pearson correlation analysis. A two-tailed p value less than 0.05 ($p<0.01$) was considered significant.

Results

The description of seminal fluid macroscopically analysis between infertile and fertile men:

The results of semen examination are crucial in diagnosing male factor infertility, monitoring the effects of medical and surgical interventions to improve male fertility, and guiding the selection of treatment in assisted reproduction. Semen analysis is an essential tool for assessing male fertility potential and understanding the health of the male genital tract.

The macroscopic evaluation of seminal fluid properties, including volume, color, viscosity, pH, and liquefaction time, was conducted and the results are presented in Table 1. The semen samples obtained from infertile men exhibited a whitish-grey hue. The majority of the samples displayed a moderate level of viscosity. The volume of the samples ranged from 1 to 3 milliliters. The average pH level was 8, and the time required for liquefaction varied between 30 and 60 minutes.

Conversely, the macroscopic analysis of seminal fluid samples from fertile males revealed a whitish-grey hue, typical appearance, volume ranging from 2 to 6 milliliters, an average pH of 8, liquefaction time between 15 to 30 minutes, and all other seminal fluid characteristics were within the usual range.

Table 1. Seminal fluid macroscopic analysis

Sperm Parameters	Infertile men (n=50)	Fertile men (n=50)
Volume ml (rang)	1-3	2-6
Color	Whitish -grey	Whitish-grey
PH (mean ± SD)	7.9 ± 0.07	8.05 ± 0.15
Viscosity	moderate	normal
liquefaction time (min)	30-60	15-30

The parameter values of seminal fluid microscopical analysis between infertile and fertile men

The majority of male infertility diagnoses are based on a descriptive study of human sperm, which includes the number of spermatozoa per ejaculate, sperm motility (rapid linear progression slow linear or non-linear, non-progressive motility, and immotile spermatozoa. Table 2 displays various sperm parameters from fertile men and infertile patients. This Table 2 shows that the microscopic semen parameters revealed a highly significant difference between the infertile and healthy control groups **(13)**.

The mean sperm concentration (m/ml) in the fertile control group (87.7 ± 42.9) was statistically significantly ($p<0.00004$) higher than that of infertile men groups (40.90 ± 43.76).

The rapid linear progression (%) and slow linear or non-linear (%) of the fertile men (14.7 ± 13.05, 25.7 ± 7.36) was significantly higher compared to infertile men groups (3.90 ± 5.69, 12.08 ± 13.53), as shown in Table 2.

On the other hand, when comparing the percentage of non-progressive motility, it has been found that the percentage for the fertile men was (26 ± 16.69), while it's for infertile men groups were (17.40 ± 15.69).

The mean of Immotile sperm (48.6 ± 7.12) in fertile men was significantly different compared with infertile groups (66.62 ± 32.42).

The last parameters in this Table 2 were the percentage of IgA & IgG that were investigated in the seminal fluid of infertile and fertile male groups, the percentage of IgA for the infertile male group was (6.25±2.21) while for the fertile group was (5.39±2.47), on the other hand the percentage of IgG for infertile male group was (5.63±2.68) while for fertile men group was (6.15±2.19).

Data were expressed as counts with percentages in mean ± SD; ‡Statistical analyses were performed by ANOVA, Statistical analyses performed by the Chi-squared test; NS, no significant difference; Significant values are bolded.

Detection HSV in semen sample

The statistical analysis of the results indicates that HSV significantly contributes to male infertility by exerting a passive influence on semen characteristics. It was discovered that HSV had a notable impact on both the quantity of sperm and the ability of the sperm to move (progressively, non-progressively, or not at all). The investigation revealed that 13 out of 100 samples tested positive for HSV, accounting for 13.00% of the total. Conversely, 37 out of 100 samples tested negative for HSV, representing 37.00% of the total in Table 3.

Table 2. **The microscopic semen parameters of the infertile and healthy fertile control groups.**

Parameters	Infertile male± SD	Fertile male ± SD	P value
sperm concentration (million/ml)	40.90 ± 43.76	87.7 ± 42.9	0.00004**
rapid linear progression (grade A)%	3.90 ± 5.69	14.7 ± 13.05	0.01*
slow linear or non-linear (grade B) %	12.08 ± 13.53	25.7 ± 7.36	0.026*
non-progressive motility (grade C) %	17.40 ± 15.69	26 ± 16.69	0.19
immotile spermatozoa (grade D) %	66.62 ± 32.42	48.6 ± 7.12	0.09
IgG %	5.63±2.68	6.15±2.19	0.87
IgA %	6.25±2.21	5.39±2.47	0.80
Dunnett T-tests		infertile compared with fertile groups	

***(P≤0.05) is significant.**

Table 3. **Distribution of HSV in samples.**

HSV	Total No.	Patients (No = 50)		Control (No = 50)	
		No	%	No	%
Positive	13	13	26	0	0
Negative	87	37	74	50	50

Pearson correlation analysis of infertile and fertile male group

To investigate the correlation between some important factors included in this study, correlation coefficient analysis for healthy fertile men was done, and the results are shown in Table 4. There was a highly significant correlation between sperm concentration and the percentage of rapid linear progression motility (r=0.478**) and (P=0.0001) of infertile men. There was a strong significant correlation between the percentage of sperm concentration with the percentage of slow linear or nonlinear (r =0.681**), (P≤0.0001) and with the percentage of non-progressive motility (r =0.604**) and (P≤0.0001) finally, there was a significant negative correlation between sperm concentration and immotile spermatozoa (r = -0.725**) and (P≤0.0001).

On the other hand, there was a highly significant correlation between rapid linear progression and slow linear or nonlinear (r = 0.413**) non-progressive motility (r=0.704**) and (P≤0.0001), respectively. The high negative significance between rapid linear progression and immotile spermatozoa. The correlation between slow linear or nonlinear and non-progressive motility was highly significant (r=0.555**), (P≤0.0001) and negative significant between slow linear or nonlinear and immotile spermatozoa.

Finally, there was a significant negative correlation between non-progressive motility and immotile spermatozoa (r= -0.813**) and (P≤0.0001).

Table 4. correlations between viral load and semen parameter in infertile and fertile male

		Viral loud	sperm concentration (million/ml)	rapid linear progression (grade A) %	slow linear or non-linear (grade B) %	non-progressive motility (grade C) %	immotile spermatozoa (grade D)%
Viral loud	**Pearson Correlation**	1	-.049	.110	.020	-.058	.013
	Sig. (2-tailed)		.875	.721	.950	.850	.966
sperm concentration(million/ml)	**Pearson Correlation**		1	.478**	.681**	.604**	-.725**
	Sig. (2-tailed)			.000	.000	.000	.000
rapid linear progression (grade A) %	**Pearson Correlation**			1	.413**	.704**	-.547**
	Sig. (2-tailed)				.000	.000	.000
slow linear or nonlinear (grade B%	**Pearson Correlation**				1	.555**	-.906**
	Sig. (2-tailed)					.000	.000
non-progressive motility (grade C) %	**Pearson Correlation**					1	-.813**
	Sig. (2-tailed)						.000
immotile spermatozoa grade D%	**Pearson Correlation**						1
	Sig. (2-tailed)						

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

Under normal conditions, an ejaculate will typically become fully liquid after 30 minutes at a temperature of 37°C. During ejaculation, the initial portions of the ejaculate, which include a high concentration of sperm, are primarily composed of acidic prostatic fluid. Subsequently, the latter portions of the ejaculate have fewer sperm and are primarily composed of alkaline seminal vesicular fluid. The subsequent portions comprise the gelatinous texture of the ejaculate within the specimen container in the laboratory. The gel is subsequently converted into a liquid state mostly by the action of prostatic enzymes. Non-pathological variation in male sexual function can be attributed to factors such as the length and intensity of sexual excitation, as well as the frequency of ejaculation (including the time of abstinence between ejaculations) **(14)**.

The most essential indicator of sperm quality is its progressive motility. The proportion of sperm that has forwarded progression is one of the most critical criteria impacting both natural and assisted pregnancy **(15)**.

This result agrees with **(16)** who demonstrated during their study the variation of parameters which conceded necessary to diagnose infertility problems such as sperm motility and sperm concentrations.

These findings were corroborated by other researchers. Significant disparities in microscopic semen parameters were identified when comparing healthy reproductive men to the infertile group. The factors contributing to the likely decrease in the quality of male reproductive function have not yet been determined. There is a suggestion that the rise in male reproductive abnormalities, namely reduced sperm motility, could be attributed to environmental or lifestyle factors such as exposure to environmental and occupational hazards, medication use, and sexually transmitted diseases. Excessive levels of oxidative stress (OS) caused by an overabundance of reactive oxygen species are also significant factors contributing to reduced sperm motility by inducing defects in sperm chromatin. Human spermatozoa travel with little or no motility from the testis through the epididymis. As they migrate through the epididymis, the proportion of motile sperm increases progressively. Therefore, the previous conditions had an adverse influence not just on sperm motility. However, the same factors impact sperm concentration and shape of infertile males **(17)**.

Antisperm antibodies, when present on the tail of sperm, can lead to immobilization or clumping of the sperm. Similarly, the presence of antisperm antibody on the head of sperm can hinder its ability to navigate through a woman's cervical mucus towards the ovum,

thereby affecting the spermatozoa's fertilization capability **(18)**.

The majority of males with anti-sperm antibodies had both IgG and IgA on their spermatozoa. However, it has been proposed that IgA linked to the surface of sperm cells is more significant than IgG in hindering penetration. Multiple lines of evidence suggest that ASA may impede the recognition of sperm binding sites on the zona pellucida of the oocyte, thereby diminishing the fertilization process. Disruption of the immune balance might lead to "immunological infertility" **(19)**.

Sperm parameters are crucial in determining the cause of infertility from a physiological standpoint. The sperm parameters have a direct correlation with the fertilization process. Fertility is adversely impacted when these characteristics decrease below a predicted threshold. There is a clear inverse correlation between sperm concentration and both the percentage of progressive motility and the presence of non-progressive and immotile sperm **(20, 21)**. The sperm concentration, active sperm motility, and sperm morphology are all indicative of the normal fertilization capacity in healthy men **(22)**.

The presence of herpes simplex virus type 1 (HSV-1) and herpes type 2 (HSV-2) has been extensively observed in human semen, with varied occurrence rates. Bai, et al. **(23)** reported a prevalence of HSV-1 and HSV-2 in 2-50% of men with infertility. The origins of HSV seminal DNA are still unclear, however, it is shown that HSV-2 can be absorbed into viable, mobile sperm and is likely to directly harm male germ cells. HSV infections are linked to atypical sperm characteristics and male infertility, which ultimately lead to immunological reactions. An evident correlation has been documented between HSV infection and abnormalities in seminal parameters, such as hematospermia, oligospermia, and elevated apoptotic cells.

Men infected with HSV-2 have been found to experience a decrease in the amount of semen produced and an atypical thickness, suggesting dysfunction in the prostate gland. Kurscheidt, et al. **(24)** found that men infected with HSV had significantly decreased sperm concentration and motility, as well as lower levels of citrate and neutral α-glucosidase. These findings indicate impaired function of the epididymis and prostate. Multiple studies have examined the influence of HSV infection on male fertility, although the findings remain contentious. The virus has unequivocally been identified in the seminal fluid of men with infertility. HSV-2 showed little affinity for sperm in the ejaculate during in vitro tests, perhaps due to the presence of seminal fluid. Studies conducted on transgenic mice indicate that HSV is linked to many abnormalities in sperm structure, including as acrosomal aberrations, abnormalities in the neck and flagella, and developmental stoppage. Research conducted on transgenic rats, which have the HSV-1 thymidine kinase gene expressed specifically in their sperm cells, has revealed the following effects: degeneration of cells involved in sperm production, disruption of the link between Sertoli cells and germ cells, and programmed cell death of germ cells **(25)**.

Conclusion

HSV significantly impacts male infertility by passively influencing semen characteristics. It was discovered that HSV had a notable impact on both sperm count and sperm motility, including progressive, non-progressive, and immotile sperm.

Acknowledgments

Not applicate.

Conflict of Interest

No conflict of Interest.

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