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Male Infertility and Viral Infection: Interference Role of the Human Herpesvirus types (3 – 6) with Disturbances Effects of Some Cytokines Hypersecretion and Seminal Oxidative Defense System in the Infertility Etiopathogenesis of Some Idiopathic Infertile Iraqi Patients

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To study the role of potential relationship of chronic human herpes virus types (3 – 6) infection and their correlation with the up-regulation of some cytokines (IL-2, IL-10 and IFN gamma) and effect of (8-OHdG) and (TAOC) levels onto male infertility. A Case – control study with semen samples which were collected by masturbation during the routine semen analysis of a total ninety age-matched participants as; fertile males 35 (38.9%) with proven fertility as a normal semen quality and infertile males 55 (61.1%) with at least one year of infertility and poor semen quality. All semen biomarkers of Human herpes viruses (HHVs): Varicella zoster virus (VZV-IgG), Epstein-Barr virus (EBV-IgG), Cytomegalovirus (CMV-IgG), Human herpes virus type 6 (HHV6-IgG), Interlukin-2 (IL-2), Interlukin-10 (IL-10), Interferon-gamma (IFN-ā), 8-hydroxy-2'-deoxy guanosine (8-OHdG) andTotal Antioxidant Capacity (TAOC) which were included in the study had been estimated by quantitative ELISA based method and the correlations with sperms parameters were evaluated. The main significant outcomes in this study of the infertile males group 55 (61.1%) were: high percentages of seminal IgG;N (%; Mean±SD) were detected of HHV6, 48 (53.3%;1.26± 0.51) then CMV, 39 (43.3%;1.51± 0.95) followed by EBV, 34 (37.8%; 2.20±1.47), and the highest abnormal cytokines levels were estimated in; 44 (48.9%; 112.62±38.64) and 40 (44.4%; 22.75±10.65) for IFN-ā and IL-10 respectively. Furthermore, High 8-OHdG level was detected in 47 (52.2%;7.29±2.15) and very low level of TAOC was detected in 16 (17.8%;19.34±12.17).Significant negative correlation between semen biomarkers and standard sperms parameters was found which were represented by: 45 (50.0%) of total sperms count less than (33 million \ ml), 52 (57.8%) of progressive motile sperms less than (31%). Finally, about 33 (24.4%) of abnormal sperm morphology was detected. Our results hypothesized that chronic asymptomatic viral infection with increasing of cytokines concentrations consequently disturbance the semen oxidative status, antioxidant defense systems that induce sperms DNA damage then might be collectively act as a co-factors on the etiology of the male infertility.

Key words: Antioxidant status, Cytokines, Human herpes virus, Infertility, Oxidative stress.

Male infertility is a multifactorial challenging with a big concern and a significant clinical problems worldwide, which affects about (8–12%) of partners globally. “Male factor” take about a fifty percent of all infertility cases, which exhibit abnormal sperm parameters in about

two percent of them which represented by; low sperm concentration, changing of semen pH, poor progressive sperms motility, abnormal morphology, defect of vitality or a combination of them or even failure to produce sperms and erectile dysfunction. Even more the average of infertility cases in less developed countries are higher and it noticed that infectious diseases were took a huge percentage of these cases[1]. Additionally, most of acute or chronic infections in the seminal tract are among the most communal reasons of male infertility. Besides, some of the infertile cases had been with asymptomatic infection and about a fifty percent of infertility cases are idiopathic[2].

Human herpes viruses (HHVs) are a common sexually transmissible viruses with possible effect on male infertility factor either directly by the viral toxic effect onto genital tract cells or indirectly by local or systemic infectious or immunological responses. So that, (EBV), (CMV) and (HHV-6) of *herpesviradae* family are commonly found in semen, but the influence of these viral agents on male fertility has not been broadly explored. Several studies have estimated the CMV prevalence in about (0 - 62.5%) of human semen samples in a many countries[3]. Another study determined the incidence of viral DNA was 3.4% for EBV, 5.2% for CMV, 6.5% for HHV-6 with different values for viral co-infection among them[2]. Few studies had examined the existence of VZV in semen samples of infertile men, so it had been indicated that the male genital tract may act as a reservoir for variable infectious agents[4]. Despite of these significant outcomes still the main role of HHVs in male infertility unexplained clearly.

Furthermore, cytokines are regulatory proteins involved in multiple immune responses with controversial effects onto testicular cells functions which are physiologically produced up there. And, there is compelling evidence that most of cytokines, play a significant regulatory role in the testis development and normal function of the testicular cells. Pro-inflammatory cytokines such as IL-2 and IFN-ã have direct effects on differentiation of spermatogenic cell, testicular steroidogenesis and spermatogenesis while anti-inflammatory cytokines such as IL-10 also associated with testicular development. Thus, dysregulation of cytokine expression during infection or illness may participate into the disruption of testicular function and effect on male fertility

Moreover, oxidative stress is a significant factor and effects on the rates of male fertility. High seminal Reactive Oxygen Species (ROS) concentration in semen can effect on the antioxidant defense system of total antioxidant capacity (TAOC) in seminal plasma and spermatozoa to produce oxidative stress and may influences on sperm function. Many studies proposed that ROS interfere with DNA sperms integrity, therefore, sperm DNA damage is closely associated with male infertility as 8-hydroxy-22 -deoxyguanosine (8-OHdG) which was considered a sensitive indicator of oxidative spermsDNA damage caused by ROS in human semen samples[7-9].

Therefore, the aims of this study were to provide a knowledge about the interferences of some human herpes viruses (HHVs) as chronic infection or with reactivation cycle and up-regulation of some cytokines expression in accordance with uncontrolled oxidative stress which maybe collectively act as a co-factors in the causes of infertility in a randomized asymptomatic infertile males.

MATERIAL AND METHODS

Participants and sampling

Seminal fluid samples were collected from ninety volunteers who were attending Kamal El-Samurai Hospital (Baghdad) from November 2018 to February 2019, (aged 23 – 44): consisting of (n=35; 38.9%) healthy controls who were experienced the fatherhood and (n=55; 61.1%) infertile patientswith primary infertility (had no baby after one year of unprotected intercourse; idiopathic infertility) as a case-control study. Participants were instructed on how to collect the sample and deliver to the laboratory within one hour of production and they were sexually abstinent for 2-7 days before the semen collection by masturbation in the private room of the laboratory using clean wide-mouthed glass and plastic containers, then kept in 37C temperature. Each sample was divided into two parts; one for biomarkers estimations and the other one for semen routine analysis.

Semen examination

Based on the requirements of World Health Organization (WHO) 2010 manual regarding to the examination and processing of human semen some of basic sperms parameters were estimated after a complete liquefaction which had been included; analysis of total sperms number (million \ ml), percentages of progressive motility, finally making a dried-fixed semen smears stained with modified Papanicolaou stain to evaluated the sperms morphology percentages of normal and abnormal (head, neck and tail) sperms microscopically. The study protocols approved by the local Ethics Committee which granted permission for each patients' participation. The classification of sperms parameters abnormalities in the participants were classified into: -

*A = Normozoospermia: 35 (38.9%)
*B = Asthenoteratozoospermia: 12 (13.3%)
*C = Oligoasthenozoospermia: 23 (25.6%)
*D = Oligoasthenoteratozoospermia: 20 (22.2%)

Biomarkers Estimation

All seminal fluid samples were centrifuged (approximately 20 minutes at 1000×g (or 3000 rpm) within 30 minutes after collection from completely liquefied ejaculates and semen plasma was isolated to be ready for assay immediately or store samples at -20 C.

Kits of commercial enzyme linked immunosorbent assay (ELISA) were used to detect all the studied biomarkers in the semen samples and the analysis steps were accomplished according to manufacturer manuals as explained in table (1) below: -

Table 1. Enzyme linked immunosorbent assay (ELISA) kits for detection of (viral-IgGs, cytokine levels and antioxidants system) in seminal plasma

Biomarkers	Supplier	Sensitivity: Detection range
VZV-IgG	MyBioSource Inc., USA	≥ 1.0
EBV-IgG	MyBioSource Inc., USA	≥ 2.1
CMV-IgG	MyBioSource Inc., USA	≥ 1.0
HHV6-IgG	MyBioSource Inc., USA	≥ 0.10
IL-2	Elabscience Biotechnology Inc., USA	4.69 pg\ml: 7.81-500 pg\ml
IL-10	Elabscience Biotechnology Inc., USA	4.69 pg\ml: 7.81 -500 pg\ml
IFN-γ	Elabscience Biotechnology Inc., USA	9.38 pg\ml: 15.63-1000 pg\ml
8-OHdG	MyBioSource Inc., USA	0.05ng\ml: 10 - 0.15ng\ml
TAOC	MyBioSource Inc., USA	0.14ng\ml: 1.56 - 50 U\ml

Table 2. Baseline data of participants

Characteristics		Study Groups				
		Healthy (Fertile) Men N= 35 (38.9%)		(Infertile) Men N= 55 (61.1%)		
		N (%)	Mean±SD	N (%)	Mean±SD	*p.value*
Age (Years)	(23 – 32)	19 (21.1%)	31.66±5.27	23 (25.6%)	33.29±5.26	0.15 NS
	≥ 33	16 (17.8%)		32 (35.6%)		
*BMI (kg/m^2)	Normal	29 (32.2%)	22.16±3.99	9 (10.0%)	27.47±3.85	< 0.001 HS
	Abnormal	6 (6.7%)		46 (51.1%)		
Total sperms numbers	< 33	0 (0.0%)	51.86±14.66	45 (50.0%)	25.31±15.83	< 0.001 HS
	≥ 33	35 (38.9%)		10 (11.1%)		
Progressive motility (%)	< 31	0 (0.0%)	45.86±11.95	52 (57.8%)	17.56±10.77	< 0.001 HS
	≥ 31	35 (38.9%)		3 (3.3%)		
Morphology (%)	< 3	0 (0.0%)	19.57±5.22	33 (36.7%)	8.29±9.34	< 0.001 HS
	≥ 3	35 (38.9%)		22 (24.4%)		

* BMI (kg/m^2) = Body mass index (Kilogram/meters squares)

Statistical analysis

All data was firstly enteredto Microsoft Excel (2013) and then submitted coded into statistical package for social sciences (SPSS) version 25 (IBM Inc., USA) for final statistical analysis, frequencies, mean and standard deviation (mean ± SD) were used to express into continuous quantitative data. Categorical variables (qualitative data) were compared using Chi-squared test (÷2) and independent t- Test for means comparison, Pearson's correlation was applied to examine relation between dependent and independent variables. Furthermore, multiple linear regression was used as a predictive analysis, to explain the relationship between one continuous dependent variable and two or more independent variables. *p.values*<0.05 were considered statistically significant.

Demographic characteristics of the recent study consisted of two groups of fertile and infertile participants according to the table (2), there was no significant difference (p>0.05) between age categories and study groups and most of the infertile men were in (e" 33) of age interval as (32 /35.6%; 33.29±5.26) mean of comparison. In contrast, it had been detected a high significant difference (p>0.001) between infertile men and abnormal BMI as (46 / 51.1%; 27.47±3.85) of mean comparison. In addition to, all semen parameters of total sperms numbers, motility and morphology data reflect high significant differences between the fertile and infertile individuals (p>0.001).

Highly Significant differences (p < 0.001) had been detected of positive CMV, HHV6 IgGs in semen samples of infertile patients as (39; 43.3%) and (48; 53.3%) respectively comparing to the fertile samples. Moreover, there was a high significant differences of abnormal IL-10 (40; 44.4%) and IFN-ã (44; 48.9%) concentrations of infertile semen samples comparing to abnormal cytokines levels in the fertile semen samples (p < 0.001). Finally, high 8-OHdG seminal concentrations revealed as 47 (52.2%) in infertile men (p < 0.001) and about 16 (17.8%) of low TAOC in infertile group (p<0.05) table (3).

Table (4) revealed highly significant means of comparison (p<0.001) between estimated seminal biomarkers and study groups (fertile and infertile participants) except in VZV-IgG and IL-2 there was no significant comparison of means levels among volunteers' samples as (0.52±0.46), (6.60±4.61) for infertile men and (0.66±0.38), (5.78±3.76) for fertile men respectively (p>0.05).

Table 3. Association of studied biomarkers with study groups

Biomarkers		Study Groups N (%) Healthy (Fertile) Men N= 35 (38.9%)	(Infertile) Men N= 55 (61.1%)	*p.value*
VSV-IgG	Positive	4 (4.4%)	5 (5.6%)	0.71 NS
	Negative	31 (34.4%)	50 (55.6%)	
EBV-IgG	Positive	14 (15.6%)	34 (37.8%)	0.04 S
	Negative	21 (23.3%)	21 (23.3%)	
CMV-IgG	Positive	5 (5.6%)	39 (43.3%)	< 0.001 HS
	Negative	30 (33.3%)	16 (17.8%)	
HHV6-IgG	Positive	11 (12.2%)	48 (53.3%)	< 0.001 HS
	Negative	24 (26.7%)	7 (7.8%)	
IL-2	Normal	30 (33.3%)	41 (45.6%)	0.20 NS
	Abnormal	5 (5.6%)	14 (15.6%)	
IL-10	Normal	31 (34.4%)	15 (16.7%)	< 0.001HS
	Abnormal	4 (4.4%)	40 (44.4%)	
IFN-ã	Normal	33 (36.7%)	11 (12.2%)	< 0.001 HS
	Abnormal	2 (2.2%)	44 (48.9%)	
8-OHdG	Normal	29 (32.2%)	8 (8.9%)	< 0.001HS
	High	6 (6.7%)	47 (52.2%)	
TAOC	Normal	34 (37.8%)	39 (43.3%)	0.002 S
	Low	1 (1.1%)	16 (17.8%)	

Table 4. Comparison association of the mean levels among studied biomarkers according to the study groups

Biomarkers	Study Groups (Mean±SD) Healthy (Fertile) Men N= 35 (38.9%)	(Infertile) Men N= 55 (61.1%)	p.value
VZV-IgG	0.66±0.38	0.52±0.46	0.14 NS
EBV-IgG	1.28±0.91	2.20±1.47	< 0.001HS
CMV-IgG	0.48±0.53	1.51±0.95	< 0.001 HS
HHV6-IgG	0.66±0.66	1.26±0.51	< 0.001 HS
IL-2	5.78±3.76	6.60±4.61	0.38 NS
IL-10	9.21±5.12	22.75±10.65	< 0.001 HS
IFN-ã	36.52±18.52	112.62±38.64	< 0.001 HS
8-OHdG	3.29±2.61	7.29±2.15	< 0.001 HS
TAOC	28.96±5.93	19.34±12.17	< 0.001 HS

According to the results of the Pearson correlation test in table (5)there was a negative significant ($p<0.05$) and highly significant ($p<0.001$) correlations among all estimated seminal biomarkers (viral –IgGs, cytokines and oxidants-antioxidants status) with semen parameters (total sperms numbers, motility and morphology), except there was a positive significant and non-significant ($p>0.05$) correlations with TAOC and VZV-IgG respectively.

Regression analysis between study groups (Fertile and infertile participants) with all the explored seminal biomarkers concentrations which were included (viral –IgGs, cytokines and oxidants-antioxidants status) and semen parameters (total sperms numbers, motility and morphology) had been predicted that the CMV-IgG was the only significant and effective viral IgG concentration in semen samples comparing to all estimated viral –IgGs with (*t*.Test= 2.965; $p < 0.05$), also IFN-ã abnormal semen concentration was the only significant expressed cytokine comparing to the IL-2 and IL-10 levels with (*t*.Test= 3.888; $p < 0.001$). Furthermore, there was a significant and effective high 8-OHdG and low TAOC semen concentrations in samples between study groups that maybe effect of sperms DNA damage then subsequently into the male infertility with (*t*.Test= 1.950; $p = 0.05$) and (*t*.Test= -3.275; $p < 0.05$) respectively. Finally, it had been significantly

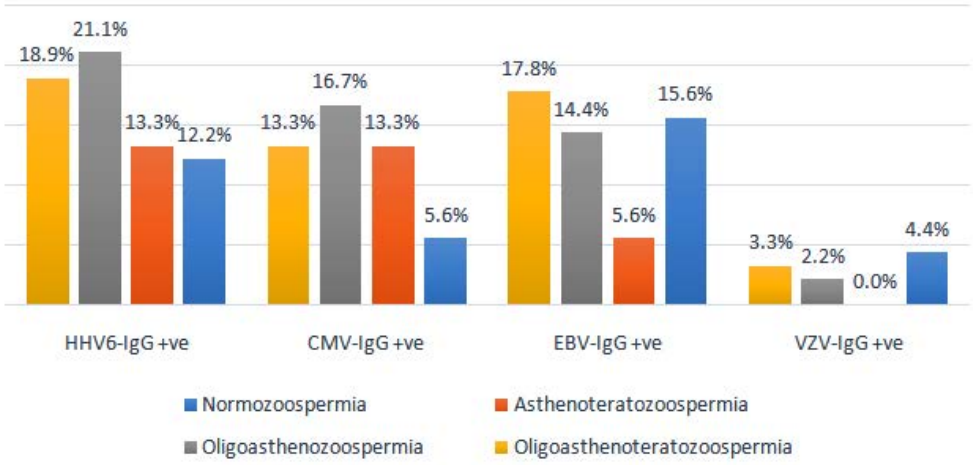

Fig. 1. Frequency distribution of sperms abnormalities onto abnormal Viral-IgGs semen concentrations

predicted that all the examined semen parameters effect on the infertility issues between study groups table (6).

The correlation between sperms parameters abnormalities and studied biomarkers revealed highly significant and negative associations regarding to theabnormal seminal levels of CMV-IgG and HHV6-IgG as (r=0.37 \ p=0.001) and (r=-0.46 \ p=0.001) respectively. Furthermore, there was a highly significant positive association with IL-10 as (r=0.58 \ p=0.001) and IFN-ã as (r=0.37 \ p=0.001) also, and there was positive association with oxidative stress and antioxidants markers of 8-OHdG and TAOC, table (7). The VZV-IgG and IL-2levelsdidn't show significant differences in sperms parameters abnormalities (p>0.05).

Figure (1) presented a highly significant association of sperms abnormality defects of Oligoasthenozoospermia, Oligoastheno teratozoospermia, Asthenoteratozoo spermia as 19 (21.1%), 17 (18.9%) and 12 (13.3%) respectively with the positive semen HHV6-IgG concentration (p<0.01). Followed with positive semen CMV-IgG concentration as 12 (13.3%) for each Oligoasthenoteratozoospermia and Asthenoteratozoospermia and 15 (16.7%) for

Table 5. Correlation of examined seminal fluid parameters with estimated biomarkers

Biomarkers	Seminal Fluid parameters (r \ *p.value*)		
	Total Sperms numbers	Progressive Motility (%)	Morphology (%)
VZV-IgG	0.088 \ 0.20	0.17 \ 0.09	0.02 \ 0.85
EBV-IgG	- 0.37 \ 0.001	- 0.18 \ 0.09	- 0.21 \ 0.04
CMV-IgG	- 0.19 \ 0.03	- 0.32 \ 0.02	- 0.32 \ 0.002
HHV6-IgG	- 0.33 \ 0.001	- 0.36 \ 0.001	- 0.24 \ 0.02
IL-2	- 0.10 \ 0.15	- 0.003 \ 0.49	- 0.14 \ 0.09
IL-10	- 0.50 \ 0.001	- 0.38 \ 0.001	- 0.23 \ 0.01
IFN-ã	- 0.53 \ 0.001	- 0.59 \ 0.001	- 0.37 \ 0.01
8-OHdG	- 0.41 \ 0.001	- 0.45 \ 0.001	- 0.46 \ 0.001
TAOC	0.33 \ 0.001	0.32 \ 0.002	0.19 \ 0.05

Table 6. Summary of multiple linear regression analysis of the studied biomarkers and semen parameters according to study groups

Studied biomarkers and semen parameters		Standardized Coefficients Beta	Std. Error	*t*.Test	p.value
Viral-IgGs	VZV-IgG	-0.018-	0.042	-.486-	0.629 NS
	EBV-IgG	-0.014-	0.016	-.308-	0.759 NS
	CMV-IgG	0.129	0.022	2.965	0.004 S
	HHV-6-IgG	0.074	0.035	1.598	0.114 NS
Cytokines	IL-2	0.003	0.004	0.071	0.943 NS
	IL-10	0.109	0.002	2.184	0.032 NS
	IFN	0.199	0.001	3.888	< 0.001 HS
Antioxidants	8-OHdG	0.097	0.008	1.950	0.05 S
System	TAOC	-0.135-	0.002	-3.275-	0.002 S
Semen	Total sperms numbers	-0.173-	0.001	-3.589-	< 0.001 HS
parameters	Progressive motility (%)	-0.326-	0.001	-6.713-	< 0.001 HS
	Morphology (%)	-0.182-	0.002	-4.316-	< 0.001 HS
Total	N = 90	R = 0.95	Std. Error	F = 61.16	
		R2 = 0.90	of the	df = 12	
		Adjusted R2 = 0.89	Estimate = 0.16		

Oligoasthenozoospermia (p<0.01). Furthermore, it had been detected a significant association of positive semen EBV-IgG level with sperms abnormality defects (p<0.05). Finally, positive semen VZV-IgG levels showed no significant association with examined sperms abnormality (p>0.05).

It had been detecting that Oligoasthenozoospermia significantly (p<0.01) associated with abnormal IFN-g" and IL-10 semen levels as 20 (22.2%) and 19 (21.1%) respectively. While it had been estimated that Oligoasthenoteratozoospermia significantly related with abnormal IFN-g" and IL-10 semen

Table 7. Correlation of sperms parameters abnormalities with studied biomarkers

Studied biomarkers			Sperms parameters abnormalities				
			A*	B*	C*	D*	(r \ *p.value*)
Viral-IgGs	VZV-IgG	Positive	4 (4.4%)	0 (0.0%)	2 (2.2%)	3 (3.3)	-0.03 \ 0.57 NS
		Negative	31 (34.4%)	12 (13.3%)	21 (23.3)	17 (18.9%)	
	EBV-IgG	Positive	14 (15.6%)	5 (5.6%)	13 (14.4%)	16 (17.8%)	-0.29 \ 0.03 S
		Negative	21 (23.3%)	7 (7.8%)	10 (11.1%)	4 (4.4%)	
	CMV-IgG	Positive	5 (5.6%)	12 (13.3%)	15 (16.7%)	12 (13.3%)	-0.37 \ 0.001 HS
		Negative	30 (33.3%)	0 (0.0%)	8 (8.9%)	8 (8.9%)	
	HHV6-IgG	Positive	11 (12.2%)	12 (13.3%)	19 (21.1%)	17 (18.9%)	-0.46 \ 0.001 HS
		Negative	24 (26.7%)	0 (0.0%)	4 (4.4%)	3 (3.3%)	
Cytokines	IL-2	Normal	30 (33.3%)	11 (12.2%)	18 (20.0%)	12 (13.3%)	0.22 \ 0.09 NS
		Abnormal	5 (5.6%)	1 (1.1%)	5 (5.6%)	8 (8.9%)	
	IL-10	Normal	31 (34.4%)	6 (6.7%)	4 (4.4%)	5 (5.6%)	0.58 \ 0.001 HS
		Abnormal	4 (4.4%)	6 (6.7%)	19 (21.1%)	15 (16.7%)	
	IFN-ã	Normal	33 (36.7%)	6 (6.7%)	3 (3.3%)	2 (2.2%)	0.37 \ 0.001 HS
		Abnormal	2 (2.2%)	6 (6.7%)	20 (22.2%)	18 (20.0%)	
Antioxidants System	8-OHdG	Normal	29 (32.2%)	1 (1.1%)	5 (5.6%)	2 (2.2%)	0.59 \ 0.001 HS
		High	6 (6.7%)	11 (12.2%)	18 (20.0%)	18 (20.0%)	
	TAOC	Normal	34 (37.8%)	11 (12.2%)	15 (16.7%)	13 (14.4%)	0.37 \ 0.003 S
		Low	1 (1.1%)	1 (1.1%)	8 (8.9%)	7 (7.8%)	

*A = Normozoospermia *B = Asthenoteratozoospermia*C = Oligoasthenozoospermia*D = Oligoasthenoteratozoospermia

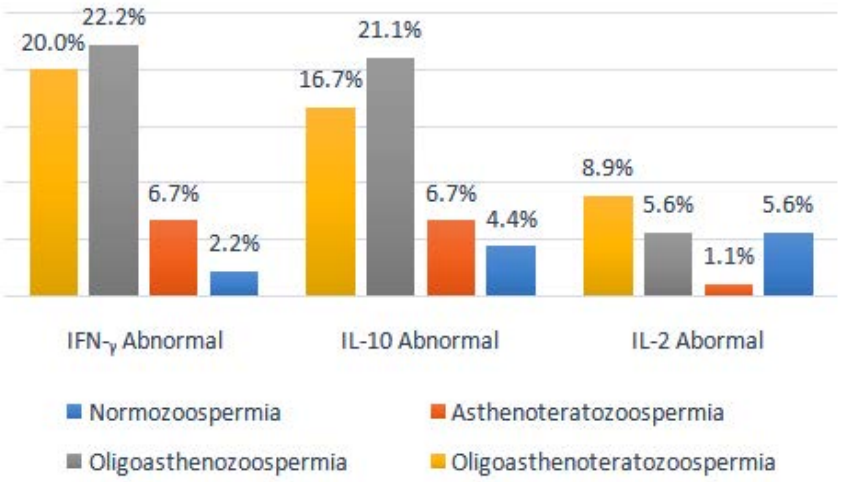

Fig. 2. Frequency distribution of sperms abnormalities onto abnormal cytokines semen concentrations

concentrations as 18 (20.0%) and 15 (16.7%) respectively. Moreover, IL-2 expressed no significant association with investigated sperms abnormality ($p>0.05$) as shown figure (2).

Figure (3) illustrated the high significant association ($p<0.001$) of sperms abnormality defects with oxidative stress system that represented by high 8-OHdG semen concentration in infertile samples that might be induced sperms DNA damage as 18 (20.0%) for each Oligoasthenozoospermia and Oligoasthenoteratozoospermia and about 11 (12.2%) in Asthenoteratozoospermia, with significant association of oxidative defense system that represented by low TAOC semen concentration in infertile samples ($p<0.05$).

DISCUSSION

This is the first Iraqi study that estimate the viral infection with cytokines expression and oxidant-antioxidants status in semen samples all togethers as a co-factors in the idiopathic male infertility, but our recent study was subjected to several limits. First of all, small sample size of infertile men with a one infertile clinic that had been involved in this study. Besides, this study did not investigate the follow-up medical status of the infertile males which maybe maximize the ability to diagnose the outcomes of selected cases. Lastly, a genetic controversial study in a different group of multiple infertility reasons should be carried.

The current study estimated the impact of the chronic HHVs infection by the detection of some viral-IgGs in idiopathic male fertility and the consequence showed the CMV-IgG, HHV6-IgG and EBV-IgG levels significantly effect on infertility cases but not VZV-IgG as shown in tables (3-7) and figure (1).The potential presence of VZV had been investigated in semen only in a few studies. Excluding for a one Greek study[4]. A study agrees that VZV is notexisting in semen samples from fertility-clinic patientsor healthy sperm donors[10,12]. EBV in semen was detected significantly and in blood samples of HIV-infected patients suggesting virus reactivation[13], EBV also can be transmitted through genital secretions to interfere with infertility issues[14,15]. Since then, studies on HCMV detection in semen showed incidence of about 6% in men from different Europeans countries. In contrast, high prevalence of HCMV between (21.6%) to (56.9%) was reported in several studies on semen of infertile participants from some Asian countries, Spain, and Greece[4,11,16], however another Greek study found HCMV in semen only as (7.1%)[9]. A comparison study of HCMV incidence in semen samples from fertile and infertile males in two reports were found similar incidences within the two studies[18]. Few studies are available on the incidence of HHV-6A/B in semen as 4.0% (German), 3.7% (American), 2.0% (China) and 13.5% (Denmark) of infertile population[10,12,16,19]. A study was used nested PCR

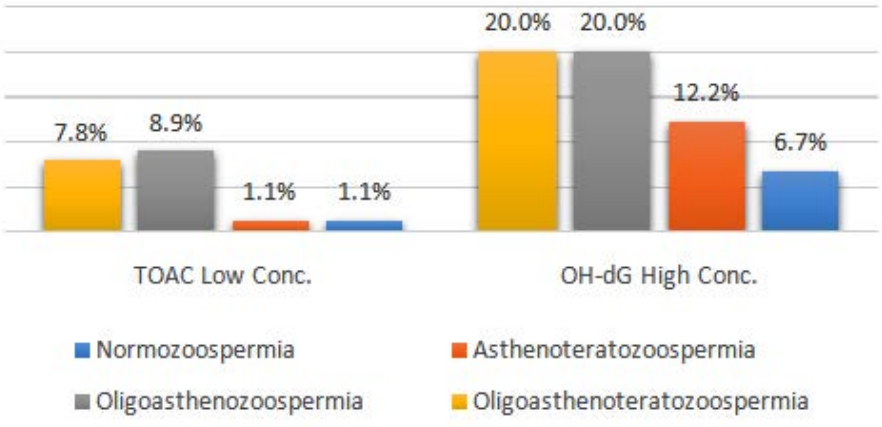

Fig. 3. Frequency distribution of sperms abnormalities onto High 8-OHdG and Low TAOC semen concentrations

reported 66.8% of HHV-6 among men attending a fertility clinic at Crete[4]. However, it'sobvious that HHV-6A/B infection doesn't directly affect spermiogram[4,10]. But, interestingly that HHV-6A/B infection is related with the sperm acrosome[12].

IL-2 as proinflammatory cytokine of humoral and cellular immune defense was insignificantly not associated with infertility rate of men in comparison with high significant levels of INF-g" and anti- inflammatory cytokine IL-10 in an infertile men, tables (3-7) and figure (2). Our data can serve as reference values for future studies on the role of cytokines concentration in idiopathic male infertility. But, further studies should be done to investigate the immunological reasons for infertility development.Generally, there are influence of various cytokines hypersecretion on the semen quality and sperms function then onto fertility process at the same times, many studies indicate the lack of any linking between the cytokine over expression and semen quality or infertility status, studies demonstrate that IFN-ã has an ability to inhibit sperm motility[20].

A study significantly detects high IL-2 levels of seminal plasma in infertile subjects (444.3 +/- 40.5) comparing in those of fertile individuals (251.3 +/- 42.7) with a significant negative correlation between IL-2 levels and spermiogram[21]. Significant negative correlations between some cytokine levels and semen parameters was detected in a variety of studies and shown the usefulness of these cytokines as clinical biomarkers of male infertility[22]. IL-10 from the contents in seminal plasma, so the hypersecretion or hyposecretion of this cytokine reflect may be effects on the status of immunity and/or infection of the male reproductive tract then is closely associated to male reproductive ability and might be influence on sperm function and semen quality[20,22,23]. Another study revealed a strong positive correlation between concentration parameters of IL-10 (r = 0,72) with semen ejaculates and indicate high IL-10 concentration in the ejaculates can effect on the parameters of seminal fluid[24]. IFN-ã is produced by several testicular cells, mainly somatic cells, and particularly during viral infections and it is implicated in protecting the testis from virus infection[25]. INF ã significantly detected in a higher level in the seminal plasma of infertile subjects (6.36±0.72) compared to fertile subjects (3.68±0.30) with a significant negative correlation between INF ã and spermiogram[25].

Regarding to evaluate the oxidative stress (marker of DNA damage of fragmentation) and antioxidants (free radicals scavenger), 8-OHdG and TAOC seminal concentrations were estimated to reveal a strong correlation with spermiogram parameters of infertile individuals as shown in tables (3-7) and figure (3).Oxidative stress considers as a causative factor for many diseases and male infertility is not an exception. Reactive oxygen species (ROS) have a role in spermatogenesis and reproduction process and basically a normal spermiogram parameters and good semen quality may require a balance between oxidative and antioxidant factors[26].Chronic viral infections may also promote spermsoxidative damage. The association between common viral infection such as CMV, HSV and EBV and oxidative status has been studied by some groups by detecting of HSV-DNA in semen (4–50%) of infertile males[17,19,27], with detection of linkage between ROS levels and leukospermia, together with decrease of sperms motility[17]. A study indicated that, pro-inflammatory cytokines, such as IL-2 with ROS increasinglevels that have been correlated with decreased sperm motility as unknown mechanism and is poorly understood. Thus, many hypotheses have been explained the relation between ROS and decreased motility[28,29]. Increase the intensity of oxidative stress in seminal fluid and subsequently effect onto semen quality and sperms function. Interestingly sperm plasma membrane has high concentration of polyunsaturated fatty acids and that mostly susceptible to lipid peroxidation by ROS, which ultimately lead to the loss of membrane integrity; hence lose their competence for the membrane fusion events during fertilization. So that, a study showed higher levels of oxidative stress in the different forms in infertile men compared with fertile men. Another study had been stated high seminal MDA concentration in oligozoospermic and azoospermic patients[29-31].

Furthermore, oxidative stress with pro-inflammatory cytokines after vasectomy or varicoceles has been anticipated to act as significant cause for infertility reversal because the disruption of the normal blood-testis barrier and increased (8-OHdG) levels, leading to activation of immune

responses against sperm and losing of immune privilege[32,33]. Cytokines liberate by T cells such as IFN-ã activate leukocytes and chemotaxis, leading to high seminal oxidative stress levels[34]. Besides, DNA damage in the Y chromosome also can cause gene deletion in the Y chromosome of the offspring, leading to infertility[35]. A study showed a negative correlation of sperm parameters of motility, sperm count and morphology with (8-OHdG) while positively correlated with TAS, these outcomes proposed that high oxidative stress levels induced DNA damage of sperms might have a significant effect on the etiopathogenesis of male fertility[36]. Hence, evaluation of ROS status, antioxidant defense systems, damage of sperms DNA, pathway of the semen cytokines and herpes viruses infection, collectively with spermiogram might be a useful tool for diagnosis, prognosis and treatment of some etiology in male infertility.

CONCLUSION

It had been concluded, viral infection of seminal fluid by EBV, CMV and HHV6, but not by VZV maybe interfere with male infertility problems through multiple pathological strategies. These viruses, which may cause impairing of semen parameters of total sperms count, motility or morphology. In addition to, the semen plasma had been estimated with significant positive correlation of low TAOC levels (oxidative defense system) and significant negative correlation of high 8-OHdG levels (oxidative stress status) regarding to sperm parameters that maybe induced sperms DNA damage. On the other hand, a significant detection role of immunological parameters changes by detecting of IL-10 and IFN-ã in seminal plasma of infertile patients that may contribute with viral-IgGs and oxidative-antioxidants system in the male infertility as a co-factors. Finally, the assessment of all the above parameters, together with spermiogram may play a significant role in the diagnosis and treatment of male infertility

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