Serum levels of IL-23, IL-8 and IL-10 to CMV, HHV-6, and IBV infection and Male Unexplained Infertility

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Abstract

Back ground: Infertility is a reproductive disorder After 12 months or longer of frequent sexual intercourse Infertility affects 60–80 million people worldwide. Unexplained or idiopathic infertility is a condition, in which couples are not able to conceive without any definite causes. Viruses, as well as many other microorganisms, are able to interfere with the reproductive function and suspected to be involved in development to infertility .Cytomegalovirus (CMV), human herpes virus type 6 (HHV-6) and Epstein–Barr virus (EBV) are also frequently discovered in sperm samples. Pro-inflammatory cytokines like IL-8 and IL-23 have direct effects on spermatogenic cell differentiation, testicular steroidogenesis, and spermatogenesis, Whereas anti-inflammatory cytokines like IL-10 are also linked to testicular development

Aim of study : To detect the levels of interleukin -23,IL-8 and IL-10 levels as immune response's for Un explained infertile men infected byCMV,HHV-6and EBV.

Materials and methods: blood sample (3.5) ml taken from twenty five patients of unexplained infertile males Herpes viruses (CMV ,HHV-6and EBV) with 20 appearance healthy fertile male as control in Iraq's Diyala province were studied From November 2020 to April 2021 measurement for them the Interlukin-23, IL-8, and IL-10 were measured by using an ELISA kit from My Bio Source in the United States. The statistical analysis was carried out using the software GraphPad Prism version 3.06 and SPSS version -26.

Results: In this study we observed that IL-23 in positive infertile male infected by CMV had significantly higher of IL-23 in plasma than CMV-seronegative recipients (P = 0.001),its respectively(942.57±205) and (714.5±35). Infertile men positive to EBV had IL-23 (951.51±432) while not infective by EBV (793±148) , no significant different appear between them . IL-23 was male (924.9±227) in unexplained infertility men positive to HHV-6 ,not infective males by HHV-6 (775.9±148) ;no significant different between the level of IL-23 in study group. Chemokine IL-8 mean of infertile males who positive to CMV was (213.762±64) and while negative results to CMV(118.9±49) with high significant relation between positive and negative results ,IL-8 in infertile male had EBV (222.85±75) and where is non infective unexplained infertile males mean (151.12±71), With no significant different .IL-8 arising in male infertile with positive result to HHV-6 (221.9±63) and lowered in negative HHV-6 (140.6±66),with high significant different between positive and negative results in patients.Anti- inflammatory interleukins-10 found in serum of infertile males positive CMV (152.25±46) and negative CMV infected males (88.8±21) ,with significant different. EBV had IL-10 mean (137.79±45) in infertile male while not infected by EBV had (112.12±46),no significant different appear between them .IL-10 in unexplained male positive result to HHV-6 was higher level form those who had negative result to HHV-6, and its (153.1±45) ,(104.4±41) respectively .with higher significant different appear between them ,no one of control infected by HHVs study in thes investigation and appear lower level of interleukins' than infertile and there mean was for IL-23 (645.2±37),for IL-8 was (86.4±10) and IL-10 was (75.1±9). In conclusion, this study found most infertile male had showed a significant increase in IL-23,IL-8 and IL-10 to infection by human herpes virus (CMV ,HHV-6and EBV) because these virus could modulated immune system and there cytotoxicity that causes rising of expression of cytokine .

Key words: Male Unexplained Infertility,CMV ,HHV-6,EBV, IL-23, IL-8 ,IL-10

1. Introduction

Infertility is a reproductive disorder After 12 months or longer of frequent sexual intercourse, the failure to achieve a clinical pregnancy is characterized as a system (as there is no other reason, such as breastfeeding or postpartum amenorrhea.(WHO;2020). It is estimated that 10–15 %of the world's reproductive-age population suffers from infertility[1]. Infertility can be classified as either primary or secondary. Primary and secondary infertility. Primary infertility is defined as the lack of a live birth in a woman who wants to have a child and has been in a relationship for at least 12 months without using contraception .[2, 3]a woman who is

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unable to achieve a clinical pregnancy but has previously been diagnosed with a clinical pregnancy is said to have secondary female infertility[4].

A form of infertility known as unexplained infertility (UI) occurs when the results of routine tests, including as ovulation tests, tubal and uterine patency, and semen analysis, are all normal. [5]. In conventional diagnostic testing indicates no cause in 10-15% of infertile couples. Men are shown to be the cause of infertility in 45-50 % of cases. The reason for 30-45 %of them is still unknown (idiopathic male infertility). Existing co-morbidities, such as general/systemic illnesses, malignancies (including lymphomas and leukemias), obesity, and other factors, as well as substantial somatic and genetic variables, can all contribute to male infertility[6, 7] .

Herpesviruses are large DNA viruses classified into three families: α-herpesviridae, β-herpesviridae and γ-herpesviridae .Herpesviruses infect their hosts for life and are widespread in the human population. In healthy people, these infections are frequently asymptomatic[8 ,9] . Human herpes viruses (HHVs) are sexually transmitted viruses that can cause viral toxicity in genital tract cells or stimulate local or systemic infectious or immunological responses. In sperm samples, cytomegalovirus (CMV), human herpes virus type 6 (HHV-6) and Epstein–Barr virus (EBV) are frequently found.,[10,11, 12]. A healthy pregnancy requires the presence of the individual immune system (innate or adaptive). [13]. Cytokines are regulatory proteins that have a role in a variety of immunological responses and have an effect on the functioning of testicular cells. Cytokines have an important regulatory role in testis formation and normal testicular cell function.[14] . Anti-inflammatory cytokines like IL-10 was associated to testicular development, while pro-inflammatory cytokines like IL-23 and IL-8 have direct effects on spermatogenic cell differentiation, testicular steroidogenesis, and spermatogenesis.t[15] .IL-10:also known as Human cytokine synthesis is a cytokine inhibitory factor that inhibits the Synthesis of cellular motility and inhibits inflammatory producing.[15]. Despite these important findings, It's still not clear what HHVs' main role is in male infertility and their impact on male fertility has not been thoroughly investigated and there relation to interleukin 23, IL-8and IL-10 level as a immune response has not been well studied. As a result, we are attempting to explain the relationship form between them .

2.Materials and Methods

2. 1. Participants in study

The current study took place in Diyala province from November 2020 to April 2021. A total of 45 clinical samples were collected from A-Batol teaching hospital (gyncology department) . Control with no fertility problems were recruited. The participants were clinically diagnosed with idiopathic infertility; the selected cases were divided into two groups, first group consisted of 25 infertility males.

2.2. Serum collected

Blood samples (3.5 ml) were collected in a gel tube to get serum to perform immunological examinations from infertile males and fertile males whom samples were taken from them to test for virus infection by CMV,HHV-6Aand EBV detection by Multiplex Real Time PCR by using CMV/EBV/HHV6 Quant Real-TM kite (Sacace Biotechnology, Italy, cat : TV48-100FRT. Blood sample were placed at room temperature for 2 hours before centrifuging it at 1000rpm for 20 minutes depended on Instructions of human ELISA kit from My Bio Source in the United States .the collected serum stored at (-20°C) until immunological .

2.3. Determination of IL-23, IL-8 and IL-10 in serum

Interleukin -23, IL-8 and IL-10 in serum are determined in serum for all samples patients and controls according to the manufacturer's instructions of Human IL-23 Catalogue No: RDEEH3270),human IL-8(Catalogue No:RDEEH0205)and human IL-10(Catalogue No: RDEEH0173) ELISA Kit from My bio source \USA.

2.4.Statistical analyses

Statistical analyses were performed using GraphPad Prism version 3.06. and SPSS version -26 . Standard deviations are plotted as error bars for the data points on all figures Two-way ANOVA, One-way ANOVA done to establish relationships of expression immunological variables levels according to the ELISA test results between infertile male/ female and control.

2.5. Ethic approves

This study was approved by the Ethics Committee of Diyala Health Department / Training and Human Development Center / Research and Knowledge Management Division (No. 48950. Date 12/17/2020) and also by the Committee of the Diyala University/College of Education for Pure Science. Written informed consents were also obtained from all participants prior to registration.

3.Results and discussion

In present study we found not all infertile male infected by CMV, HHV-6 and EBV, and there wasn't found any infection by CMV,HHV-6 and EBV in healthy fertile males(control).

4.1. Effect of human herpes viruses (CMV,HHV-7 and EBV) on interleukins(IL-23,IL- and IL-10) level

4.1. Proinflammatory cytokine against CMV, EBV and HHV-6

4.1.1. Level of IL-23 in unexplained infertile males infected with CMV, HHV-6 and EBV

A novel study for CMV related to pro-inflammatory IL-23 in serum of unexplained infertile male positive infected with CMV recipients had significantly higher levels of IL-23 in their plasma than CMV-negative of

infertile men ,IL-23 was (942.57±205) and (714.5±35) respectively . EBV had IL-23 mean (951.51±432) was higher than non-infective by EBV (793±148) , no significant different appear between them . Also new found appear IL-23 was higher infertile male positive HHV-6 in (924.9±227) ,and lower in negative to HHV-6 (775.9±148) ,in control was lower than fertile males (645.2±37)with no significant different between the level of IL-23 in study groups, , show table (1).

No study mention to related between IL-23 and infertile males infected by CMV HHV-6 and EBV. IL-23 play roles as pro-inflammatory cytokines, and resistance to both viruses and bacteria[16], and level of IL-23 rising in asymptomatic genital tract viral infections ,for this reason may induce the increase in IL-23 which is secreted by people who have abnormal semen quality, and IL-23 might influence the development, structure, and function of sperm in cytokines.[17].

The Herpesviridae enhanced the amounts of IL-23 mediators in general, showing that EBV and HHV-6A DNA have a unique ability to induce Th17 responses. This suggests that copies of EBV DNA cause IL-23 levels high enough to enhance IL-17 production. The levels of IL-23 were nearly identical to those of IL-17, and they when EBV DNA copies were high. [18] . previous study refer also that latent CMV induces IL-23 because IL-23 as an inflammatory mediator of latent CMV infection in patients. [19] .we did not agree with study finding that Positive serology for anti EBV Ig was reported in (71 %) of IL-23 deficient [21] . Human herpes viruses (HHV-6) may cause male infertility by generating viral toxicity in genital tract cells or by inducing local or systemic infectious or immune responses [10].IL-23 have direct effects on spermatogenic cell differentiation, testicular steroidogenesis, and spermatogenesis[15]. As result of all that may be that the reason of present the higher level of IL-23 in infertile males.

4.1.2. IL-8 level in unexplained male infected with CMV, HHV-6 and EBV

In present study we found that interleukin-8 mean of infertile males who positive to CMV was (213.762±64) and while negative results to CMV(118.9±49) and appearance high significant relation between positive and negative results with CMV of infertile males. Also new study for immune responses by IL-8 to infertile male infected with IBV found that IL-8 in positive male infected with IBV was (222.85±75) and not infective infertile males was (151.12±71), With no significant different between them (P=0.2). IL-8 arising in male with positive result to HHV-6 (221.9±63) and lowered in negative HHV-6 in male with unexplained infertility (140.6±66),with high significant different between positive and negative results in patient. In control infertile male was IL-8 was (86.4±10).Show table (1).

CMV is common in infertile patients' genital tracts, however sexual transmission is not a common route of infection or a major cause of infertility[22].CMV infection increases trans endothelial neutrophil migration by upregulating IL-8 gene expression[23]. Before DNA synthesis, IL-8 can increase CMV late antigen protein synthesis and transcription. IL-8 may increase CMV replication in fibroblasts by interacting with CXCR-1[24]. IL-8 concentrations may change during pathological conditions, affecting sperm production and quality[25],also Genetic factor may be substantially affect cytokine production[26]..our results supported by study to in semen CMV shedding found that proinflammatory IL-8 was higher with significant relation ship (p>0.001)[27]. Moreover, another study refer the enhancing of IL-8 after infection by CMV from at 0.1 ng/mL to maximal at 10 ng/mL[28]and that prove that IL-8 increase with CMV . No study refer to relation between EBV the level of IL-8 ,the reason may be EBV promote the expression of IL8, IL10 in immortalized B cells, because predicted structure of is highly homologous to the receptor for IL-8 (IL8R)[29] , and IL-8 is a converged target gene of gamma herpesviruses in both latent and lytic infection states. EBV utilizes the lytic protein Zta and the latent protein LMP1 to induc IL-8 expression [30]. Also no document reported on relationship between IL-8 and unexplained infertile male infected by HHV-6 .

We think because the endothelial cell damage that is caused by HHV-6's stimulation of inflammatory processes and the local immune response, the virus enhances the production of the inflammatory cytokine IL-8[31, 32].

4.2. Anti -inflammatory cytokine

4.2.1. IL-10 level in male infected by CMV, HHV-6 and EBV

As shown in table (1) ,the present study found that serum of positive result to CMV of infertile males had higher level of IL-10 (152.2±46) and while males negative CMV was(88.8±21) ,with significant different between them (P=0.001). EBV in infertile males had IL-10 mean (137.79±45) while infertile males negative to EBV(112.12±46),no significant different appear between them . IL-10 in unexplained male positive result to HHV-6 was (153.1±45) and who had negative result to HHV-6, was(104.4±41) ,with higher significant different appear between them (P=0.03),and the control had lower level (75.1±9) when compeer with infertile male

One of the strategies used by Human Cytomegalovirus and similar to other herpesviruses, during coevolution with its host to disable the immune system is to manipulate the immunoregulatory functions of cellular anti-inflammatory by encodes an IL-10 homolog with 27% identity to human IL-10 [33,34]. Human IL-10 and CMV IL-10 exhibit similar immunosuppressive and stimulatory characteristic by inhibiting LPS-induced DC maturation, cytokine production and upregulation of multiple T cell co-stimulatory molecules[35]. As a result, CMV IL-10 may enhance the spread of HCMV while simultaneously suppressing the early immune response [36].HCMV IL-10 is

upregulated during both latent and lytic phases of HCMV replication specially during transplant Furthermore, elevated levels of cIL10 associated with high viral loads[37 ,35, 38].

.Also EBV can be transferred through genital secretions, causing infertility problems, according to a previous study by induced B cells to produce the growth factor IL-10 [39 ,40] . EBV encodes 3 host cytokine or chemokine receptor mimics one of them are an interleukin -10(IL-10) homolog encoded by BamHI-C fragment rightward reading frame 1 (BCRF1) , The EBV BCRF1 gene product Viral IL-10 (vIL-10) shares 85%[41] and 80% amino acid sequence . viral -IL-10 protein is expressed late during the lytic life cycle and expressed throw the first(6–9 h), after infection of human B cells, therefore vILs as well as the viral chemokine receptor homologs are typically classified as ''lytic'' cycle viral genes, they are also transcribed in response to specific host cell signaling events outside the rigid framework of the canonical transcriptional cascade that governs herpesvirus lytic replication . Thus, one might speculate that they have a purpose in latent persistence in addition to their role in support of viral replication and progeny production..[42],in another hand HHV-6 , increase of IL-10 may be due to infections can modulate the profile of cytokine and chemokine production by different cell types. HHV-6 induced the production of inflammatory cytokines such as , IL-8 and increased and Production of IL-10 has been documented as a marker of cell-mediated immune response to HHV-6[43] ,and another researcher observed that these viruses induce a cytokine imbalance with a switch from an antiviral Th1-polarized cytokine profile to a Th2 profile, since they have been shown to downregulate IL-12 and IFN-ɣ, while upregulating IL-10 [44,45]. Our result was supported by a previous study, Muhsin discovered that when it came to the relationship between chronic human herpes virus types CMV, IBV, and HHV-6 infection and the up-regulation of some cytokines, IL-10 had the highest abnormal cytokine levels (44.4%; 22.75±10.65)[14] .

Table(1): a comparison of expression immunological variables levels according to the elisa test results between positive(10\40%) and negative infertile male(15\60%) infected with cmv,ebvand hhv-6.

Variables			IL-8 (mean ±SD)	IL-23 (mean ±SD)	IL-10 (mean ±SD)
Infertile male	CMV	Positive	213.762±64	942.57±205	152.25±46
		Negative	118.9±49	714.5±35	88.8±21
		P.value	**0.001**	**0.001**	**0.001**
	EBV	Positive	222.85±75	951.51±432	137.79±45
		Negative	151.12±71	793±148	112.12±46
		P.value	**0.2**	**0.2**	**0.5**
	HHV-6	Positive	221.9±63	924.9±227	153.1±45
		Negative	140.6±66	775.9±148	104.4±41
		P .value	**0.02**	0.08	**0.03**
total		Infertile (total)	156.9±72	805.7±172	114.2±45
		Fertile	86.4±10	645.2±37	75.1±9
		Total P .value	**0.001**	**0.001**	**0.002**

Anova used to compare means between different groups and results are expressed as mean ±SEM : using P-value <0.05 was considered statistically significant (are shown in bold)

5. Conclusion

This study reports for the first time, relation between human herpes virus (CMV, HHV-6 , and EBV) in un explained infertile males in Diyala province-Iraq. This study found most infertile male had showed a significant increase in IL-23,IL-8 and IL-10 to infection by human herpes virus (CMV ,HHV-6and EBV) because these virus homologous to IL-0 and could modulated immune system and there cytotoxicity that causes rising of expression of cytokine .

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7. Conflict of interest statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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