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RESEARCH ARTICLE

**DETECTION OF HUMAN CYTOMEGALO VIRUS AMONG MALE WITH INFERTILITY DISORDERS
 IN KHARTOUM STATE, SUDAN.**

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Abstract

Background: Human pathogens have been recognized as having a considerable possible effect on male infertility. Recent studies have shown the role of viral infections as an idiopathic pathogenesis of male infertility including cytomegalovirus. This study aimed to detect human cytomegalovirus antibody in serum among infertile male and to find out the association of this virus with seminal abnormality.

Materials and Methods: From a known ninety infertile men, serum samples were collected and tested for anti-CMV IgG and IgM using enzyme-linked immune sorbent assay (ELISA). Personal and clinical data were obtained. Comparison between anti-CMV IgG and IgM results and abnormal semen parameters were performed.

Results: In this study, Anti-CMV IgM, anti- CMV IgG and both were detected in 14 (15.5%), 83 (92.2%), and in 14 (15.5%) of serum samples, respectively. This study revealed that there was significant association between anti-CMV IgG and IgM results and azoospermia, oligozoospermia, asthenozoospermia and teratozoospermia. However, there was no significant association between demographic data and anti-CMV IgG and IgM result.

Conclusion: Human cytomegalovirus was present in high percentage among infertile men and the virus had many effects on semen parameters. CMV seems to play an important role in male infertility.

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Introduction:

There is growing evidence that viral infections may contribute to male fertility disorders (Naumenko, *et al.* 2014). Recent studies have shown the role of viral infections as an idiopathic pathogenesis of male infertility (Habibi, *et*

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al.2014), The impact of viruses in the genital tract on male and female reproductive health has been a neglected field of interest (Eggert-Kruse, et al. 2009). Common infections include human cytomegalovirus, which is a herpes virus belonging to the beta herpesvirinae subfamily (Baghdadi, et al. 2016). Several studies revealed the role of viral infections in male infertility. There is an increasing evidence that viral infections play some important role in the pathogenesis of male infertility. The aim of the current study was to determine *cytomegalovirus* seropositivity among male with fertility disorder. And to correlate the human *cytomegalovirus* antibodies with semen parameters including (count, motility and morphology) and their contribution to male infertility disorders.

Materials and Methods:

This is a clinical based descriptive cross-sectional study conducted in Banoon center for assisted reproduction obstetrics and gynecology in Khartoum state, Sudan. This study was carried out during the period of March to August, 2017. A ninety persons whom considered as known infertile attending Banoon center in Khartoum state, Sudan, were randomly selected to participate in this study. They are diagnosed as infertile according to American guidelines of semen analysis (Petak, et al. 2002). Primary data were obtained from the medical records of the patients using a constructed questionnaire especially designed for this purpose. These data include age, residents, married duration and occupation and also laboratory result of the semen analysis (Count, motility and Morphology). However, those who are treatment with antiviral therapy or any type of malignant disorders and metabolic disorders were excluded. Under a septic conditions three milliliters of blood samples was obtained via vein puncture in plain tubes and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 round/minute for 5 minutes, then transferred in plain sterile containers and kept at -20°C ready for use. The Enzyme-linked Immuno-Sorbent Assay (ChemuxBioScience, Inc, USA) was used in evaluating a patient's serologic status to specific cytomegalovirus IgG and IgM. Normal clinical laboratory safety procedures were maintained. All specimens and kit reagents were brought to room temperature before beginning the procedure and gently mixed. The washing buffer diluted by adding distilled water to 20x (H) wash concentrate to a final volume of 1 liter. The sample, negative control, positive control, and calibrators were diluted by adding 5 µL of them to 200 µL of sample diluent and mixed well.

According to manufacturer guidelines (ChemuxBioScience, Inc USA) the following steps were followed (see appendix). A 100 µL of diluted sera, calibrator, and controls were transferred into the appropriate wells. For the reagent blank, 100µL of sample diluent were transferred in 1A well position. The holder was tapped to remove air bubbles, mixed well and incubated for 30 minutes at room temperature. Liquid removed from all wells and washed three times by washing buffer using automatic washer to remove unbounded components. Then 100µL of enzyme conjugate were added to each well and incubated at room temperature for 30 minutes. After another three times washing step, 100µL of TMB chromogenic substrate were added to each well and the plate was incubated for 15 minutes at room temperature, and then 100µL of stop solution were added to stop reaction. The optical density was then read at 450 nm with a micro well reader. The CMV (IgM and IgG) index is calculated according to the following formula:

$$\frac{\text{Optical density values of each sample}}{\text{cut - off calibrator Optical density value}}$$

The CMV Index of 0.90 or less were considered seronegative for IgG or IgM antibody to CMV. The CMV Index of 1.00 or greater were considered seropositive IgG or IgM antibody to CMV.

Data analysis:-

Data were analyzed using statistical package for social science (SPSS) software for Windows, version 21. Chi-square test was used to test the difference of significance. *P* value of ≤ 0.05 was considered statistically significant.

Ethical consideration:

Permissions to carry out this study were obtained from the Alzaem Alazhari University, Faculty of Graduate Studies, medical laboratory sciences department of microbiology and immunology and Banoon center for assisted reproduction obstetrics and gynecology.

Results:-

In this study, the percentage of anti-CMV IgM antibodies seropositivity was 15.5% (14 samples) out of the total infertile men involved, while the percentage of anti-CMV IgM antibodies seronegativity was 84.5% (76 samples). It was also found that, 92.2% (83 samples) were seropositive for anti-CMV IgG, while 7.7% (7 samples) were found

to be seronegative. Also, the prevalence of both of HCMV IgG and IgM antibodies were 15.5% (14 samples) in serum samples of infertile men.

Table 1:-Married Duration of Study Group.

Married duration	Frequency	Percent
0-2 year	19	21.1%
3-5 year	33	36.7%
Above 5 year	38	42.2%
Total	90	100%

Table 2:-Description of semen analysis parameters.

Semen parameters	Abnormal	Normal	Total
Count	58 (64.4%)	32 (35.6%)	90 (100%)
Motility	69 (76.7%)	21 (23.3%)	90 (100%)
Morphology	80 (88.9%)	10 (11.1%)	90 (100%)

Table 3:-Association between CMV IgM and IgG result with age.

Age	CMV IgG antibody			<i>P</i> value	CMV IgM antibody			<i>P</i> value
	Negative	Positive	Total		Negative	Positive	Total	
20-35	2	25	27	0.962	23	4	27	0.349
	(2.2%)	(27.8%)	(30%)		(24.4%)	(5.6%)	(30%)	
36-50	4	49	53		44	9	53	
	(4.4%)	(54.4%)	(58.9%)		(48.9%)	(10%)	(58.9%)	
51-66	1	9	10		10	0	10	
	(1.1%)	(10%)	(11.1%)		(11.1%)	(0%)	(11.1%)	
Total	7	83	90		76	14	90	
	(7.8%)	(92.2%)	(100%)		(84.4%)	(15.6%)	(100%)	

Table 4:-Association between CMV IgM and IgG result with occupation.

Occupation	CMV IgG antibody		<i>P</i> value	CMV IgM antibody		<i>P</i> value
	Negative	Positive		Negative	Positive	
Driver	1	8	0.907	6	3	0.055
	(1.1%)	(8.9%)		(6.7%)	(3.3%)	
Lobar intensive work	2	22		18	6	
	(2.2%)	(24.4%)		(20%)	(6.7%)	
Other	4	53		52	5	
	(4.4%)	(58.9%)		(57.8%)	(5.6%)	
Total	7	83		76	14	
	(7.8%)	(92.2%)		(84.4%)	(15.6%)	

Table 5:-Association between CMV IgM and IgG result with residence

Residence	CMV IgG antibody		P value	CMV IgM antibody		P value
	Negative	Positive		Negative	Positive	
Urban	4	58	0.485	54	8	0.302
	(4.4%)	(64.4%)		(60.0%)	(8.9%)	
Rural	3	25		22	6	
	(3.3%)	(27.8%)		(24.4%)	(6.7%)	
Total	7	83		76	14	
	(7.8%)	(92.2%)		(84.4%)	(15.6%)	

Table 6: Association between CMV IgM and IgG result with married duration.

Married duration	CMV IgG antibody		P value	CMV IgM antibody		P value
	Negative	Positive		Negative	Positive	
0-2 year	1	18	0.884	14	5	0.343
	(1.1%)	(20.0%)		(15.6%)	(5.6%)	
3-5 year	3	30		29	4	
	(3.3%)	(33.3%)		(32.2%)	(4.4%)	
Above 5year	3	35		33	5	
	(3.3%)	(38.9%)		(36.7%)	(5.6%)	
Total	7	83	76	14		
	(7.8%)	(92.2%)	(84.4%)	(15.6%)		

Table 7:-Association between CMV IgM and IgG with abnormal semen parameters.

Abnormal variables	CMV IgG antibody		CMV IgM antibody	
	Negative	Positive	Negative	Positive
Oligozoospermia	3	26	23	6
	0.000		0.002	
Asthenozoospermia	3	37	31	9
	0.000		0.000	
Teratozoospermia	1	50	43	8
	0.000		0.001	
Azoospermia	3	26	26	3
	0.000		0.000	

Discussion:-

This study showed a high prevalence of anti-CMV IgG antibodies (92.2%). This result was higher than the result of (Yasir, *et al.* 2014), they found that, the anti-CMV IgG antibodies seropositivity (78%). In this study, the percentage of anti-CMV IgM antibodies seropositivity was (15.5%) out of the total infertile men. These data correspond to the results of (Yasir, *et al.* 2014), CMV IgM antibodies seropositivity were (% 11). Also, it is found that there was a high prevalence of both of HCMV IgG and IgM antibodies (15.5%) in serum samples of infertile men. This result appears to be very high when compared with result reported by Levy, *et al.* (1997), they found only one case with IgM and IgG antibodies detected as positive. A possible explanation for this high percentage could be the examination of a small sample size compared with most of the previous studies and this could be a reason for the higher viral prevalence. Eldowma, 2004, detected IgG antibodies of CMV among blood donors and antenatal women in Sudan with 77% and 95%, respectively. Our study showed that there was no significant statistical association with occupation, resident and married duration. Similar findings were stated by Habibi, *et al.* 2014, in Iran which conclude that, there were no significant association between the age, duration of infertility and job with CMV infection.

Our results showed a significant correlation between CMV IgG and IgM results with the zero sperm count (azoospermia) results. This finding was not in agreement with the reports by Eggert-Kruse W *et al.* (2009), where his study revealed that, no significant association between CMV and azoospermia. Also, this study showed that, there was significant association between CMV IgG and IgM results with low sperm count (Oligozoospermia). This

finding agreed with previous study done by Naumenko, et al. (2014), which they found that, CMV infection was associated with a reduced sperm cell count. Although our results showed a significant association between CMV IgG and IgM results with abnormal sperm motility (Asthenozoospermia) and morphology (Teratozoospermia). This was inconsistent with study conducted by (Kapranos, et al. 2003; Habibi, et al. 2014; Baghdadi, et al. 2016), they reported that, the infection with CMV did not show any association with poor sperm motility or abnormal sperm morphology. The disparity of results reported by different studies might be due to the differences in testing techniques applied and the study populations.

Conclusion:

Findings of the present study indicate that, a high percentage of CMV antibody among infertile men was enrolled. Also, the study concluded that, CMV may play an important role as one of the causes of infertility in the study group.

Recommendations:-

Further studies should be done with larger sample size which is critical for better result. Further studies are needed to detect CMV in semen of infertile male using sensitive and specific methods like nested-PCR technique, a strong molecular technique for identifying viral DNA and detect the possibility of infertility caused by the virus. Performing routine initial screening test for the virus, to reduce the subsequent risks associated with the virus. Further studies are needed to investigate the relation between CMV in semen of infertile male and its effect on fertility hormones.

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