

Serofrequency of Cytomegalovirus infection in women with Bad Obstetric History attending routine antenatal clinic at Omdurman Military Hospital

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Abstract:

Bad obstetric history (BOH) implies for previous unfavorable foetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, still birth, early neonatal death and congenital anomalies. To determine the frequency of cytomegalovirus infection in pregnancy wastage in women at childbearing hood with BOH. This was a case control descriptive prospective study conducted in routine antenatal clinic at Omdurman military hospital, Khartoum, Sudan; A 88 pregnant women with age range from 15 to 45 years were included in the study. Of the total, 35 women were with bad obstetric history (BOH) and 53 women with normal previous pregnancy as control group. All the serum samples collected from the study and control groups were tested for CMV IgM and IgG antibodies by ELISA . Seropositivity of test group was 30 (85.7%), 4 (11.4%) for IgG, IgM respectively, and among the control group it 47(88.7%), 3(5.7%) for IgG and IgM respectively. CMV IgM seroprevalence was higher in women

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with bad obstetric history while CMV IgG seroprevalence was with no significant difference between bad obstetric history and control.

Key words: cytomegalovirus, IgM-IgG, bad obstetric history, pregnancy, ELISA, Khartoum, Sudan.

Introduction:

Viral infections during pregnancy carry a risk for intrauterine transmission which may result in fetal damage. Bad obstetric history (BOH) implies for previous unfavorable foetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, still birth, early neonatal death and congenital anomalies. There might be different causes of BOH like genetic, hormonal, abnormal maternal immune response and maternal infection. ⁽¹⁾ Primary infections caused by the TORCH complex (also known as STORCH, TORCHES or the TORCH infections) – Toxoplasma gondii , rubella virus, cytomegalovirus (CMV) and herpes simplex virus (HSV) are the major cause of BOH ⁽²⁾. They are a group of viral, bacterial, and protozoan infections that gain access to the fetal bloodstream transplacentally via the chorionic villi. ⁽²⁾

CMV is the most frequent cause of congenital infection in humans. About 10 to 20 percent of infected infants may suffer sensor neural hearing loss, ocular damage or impairment of cognitive and motor function. ⁽³⁾ The common modes of infection of this DNA virus are through saliva (kissing), urine, stool, breast milk and unscreened blood transmission. For most healthy people who acquire CMV infection after birth or through blood transfusion, there are few symptoms and no long term sequel. Therefore for the vast majority of individuals, CMV infection is innocuous. However, foetal damage is more likely to be severe when maternal infection occurs early in

pregnancy because of their immunocompromised state and risk of infection to the fetus whose immune system is not fully developed. ⁽⁴⁾ Not all maternal infections result in fetal transmission and damage. Only 35 to 50 % of maternal primary infections and 0.2-2% of the secondary infections lead to fetal infection, out of which only 5-15% in primary infection and about 1% in secondary infections are clinically affected.

The seroprevalence of CMV among women of childbearing age ranges from 30% to 90% in different countries especially in developing countries with lower socioeconomic conditions. ⁽⁵⁾ Since the prevalence of congenital infection varies with the prevalence of infection in population, the need to determine the seroprevalence of CMV antibody in pregnant women cannot be overemphasized. Irrespective of the number of babies affected, CMV embryopathy (sensory neural hearing loss, chore retinitis, mental retardation and fetal death) should be a major concern for public health. Screening of pregnant mother is necessary to avoid the transmission of CMV. Hence we aim to determine the possible involvement of CMV determine the possible involvement of CMV infection by measuring seroprevalence of this viral infection among pregnant women with BOH.

Cytomegalovirus (from the Greek *cyto*-, "cell", and *megalo*-, "large") is a viral genus of the viral family known as *Herpesviridae* or herpes viruses. It is typically abbreviated as CMV.

The species that infects humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV-5), and is the most studied of all cytomegaloviruses.⁽⁶⁾ Within *Herpesviridae*, CMV belongs to the *Betaherpesvirinae* subfamily, which also includes the genera *Muromegalovirus* and *Roseolovirus* (*HHV-6 and HHV-7*).⁽⁷⁾ It is related to other herpes viruses within the subfamilies of *Alphaherpesvirinae* that includes herpes simplex viruses (HSV)-1 and -2 and

varicella-zoster virus (VZV), and the *Gammaherpesvirinae* subfamily that includes Epstein–Barr virus.⁽⁸⁾

All herpes viruses share a characteristic ability to remain latent within the body over long periods. Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands in humans and other mammals.⁽⁹⁾ Other CMV viruses are found in several mammal species, but species isolated from animals differ from HCMV in terms of genomic structure, and have not been reported to cause human disease.

HCMV is found throughout all geographic locations and socioeconomic groups, and infects between 50% and 80% of adults in the United States (>90% worldwide ⁽¹⁰⁾) as indicated by the presence of antibodies in a majority of the general population.⁽²⁾ Seroprevalence is age-dependent: 58.9% of individuals aged 6 and older are infected with CMV while 90.8% of individuals aged 80 and older are positive for HCMV.⁽¹¹⁾ HCMV infection during pregnancy results in transmission to a developing fetus. Between 0.2% and 2% of newborns are infected in studies that have been carried out worldwide. HCMV infection occurs earlier in life and is more widespread in developing countries and, in developed countries, in communities with lower socioeconomic status. HCMV represents the most significant infectious cause of birth defects in industrialized countries. Congenital HCMV "infection is the leading infectious cause of deafness, learning disabilities, and mental retardation in children. ⁽¹²⁾ CMV infection may also "have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality."⁽¹³⁾

Materials and methods:

This was a descriptive cross control study which had been conducted in Khartoum state during period from January to

February 2015; 88 pregnant women with age range from 15 to 45 were included in the study. Of the total, 35 women were with bad obstetric history (BOH) and 53 women with normal previous pregnancy as control group, data was collected by using direct interviewing questionnaire, ethical clearance was obtained from research ethical committee of Faculty of Graduate Studies Al-Neelain University and Ministry of Health Khartoum State, written consent also was obtained from pregnant women.

Experimental work and specimen collection:

Blood samples were collected from 88 females , under direct medical supervision by medical vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 minutes, serums was kept in -20°C till serological study was performed, Specimens were processed by ELISA (4th generation ELISA) (fortress-diagnostics limited, United Kingdom) for detection cmv IgM, IgG antibodies.

All reagents and samples were allowed to reach room temperature for 15 minutes before use, washing buffer was prepared 1:20 from buffer concentrate with distilled water, 20µl of sample diluents was added into appropriate wells except the blank well and negative well, 100µl from each sample was added to the appropriate wells and mixed by tapping the plate gently, 100µl from negative and positive control was dispense and added to the negative and positive wells separately without dispensing liquid into the blank control well, then plate was covered and incubated for 60 minutes at 37°C, plate cover was removed and discarded, each well washed 5 times with diluted wash buffer each time (washing 1), 50µl of Horseradish Peroxides (HRP)-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 30 minutes at 37°C.

The plate cover was removed and discarded, the liquid was aspirated and each well was rinsed in wash buffer (washing 2), this step was repeated for 5 times until each well became dry, 50µl of chromogen A and 50µl of chromogen B solution were added in to each well including the blank and mixed by tapping the plate gently, the plate was incubated at 37°C for 15 minutes, 50 µl of Stop solution was added into each well and mixed gently, blue color developed in a positive control and cmv antibodies positive samples wells.

Measuring the absorbance:

The plate reader was calibrated with blank well and the absorbance was read with micro well reader at 450 nm, the results were calculated by relating each sample optical density (OD) value to the Cut off value of plate.

Calculation of Cut off (C.O) value: $C.O = *Nc * 2.1$

*Nc = the mean absorbance value for the three negative controls.

Interpretation of results:

Negative results: samples giving absorbance less than Cut-off value are negative for this assay.

Positive results: samples giving absorbance equal to or greater than Cut-off value considered initially reactive.

Borderline: samples with absorbance $O.D. \leq \text{Cut-off} * 2$ are considered borderline and retesting of those samples in duplicates is recommended.

Data analysis:

Data was analyzed by SPSS (Statistical Package of Social Science) software program version 13.

Result:

A total of 88 pregnant women attending Omdurman Military Hospital with age range from 15 to 45 years (the mean age is 25.7) were enrolled to the study to detect the Serofrequency of Cytomegalovirus infection. Out of them, 35 were with bad obstetric history (the tested group) among them 30 (85.7%) and 4 (11.4%) were seropositive for CMV IgG and IgM, respectively. While among 53 pregnant women (the control group) were with normal pregnancy history out of them 47(88.7%) and 3(5.7%) were seropositive CMV IgG and IgM, respectively. Statistical analysis showed significant relation between bad obstetric history and CMV IgM infection (P value 0.03) and insignificant relation among CMV IgG (P value 0.45) tables (1,2).

Most of the pregnant women aged from 25-34 years old (62\88,70.45%), and most of positive results was also observed among this age group, (Table-3).

Among the total population 53 pregnant women (the control group) were with no previous history of abortion, intrauterine foetal death, intrauterine growth retardation, still birth, early neonatal death and congenital anomalies. And among 35 pregnant women (the test group) all of them had history of abortion once, twice, three times (table 4, 5), (3\35\8.6%) had still birth history, and all of them had no congenital anomalies, intrauterine fetal death, and intrauterine growth retardation.

Table (1) Serofrequency of IgM among pregnant women with BOH and control group

	IgM CMV		Total
	Positive	Negative	
Test	4 (11.4%)	31(88,6%)	35
Control	3(5.6%)	50(94.3%)	53
Total	7	81	88

P. value = 0.03

Table (2) Serofrequency of IgG among pregnant women with BOH and control group

	CMV IgG		Total
	Positive	Negative	
Test	30(85.7%)	5(14.3%)	35
Control	47(88.7%)	6(11.3)	53
Total	77	11	88

P. value = 0.45

Table (3) Serofrequency of CMV among study population (n=88) in relation to their age

Age range	Frequency	IgM + ve	IgG +ve
35-45 years	10 (11.1 %)	1	13
25-34 years	62(70.45%)	5	47
15-24 years	16(17.8%)	1	17
Total	88(100%)	7	77

Table (4) Cross-tab between number of abortion & IgM results among the TEST group

Number of abortion	IgM		Total
	Positive	Negative	
once	2	20	22
twice	2	7	9
three	0	4	4
Total	4	31	35

P. value = 0.379

Table (5) Cross-tab between number of abortion & IgG results among the TEST group

Number of abortion	IgG		Total
	Positive	Negative	
once	17	5	22
twice	9	0	9
three	4	0	4
Total	30	5	35

P. value = 0.077

Discussion:

Infection with cytomegalovirus can be disastrous in early gestation. The virus may affect all organs and cause a variety of congenital defects. Infection may lead to intra uterine death,

spontaneous abortion, or preterm delivery. Infection with cytomegalovirus is initially unapparent and asymptomatic and it is difficult to diagnose on clinical grounds ⁽¹³⁾.

The present study shows high CMV IgG overall seroprevalence (90.0%), with no significant differences between rate in women with BOH and that with normal pregnancy. While it shows a low CMV IgM overall seroprevalence 46.2 %.

The result revealed seropositivity of test group 30 (85.7%),4 (11.4%) and control group 47(88.7%) 3(5.7%) for IgG, IgM respectively, these findings were similar to that obtained in Kenya CMV IgG 77.3% and IgM 8.1% ⁽¹⁴⁾. Contrary to previous studies conducted in Africa, higher rates have been reported, in Benin (97.2%) ⁽¹⁵⁾ , Egypt (96%) ⁽¹⁶⁾, Gambia (87%) ⁽¹⁷⁾ , South Africa (86.4%) ⁽¹⁸⁾, Nigeria (100%), ⁽¹⁹⁾, 87% ⁽¹⁷⁾ Dares Salaam, Tanzania (6) and also in South East Asia ⁽²⁰⁾. However, in some of European countries, low CMV infection rates have been reported, Australia (56.9%) and France (46.8%) ⁽²¹⁾. The low prevalence rates could be due to the inclusion of CMV screening among the antenatal profile tests and better hygienic standards ⁽²²⁾. The low prevalence rates of CMV in this study compared to the rest of the studies in African countries, could be due to diverse HIV infections (which is an important confounders with CMV) ⁽²³⁾, diverse socio-demographics, diverse cultures, population behavior, child cares, breast feeding and sexual activity ⁽²⁴⁾.

Conclusion:

This study shows seroprevalence of test group 30 (85.7%),4 (11.4%) and control group 47(88.7%) 3(5.7%) for IgG, IgM respectively, similarly to those obtain from other countries with those married aged and with high parity being at a high risk to CMV infections. This study concurs with previous studies that have suggested all women of the child bearing age to be incorporated in routine antenatal screening profile.

Recommendations:

Diagnosis of primary maternal cytomegalovirus (CMV) infection in pregnancy should be based on de-novo appearance of virus-specific IgG in the serum of a pregnant woman who was previously seronegative, or on detection of specific IgM antibody associated with low IgG avidity.

Further recent confirmatory techniques like Western blot, Southern blot and Polymerase Chain Reaction (PCR) should be considered.

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