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Original Research Article

Studies on the prevalence of cytomegalovirus (IgM) antibodies among pregnant women attending ante-natal clinic at a specialist hospital North Eastern Nigeria

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Abstract: Human cytomegalovirus (CMV) is a leading cause of congenital infections worldwide Women infected for the first time during pregnancy are especially likely to transmit CMV to their fetuses and has been proposed as a risk factor for preterm birth. The seroprevalence of CMV in adults and the incidence of congenital CMV infection are highest in developing countries (1 to 5% of births) 90% of infected infants are asymptomatic at birth and are not recognized as at risk for CMV-associated infection. This study was carried out to determine the prevalence rate of CMV infection among pregnant women attending an antenatal clinic. In this study, Two-hundred and eighty eight (288) pregnant women were enrolled. Questionnaires were issued to volunteer subjects after due consent was sought, to determine demographic and other relevant data. 5mls of blood was collected by venous puncture from the antecubital fossa and dispensed into plain containers; sera were collected after centrifugation of the blood. Sera obtained were screened for the presence of CMV (IgM) antibodies using ELISA technique (Clinotec Laboratories Canada).Result showed that out of the 288 women tested, 54 (18.8%) were positive for CMV antibodies while 234 (81.2%) tested negative. With regards to age group distribution, women within 15 – 20 years had a prevalence rate of 4.5%, 21 – 30 years both had 3.5%, 31 – 35 had 3.1%, 36 – 40 years had 1.3% while 41 – 45 years had a prevalence rate of 2.8%, all the age groups had no statistical significant ($P>0.05$) result. With regards to trimester of the volunteer subjects screened, 1st, 2nd and 3rd trimester subjects recorded a prevalence rate of 3.1%, 9.4% and 6.2% respectively which was statistically significant ($P<0.05$).Occupational status of subjects studied recorded no statistical significant ($P>0.05$) result obtained showed that unemployed subjects recorded a prevalence of 5.6% compared to the self-employed with 4.9%, farmers recorded 3.8%, while students had a prevalence of 1.7% however subjects who are civil servants recorded a prevalence of 2.8%. Location of volunteer subjects studied showed that pregnant in rural areas had a prevalence rate of 12.2% while those living in urban areas recorded 6.6% prevalence without any statistical significant ($P>0.05$).Records from this study indicates the of presence of CMV (Igm) antibodies amongst the subjects screened. Hence the need for early detection of the virus in pregnant women.
Keywords: Cytomegalovirus, Antibodies, Pregnant Women

INTRODUCTION

CMV is a host-restricted member of the Herpesviridae family of viruses [1]. Primary infection is characterized by a period of active virus replication with virus shedding in saliva, urine, milk, and genital secretions, a viremic phase, and, in some, an infectious mononucleosis syndrome [1, 2]. This is followed by the development of a broad immune response involving all arms of the adaptive immune system, and after several weeks, viral latency is established[1].The disease is usually asymptomatic, and is found universally throughout all geographic locations and socio-economic groups although it is more common in developing countries and areas of lower socio-economic conditions [3]. In immunocompetent mothers, reactivation of endogenous virus and/or reinfection with new strains occurs periodically, and DNAemia and viruria may be present in both [2] Indeed, CMV causes more cases of congenital disease than the combination of 29 currently screened conditions in most American states [4] and is more common than several disorders included in newborn screening in European Union countries [5].

CMV can be shed in various bodily secretions, particularly urine and saliva [6]. CMV is transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation [6]. For pregnant women, important sources of infection include sexual activity and contact with the urine or saliva of young children, especially their own children [7].Intrauterine CMV infection occurs in approximately 1% of all live births, with up to 15% of congenitally infected infants showing symptoms at birth [8,9,10]. These symptoms include any combination of microcephaly, intracranial calcification, chrioretinitis, jaundice, low birth weight, hepatosplenomegaly and purpura [11]. The mortality rate among symptomatic infants can be as high as 30%; these symptomatic infants who survive are likely to develop long-term neurologic sequelae including hearing loss, visual impairment, psychomotor delay and mental retardation [11].

The risk for long-term outcomes appears to be highest in infants born to mothers with primary infection in the first half of pregnancy [12, 13]. Following first-trimester maternal CMV infections, about a quarter of infants (20 to 25%) who are congenitally infected will develop sensor neural hearing loss (SNHL), and 30 to 35% will suffer some form of central nervous system (CNS) sequelae [13]. However, maternal CMV reactivation or reinfection with a different CMV strain can also lead to fetal infection [14]. Approximately 10 percent of congenitally infected infants are symptomatic at birth, and of the 90 percent who are asymptomatic, 10–15 percent will develop symptoms over months or even years [14].Recent studies report lower transmission rates in early pregnancy (in comparison to later gestation)[15,16], with maternal primary infection leading to infection in 30 to 35% of fetuses and nonprimary infection having a transmission rate of 1.4% in study populations predominantly from industrialized countries (1.1 to 1.7%) [17]. Data from screened populations indicate that while only one in 10 newborns infected in utero have obvious clinical signs of congenital infection [18,19] 10% to 15% of those without clinical findings (here referred to as having symptomatic and asymptomatic congenital CMV infection, respectively) develop long-term neurological sequelae [19].Although, exposure to young children and sexual activities have been linked with increased risk of CMV, it is unlikely that avoidance of either of these activities will be a practical means of preventing CMV infection for women of childbearing age. Maternal (prenatal) screening may permit early identification of at-risk pregnancies or infected infants and thus the use of interventions to reduce morbidity has attracted increasing interest in recent years [20]. The majority of congenital CMV infections are asymptomatic at birth, and the diagnosis of intrauterine infection relies on virus detection by culture-based methods or PCR. Saliva or urine specimens should be obtained within the first 2 weeks of life [21]. Studies have revealed that Cytomegalovirus is found throughout all geographic locations and infect between 50% and 80% of adults in the United States as indicated by the presence of antibodies in much of the general populations [22].In Nigeria, a study conducted in 2008, reported a prevalence of 45.0% and 33% IgM antibodies among breastfeeding mothers and of the infants [23]. Similarly, Okwori *et al.;* in a study among expectant mothers in Bida, Nigeria, reported IgG antibodies prevalence of 86.1% among multigravid women and 77.1% among primigravid women [24].

MATERIALS AND METHOD

Study design

The study was a cross sectional study which lasted for three months. Data was collected from consenting volunteers after obtaining due ethical permits from the relevant bodies.

Study area and study Population

The study was carried out among pregnant women attending antenatal clinic at Specialist Hospital Gombe. These were the inclusive criteria while non-pregnant women were excluded from the study. The population used for this study was two hundred and eighty eight (288) pregnant women whose ages range between 15 and 45 years.

Sample collection and processing

A well-structured questionnaire was used to obtain bio data and risk factors from the pregnant women screened. Five (5mls) of blood was collected aseptically by venipuncture from the volunteer subjects according to the method of [25].Sera obtained were separated, dispensed into a clean container and stored at -20^0C until they were ready for the assay.

Test methodology

Sera samples were screened for the presence of CMV IgM antibodies using Enzyme linked immunosorbent Assay (ELISA) Diagnostic Antigen kit by Clinotec Laboratories.

Principles

Cytomegalovirus antigens are fixed to the interior surface of microwells, patient's serum is added and any antibody present to CMV will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with Horse radish peroxidasae enzyme are directed against human 1gM are added and will in turn bind to any human 1gM present. The microwells are washed to remove unbound conjugate and then chromogen/substrate is added. In the presence of peroxidase enzyme, the colourless substrate is

hydrolyzed to a coloured end product. The colour intensity is proportional to the amount of antibodies present in the patient's serum.

PREPARATION FOR ASSAY

All reagents were brought to room temperature and gently mixed. The wash buffer was diluted (1:30) with distilled water and mixed well.

Assay Procedure:

A 1:51 dilution of sample was done by adding 5µl of test sample to 250µl of sample diluents into separate tubes. Using a multichannel pipette 100µl of prediluted negative control, positive control, calibrated (prediluted by the manufacturer) and each diluted sample was transferred from the tubes to the wells. The wells were covered and incubated at 37^{o}C for 30 minutes. The wells were vigorously shaken to move out liquid and each well were washed 5 times with 250 - 300µl diluted wash buffer 100µl of Horse Radish Peroxidase (HRP) conjugate was added to each well and incubated for 30minutes at 37^{o}C. The wells were washed 5 times again with 250 - 300µl diluted wash buffer after removing excess liquid and 100µl of TMB, substrate solution was added to each well and incubated for 10minutes at room temperature. 100µl of stop solution was added to each well and gently shaken; the absorbance of each well was read at a wavelength of 540nm with the aid of an Elisa technique assay.

RESULTS

Out of the 288 sera samples screened, 54 (18.8%) tested positive while 234 (81.2%) tested negative. Table 1. The distribution of human CMV with respect to age group showed that women within the age group of 15 – 20 years had the highest prevalence rate of 13(4.5%) followed by those within the age range of 21 -25 years with 109(3.5%) prevalence. Subjects within 26-30 years of age recorded a prevalence of 10(3.5%). Women within 31 – 35 years had 9(3.1%).While those within 36-40 years of age recorded a prevalence of 4(1.3%) as compared to subjects aged 41 – 45years with a prevalence of 1.4%. Table 2

Table 1: Overall result of HCMV (Igm) Screening

No. of Samples	No. Positive	% Positive	No. Negative	% Negative	P- value
288	54	18.8%	234	81.2%	0.317(P>0.05)
Total	**288**	**54**	**18.8%**	**81.2%**	

Table 2: Distribution of HCMV based on age group (years)

Age group	No of samples	No Positive	% Positive	P-value
15 – 20	59	13	4.5%	0.145(P>0.05)
21 – 25	73	10	3.5%	0.145(P>0.05)
26 – 30	68	10	3.5%	0.279(P>0.05)
31 – 35	52	9	3.1%	0.698(P>0.05)
36 – 40	24	4	1.3%	0.560(P>0.05)
41 – 45	12	8	2.8%	0.060(P>0.05
Total	**288**	**54**	**18.8%**	

Distribution in relation to gestational age of the women showed that those in their second (2nd) trimester recorded the highest seroprevalence of 27(9.4%) followed by those in their third (3rd) trimester with 18(6.2%) and lastly those in their first (1st) trimester with 9(3.1%). Table 3.

Table 3: Distribution of HCMV with respect to gestational Age of the women

Trimester	No of samples	No. Positive	% Positive	P-value
1st Trimester	23	9	3.1%	0.016(P<0.05)
2nd Trimester	149	27	9.4%	0.016(P<0.05)
3rd Trimester	116	18	6.2%	0.008(P<0.05)
Total	**288**	**54**	**18.8%**	

Distribution based on occupational status of the women showed that those who are unemployed had the highest prevalence of 16(5.6) followed by those who are self-employed with 14(4.9%). Civil servants had 8(2.8%), farmers 11(3.8%) and students recorded the lowest prevalence of 5(1.7%). Table 4.

Table 4: Distribution of HCMV in relation to occupation

Occupation	No of samples	No Positive	% Positive	P-value
Civil Servant	81	8	2.8%	0.666(P>0.05)
Students	29	5		0.666(P>0.05)
Farmers	27	11		0.066(P>0.05)
Self employed	68	14		0.277(P>0.05)
Unemployed	83	16		0.352(P>0.05)
Total	**288**	**54**	**18.8%**	

Women who reside in rural areas had a prevalence rate of 35(12.2%) compared to a prevalence rate of 19(6.6%) in those who reside in urban areas. Table 5

Table 5: Distribution of HCMV in relation to geographical location

Geographical Location	No of samples	No Positive	% Positive	P-value
Urban	201	19	6.6%	0.718(P>0.05)
Rural	87	35	12.2%	0.259(P>0.05)
Total	**288**	**54**	**18.8%**	

DISCUSSION

From the results obtained in this study, prevalence rate showed that over 18% of the women tested were seropositive for CMV (IgM) antibodies while 81% were seronegative. The result obtained in this study differs from work of Munro, *et al.;* [26] who recorded a low prevalence rate of 5.5% among pregnant women in Australia. Arakpour, *et al.;* [27] equally recorded a low prevalence rate of 5.4% in women of childbearing age in a study conducted in Iran while Kassim, *et al.;* [28] recorded a higher prevalence of 45% among Nigerian mothers.

The difference in prevalence rates could be attributed to differences in geographical locations, socio-economic status and strata of the women at various locations where the studies/research was carried out [29]. In a similar studies carried out in Kaduna State by Edward *et al.;* [30] a prevalence of 10.5% was recorded which is lower than what obtains in this study.

From this finding a high prevalence was observed among all the age groups. However, the low prevalence rates recorded among those aged 36 – 45 agrees with the fact that the rate of CMV (Igm) prevalence decreases with advance in age [31]. similarly in the work of Ndako *et al.;* [32] Age variation showed the highest prevalence rate of 14.7% among pregnant women aged of 20-34 years (χ^2 = 1.333, P>0.05).Moreso, the increase in seroprevalence with age as adduced in other studies, is due to the fact that majority of the women have already been exposed and recovered from primary infection by the time they reach childbearing age

The high prevalence rates seen among subjects aged, 15 – 30 years could also be attributed to the fact that subjects within this age group are among the sexually active age hence the likelihood of being infected through the sexually transmitted route [32].This also agrees with the work of [33] that the prevalence of CMV infection is higher among women attending clinics for sexually transmitted diseases and also among sexually active adolescents.

Our findings showed that subjects in their second trimester of pregnancy recorded the highest prevalence followed by those within the third trimester and lastly those in first trimester. However the result obtained from all trimesters are statistically significant $\chi 2=0.016$;($P<0.05$).The result obtained in this study with regards to gestational period agrees with the work of Okwori *et al.;* [34] where Pregnant women in their second trimester showed the highest seroprevalence (86.2%) of Cytomegalovirus antibodies followed by subjects in their third trimester with 75.9% prevalence [24]. This result from our study is however is in contrast with the result obtained by [30] where women at their third trimester have the highest prevalence of 12.5% followed by the first and second trimester with

prevalence of 11.1% and 9.7% respectively (P>0.05) Women at all stages of pregnancy could be at high risk of intrauterine transmission but those at higher risk are those who were infected within the first 20 weeks of pregnancy [34, 35]. Babies born to these women in their second trimester are at risk of getting congenital CMV infection [36].

This poses a serious threat to the foetus in utero as stated in the report of [36] that women infected with CMV during late gestation are more likely to transmit the virus to their unborn babies compared to women who are infected at early gestation. Similarly, high rates observed amongst subjects at the second and third trimesters could be as a result of advancement in foetal age making such women heavier and careless to personal hygiene thereby predisposing them to infection and a high risk of intrauterine transmission [37].

Occupational distribution of subjects showed that there was no association between maternal immunity and social class. The rate of prevalence seen in all the groups were not statistically significant (P>0.05) but high percentages seen in the unemployed, farmers and self employed could be due to the fact that CMV infection is most likely acquired among those at the lower socio-economic strata in developing countries (Jawetz, et al 2007).This finding agrees with a previous study, which demonstrated that CMV infection was higher in the lower socioeconomic class [38, 39].

High prevalence rate was recorded among women living in rural areas compared to those living in urban areas, though the prevalence were not statistically significant (P>0.05), it is however in accordance with the work documented by [3, 29] that although prevalence of CMV infection increases within every group, the overall prevalence of infection and the age of initial acquisition of the virus varies greatly according to living environment of the individuals. Antibody prevalence may be moderate in 40 – 70% of adults found among the high socio-economic groups in developed countries in contrast to a prevalence rate of 90% in children and adults living in underdeveloped nations and in low socio-economic groups

The greatest risk connected to CMV is the probability of congenital defects. Congenital infection occurs in foetus if the mother has a primary infection or reactivation during pregnancy. A Clinical symptom of this takes the form of severe generalized or cytomegalic inclusion disease in which the infants usually have jaundice, hepatosplenomegaly, thrombocytopenia, haemolytic anaemia. The brain is almost always involved with microcephaly and motor disorder. Most infants with these symptoms do not survive. Infants are usually deaf and mentally retarded [40].

CONCLUSION

In conclusion, reports obtained from this work showed the presence of CMV (IgM) antibodies amongst the pregnant women screened, thereby indicating a current infection and likelihood of transmission in utero. Since the seroprevalence of CMV infection increases with lower socio-economic status of individual subjects coupled with the risk of intrauterine transmission with advance in the gestational age of pregnant women, thereby making the tendency of transmitting the virus in utero high, calls for an urgent need for women of childbearing age to be early diagnosed. Since the resultant congenital infection could be asymptomatic or symptomatic. Asymptomatic infants serve as a source of infection to other children and those handling them could still develop clinical sequelae later in life. This calls for closer monitoring among this group as they grow, so as to decrease the rate of transmission and infection within the population.

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