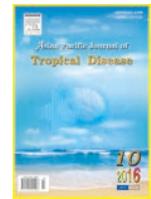




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Microbiological research

doi: 10.1016/S2222-1808(16)61137-4

©2016 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Detection of primary CMV infection in Sudanese pregnant women by IgG avidity test

Mohammed Abubaker Altayeb¹, Shamsaldin Ibrahim Mokhtar¹, Mona Eltahir Adam¹, Salahaldeen Ismail Mohammed², Hassan Hussein Musa^{1*}

¹Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum State, Sudan

²Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Omdurman, Sudan

ARTICLE INFO

Article history:

Received 11 Jul 2016

Received in revised form 21 Jul, 2nd revised form 8 Aug 2016

Accepted 14 Aug 2016

Available online 17 Aug 2016

Keywords:

CMV

Avidity index

Pregnant women

Sudan

ABSTRACT

Objective: To diagnose primary cytomegalovirus (CMV) infection in pregnant women by determining CMV immunoglobulin G (IgG) avidity index.

Methods: Ninety pregnant women with an average age of 23 years were studied. Their demographic data were obtained along with blood samples. The anti-CMV immunoglobulin M (IgM) antibodies were determined on fully automated immunoanalyzer, while the CMV IgG avidity testing was carried out using avidity ELISA assay.

Results: Among the pregnant women, 15.6% were in the first trimester, 30.0% in the second, and 54.4% in the third trimesters. Besides, 40.0% had chronic disease, while the other 60.0% did not. Anti-CMV IgM antibodies and CMV IgG avidity test showed that 1.1% of the pregnant women were CMV IgM positive and 98.9% were CMV IgG positive, respectively. The avidity test revealed the presence of low avidity of CMV IgG antibodies in 1.1% cases while 98.9% cases exhibited high avidity of CMV IgG antibodies.

Conclusions: CMV IgG avidity test was important to distinguish between CMV recent and past infection rather than relying on IgG and IgM only, and IgM is not always indicative for recent or primary CMV infection.

1. Introduction

Human cytomegalovirus (CMV) causes congenital malformation that commonly infects individuals from diverse geographical and socio-economic backgrounds[1]. The seroprevalence of human CMV is high in developing countries and those of lower socio-economic status in developed countries[2]. Although most CMV infections are asymptomatic, some patients are at risk of developing serious illnesses and long-term sequelae. Its clinical manifestations include asymptomatic forms, severe fetal damage and death in rare cases due to spontaneous abortion. Primary CMV infection occurs in 0.15%–2.00% of all pregnancies and may be transmitted to the fetus in 40% cases[3]. Therefore, discrimination between recent primary and past CMV infection may play a role in the clinical management of pregnant women[4].

In the absence of acute clinical manifestations, the diagnosis of CMV infection is usually done by the detection of specific CMV

immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies. Antibody avidity is an indirect measure of the tightness of the antibody binding to its target antigen, and it increases in the first week after a primary infection. Low-avidity IgG antibodies to CMV persist 20 weeks after a primary CMV infection, and then are replaced by high-avidity antibodies[5]. The combination of the presence of anti-CMV IgM antibodies and low-avidity anti-CMV IgG antibodies with maternal or fetal symptoms is used for the diagnosis of a primary maternal infection[6].

A previous study showed that 56.8% of women were CMV IgG positive at pregnancy[7]. In Sudan, the age was significantly associated with CMV IgM and history of miscarriage was associated with CMV IgG positive women, while parity, congenital abnormalities, educational level and occupation were not significantly associated with CMV infection ($P > 0.05$)[8]. Studies on the prevalence of primary maternal CMV infection were few in Sudan due to the absence of an accessible method to identify primary CMV infection. The aim of this study was to detect the seroprevalence of CMV antibodies among Sudanese pregnant women using avidity test.

2. Materials and methods

2.1. Study population

A cross-sectional study was conducted on 90 pregnant women

*Corresponding author: Hassan Hussein Musa, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum State, Sudan.

Tel: 00249-906547116

E-mail: hassanhm@uofk.edu

The study protocol was performed according to the Helsinki declaration and approved by the ethical board of the Ministry of Health. A verbal consent was obtained from all participants.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

from Soba University Hospital and Omdurman Maternity Hospital in Sudan. The ethical approval was obtained from the ethical board of the Ministry of Health and a verbal consent was obtained from all participants. Demographic data were collected in a predesigned sheet, and blood samples were collected in ethylene diamine tetra acetic acid containers.

2.2. Anti-CMV IgM antibody test

Anti-CMV IgM antibodies were tested for each samples on a fully automated immunoanalyzer (Roche, Cobas e411 immunoanalyzer) utilizing micro capture assay to detect the specific target IgM by means of biotinylated anti-human IgM antibodies, with the target CMV IgM interacting with ruthenylated recombinant CMV (pp150, p52) antigens, streptavidin coated paramagnetic micro particles were subsequently form stable immune complex. The reagent mixture was then transferred to a measuring cell, where the micro particles were bound to the electrode surface by magnetic reaction. The unbound substances were subsequently removed. Luminescence was then induced by applying a voltage and measured with a photomultiplier. The signal yielded depended on the properties of the antibodies in the sample.

2.3. CMV IgG avidity test

The avidity index is a ratio of the relative fluorescence value obtained for the sample with strip containing urea buffer to those without urea buffer. The IgG avidity test for CMV was performed using kits (Euro immune, CMV IgG avidity testing) which measured the avidity utilizing urea as a denaturing agent. The test was performed according to manufactures instructions. Each diluted sample or control was pipetted into two adjacent wells and incubated for 30 min, followed by washing for only one time, then each sample or control was treated with 200 μ L of urea in one well and 200 μ L of buffer into the parallel well and then incubated for 10 min and washed three times. Samples were incubated for 30 min by anti-human IgG antibody enzyme-conjugate, and 15 min by chromogen substrate solution, and then the reaction stopped by stop solution. Photometric measurement of the color reaction was made at 450 nm and a reference at 620 nm wavelength. The avidity index for each sample or control was calculated using the following formula:

Relative avidity index (RAI) (%) = Extinction of sample with urea treatment/Extinction of sample without urea \times 100

The results were interpreted according to the manufacture in structure as follow: RAI < 40%, indicated low avidity antibodies, RAI 40%–60% indicated gray zone and RAI > 60% indicated high avidity antibodies.

2.4. Data analysis

Data was analyzed by SPSS software. The significant of results were determined using the *Chi*-square test, and $P < 0.05$ was considered statistically significant.

3. Results

Ninety pregnant women with an average age of 23 years were

studied. Among them, 55.7% of them were from Omdurman Maternity Hospital and 44.3% were from Soba University Hospital. About 15.6% of the pregnant women were in the first trimester, 30.0% in the second, and 54.4% in the third trimesters. Forty percent of the studied women had chronic diseases, while the other sixty percent did not. The age of the most women was ranged from 14–24 years (Figure 1).

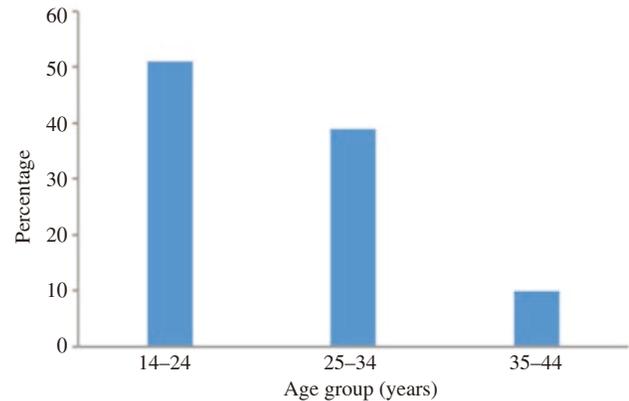


Figure 1. Distribution of age group in the study population.

Anti-CMV IgM antibodies and CMV-IgG avidity were determined, CMV IgM was found positive in only one pregnant woman (1.1%) (Figure 2). While the reference (the buffer-treated wells) CMV IgG was found positive in 89 women (98.9%) (Figure 3). The avidity index calculation revealed the presence of low avidity CMV IgG antibodies in one case (1.1%) while 88 cases (97.8%) exhibited high avidity CMV IgG antibodies and one case was excluded from avidity testing for being sero-negative for CMV (Figure 4).

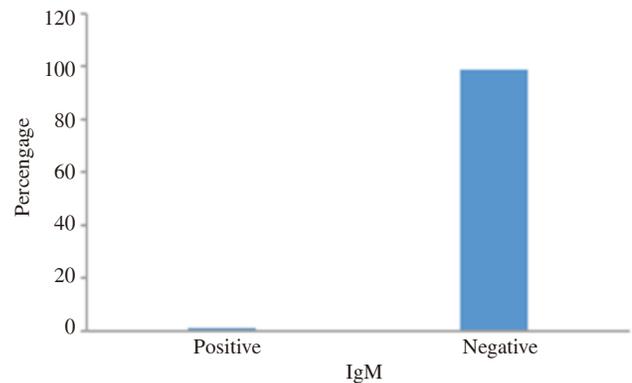


Figure 2. Seroprevalence of IgM in the pregnant women.

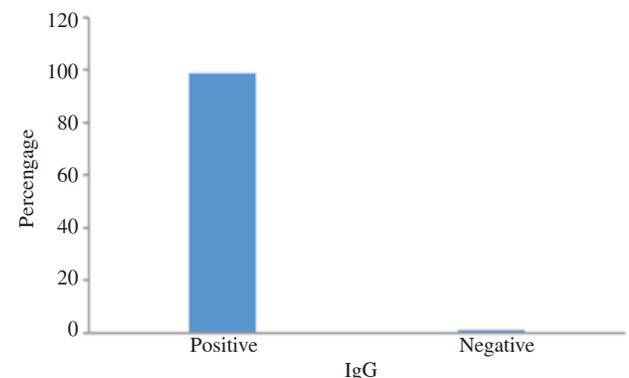


Figure 3. Seroprevalence of IgG in the pregnant women.

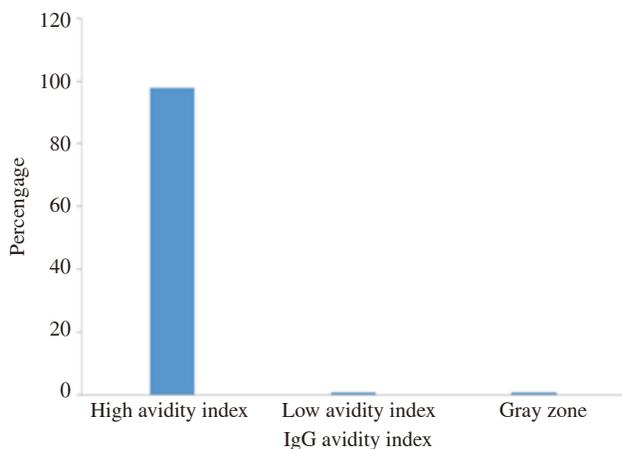


Figure 4. Seroprevalence of IgG avidity index in the pregnant women.

4. Discussion

CMV infection remains of great concern in pregnant women and infants' health settings. The diagnosis of CMV infection is usually done by serology utilizing the conventional approach (CMV-IgG/CMV-IgM). However, CMV-IgG avidity test was a better marker for the diagnosis of CMV infection. The present study revealed a high seroprevalence 98.9% of CMV IgG among pregnant women, while the CMV IgM prevalence was significantly rare. CMV seroprevalence among antenatal women in Sudan was 84.0%[9], and in Western Sudan the prevalence of CMV IgG was 72.2% and CMV IgM was 2.5%[10]. In other studies, the overall CMV IgG seropositivity was 70.7% and the IgM seropositivity was 4.0%[11]. However, Kamel *et al.*[12] stated that 100% of pregnant women enrolled in his study were CMV IgG positive. The differences in percentages may be attributed basically to differences in regions and sample sizes. Previous studies indicated that 50.0% of CMV IgM-positive individuals have primary infection, this is because IgM can be produced during viral reactivation with different CMV strain[13].

The avidity test revealed the presence of low avidity CMV IgG antibodies in 1.1% case while 98.9% cases exhibited high avidity CMV IgG antibodies. Kamel *et al.*[12] found that sera from 40 pregnant women have shown a high or intermediate CMV IgG avidity in accordance with our results in which pregnant women with both IgM and IgG were detected with higher IgG avidity. This conflicting finding indicated that about 50.0% of CMV IgM positive cases may not actually be experiencing new infections. On the other hand, we had one pregnant woman with negative IgM finding revealed significantly low avidity index indicating a quite probability of new infection which would be missed when solely depending on the IgM test, this may be due to the weak IgM response in some individuals. Recent primary infection of CMV was only confirmed in 2 out of 26 IgM-positive women using IgG avidity and the rest 24 IgM-positive women had high avidity index indicating recurrent or past CMV infection[11].

In the present study, the age of the most pregnant women was ranged from 14–24 years, and there was no significant association between CMV infection and age or gestational period. However, the association was found between the maternal age of 25 and congenital CMV infection in a large cross-sectional study[14]. In addition, a significant age association was observed in blood donors, where the rate of CMV IgG seroprevalence has shown an increase age from 34.9% at less than 20 years of age to 72.4% after the age of more than 50 years in an Australian population[7]. Finally, we conclude that the determination of CMV IgG avidity is very important

to distinguish between recent CMV infection and past infection rather than relying on IgG and IgM only, and the IgM is not always indicative for recent or primary CMV infection.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge the pregnant women who participated in the study and the manager of Total Lab Care Laboratories for his support to conduct the practical work.

References

- [1] Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. *Clin Microbiol Rev* 2013; **26**: 86-102.
- [2] Neirukh T, Qaisi A, Saleh N, Rmaileh AA, Zahriyeh EA, Qurei L, et al. Seroprevalence of cytomegalovirus among pregnant women and hospitalized children in Palestine. *BMC Infect Dis* 2013; **13**: 528.
- [3] de Vries JJ, Korver AM, Verkerk PH, Rusman L, Claas EC, Loeber JG, et al. Congenital cytomegalovirus infection in the Netherlands: birth prevalence and risk factors. *J Med Virol* 2011; **83**: 1777-82.
- [4] Carlson A, Norwitz ER, Stiller RJ. Cytomegalovirus infection in pregnancy: should all women be screened. *Rev Obstet Gynecol* 2010; **3**: 172-9.
- [5] Berth M, Grangeot-Keros L, Heskia F, Dugua JM, Vauloup-Fellous C. Analytical issues possibly affecting the performance of commercial human cytomegalovirus IgG avidity assays. *Eur J Clin Microbiol Infect Dis* 2014; **33**(9): 1579-84.
- [6] Adler SP. Primary maternal cytomegalovirus infection during pregnancy: do we have a treatment option. *Clin Infect Dis* 2012; **55**(4): 504-6.
- [7] Walker SP, Palma-Dias R, Wood EM, Shekleton P, Giles ML. Cytomegalovirus in pregnancy: to screen or not to screen. *BMC Pregnancy Childbirth* 2013; **13**: 96.
- [8] Khairi SI, Intisar KS, Enan KH, Ishag MY, Baraa AM, Ali YH. Seroprevalence of cytomegalovirus infection among pregnant women at Omdurman Maternity Hospital, Sudan. *J Med Lab Diag* 2013; **4**(4): 45-9.
- [9] Kafi SK, Eldouma EY, Saeed SBM, Musa HA. Seroprevalence of cytomegalovirus among blood donors and antenatal women attending two hospitals in Khartoum State. *Sudan J Med Sci* 2009; **4**: 399-402.
- [10] Hamdan HZ, Abdelbagi IE, Nasser NM, Adam I. Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. *Virol J* 2011; **8**: 217.
- [11] Nasir IA, Babayo A, Shehu MS. Clinical significance of IgG avidity testing and other considerations in the diagnosis of congenital cytomegalovirus infection: a review update. *Med Sci* 2016; doi:10.3390/medsci4010005.
- [12] Kamel N, Metwally L, Gomaa N, Sayed Ahmed WA, Lotfi M, Younis S. Primary cytomegalovirus infection in pregnant egyptian women confirmed by cytomegalovirus IgG avidity testing. *Med Princ Pract* 2014; **23**(1): 29-33.
- [13] Prince HE, Lapé-Nixon M. Role of cytomegalovirus (CMV) IgG avidity testing in diagnosing primary CMV infection during pregnancy. *Clin Vaccine Immunol* 2014; **21**(10): 1377-84.
- [14] Bonalumi S, Trapanese A, Santamaria A, D'Emidio L, Mobili L. Cytomegalovirus infection in pregnancy: review of the literature. *J Prenat Med* 2011; **5**(1): 1-8.