Original Article

Seroprevalence of cytomegalovirus Antibodies among pregnant women and it's correlation with spontaneous abortion in Khartoum state

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ABSTRACT

Background: Cytomegalovirus is a common virus that infects most people at some time during their lives. It becomes dormant for a while and may reactivate later. In pregnant women, intrauterine infection may be associated with congenital abnormalities, intrauterine growth retardation and intrauterine death of the fetus as well as late sequelae such as developmental delay, blindness and congenital deafness.

Objectives: The aim of this study was to determine the seroprevalence of CMV infection among women presenting with spontaneous abortion to major hospitals in Khartoum State and to find out the correlation between CMV infection and spontaneous abortion in the group under study.

Methodology: In this study a total of 180 spontaneously aborted females, and 80 normally delivered females (control) were included. Three mls of venous blood were collected from each subject under study in a plain container allowed to clot and after clot retraction centrifuged at 4000rpm. The sera were then separated and stored at -20c^0 in a deep freezer. The stored sera were tested for CMV IgG and IgM antibodies using Enzyme Linked Immunosorbent Assay (BIOTECH – ENGLAND).

Results: In the case group, 176 (97.8%) women were positive for Anti-CMV IgG and 69 (38.3%) for Anti-CMV IgM. The CMV antibodies significantly co related with increasing age (P-value = -0.0185), the number of abortion (P-value = -0.0177) and congenital malformation in children (P value= 0.037).

Conclusion: Seroprevalence of CMV antibodies was found to be 97.8% and 38.3% for IgG & IgM respectively. There was significant association between CMV infection and frequency of abortion, age and congenital malformation in children.

Key words: CMV, seroprevalnce, pregnancy, spontaneous abortion, Sudan.

Cytomegalovirus (CMV) causes infection in immunocompromised patients. Intrauterine infections were found to be associated with congenital abnormalities, intrauterine growth retardation and intrauterine death of the fetus, as well as late sequelae such as developmental delay, blindness and deafness of the infected child.

Cytomegalovirus (CMV) infection during pregnancy is far more complex than other infections, due to the ability of the virus to be frequently reactivated during the child bearing age and be transmitted to the fetus in spite of maternal immunity [1].

Pregnant women are physiologically immunocopromized and CMV is a latent virus that can be reactivated during this periods of low immunity and create many types of clinical diseases for the mothers and their babies, ranging from mild to serious complications. It may cause abortion (miscarriage) or stillbirth. Previous studies conducted in London and Iran revealed statistically significant association between CMV infection and Ethnic group and a high

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seroprevalance of CMV IgG antibodies among women with spontaneous abortion [2,3].
A study conducted in Sudan showed a high seroprevalance of CMV IgG antibodies among antenatal women compared to blood donors (95% & 77%, respectively) [4].

Methods:

Specimen collection & processing:

Three mls of venous blood were collected from each subject under study in a plain container allowed to clot and after clot retraction centrifuged at 4000 rpm. The sera were then separated and stored at -20c^0 in a deep freezer.

Laboratory Examination:

The stored sera were tested for CMV IgG and IgM antibodies using ELISA (BIOTECH, UK) as follows:

- The required number of microwell strips (180) were removed from the kit and left at room temperature for 30 minutes before use.
- The washing buffer by diluting the rinsing buffer (100mLs buffer + 900mL H_2O)
- Samples were diluted 1:101 by adding 10 μL of serum into 1mL of the diluents.
- 100 μL of each diluted sample were dispensed into the corresponding wells.
- Undiluted calibrators were added to 5 wells in a strip (100 μL in each well) and one well was left for the blank.
- Strips were covered with protective foil and incubated for 45 minutes at 37^{0}C.
- After incubation washing was performed four times by adding 300 μL of washing buffer to all wells, left for 30 seconds then aspirated.
- 100 μL of conjugate were added to each well and incubated again for 45 minutes at 37^{0}C covering the wells with protective foil.
- The plate was washed again 4 times as described above.
- Finally the substrate was added (100 μL/well) and kept for 15 minutes at room temperature.
- The enzymatic reaction was stopped with 100 μL of stop solution.
- The optical densities (O.D.) were read at 450 nm within 30 min.
- Reading was repeated again at 405 nm if OD was higher than 2,000.

Validation of test:

The test was considered valid and hence accepted when:

- The O.D. of the negative control was ≤ 0.6 times the O.D of calibrator 2.
- The O.D of the calibrator 2 was ≥ 0.2 at 450 nm; ≥ 0.16 at 450/620 nm.
- The O.D of the calibrator 5 was ≥ 1.5

Calculation:

Conversion of the O.D into units/mL: The Anti-CMV antibodies were expressed in EU/mL (arbitrary units) or in IU/mL (proposed anti CMV antibodies WHO standard), by interpolating the results of the 6 calibrators and comparing the O.D of the sample with the curve obtained.

Calibrator values in arbitrary and international units.

	EU/mL	IU/mL
Calibrator 0	0	0
Calibrator 1	5	0.5
Calibrator 2	10	1
Calibrator 3	50	5
Calibrator 4	100	10
Calibrator 5	200	20

Interpretation of the result:

The ratio between OD value of sample and that of the cut-off (calibrator 2) was calculated, and the sample was considered:

Positive: if the ratio is > 1.2
Doubtful: if the ratio is >= 0.8 - <= 1.2
Negative: if the ratio is <0.8

Specimens with doubtful results were retested using the same laboratory test.

Results:

In this study a total of 180 spontaneously aborted and 80 normally delivered females (control) were included. The distribution of the cases according to hospital was; 60 from each of Khartoum teaching and EL-Saudi hospitals and 30 from each of Khartoum north and Umbada hospitals.

The overall seroprevalence of Cytomegalo virus antibodies was found to be 97.8% and 38.3% for CMV IgG & IgM, respectively. The highest prevalence of CMV IgG and IgM antibodies was found in the age group 41—50

years (being 100% & 66.6% respectively) (table 1). This result revealed a significant association between CMV infection & age (P-value <0.0185). The frequency of abortion was found to be significantly associated with the rate of CMV IgM antibodies (P value =0.017) (table 2). Thirty subjects (16.6%) gave history of having a congenitally malformed child in form of deafness, blindness and mental retardation. All of them were found to be positive for CMV IgG antibodies and 83.3% for CMV IgM antibodies. The highest percentage of CMV IgM antibodies (100%) was found in women with mentally retarded babies (table 3). The study showed significant association between congenital malformation and CMV infection (P value = 0.037).

Table 1: Distribution of cytomegalovirus positive cases according to the age group.

Age-group years	Total tested	Anti-IgG +ve		Anti-IgM +ve	
		frequency	%	frequency	%
20-30	89	86	96.6	28	31.5
31-40	76	75	98.7	31	40.8
41-50	15	15	100	10	66.7
Total	180	176	97.8	69	38.3

Table 2: distribution of CMV antibodies according to the frequency of abortion.

No of Abortions	Total tested	Anti-IgG +ve		Anti-IgM +ve	
		frequency	%	frequency	%
1	88	86	97.7	28	31.8
2	52	51	98	21	40.4
3	40	39	97.5	20	50
Total	180	176	97.8	69	38.3

Table 3: distribution of CMV antibodies in relation to history of congenital malformation in children.

Type of malformation	Total tested	Anti-IgG +ve		Anti-IgM +ve	
		frequency	%	frequency	%
Deafness	16	16	100	14	87.5
Blindness	11	11	100	8	72.7
Mental retardation	3	3	100	3	100
Total	30	30	100	25	83.3

Discussion:

In this study the seroprevalence of CMV antibodies was found to be 97.8%, 38.3% for IgG and IgM respectively. This result is in agreement with what was obtained by Kafi and his co-workers in 2003. They found seroprevalence of CMV to be 95%, 77% among antenatal women and blood donor's respectively[5] .The percentage of CMV IgG antibodies obtained in the current study was higher than what was reported by Rubine Lone, and his team in Kashmir[6.] However the seroprevalence of CMV IgM was in agreement in both studies. This study showed significant association between CMV infection and spontaneous abortion which is in agreement with a previous result reported by Abdolreza Soloodeh and his team In South Iran[7]. The current study agrees also with the finding of Lueri M, and his co-worker in Germany, which showed seroprevalence of cytomegalovirus (CMV) IgG antibodies ranging between 64.3% and 92% however none of the control subjects was found to be IgM-positive [8]. Munro, in Australia found 5.5% of his subjects to be CMV IgM positive which is much lower than the figure obtained in this study [9].

All women (100%) with history of mentaly retarded children were found to be CMV

Anti-IgG & Anti-IgM +ve. Women with history of children with congenital deafness and blindness were 100% positive for CMV IgG antibodies but 87.5% and 72.7% for IgM, respectively. The overall seroprevalence of CMV IgG and IgM antibodies among women with the three congenital anomalies collectively were found to be 100% and 83%, respectively. These findings reveal statistically significant association between CMV infection and the above congenital abnormalities (P value = 0.01)

Conclusion:

Among women attending the four major hospitals in Khartoum state with spontaneous abortion, the seroprevalence of CMV antibodies was found to be 97.8% and 38.3% for IgG & IgM respectively.

The highest prevalence of CMV IgG & IgM antibodies was found in the age group 41—50year. Infection rate was high in patients inhabiting Khartoum town.

Prevalence of CMV IgM antibodies was found to be significantly associated with the number of abortions.

Congenital malformation (mental retardation, deafness, blindness) were found to be associated with CMV infection.

There was significant association between CMV infection and congenital anomalies in children born to CMV infected women.

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