The Seroprevalence of IgM Among Iraqi Aborted Women Infected with Human Cytomegalovirus

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ABSTRACT:

BACKGROUND:

Fetal loss and abortion are responsible for significant emotional distress for couples desiring children. There are many documents which support the role of some certain asymptomatic infections such as Cytomegalovirus (CMV) in spontaneous abortion.

OBJECTIVE:

This study was aimed to evaluate the prevalence of seropositivity of specific IgM antibody for CMV by ELISA in the sera of (108) women with recurrent abortion associated with obstetric complications.

METHODS:

This study was carried out in two central hospital in Baghdad (Al-Elwea& Baghdad teaching hospitals)during (2009-2010).A number of (108) women with definite diagnosis of previous abortion ,35women with recurrent abortion , 30 women with premature delivary and 43women with intra uterine death .compered to (50)women with history of normal full term delivary and negative history of miscarrage as control groups .Serum samples were collected and then tested by using ELISA for detection of anti-CMV IgM antibodies .

RESULTS :

Cytomegalovirus (CMV)specific IgM antibody was detected in (15.7%) of the 108 women studied ,while the women with obstetric complications were positive for CMV specific IgM antibody these compriesd
of(16.6%)with premature delivary ,(14%) with recurrent abortion and (16.2%) with intra uterine death . these result statistically significant ($P<0.05$) .Our data faild to found asignificant association between the Cytomegalovirus infection with age and residence of patients ($p>0.01$).

CONCLUSION :

Higher seropositivity for cytomegalovirus (CMV)in women with spontaneous abortion compared to women with normal obstetric history suggests that cytomegalo virus Plays a sigificant role in abortion

KEY WORDS: cytomegalovirus ,fetus death ,congenital infection .

INTRODUCTION:

Human Cytomegalovirus (CMV) is the most common cause of congenital malformation in developed countries its clinical manifestations range from asymptomatic infection to severe fetal damage [(1)].Up to 15% of intrauterine CMV infections result in symptomatic congenital disease at birth and 10 to 15%of those born with asymptomatic congenital CMV will develop significant clinical squale in infancy [(2)].

The presence of CMV-specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and re infection [(3)].Some researchers showed significant relation between CMV infection and spontaneous abortion [(4)]. There are also evidences which suggest that CMV will lead to complicated pregnancies [(5)].It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy [(6)].

The prevalence of congenital infection ranges from 0.2% to 2.5% in different populations[(7)] , in which the risk factors include particular races or ethnic groups, a low socioeconomic status, premature birth, and admission to an intensive care unit [(8)].

In India, serological surveys have shown the prevalence of CMVantibodies in adult population to be about 80-90%(3,4). However, the data regarding the occurrence of CMV infection in pregnant population is sparse. The aim of this study was; to determine the seropositivity of CMV IgM infection in studied group, in relation to their age and residence of patients[(9)] .

As far as prevention is concerned, in addition to health education campaigns, the serological

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screening of pregnant women has been proposed. However, there is no consensus in the scientific community concerning the implementation of screening [10].

In Iraq, studies have revealed that the majority of women of childbearing Age are seropositive for CMV and that they contract the infection either through prenatal or postnatal transmission during early childhood [11]. Sexual transmission and blood transfusion are other sources of infection .

Primary CMV infection has been found to be more prevalent in pregnant women than non –pregnant

This difference may be attributed to the susceptibility of seronegative women ,at the onset of pregnancy ,to the first CMV infection [12].

Transmission of CMV infection to the fetus has been identified in all trimesters of pregnancy . Abortion can result from ascending CMV endometritis and the virus has been isolated from post- abortion uterine discharge [13].

MATERIAL AND METHODS:

During the period between January 2009 to May 2010. (108) women in Gynecology and Obstetric department of Al- Elweya hospital and Baghdad hospital were subjected for the detection of CMV specific IgM antibody by ELISA test. These included (108) women with previous history of recurrent abortions, intrauterinedeath (IUD), and premature deliveries. Compared with 50 women with normal deliveryand negative IgM for CMV as control group,5 mls of blood samples were collected from all patients during clinical illness and control group for detection of CMV IgM by enzyme linked immunosorbent assay(ELISA).

Serum separation was done by centrifuging of whole blood samples at 2000xg for 20 min, and the serum samples were kept with -4C°.

the ELIZA technique was performed using kits intended for estimating concentration of specific CMV-IgM Markers.Cytomegalovirus IgM (CMV IgM) ELISA Kit(Cat# 1202Z: REF=1201Z :cod# 9-D3-022).

Company Sigma /USA.The ELISA technique was performed according to the instruction of the manufacturer.

Principle of Enzyme Linked Immuonosorbent Assay (ELIZA) for cytomegalovirus IgM :

Wells of microtiter plate were coated with recombinant antigen representing epitops of CMV . Serum samples were added to the wells.If antibodies specific for CMV are present in the sample they will form stable complexwith CMV antigen .After washing peroxides was added .

IF the Ag-Ab complex is present ,the conjugate will bind to the complex . After a second wash an enzyme substrate solution containing chromogen was added .Ablue color were developed that turen to yellow after H2So4 addition as a bloking solution .The intensity of it is proportional to CMV-IgM antibodies concentration in the sample .Wells containing negative samples remaind un changed.

Data analysis :

For the asscessment of risk factors for CMV infection ,characteristics of case patients and control group were

Examined using a two-sample student t-test .Cross-tabulation and chi-square were used to examine the

Relationship between variables using a 95% confidence interval as a measure of association .

RESULTS:

The age of the patients were range from(20-30)years with mean age (25.3±SD)in the pregnant women with abortion ,the mean gestational age was 8 weeks ,there is no significant difference between CMV infection and age (p=0.69).

According to prevalence of seropositivity for CMV antibodies, about (15.7%)of the subjects were divided in recurrent abortion ,premature delivery and intra uterine death .

The results of serological assay we can divided in 3 groups:

Group 1: women suffering from premature delivary :

In table (1) : out of (30/108) suffering from premature delivary ,only (5/30)were positive IgM to CMV

(16.6%) ,while (25/30) were negative IgM to CMV (83.3%).

Group 2: women suffering from Recurrent abortion:

In table (2) :out of (35/108) suffering from recurrent abortion ,only (5/35)were positive IgM to CMV

(14.3%) ,while (30/35) were negative to CMV (85.7%).

Group 3: women suffering from intra uterine death:

Out of (43/108) women suffering from intra uterine death ,only (7/43) were positive to IgM to CMV (16.3%)

,while (83.7%)were negative to IgM to CMV

table (4) Show the ELISA result seropositivity of all the cases mention before ,our result showed their is a significant association between recurrent abortion ,premature delivary and intra uterine death.

Our results showed the positive IgM (17/108) ,7/ 17 from rural areas (41.2%) while,10/17 from urban areas (58.8%),more over 55/108 (60.4%)negative IgM from rural and 36/108 (39.6%) from urban areas .these results showed no significant association between residence of patients and infections with virus ,as showen in table (5).

Table 1: Relation between CMV infection with premature delivery according according to age of patients.

Age group/ Year	No.of patients with premature delivery	+ve IgM to CMV No. (%)	
(20-25)	15	4	13.3%
(26-30)	10	1	3.3%
(31-35)	3	0	
(36-40)	2	0	
Total	30	5	16.6%

P-Value =0.847.

Table 2: Relation between CMV infection with Recurrent abortion according to the age of the patients.

Age group/ Year	No.of patients with recurrent Abortion	+ve IgM to CMV No. (%)	
(20-25)	20	3	8.57%
(26-30)	11	2	5.71%
(31-35)	3	0	
(36-40)	1	0	
Total	35	5	14%

P-Value =0.696.

Table 3: Relation between CMV infection with Intra uterine death according to the age of the patients .

Age group/year	No.of patients with intra uterine death	+ve IgM CMV NO. (%)	
(20-25)	20	4	9.3 %
(26 -30)	18	2	4.6 %
(31 -35)	4	1	2.3 %
(36 -40)	1	0	
Total	43	7	16.2 %

P-Value =0.808 .

Table 4: ELIZA positivity for CMV IgM antibody in different group.

Group	No.of sample tested	ELIZA positivity CMV No. (%)	
Recurrent abortion	35	5	14 %
Intrauterine death	43	7	16.2%
Premature delivery	30	5	16.6 %
Total	108	17	15.7 %

P – Value = 0.085.

Table 5: Relation ship between CMV infection and Residance of patients .

CMV IgM	Rural No.	(%)	Urban No.	(%)
Positive IgM	7	(41.2%)	10	(58.8%)
Negative IgM	55	(60.4%)	36	(39.6%)
Total	62	(57.4%)	46	(42.6%)

P-value = 0.14

DISCUSSION:

CMV is the most common congenital infection &its incidence has been estimated between 0.2-2.2% of all live births in different parts of the world[14] . In 1994,a study conducted in Malaysia involving 1688 infants with congenital CMV infection &it was detected in 11.4 %of The infants ,which was much higher than other intrauterine infections like congenital toxoplasmosis (1%) and congenital rubella infection (2.7%). [15].Primary CMV infection in an individual can be detected by demonstration of CMV specific IgM antibody [16].However ,infected infants can be asymptomatic at birth with 10-15% of these subsequently developing late sequelae such as visual &auditory defects[17].

Congenital intrauterine infections have been associated with congenital abnormalities ,intrauterine growth retardation and intra uterine death of the fetus ,as well as late sequelae such as developmental delay ,blindness and deafness of the infected child [18].Cytomegalovirus (CMV) infection during pregnancy is more complex than other infections,due to virus reactivation during the child bearing age and be transmitted to the fetus inspite of maternal immunity [19].

Risk factors for CMV infection have been correlated with the socioeconomic status within a community [20,21].Other studies showed that elderly persons seem to be well protected against symptomaticCMV disease due to accumulation of CD28 effector cytotoxic T lymphocytes. This is a characteristic feature of all age groups but is most pronounced in elderly persons[22].As a consequence of placental infection, HCMV impairs cytotrophoblast differentiation and invasiveness; this could explain early abortion occurring in women with primary infection. In addition, HCMV infection impairs cytotrophoblast expression of HLA-G, thus activating the maternal immune response against the cytotrophoblast subpopulation expressing this molecule[23].

Further more , Failure of the systemic and uterine vasculature to adapt during pregnancy leads to several complications, including preeclampsia and intrauterine growth retaedation (IUGR) [24].CMV is a virus that can affect the fetal organs through out the whole pregnancy. The damage seems to be more severe in infections occurring during the first half of the pregnancy, while infections in the second half would result in reduced morbidity [25].Various ways of transmitting the virus to the fetus have been suggested, whereas the hematogenious spreading across the placenta with subsequent infection of placental and amniotic tissue seems to be the most common transmission way. [26]. In the present study ,CMV specific IgM antibody was detected in (15.7%) of all pregnant women tested indicating the substantial prevalence of infection in local population .

In table (1) : out of (30/108) suffering from premature delivary ,only (5/30)were positive IgM to CMV
(16.6%) ,while (25/30) were negative IgM to CMV (83.3%).And out of (35/108) women suffering from recurrent abortion ,only (5/35)were positive IgM to CMV (14.3%) ,while (30/35) were negative to CMV (85.7%).as in table (2).Further more , Out of (43/108) women suffering from intra uterine death ,only (7/43) were positive to IgM to CMV (16.3%),while (83.7%)were negative to IgM to CMV .In addition ,
the ELISA result seropositivity of all the cases mention befor , showed their is asignificant association between recurrent abortion ,premature delivary and intra uterine death and cytomegalovirus infections .

Ahypothesis for the probable role of geographical influence upon CMV seroprevalence might be the route of infection .In rural areas saliva is propabably the main route through which the virus is transmitted postnatally .This is likely to be the route through which the virus is transmitted early in life amongst infants and young children due to poor sanitation [27].On the other hand in urban areas sexual transmission seems to be the major route of infection later in life during childbearing age.

In addition ,our result showed that there is no correlation between CMV infections with the residence of patients. Its statistically no significant association (p>0.01) between the rural and the urban residence like ,
in(table 5).Another factor that may contribute in human CMV infection prevalence is geographical distribution [28,29,30]. Unlike previous studies data showed that CMV IgM seroprevalence had no significant correlation with geographical location ,even if CMV seroprevalence had higher value in rural as compered to those of urban areas .On the other hand primary infections rate was higher through out urban areas [31].
CMV infection is endemic in Iraq in (2002);the prevalence rates of human cytomegalovirus IgM and IgG in non pregnant women have been reported to be 1% and 84% respectively ,and 2.5% and 90% in pregnant women [32].
In the present study ,CMV specific IgM antibody was detected in (15.7%) of all pregnant women tested indicating the substantial prevalence of infection in local population .our result is lower than the result reported by alwam (2011); (46.6%),this different may be due to small sample size [33].
Our result was similar to result (15.98%)of Rubina et al (2004) [34]. In our study the prevalence of seropositivity for CMV was lower than western Europe,America and Australia (Munro et al ,2005) [35] ,In India ,the serological surveys have showen the prevalence of CMV antibodies in adult population to be about 80-90 % [36] .
However ,our result is higher than the result of arti kapil et al that reported CMV specific IgM antibody in (12.9%) of pregnant women with complication [37].All these findings indicate that CMV infection is not un common in our local population .
This high seroprevalence reflects the low hygienic standards&practices in our part. Also CMV can lead to substantial damage to the fetus &as the damge done in utero cannot be reverted ,control of intrauterine CMV infection CMV infection is important .Hence prevention of CMV infection ,especially in the pregnant women is essential by screening of pregnant women ,althoughthe measures ,can not change the out come of the disease but may be useful in altering the physician for possible infection to the baby ,hence routine screening of females of child bearing age for CMV infection is desired in order to reduce the fatal out come of the pregnancy occurring due to the CMV infection .

CONCLUSION :

We can diagnose high risk pregnancy even with serological tests in areas with insufficient equipment,we recommend pregnant women should be attentive of diseas prevention guidlineson personal hygien during
Pregnancy,especially hand washing after handling diapers or oral secretions.But werecommend high risk pregnant women for example mothers that working in day care center or health care worker should be screend for CMV serological test during pregnancy .All pregnant women especially with bad obstetric history should be
Screened for the presence of CMV infections as well as screening against other maternal infection to exclude any congenital infection such as (S TO R CH) is manidatory .

REFERENCES:

1. Demmler, G.J.,. Infectious diseases society of America and centers for disease control: Summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev. Infect. Dis., 1991;13:315-29. PMID: 1645882.
2. Dopana, S.B., R.F. Pass, W.J. Britt, S. Stagno and C.A. Alford,. Symptomaticcongenital cytomegalovirus infection: Neonatal morbidity and mortality. Pediatr. Infect. Dis. J., 1992;11:93-99. PMID: 1311066
3. Nielsen, S.L., I. Sorensen and H.K. Andersen, Kinetics of specific immunoglobulin M, E, A, and G in congenital, primary, secondary cytomegalovirus infection studied by antibody capture enzyme-linked immune sorbent assay. J. Clin. Microbiol., 1988;26: 654-61. PMID: 2835388
4. Enders, G., U. Bader, L. Lindemann, G. Schalasta andA. Daiminger,. Prenatal diagnosis ofcongenital cytomegalovirus infection in 189pregnancies with known outcome. Prenat. Diagn., 2001;21:362-77. PMID: 11360277
5. Hammouda, N.A., W.M. El-Gelbaly and S.M. Sadaka,. Seroprevalence of toxoplasma and cytomegalovirus in complicated pregnancies. J. Egypt. Soc. Parasitol., 1993;23:865-70. PMID: 8308361
6. S. Stagno, R. F. Pass, G. Cloud, et al., “Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome,” *Journal of the American Medical Association*, 1986; 256: 1904–8.

7. G. J. Demmler, "Infectious Diseases Society of America and Centers for Disease Control: summary of a workshop on surveillance for congenital cytomegalovirus disease," *Reviews of Infectious Diseases*, 1991;13: 315–29.
8. S. C. Dollard, S. D. Grosse, and D. S. Ross, "New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection," Reviews in Medical Virology, 2007; 17: 355–63.
9. G. Malm and M.-L. Engman, "Congenital cytomegalovirus infections," Seminars in Fetal and Neonatal Medicine, 2007; 12:154–59.
10. M. G. Revello and G. Gerna, "Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant," Clinical Microbiology Reviews, 2002;15: 680–715.
11. Ali HYM,Yaseen SA,and Najem SN .Prevalence of cytomegalovirus infection in child bearing age women in Mosul.Jord.Med.L.,(1992),26:53-8
12. Stagno S *et al* Congenital Cytomegalovirus infection : the relative importance of primary and recurrent maternal infection .New England Journal of medicine , (1982),306:945-9.
13. Dehner LP,and Askin FB . Cytomegalovirus endometritis :report of acase associated with spontaneous abortion .Obstetrics and gynecology 1975;45:211-4.
14. Chung-Hua F, Chan K, Tsa C effects of cytomegalovirus infections in pregnant women to fetuses: study with DNA–DNA hybridization method (with English abstract). Chin J ObstetGynecol 1992;380:355–58.
15. WongA,Tank KH,Tee CS,YeoGSH.Seroprevalence of cytomegalovirus ,toxoplasma and parvovirus in pregnancy .singapore Med J 2000;41:151-55.
16. Balasubramanium V,Simmrah M ,Tan DS ,Redzwan G,Loman SG. The role of cytomegalovirus infection in conginetal disease in Malaysia .Med J Malaysia 1994;49:113-16
17. Griffiths PD,Stagno S, Pass RF etal .Infection with cytomegalovirus during pregnancy .Specific IgM antibodies as amarker of recent primary infection .JInfectDes 1982;145:647-53.
18. Brown HI,Abernathy MP. cytomegalovirus infection.Semin pernatol 1998;22:260-66.
19. Mukundan P,Jadvan M and John TJ.Prevalence of cytomegalovirus antibody in young children in vellore .Indian JMed Res 1977;65:589-92.
20. Fowler, K. B., S. Stagno, and R. F. Pass.. Maternal age and congenital cytomegalovirus infection: screening of two diverse newborn populations, 1980-1990. J. Infect. Dis. 1993;168:552-56.[Abstract/Free Full Text]
21. Fowler, K. B., S. Stagno, and R. F. Pass. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA . 2003;289.
22. Pedron B, Guerin V, Jacquemard F, Munier A, Daffos F, Thulliez P, Aujard Y, Luton D, Sterkers G. 'Comparison of CD8+ T Cell responses to cytomegalovirus between human fetuses and their transmitter mothers'.J Infect Dis. 2007;196:1033-43.
23. tagno,S.R.F.,Pass,G.,Cloud,W.J.,etalPrimar Cytomegalo virus infection in pregnancy incidence,transmission to fetus ,and clinical outcome .JAMA-Journalof the American Medical Association .1986;258:1904-8.
24. Fisher, S., O. Genbacev, E. Maidji, and L. Pereira.. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J. Virol. 2000;74:6808-20.[Abstract/Free Full Text] .
25. PH, Walters WA. The effects of chronic maternal hypotension during pregnancy. Aust N Z J ObstetGynaecol 1992;32: 14–16,.CrossRef
26. Pass RF, Boppona S Cytomegalovirus. In: Viral infections in obstetrics and gynecology, 1999; 35–56.
27. Gaytant ,M.A.,Steegers,B.A.,Semmekrot,H.M.,et al.Congenital Cytomegalovirus infection : Review of the epidemiology and out come .Obstetrical&Gynecological Survey .2002;57:245-56.
28. Wen ,L,Wu,S.and Lu,S.The epidemiological study on human cytomegalovirus infection in pregnant women and maternal-fetal transmission in three Chinese metropolis .Chung Hua Fuchar Ko Tsa Chih .1996;31:714-17.

29. Gratacap,C.B.,Bosson ,J.L.,Morand ,P.etal. Cytomegalovirus seroprevalence in French pregnant women :Parity and place of birth as major predictive factors ,European Journal of Epidemiology .1998;14:147-52.

30. Mustakangas,P.,Saran ,S. and Ammaia,PHuman Cytomegalovirus seroprevalence in three different socioeconomically different urban areas during the first trimester :apopulation based cohort study .International Journalof Epidemiology.2000;29:585-91.

31. Forbes ,B.AAcquisition of Cytomegalovirus infection :an Update.Rev Clini Microbial2. 1998:204-16.

32. Alwan, A.Sera interleukin-10 and interlukin-12 level among Iraqi aborted women infected with human cytomegalovirus .Higher Diploma thesis.Health and Medical Tachnical College .Foundation of Technical Education 2011 .

33. Brooks,G.F.,Butel,J.S.and Morse,S.AHerpes viruses cahpter33 in Jawetz ,Melinck and Adelbergs Medical Microbiology22nd Edn.Lang Medical Books/McGraw-Hill,USA 2001:382-86.

34. Rubina Ione ,MD;Bashir A.Fomda,MD;Manzoor Thokar,MD;Tehmeena Wani,MD; Rubina Shaheen ,MD;Asifa Nazir ,MBBS .Seroprevalence of cytomegalovirus (CMV) In Kashmir valley Apreliminary study .JK-Practitioner 2004;11:261-62.

35. Munro,S.L.,B.Hall and L.R. Whybin .Diagnosis of and screening for cytomegalovirus infection in pregnant women .J.Clin.Microbiol., 2005;43:4713-18.PMID:16207970.

36. Mathur A,Jindal L and Chaturvedi VC .Aserological study of cytomegalovirus infection at Lucknow .Indian JMed Res 1981;73:678-81.

37. KapilA and Boor S . Primary cytomegalovirus infection in pregnant women in India . Ind JMed Microbiol 1992;10:53-55.