Iranian Red Crescent Medical Journal

ORIGINAL ARTICLE

Human cytomegalovirus infection in women of childbearing age, Fars Province: a population-based cohort study

M Arabpour[1], K Kaviyanee[1], A Jankhah[2], R Yaghobi[3]*

[1]Diagnostic Medical Laboratory, Pars Hospital, [2]Health Genetic Consulting Center, [3]Shiraz Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Human cytomegalovirus (HCMV) is a ubiquities infection in women of childbearing age and is the most common cause of congenital malformation resulting from viral intrauterine infections either in developed or developing countries**.** This study was performed to evaluate the seroprevalence of HCMV-IgM and IgG antibodies and some of epidemiological risk factors in women of childbearing age in southern Iran.

Methods: 844 women of childbearing age from Fars Province, southern Iran who referred to Health Genetic Consulting Center of Shiraz from 2001-2005 were enrolled. The serum of the collected blood samples were separated and stored at -20°C and HCMV-IgM and IgG antibodies were detected by IgM capture and IgG ELISA tests.

Results: 45 (5.4%) samples were seropositive and 778 (94.6%) were seronegative for HCMV-IgM antibodies, respectively. HCMV-IgG antibodies were seropositive in 764 (93%) and seronegative in 8 (7%) blood samples. A negative significant correlation was observed between IgG titer of HCMV and previous abortions. A significant positive trend was detected between HCMV-IgG titer and increase in age. Also, a higher sensitivity to primary infection was observed in Shiraz population than the other cities of this province.

Conclusion: For the negative correlation of HCMV-IgG titer with the history of abortion, and for protection of women of child bearing age, the design of effective vaccines may be useful for provoking IgG titer against HCMV infections.

Keywords: Human cytomegalovirus; Seroprevalence; IgM; IgG; Antibodies

Introduction

Human cytomegalovirus (HCMV) seroprevalence varies in different populations and age groups. Congenital malformation is the most resulting clinical symptoms of HCMV intrauterine infection.[1-4] Primary HCMV infection occurs in 0.15-2.0% of all pregnancies and may be transmitted to the fetus in up to 40% of cases.[4] Up to 15% of intrauterine HCMV infections lead to symptomatic congenital disease at birth. Asymptomatic congenital HCMV infection will develop in 10-15% of infants.[5-7] Intra-uterine transmission of HCMV can occur during primary maternal infection, reactivation, or re-infection of seropositive mothers. Primary HCMV infections are transmitted more frequently to the fetus and it is more likely to cause fetal damage than recurrent infections.[4-8] If strategies for the prevention of HCMV disease are to be successfully developed, knowledge regarding the epidemiology of the virus is prerequisite. However, a number of HCMV features including endemic worldwide infection, complex natural history, unusual relationship with infected hosts, ubiquity of infection, and lack of clinical symptoms in most cases complicate the understanding of the epidemiology of HCMV infection.[9] In this population-based research, the seroprevalence of HCMV-IgM and IgG antibodies and some of epidemiological risk factors, as important

***Correspondence:** Ramin Yaghobi, PhD, Assistant Professor of Department of Virology, Shiraz Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-711-6276211, Fax: +98-711-6276211,
e-mail: rayaviro@yahoo.com
Received: October 24, 2007 Accepted: March 9, 2008

predictive values having close relationships with the occurrence of primary and recurrent HCMV infections, were studied in women of childbearing age in southern Iran.

Materials and Methods

A total of 844 women in child bearing age in Fars Province, southern Iran, who referred to Health Genetic Consulting Center in Shiraz, southern Iran from 2001 to 2005, participated in the present study. These women belonged to different geographical areas categorized to 3 different regions (Shiraz rural, Shiraz urban, and the other cities and villages of Fars Province). Obtaining institutional Ethics Committee, taking the patient's consent, patients' demographic data, and gathering blood samples form these women before including them in the study are the limitations of this study. Blood sample was collected from all women and the serums was separated and stored at -20°C until tested. Factors such as age, marital and pregnancy status, previous parity number of married women, number of previous abortions and child with congenital disorders, and regional distribution of the patients were included in this study as HCMV risk factors. A total and differential counting of white blood cells (WBCs) was carried out for each patient.

HCMV-IgM and IgG antibody titers were analyzed by following the manufacturer's instruction of HCMV-IgM capture ELISA and IgG ELISA Kits (Trinity Biotech Co). The results were interpreted on the basis of ISR (immune statues ratio) index calculated by dividing the specimen O.D. value by the cut off calibrator ratio according to manufacturer instruction. The tests were considered seropositive if ISR $\geq$ 1.1 and considered seronegative if ISR$\leq$ 0.9. Samples with 0.9< ISR <1.1 were considered equivocal and hence ruled out from data processing. Also those samples with IgM positive results were screened by taking another sample one week later. After the first test result had became clear, not only to confirm the IgM serostateus of the patient but also to detect a seroconversion for IgG in $IgM^{+}IgG$ patients. Only those who were double-checked were considered positive for IgM. Finally, those patients who had seroconverion for IgG and IgM and or a very high titer of IgM,[10] were considered to have primary HCMV infection.[11] Chi-Square, Fisher's Exact and Independent Sample t tests, and also Pearson Correlation were used to detect any significant relationship.

Results

Out of 844 women of childbearing age (15-44 years old), the majority of cases (407, 48.4 %) were in 20-24 years old age group. Geographical distribution of this population was 278 (33.0%) in Shiraz urban areas, 52 (6.2%) in Shiraz rural areas and 513 (60.7%) in other cities and villages of Fars Province, southern Iran. 537 (63.6%) and 307 (36.3%) women were married and unmarried, respectively. Among the married women, 121 (22.5%) had more than 2 parities and 290 (54%) had 1-2 parities, while 126 (23.5%) were nulliparous. Among those who had $\geq$1 parity number, 22.5% (94) had at least one child with congenital disorders and 220 (52.6%) had at least one abortion or child's early death because of congenital problems. Also among the married women, 85 (10.1%) were pregnant in the 9th week or more of their gestation. 4 (0.5%) cases were graduated, 170 (20%) undergraduate, 411 (48.6%) diploma or in high school educational level, 248 (29.3%) had basic education, and 11 (1.3%) cases were illiterate.

The HCMV-IgM antibodies were seropositive in 45 (5.4%) and seronegative in 778 (94.6%) cases with a mean of 0.45 ISR in Fars Province, southern Iran. From those who were seropositive for IgM, 16 (35.6 %), 2 (4.4%), and 27 (60%) were from Shiraz urban, Shiraz rural, and other cities of Fars Province respectively. The mean titer of IgM (0.34) was higher in other cities of Fars Province than Shiraz rural/urban areas. Significant correlation was not detected between higher titer of HCMV-IgM and seasonal substitution (p=0.74). The effect of seasonal fluctuation on HCMV-IgM and IgG seroprevalance were presented in Figures 1a and 1b. No significant correlation was observed between IgM seroprevalance and increase in age (p=0.65), parity number (p=0.80), previous children with congenital disorders (p=0.25), and number of previous abortion (p=0.65). Maximum IgM seroprevalance was observed in 25-29 years old age group, while minimum IgM seroprevalance was observed in those with 35-39 years of age (Figure 1c). A significant rise in IgM titer was seen from 15 to 29 years of age (*p=0.04*) but a non-significant fall in IgM titer was seen from 29 to 39 years of age (p=0.27). A significant negative correlation was detected between HCMV-IgM seroprevalance and the number of previous children with congenital disorders (p=0.005). Higher seroprevalence of HCMV-IgM was shown in Shiraz urban area than Shiraz rural and other cities of Fars Province without any significant

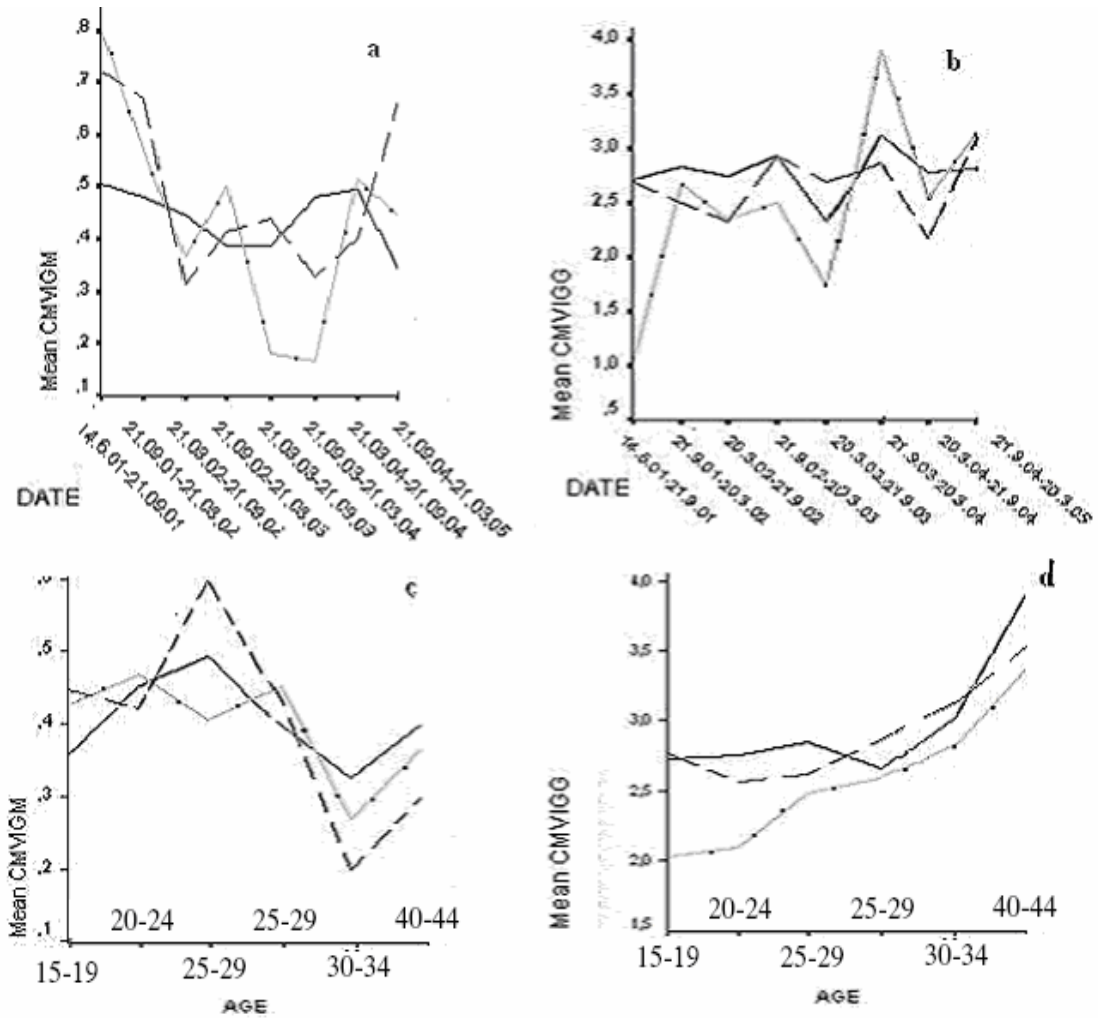

Fig 1: HCMV IgM(a) and IgG(b) seroprevalence during four consecutive years throughout Fars state: HCMV IgM(c) and IgG(b) seroprevalance in different age groups through hout three different geographical district (Dates are in AD)

correlation and also in married women than single ones. A significant higher IgM seroprevalance was observed in pregnant women than non-pregnant ones (p=0.03). Totally 2.5% of the whole society proved to have primary HCMV infection either due to a sero-conversion or a high level of IgM titer. 30% of those who had primary HCMV infection were from Shiraz urban and rural areas, but 70% of them were from other cities of Fars Province. The maximum rate (2.8%) of primary HCMV infection happened in 20-29 year old age group. The results of the statistical analysis of correlation between demographic data and hematological indexes with primary HCMV infections in women of childbearing age are presented in Table 1.

HCMV-IgG antibodies were seropositive in 764 (92.9%) and seronegative in 58 (7%) of all cases with a mean of 2.7 ISR in Fars Province. From those who were seropositive for HCMV IgG, 708 (93.8%) had no trace of IgM seropositivity. Also 47 (6.2%) cases were simultaneously seronegative for both HCMV-IgG and IgM antibodies. The group of patients with HCMV-IgG seropositive and HCMV-IgM seronegative results were from Shiraz urban [256 (33.5%)], Shiraz rural [42 (5.5%)] and other cities of Fars Province, respectively [466 (61%)]. The maximum mean of CMV-IgG antibody titer belonged to 40-44 year old age group while the minimum IgG titer was seen in <24 year old women (Table 2). No significant correlation was observed between HCMV-IgG seroprevalance with pregnancy statue (p=0.1), previous parity number (p=0.3), marital statue (p=0.1), number of previous children with congenital disorders

(p=0.67), and geographical distribution (p=0.12). A non-significant negative correlation was observed between IgG seroprevalance and the previous history of abortion *(*p=0.08*)*. A significant negative correlation was observed between the number of previous abortions with HCMV-IgG titer (p=0.03) while a very significant positive correlation was observed between HCMV-IgG titer and the number of child and increase in age (p<0.001) (Figure 1d). Statistical analysis did not detect any significant differences between the seroprevalance of HCMV-IgG in pregnant women compared to non-pregnants, in married woman in comparison to single ones, in those who lived in other cities of Fars Province than in those who lived in Shiraz, and in women who had a history of abortion or child with congenital disorders than in those who hadn't (data not shown). Unlike HCMV-IgM antibody**,** HCMV-IgG antibody titer revealed a seasonal variation during the five years of the study. An increase in IgG titer was observed during autumn and winter followed by a subsequent decrease in HCMV-IgG titer during spring and summer in all of the geographical districts (p<0.001) (Figure 1b). The statistical relation between the two serogroups, including HCMV (IgM^- and IgG^-) and HCMV (IgM^- and IgG^+), with demographic data are presented in Table 2.

Table 1: Distribution of demographic characteristic and hematological indexes of women of childbearing age with primary HCMV infection.

Demographic characteristics		**r**			**P. Value**[B]
		Primary HCMV infection [A]			
		No. of cases	**% of cases**		
Age	15-19	3	15		0.76
	20-24	11	55		
	25-29	6	30		
	30-34	0	0		
Marital Status	Single	15	75		0.35
	Married	5	25		
History of abortion and congenital disorders	Pregnant	2	10		1
	Non pregnant	18	90		
History of abortion and congenital disorders	Abortion Background	3	27.3		1
	Congenital Handicapped	1	9.1		1
No. of parity	X > 2	4	20		0.62
	X=1-2	6	30		
	X=0	10	50		
Education	Graduate	0	0		0.102
	Undergraduate	4	20		
	High school or Diploma	9	45		
	Basic Education	7	35		
Geographical District	Shiraz Urban Area	5	25		0.8
	Shiraz Rural Area	1	5		
	Other Cities of Fars State	14	70		
No. o WBC	WBC count 10^3/ml	Mean	Max	Min	
		7.14	11.6	4.8	
	Nutrophile count%	62.6	81	44	
	Eosinophile count %	3	5	1	
	Lymphocyte count%	30	54	14	
	Monocyte Count%	5.8	10	1	

A. Primary HCMV infection (IgM+IgG- with a seroconversion in IgG or IgM + and IgG+ with high IgM titer), B. Chi-Square or Fisher Exact test (when more than 5% of cells had expected count less than 5 were used to inquire significant relationships between each character and two condition or HCMV infection (α=0.05).

Table 2: Demographic characteristic distribution of childbearing age women within two from of HCMV IgG seroprevalance.

Personal Characteristics		IgG seroprevalance Statue				P value[A]	
		Susceptible to Primary HCMV (IgM- & IgG-)		Resistant to Primary[B] HCMV (IgM- & IgG+)		IgM-IgG-	IgM-IgG+
		Number of cases	Percent of cases	Number of cases	Percent of cases		
Age	15-19	11	23.4	131	18.5	0.73	0.94
	20-24	21	44.7	343	48.4		
	25-29	11	23.4	159	22.5		
	30-34	3	6.4	5+6	7.9		
	35-39	1	2.1	15	2.1		
	40-44	0	0	4	0.6		
Martial status	Married	24	51	451	63	0.08	0.07
	Single	23	49	257	36		
Pregnancy status	Pregnant	43	91.5	74	10	1.00	0.50
	Non pregnant	4	8.5	634	89		
Having abortion or child with congenital disorder history	Abortion background	10	21.3	177	25	0.02	0.01
	Congenital handicapped child	6	12.8	80	11.3	0.64	1.00
Previous parity number	X > 2	11	23.3	96	13.5	0.02	0.19
	X = 1-2	7	15	257	36.3		
	X = 0	29	61.8	355	50		
Education	Graduate & higher	0	0	4	0.6	0.60	0.96
	Undergraduate	10	21.3	148	20.9		
	Diploma or high school	19	40.4	341	48.2		
	Basic education	18	38.3	204	28.8		
	Illiterate	0	0	11	1.6		
Geographical district	Shiraz urban area	19	40.4	233	32.9	0.01	0.05
	Shiraz rural area	7	14.9	38	5.4		
	Other cities of Fars state	21	44.7	437	61.7		

A: Chi–Square or Fisher's Exact test (when more than 5% of cell had expected count less than 5) were used. (a = 0.05), B: Data that were suspected to be recurrent infection (IgM+, IgG+) and so logically couldent simultaneously primary infection excluded from this list.
* Having significant correlation (p< 0.05), ** Having very significant correlation (p< 0.01)

Discussion

The prevalence of HCMV antibodies in women in childbearing age varies greatly in different population groups. Lower rates of HCMV-IgG antibody prevalence (40-80%) have been reported in developed countries whereas higher rates of HCMV-IgG antibody prevalence (90-100%) have been reported in developing countries. These rates mostly depend on the variability of viral accessibility and its circulation rate in the community.[1] The IgG seropositivity was 93.0% in Fars Province which is in the range of developing countries and HCMV-IgM seropositivity was 5.4% in the province, which is interestingly the same as developed countries (3%-5.5%) when compared to developing countries (8%-8.5%).[12-14] Also, primary HCMV infection showed a base rate of 2.5% in the province, which is in the world rang (0-10%).[15]

Age is one of the risk factors that affect the seroprevalence of both HCMV-IgM and IgG antibodies. A direct correlation was observed between a rise in IgG seroprevalance with increase in age, and IgG titer level reaching a maximum of 3.33 ISR in 36-42 year old women (Figure 1d). These findings are in complete accordance with those of other studies which emphasize the role of age in HCMV IgG seroprevalance.[16-19] HCMV-IgM titer showed an increase until reaching its maximum rate of 0.6 ISR in 29 year old individuals in

Shiraz urban and other cities of Fars Province. The maximum rate (2.8%) of HCMV infections happened during 20-29 years of age, which is in accordance with the most frequent (60%) age of new marriages (Table 1). Besides, it was shown that IgG seroprevalance was higher in married women than among single ones, which is in concordance with previous findings which introduced sexual factor as a determinant of HCMV infection prevalence.[1,9,14] HCMV-IgM but not IgG titer significantly correlated with the pregnancy status. Also, most of pregnancies (83%) were seen during 20-34 years of age. To exclude the role of age interference on the correlation between IgM titer and pregnancy status, a Pearson partial correlation test was used (P=0.77). The result showed no significant correlation between the rate of pregnancy and HCMV infection seroprevalance. HCMV-IgM seroprevalance did not show seasonal fluctuation during the five years of study; but rather had a constant change during a very long period of time. HCMV-IgG titer revealed to have seasonal fluctuation rather than long periodical variation. Further analysis showed that IgG titer fluctuation was statistically significant (data not shown) only in those patients who had at least one child. Moreover, patients who were both IgG^- and IgM^-, revealed a significant seasonal fluctuation for primary HCMV infection ($p<0.001$). The finding about HCMV-IgG seasonal fluctuation could be explained by a combinatorial role of 2 main factors. Mothers at child bearing age, who had one or more child comprised 35% of the whole society of child bearing age women. Of this recent group, 96% were previously defined to have the lowest chance of taking primary HCMV infection because of their serological stute (IgG^+ and IgM^-). Previous findings have reported the important role of day care centers in transmitting HCMV infection from child to mothers.[9,18,19] For those mothers susceptible to HCMV primary infection, encountering a HCMV carrier child mostly ends up in a primary infection but for those mothers resistant to primary infection it usually means an HCMV-IgG titer increase to a higher level. Since day care centers and kindergartens are mostly active during winter and fall, the seasonal variation in HCMV-IgG titer might be somehow attributable to their role in transmitting HCMV infection from child to immune mothers. A history of previous abortion has no significant correlation with HCMV-IgM titer and primary or recurrent HCMV infection, but a negative correlation was observed with IgG titer especially in the case of obsessive abortion, which might be an indication of anti-HCMV maternal IgG titer in preventing serial abortion which is caused by HCMV infection.[8] Also a negative significant correlation was observed between previous abortions and IgG^-,IgM^- or IgG^+,IgM^- conditions.

Geographical distribution shows no significant correlation with HCMV-IgG and IgM titers, primary HCMV infection, and recurrent HCMV infection. IgG seroprevalance had the following order: other cities of Fars Province > Shiraz urban > Shiraz rural areas while IgM seroprevalence had the order of Shiraz urban > Shiraz rural areas = other cities of Fars Province. So, it is concluded that those who lived in the other cities of Fars Province, but not the women who lived in Shiraz may acquire HCMV infection earlier in their life while such a difference in seroepidemiology of HCMV was also noticed in other parts of the world.[19,20,21] A significant correlation was observed between primary HCMV infections with three hematological indexes of WBC, monocyte, and lymphocyte counts that are an indication of infectious mononucleosis,[1] while only monocyte and total WBC counts showed a significant correlation with recurrent HCMV infection. These findings suggest that hematological indexes of infectious mononucleosis might be worth for screening of primary HCMV infections.

Finally, the results of this study showed that the prevalence of HCMV infection among the women of childbearing age of Fars Province was in the range of worldwide HCMV infection. Significant correlations were detected between various epidemiological risk factors like; age, history of previous abortion, and number of child rather than previous parity number with HCMV infection. For no effective and safe therapeutic and prophylactic strategies against HCMV infection and disease, primary HCMV infections are very challenging health problems during pregnancy period in these women of childbearing age. Also more emphasis should be laid for women of childbearing age to include prospective screening programs for HCMV infection before pregnancy, and limited contact with HCMV infected children during pregnancy and responsible sexual practices. Also for reducing the chance of previous abortions, an effective HCMV vaccination program should be mentioned in the antiviral preventive strategies.

Acknowledgement

The authors would like to thank Dr. D. Mehrabani at Center for Development of Clinical Studies of Nemazee Hospital for editorial assistance.

References

1 Brooks GF, Butel JS, Morse SA. Herpes viruses, Cahpter 33. in*: Jawetz, Melnick and Adelberg's Medical Microbiology, 22nd Edn*. Lange Medical Books/McGraw-Hill, USA. 2001: 382-386.

2 Demmler GJ**.** Infectious Diseases Society of America and Centers for Disease Control: summary of a workshop on surveillance for congenital cytomegalovirus disease. *Infect Dis Rev* 1991;**13:**315-329.

3 Gaytant MA, Steegers BA, Semmekrot HM, Merkus HM, Galama JM. Congenital cytomegalovirus infection: review of the epidemiology and outcome. *Obstet Gynecol Surv* 2002;**57:**245-256.

4 Stagno SRF, Pass G, Cloud WJ**,** Britt, RE, Henderson PD, Walton DA, Veren F, Alford CA. Primary cytomegalovirus infection in pregnancy: incidence, transmission to fetus, and clinical outcome. *JAMA* 1986;**256:**1904-1908.

5 Boppana SB, Pass WJ, Britt WJ, Stagno S, Alford CA**.** Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr. *J Infect Dis* 1992;**11:**93-99.

6 Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. *J Am Acad Audiol* 2000;**11:**283-290.

7 Fowler KB, Mc Collister FP, Dahle AJ, Boppana S, Britt WJ, Pass RF. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus. *J infection Pediatr1997*;**130:**624-630.

8 Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody stateus*. New Engl J Med* 1992;**326:**663-667.

9 Forbes BA. Acquisition of Cytomegalovirus Infection: an Update. *Rev Clini Microbial* 1989;**2:**204-216.

10 Prince HE, Leber AL. IgM Levels Validation of an In-House Assay for Cytomegalovirus Immunoglobulin G (CMV IgG) Avidity and Relationship of Avidity to CMV. *Clini Diag Lab immiunol* 2002;**9:**824-827.

11 Revello MG, Gerna G. Diagnosis and Management of Human Cytomegalovirus Infection in the Mother, Fetus, and Newborn Infant. *Clini Micriobiol Rev* 2002;**15(4):**680-715.

12 Hizel S, Parker S, Onde U. Seroprevalence of Cytomegalovirus infection among children and females of Ankara, Turkey. *Pediatr Int* 1999;**41(5):**506-509.

13 Munro SC, Hall B, Whybin LR, Leader L, Robertson P, Maine G T, Rawlinson WD**.** Diagnosis and screening for cytomegalovirus infection in pregnant women. *J Clini Microbiol* 2005;**43(9):**4713-4718.

14 Ray K, Mahajan M. Seroprevalence of cytomegalovirus antibodies in patients attending STD and antenatal clinics. *J Commun Dis* 1997; **29(2):**85-90.

15 Turbadkar D, Mathur M, Rele M. Seroprevalance of torch infection in bad obstetric*. Indian J Med Microbiol* 2000;**21(2):**108-110.

16 Mathur A, Jindal I , Chaturvedi UC. A serological study of Cytomegalovirus infection at Lucknow. *Ind J Med Res* 1981;**73:**678-681.

17 Venkitaraman AR, Seigneurin JM, Lenoia GM, John TJ. Infections due to the human herpes viruses in Southern India: A sero epidemiological survey. *Int J of Epid* 1986;**16(4):**561-566.

18 Gambarroto K, Ranger RS, Aubard Y, Piva P, Duffetelle B, Delpeygrow C, Roussanne MC, Nicot T, Denis F. Primary cytomegalovirus infection and pregnant women: Epidemiological study on 1100 women at limoges. *Pathol Biol Paris* 1977; **45(6):**453-461.

19 Gratacap CB, Bosson JL, Morand P, Dutertre N, Chanzy B, Jouk PS, Vandekuckhove C, Cary LP, Seigneuren JM.Cytomegalovirus seroprevalence in French pregnant women: Parity and place of birth as major predictive factors. *Eur J Epidemiol* 1998;**14(2):**147-152.

20 Wen L, Wu S, 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(12):**714-717.

21 Mustakangas P, Saran S, Ammala P. Human cytomegalovirus seroprevalance in three different socioeconamically different urban areas during the first trimester : a population based cohort study. *Int J Eepidemiol* 2000;**29:**585-591.