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

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ABSTRACT

Human cytomegalovirus (hCMV) has been described as an important etiological agent of intrauterine infection in women of childbearing age that causes congenital malformation**.** In the present study we examined 844 serum samples from women of child-bearing age for the presence of IgM and IgG antibodies against hCMV by Elisa technique. 764 out of 844 (93%) of the cases were seropositive for hCMV-IgG and 45 (5.4%) cases were seropositive for hCMV-IgM. An increase in the rate of IgG seroprevalance was associated with an increase in age and parity. The IgG seroprevalance rate was inversely proportional to increasing abortions. Intrestingly seasonal variation affected IgG seroprevalance. There was an increasing trend in IgM positivity rate with age in women less than 29 years. hCMV seroprevalence rate was higher in women from rural as compared to those of urban areas. Finally hCMV primary infections occured in 2.4 % of all pregnancis and it is estimated that up to 0.3% of all congenital disorders, through out Fars province, were due to hCMV. We suggest a role of child to mother hCMV transmission and sexual maturity as the most probable epidemiological factors of hCMV seroprevalence among women of child bearing age.

Keywords: Human Cytomegalovirus, Seroprevalence, IgM and IgG antibodies

INTRODUCTION

Human cytomegalovirus (hCMV) seroprevalence varies in different populations and age groups. Congenital malformation is the most resulting clinical symptom of hCMV intrauterine infection (Stagno *et al*., 1986; Demler, 1991; Brooks *et al*., 2001; Gaytant *et al*., 2002). Up to 15% of intrauterine hCMV infections led to congenital symptomatic diseases. Asymptomatic congenital hCMV infection will develop in 10-15% of infants (Boppana *et al*., 1992; Fowler *et al*., 1997; Dahle *et al*., 2000). Intra uterus transmission of hCMV can occur during primary maternal infection, reactivation, or reinfection of seropositive mothers. Primary hCMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections (Stagno *et al*., 1986; Boppana *et al*., 1992; Fowler *et al*., 1992; Fowler *et al*., 1997; Dahle *et al*., 2000). 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 world wide infection, complex natural history, unusual relationship with infected host, ubiquity of infection, and lack of clinical symptoms in most cases, complicate the understanding of the epidemiology of hCMV infection (Forbes, 1989). In this study we examined the association of hCMV seroprevalence with some epidemiological risk factors in women of childbearing age.

MATERIALS AND METHODS

During 2001 to 2005 serum samples from 844 women at child bearing age (15 to 44 years old) that belonged to different geographical regions of Fars province were collected and stored at -20 °C until usage every week. The samples were screened for Specific IgM and IgG against hCMV antibodies according to manufacturer's instruction (Trinity Biotech co). The results were then interpreted on the basis of antibody titers as seropositive, seronegative and equivocal. Patients with positive IgM were checked for seroconversion two week later. Among patients that were positive for IgM only cases with seroconversion or high IgM titer (Prince *et al*., 2002) were considered as primary hCMV infections as previously described (Revello and Gerna, 2002). Patients that were simultaneously negative for IgM and IgG antibodies considered as susceptible to hCMV primary infection; whereas those with positive IgG and negative IgM were considered as resistant. Beside serological screening tests for hCMV; total and differential counting of white blood cells (WBCs) was carried out for each case for further analysis.

RESULTS

hCMV IgM seroprevalence

45 out of 844 (5.4%) cases were seropositive for hCMV-IgM antibodies. Among positive IgM cases 35.6 % (Pal *et al*., 1972) were from Shiraz urban area; whereas 4.4 %

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(Demler, 1991) were from rural areas, the rest of them belonged to other cities of Fars province.

Statistical analysis showed that there was no significant linear correlation (Pearson Correlation) between increasing in IgM seroprevalence and increasing in age (*p*= 0.65), increasing congenital disorders (*p*= 0.25) and increasing abortions (*p*= 0.65). On the other hand increasing in IgM seroprevalence associated with increasing gestations and parity numbers (*p<.05)*.

Maximum IgM seroprevalence rate occurred in women between 25-29 years old (Figure 1c). Further statistical analysis revealed that there was no significant linear correlation (Pearson Correlation test) between an increase in age and an increase in hMPV infection rate; although the rate of hCMV infection showed a strong correlation with age group 15-29 years (p= 0.04), versus age group29-39 years (p= 0.27).More over a soar in IgM seroprevalence associated with age until 29 years.

IgM seroprevalence rate was more in women from rural as compared to those of urban areas. In addition IgM seroprevalence rate was higher in married women in comparison with those of single ones, yet these findings were not statistically significant.

Data indicated that there was significant difference between hCMV-IgM positive rate in pregnant (in 9th Week or more of their gestations) versus non-pregnant women in favor of pregnant women (*p*= 0.03).

2.3 % (Revello and Gerna, 2002) of all cases had primary or acute hCMV infections. Among pregnant women 2.4% had primary infections. Also it was revealed that maximum primary infections rate belonged to women between 20-29 years. Primary infections rate was higher in women from urban as compared to those of rural areas; whereas the reverse was true for recurrent or reinfections rate (Table1). Demographic data and hematological indexes association with the presence of hCMV primary infection are reported in Table 1.

Figure 1: HCMV IgM(a)& IgG(b) seroprevalance during four consecutive years throughout Fars state.HCMV IgM(c)& IgG(d) seroprevalance in different age groups throughout three differenr geographical district (Dates are in A.D).

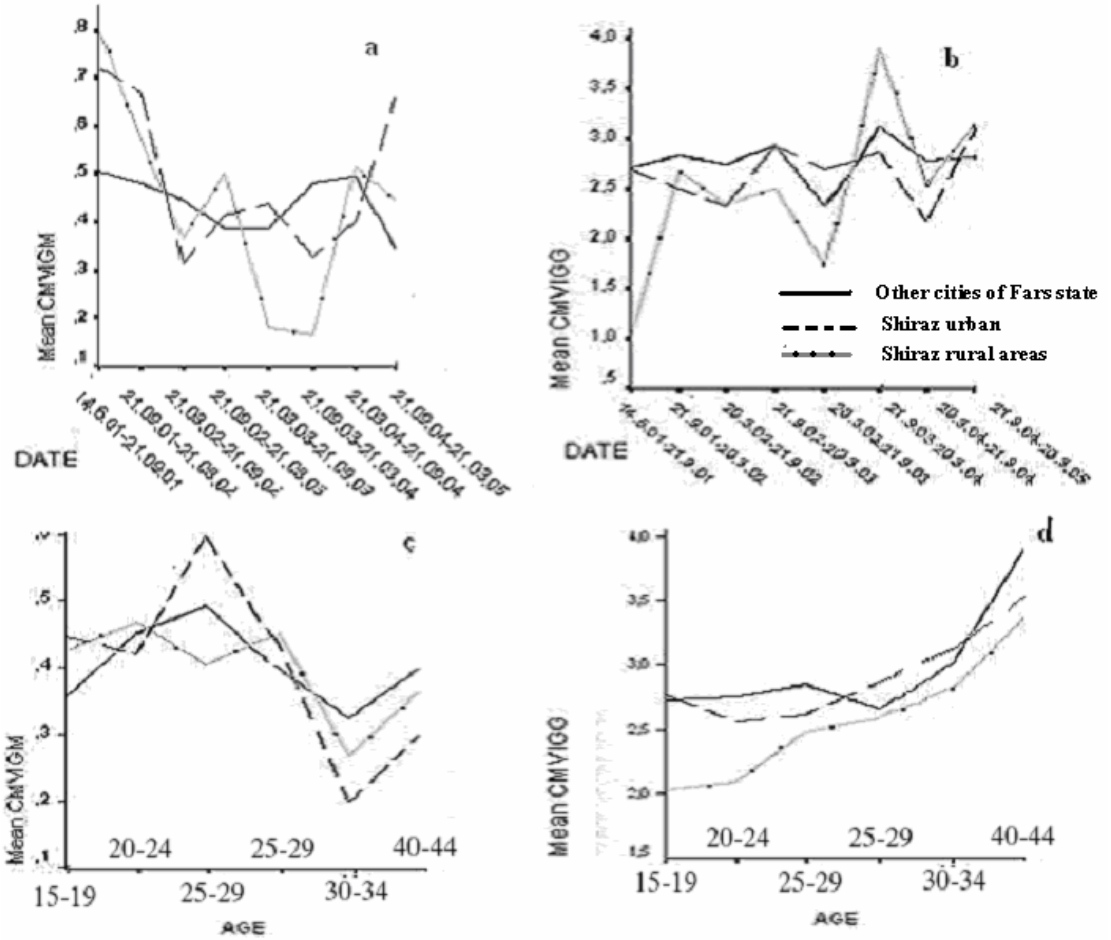

Table 1: demographic characteristic & hematological indexes distribution of childbearing age women with **Primary** HCMV infection disease.

Demographical characteristics		Primary HCMV infection [A]			P value [B]
		Number Of cases	Percent of Cases		
Age	15-19	3	15		.76
	20-24	11	55		
	25-29	6	30		
	30-34	0	0		
Marital statue	Married	15	75		.35
	Single	5	25		
Pregnancy statue	Pregnant	2	10		1
	Non pregnant	18	90		
Having abortion or child with congenital disorders history	Abortion background	3	27.3		1
	Congenital handicapped child	1	9.1		1
Previous Parity number	X>2	4	20		.62
	X=1-2	6	30		
	X=0	10	50		
Education	Graduate degree	0	0		.102
	Undergraduate degree	4	20		
	Diploma or high school	9	45		
	Basic education	7	35		
Geographical District	Shiraz urban area	5	25		.80
	Shiraz rural area	1	5		
	Other cities of Fars state	14	70		
number WBC of total differential indexes		Mean	Max	Min	
	WBC count* 10^3/ml	7.138	11.60	4.80	.00**
	Nutrophile count%	62.6	81	44	.22
	Eosinophil count%	3	5	1	.22
	Lymphocyte count%	30	54	14	.04*
	Monocyt count%	5.8	10	1	.03*

A: primary HCMV infection (IgM+IgG- wuth a sero conversion in IgG or IgM+ & IgG + with high IgM titer)

B: Chi-square or exact fisher's test (when more than 5% of cells had expected count less than 5) were used to inquire significant relationship between each character & two condition of HCMV infections (α=0.05).

* having significant correlation ($p<0.05$)

** having very significant correlation ($p<0.01$)

hCMV IgG seroprevalence

764 (92.9%) out of 844 cases were seropositive for hCMV-IgG antibodies. Statistical analysis revealed that congenital disorders (p= 0.67) wasn't associated with hCMV-IgG seropositivity. On the other hand there was an increasing trend in IgG seropositivity rate with age, increasing parity (p < 001), increasing gestations and decreasing abortion (p= 0.03). Age distribution of hCMV-IgG positive women are reported in Figure1d.

Geographical distribution of IgG positive women was stable throughout Fars province, with almost no statistical difference (p= 0.12). An analysis by two tailed independent T test showed hCMV-IgG prevalence rate among pregnant and non-pregnant patients was almost similar (p= 0.1). Also there was no correlation (p= 0.1) between marital statue and the rate of IgG seroprevalence.

Surprisingly statistical analysis during five years revealed that unlike IgM, IgG seroprevalence had a vigorous correlation (p< 0.001) with seasonal fluctuation especially in mothers: An increase in IgG positivity during fall/winter, versus a decrease during spring/summer (Figure 1a, 1b).

84% (708/844) of cases were resistant, while 5.5% (47/844) were susceptible to hCMV primary infections. A comparative correlation of two hCMV-IgG serogroups with hematological indexes and epidemiological risk factors is reported in Table 2.

DISCUSSION

The prevalence of hCMV antibodies during childbearing age is variable in the world. IgG prevalence is between 40% to 100%; while IgM seroprevalence rate is between 0% to 10%, depending on the variability of viral accessibility and its circulation rate in the community (Ray and Mahajan,1997; Mustakangas *et al*., 2000; Turbadkar *et al*., 2000; Brooks *et al*., 2001; Munro *et al*., 2005). As previously mentioned, our study indicated that IgG seroprevalence rate was 93.0 % that is comparable with those of developing countries (90% - 100%) (Brooks *et al*., 2001; Munro *et al*., 2005). In contrast, IgM seroprevalence rate 5.4% is very close to that reported in developed countries (3%-5.5%) (Ray and Mahajan,1997; Mustakangas *et al*., 2000; Turbadkar *et al*., 2000; Munro *et al*., 2005).These findings indicated the fact that by the age of 44 almost 93% of women had encountered hCMV, while active infection due to viral circulation occurred in 5.4% of the cases during child-bearing age. According to previous studies (Stagno *et al*., 1986) it is estimated that up to 1% of fetuses in pregnant women had been infected by hCMV via intrauterine infections, of whom 30% subsequently developed into congenital diseases.

As previously described, several epidemiological factors including age, parity numbers, number of gestations, geographical distribution, socioeconomic provinceus, marital provinceus and sexual maturity may contribute to hCMV seroprevalence in women of child bearing age (Pal *et al*., 1972; Gambarroto *et al*., 1977; Mathur *et al*., 1981; Venkitaraman *et al*., 1986; Griffiths *et al*., 1991; Wen *et al*., 1996; De Jong *et al*., 1998; Gratacap *et al*., 1998; Hizel *et al*., 1999).

Age is a determinant factor influencing hCMV seroprevalence. Data indicated that there was an increasing trend in IgG seropositivity rate with age that is in complete concordance with previous findings (Gambarroto *et al*., 1977; Mathur *et al*., 1981; Venkitaraman *et al*., 1986; Gratacap *et al*., 1998). Also age group 20-29 was determined as the major age group for the occurrence of hCMV primary infections (2.8%). Further analysis revealed that most marriages (60%) occurred among the recent age group. According to other studies this data raises the probable role of sexual maturity as a determinative factor of hCMV infections (Forbes, 1989; Brooks *et al*., 2001).

Table 2: demographic characteristic distribution of childbearing age women within two form 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	.73	.94
	20-24	21	44.7	343	48.4		
	25-29	11	23.4	159	22.5		
	30-34	3	6.4	56	7.9		
	35-39	1	2.1	15	2.1		
	40-44	0	0	4	.6		
Marital statue	Married	24	51	451	63	.08	.07
	Single	23	49	257	36		
Pregnancy statue	Pregnant	43	91.5	74	10	1.00	.50
	Non pregnant	4	8.5	634	89		
Having abortion or child with congenital disorder history	Abortion background	10	21.3	177	25	.02*	.005**
	Congenital handicapped child	6	12.8	80	11.3	.64	1.00
Previous Parity number	X>2	11	23.3	96	13.5	.02*	.19
	X=1-2	7	15	257	36.3		
	X=0	29	61.8	355	50		
Education	Graduate & higher	0	0	4	.6	.6	.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	.01*	.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 exact fisher's test (when more than 5% of cells had expected count less than 5) were used . (α=0.05)

B: data that were suspected to be recurrent infection (IgM+,IgG+) & so logically couldent simultaneously primary infection excluded from this list

* having significant correlation ($p<0.05$)

** having very significant correlation ($p<0.01$)

Another factor that may contribute in hCMV infection prevalence is geographical distribution (Wen *et al*., 1996; Gratacap *et al*., 1998; Mustakangas *et al*., 2000; Chakravarty *et al*., 2005). Unlike previous studies data showed that hCMV-IgG and IgM seroprevalence had no significant correlation, with geographical location, even if hCMV seroprevalence had higher value in rural as compared to those of urban areas. On the other hand primary infections rate was higher through out urban areas. A hypothesis for the probable role of geographical influence upon hCMV seroprevalance might be the route of infection. In rural areas saliva is probably 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 (Forbes, 1989). On the other hand in urban areas sexual transmission seems to be the major route of infection later in life during child–bearing age.

Other factors that may contribute to the hCMV seroprevalence are parity and gestation numbers. In our study perity and gestations numbers were correlated with hCMV seroprevalance.Further analysis with Pearson partial correlation test showed these correlations weren't due to the influence of age interference: the impact of age did not increase enhancing impact of parity ang gestation . These findings are in complete concordance with previous findings (Gaytant *et al*., 2002; Boppana *et al*., 1992; Fowler *et al*., 1992; Gratacap *et al*., 1998; Turbadkar *et al*., 2000).

In contrast with previous studies that ruled out the influence of seasonal fluctuation on hCMV seroprevalence (Brooks *et al*., 2001), hCMV-IgG seroprevalence was higher during fall/winter versus spring/summer. Further analysis revealed the difference is due probably to combinatorial role of day caring centers in transmitting hCMV infection from children to mothers (Forbes, 1989; Gambarroto *et al*., 1977; Gratacap *et al*., 1998) and periodical working seasons of day caring centers during fall/winter seasons versus spring /summer: Almost 95% of mothers population was resistant to hCMV primary infections due to preexisting immunity. Therefore hCMV infections in mother during school opening seasons mostly ended up in IgG immunity boosting, though reinfection where the hCMV strains are different cannot be ruled out (Fowler *et al*., 1992). By the way more is needed to be done for further confirmation.

An interesting finding in our study was negative association between increasing IgG and obsessive abortions. A decrease in abortions might be an indication that anti-hCMV maternal immunity could prevent abortions due to hCMV infections. Since there wasn't any significant correlation between IgG seroprevalence and congenital disorders, hCMV-IgG circulating antibody is only considered significantly protective against abortion versus congenital disorders. In accordance with previous studies (Mustakangas *et al*., 2000) these findings indicate that maternal antibodies afford substantial protection to the fetus but the protection is imperfect (Fowler *et al*., 1992; Boppana et al., 1992).

Eventually statistical analysis revealed that increasing in whole blood cells, lymphocyte and Monocyte counts significantly correlated with hCMV infections, even if lymphocyte count only correlated with primary as compared to recurrent or reinfections .These findings suggest that classic hematological patern for infectious mononucleosis could be seen only during primary infections versus recurrent or reinfections.

Considering all the epidemiologic factors that may contribute to hCMV infection among child-bearing age women through out Fars province, we suggest roles of age, parity, seasonal fluctuation, geographical position and sexual maturity as the most probable associated factors, though these factors are not totally independent and should be considered carefully for interrelated associations. Since there is no effective therapeutic and prophylactics strategies against hCMV infections, primary hCMV infections are very challenging health problem during pregnancy period. So more emphasis should be laid for women of childbearing age, including prospective screening program for hCMV infections before pregnancy, limited contact with hCMV infected children during pregnancy and responsible sexual practices. Also for reducing the chance of abortion implications an effective hCMV vaccination program should be concluded.

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