African Journal of Biotechnology Vol. 10(34), pp. 6616-6619, 11 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.162
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Prevalence and detection of cytomegalovirus by polymerase chain reaction (PCR) and simple ELISA in pregnant women

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Accepted 15 April, 2011

A total of 327 women were screened, amongst them, 7 (2.14%) were cytomegalovirus (CMV) DNA positive by polymerase chain reaction (PCR). Antibodies against toxoplasma were also detected in 106 (32.41%) women, while 54 (16.51%) were anti CMV positive. Eleven (3.36%) and thirteen (3.97%) women were anti HSV and anti Rubella virus positive, respectively. High prevalence was recorded in age group 21 to 30 years which was 2.53% (4/158), followed by the age group <20 years which was 2.27% (1/44), then in age group 31 to 40 years, 2.25% (2/89) CMV DNA were detected by PCR and 0% was recorded in age group of above 40 years. The overall prevalence of human cytomegalovirus (HCMV) infection in 16 to 45 year-old was 2.14% by PCR and the number of abortion noted was 0 to 5 times. Active infection of HCMV was observed in women with number of abortion. Force of infection was significantly higher among age group 21 to 30 and 31 to 40 years. PCR was a more sensitive, reliable and accurate method for the detection of HCMV infection in pregnant women during this study.

Key words: Cytomegalovirus, PCR, human cytomegalovirus (HCMV) and ELISA.

INTRODUCTION

Human cytomegalovirus (HCMV) is the most common source of congenital malformation resulting from viral intrauterine infection in developed countries (Jahromi et al., 2010). HCMV affects 1% of all infants born in the USA and less than 1% of all infants born worldwide. It is the leading viral cause of mental retardation and the most frequent non-hereditary cause of sensorineural hearing loss (Nassetta et al., 2009). HCMV is large, with a genome of 235 kb encoding 165 genes (Davidson et al., 2003). The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix (Chen et al., 1999). These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins (Mocarski et al., 2007).

Cytomegalovirus (CMV) infection during pregnancy can be transmitted to the fetus, resulting in a congenital infection and is a leading cause of hearing loss, vision loss and mental retardation (Soetens et al., 2008). About 58.9% of individuals aged 6 and above are infected with CMV (Staras et al., 2006). Women infected for the first time during pregnancy are likely to transmit CMV to their fetuses. More children suffer serious disabilities caused by congenital CMV than by several better-known childhood maladies such as Down syndrome or fetal alcohol syndrome (McCarthy et al., 2009). HCMV is the most common cause of congenital infection in humans and intrauterine primary infections are second only to Down's syndrome as a known cause of mental retardation (Ryan and Ray, 2004).

A number of investigators are evaluating qualitative and quantitative CMV DNA polymerase chain reaction (PCR) and antigenemia assays for their abilities to diagnose patients with CMV disease (Hansen et al., 1994; Rasmussen et al., 1995; Wetherill et al., 1996) and to

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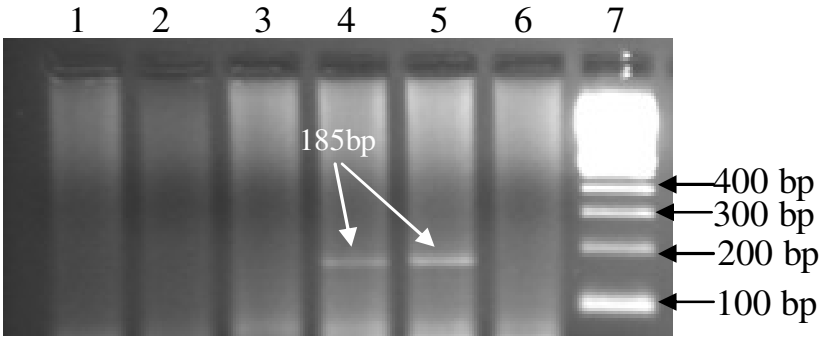

Figure 1. Gel picture of amplified DNA product of CMV. Lanes 1 to 3: Negative; lane 4: 5 CMV positive (185 bp); lane 6: negative control; lane 7: 100 bp ladder marker.

predict those at the highest level (Drouet et al., 1993; Shinkai et al., 1997). CMV infection diagnosis now relies on real-time PCR assays (Gault et al., 2001; Gouarin et al., 2004; Garrigue et al., 2006).

This study was conducted to investigate the prevalence of HCMV in pregnant women and its relation to the rate of abortions.

MATERIALS AND METHODS

Study design

HCMV infection during pregnancy can be transmitted to the fetus, resulting in a congenital infection. This study was proposed to analyze/detect the active infection of HCMV by PCR in pregnant women, which are the major contributor in congenital HCMV spread.

Source of samples

A total of 327 blood samples were collected from the gynecology wards of Lady Reading Hospital, Peshawar and Khyber Teaching Hospital Peshawar, the province large hospitals where patients visit from different areas of Khyber Pukhtoonkhwa (formerly NWFP). 5 CC of venous blood was taken in a sterile tube; serum was separated by centrifugation at 5000 rpm for 5 min. The samples were divided into two aliquots and were stored at -20°C and transported to the main Laboratory of Department of Biotechnology/Microbiology, KUST, where DNA was extracted from the serum and PCR was performed for CMV DNA amplification.

DNA extraction

DNA was extracted in biosafety hood type II from all blood samples with the help of Ultra script DNA extraction kit (Anagen, Inc. USA) according to the manufacturer procedure. 100 µl serum was used for DNA extraction and the purified extracted DNA was re-suspended in 40 µl of TE buffer and stored at -80°C in the refrigerator.

HCMV DNA amplification

Regular PCR

PCR reactions were carried out in a thermal cycler (Nyxtech USA) with *Taq* DNA polymerase (Fermentas USA). The first round of amplification was performed with 5 µl of extracted DNA by using an outer sense primer and an outer antisense primer. The reaction mixture for a single reaction consisted of 10X PCR Buffer- 2.0 µl, $MgCl_2$ (25 mM)- 2.4 µl, dNTPs (500 µM)- 1.0 µl, outer sense primer- 1.0 µl, outer antisense primer- 1.0 µl, dH_2O- 11.6 µl, *Taq.* DNA Polymérase (2 U/µl)- 1.0µ l and extracted DNA- 5.0 µl. The cycling conditions for regular PCR are denaturation temperature at 95°C, annealing temperature at 95°C for 45 s, 53°C for 45 s and 72°C for 1 min.

Nested PCR

The first round amplification product were re-amplified with nested primers in the same thermal cycler with *Taq* DNA polymerase. The second nested round was carried out to re-amplify 5 mµl PCR product of the first round by using an inner sense primer and inner antisense primer. The reaction mixture and conditions were the same as the first round, except the primers.

Electrophoresis

PCR products were electrophoreses in 2% agarose gel prepared in 0.5X TBE buffer (boiled for 2 min in microwave oven and cooled to 50°C), adding ethidium bromide (1 µg/ml) stain and evaluated under ultra violet light.

The specific DNA product for HCMV of each sample was determined by identifying the 185 bp amplified DNA bands in comparison with the 100-bp DNA ladder (Fermentas Germany), used as DNA size marker (Figure 1).

Prevalence rate

The prevalence of cytomegalovirus in pregnant women was determined by the following formula.

Prevalence (%) = (Number of anemic patients / Total number of patients) x 100

Statistical analysis

The data obtained was analyzed by using statistical software *statistix9*.

Table 1. Age wise prevalence of HCMV (no = 327).

Age group (number of individual)	TORCH				CMV DNA PCR (+ve %)
	Anti-CMV (+ve %)	Anti-HSV (+ve %)	Anti-RV (+ve %)	Anti-*Toxoplasma* (+ve %)	
1 (44)	20.45	0.00	0.00	18.18	2.27
2(158)	13.29	5.06	2.53	31.01	2.53
3 (89)	21.35	0.00	7.87	44.94	2.25
4 (36)	13.89	8.33	5.56	25.00	0.00

Age group I = <20 years; II = 21 to 30 years; III = 31 to 40 years; IV = >40 year. Roy's largest root = 0.318 ($p > 0.05$ non significant).+ve, positive.

Table 2. Abortion rate and prevalence of HCMV.

Number of abortion (number of individual)	TORCH				CMV DNA PCR (+ve %)
	Anti-CMV (+ve %)	Anti-HSV (+ve %)	Anti-RV (+ve %)	Anti-*Toxoplasma* (+ve %)	
0 (163)	6.13	0.00	0.00	16.56	0.00
1 (51)	27.45	13.73	1.96	35.29	1.96
2 (34)	17.65	0.00	11.76	61.76	2.94
3 (39)	23.08	10.26	12.82	48.72	5.13
4 (22)	45.45	0.00	9.09	54.55	4.55
5 (18)	27.78	0.00	5.56	50.00	11.11

Student's t test = 0.21 ($p > 0.05$ non significant).

RESULTS AND DISCUSSION

In this study, women that have no medical history of hypertension (high blood pressure), diabetes and renal (kidney) disease were included. A total of 327 women were screened, amongst them, 7 (2.14%) were CMV DNA positive by PCR. Antibodies against toxoplasma were also detected in 106 (32.41%) women, while 54 (16.51%) were anti CMV positive. Eleven (3.36%) and thirteen (3.97%) women were anti HSV and anti Rubella virus positive, respectively. High prevalence was recorded in the age group of 21 to 30 years which was 2.53% (4/158), followed by the age group of <20 years which was 2.27% (1/44), then by the age group of 31 to 40 years which was 2.25% (2/89). CMV DNA were detected by PCR and 0% was recorded in the age group of >40 years (Table 1). From a total of 327 individuals, 164 (50.15%) showed abortion rates of 1 to 5, while 163 (49.85%) showed no abortion. Out of the 51 (15.6%) pregnant women who had abortion once, only one (1.96% of this group) was CMV DNA PCR positive and 14 (27.45%) were CMV antibodies positive. Out of the 39 (11.93%) women who had abortion thrice, two (5.13%) women were CMV-PCR positive and nine (23.08%) were CMV antibodies positive, whereas in women who had abortion 4 times, one (4.55%) was CMV-PCR positive and ten (45.45%) were CMV antibodies positive (Table 2).

CMV the largest and most complex member of the herpesvirus family that infects humans (Gouarin et al., 2004), is named so due to the cytopathic effect resulting in enlarged cells having intranuclear and cytoplasmic inclusions (Stagno and Britt, 2006). They can be found in virtually every cell type of humans (Sinzger et al., 1995; Halwachs-Baumann et al., 1998; Hemmings et al., 1998). The most common known congenital viral infection is CMV, where in different parts of the world, its incidence has been estimated to be 0.2 to 2.2% of all live births (Fisher et al., 2000).

PCR is the method of choice for CMV DNA identification which has been used in various clinical specimens as amniotic fluid, urine, blood and cerebrospinal fluid (Ross et al., 2006). In this study, we used TORCH for antibodies detection and PCR for CMV DNA detection in blood of individuals with age range of 17 to 45 years. The CMV antibodies occurrence was estimated to be 16.51% and CMV DNA was detected in 2.14% pregnant women. The high prevalence and force of infection indicates that CMV is easily transmitted than some other infections such as measles. In USA, more than 27,000 pregnant women experience primary CMV infection and are thus at high risk of giving birth to a child with congenital CMV infection (Stehel and Sanchez, 2005). Special care and proper vaccination coverage are required to interrupt the spread of CMV (Colugnati et al., 2007). In Iran, anti CMV IgM was detected in 94% of the

women who had abortion history (Edmunds et al., 2000).

Analyzing CMV infection in different age group suggested that in women that are aged 16 to 45 years, infectivity rate is independent of age. This indicates that CMV transmission may be possible through these routes; same was also addressed by Jahromi et al. (2010). Other sources like non sexual close contact with urine and saliva may also play a major role in CMV spread (Stover et al., 2003). The screening of women before pregnancy for CMV antibodies will reduce abortion rate and also the number of congenitally infected infants. The transmission of CMV can possibly be prevented by various means (Ventura et al., 2001).

Conclusion

It is clear from this study that a lot of pregnant women are antibodies positive against CMV and *Toxoplasma* which is suggestive of putting many unborn babies at high risk of different serious disabilities.

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