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Short Communication

Detection of Cytomegalovirus in Pregnant Women of Lakki Marwat and Bannu, Khyber Pakhtunkhwa, Pakistan

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Authors' Contribution
KU and FS designed the study. FS, AK and RN collected samples and performed lab experiments, KJ and RH drafted the manuscript, AUR and AN analyzed the data, ZUR and SNK provided guidelines in collecting data and manuscript writing, IU and AF interpreted results. FUR edited the manuscript and KU approved the final draft of manuscript.

Key words
Cytomegalovirus, ELISA, PCR

ABSTRACT

Cytomegalovirus (CMV) infection is the most common cause of both congenital and intrauterine infection in developing fetus and pregnant women respectively. Proper in-time diagnosis of CMV is the most effective way to control this disease. Lack of health care facilities and proper screening of pregnant women for various congenital infections in the Sothern districts of Khyber Pakhtunkhwa, making the situation more worst. Therefore, this preliminary study was set to assess the seroprevalence and molecular detection of CMV infection among pregnant women of districts Lakki Marwat and Bannu of Khyber Pakhtunkhwa. A total of 188 blood samples were randomly collected from pregnant women of both districts and were examined through Enzyme-Linked Immunosorrbent Assay (ELISA) and Polymerase Chain Reaction (PCR) for CMV. Out of total 88 samples, 73.40% were positive for CMV through ELISA and 57.44% were positive for CMV through PCR respectively.

Cytomegalovirus (CMV), a double-stranded DNA virus belongs to family Herpesviridae. CMV infections are most prevalent in pregnant women causing auditory and mental retardation in developing fetus in developed countries and also throughout the world (Esquivela *et al*., 2018). Young infants are main source of CMV infection and occur approximately 1-4% of pregnancies. Rate of CMV transmission to developing fetus are as high as 50% in women acquired first CMV infection during pregnancy and less than 2% in women with non-primary infection (Davis *et al*., 2017). Intrauterine CMV transmission rates for primary and non-primary infections are about 30% and 0.2%, respectively. During breast feeding CMV is also transmitted from mother to child (Saldan *et al*., 2017).

Throughout the world CMV infections are present; however, it is more prevalent in Asia, South America and Africa and less prevalent in North America, England, Australia, Western Europe and Germany. In Saudi Arabia 90% CMV prevalence has been reported. Similarly, in Brazil 98.3% in non-pregnant women and 98% in pregnant prevalence of CMV antibodies were reported (Hassan *et al*., 2016; Sharghi *et al*., 2019). Pakistan is ranked high in pregnancy related deaths. In Pakistan mortality rate is 260 deaths per 100,000 live births. Moreover, in Pakistan neonatal and infant mortality rate has been estimated from 41-70 per 1000 births annually that accounts for 7% globally (WHO, 2012).

In Southern parts of Khyber Pakhtunkhwa maternal

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and child health related issues are on rising. Unhygienic practices during pregnancy also contributing to high morbidity and mortality. In the view of above mention context, the present study was conducted to find out the prevalence of CMV infection in pregnant women of Lakki Marwat and Bannu, Khyber Pakhtunkhwa.

Material and methods

The present study was carried out in districts Lakki Marwat and Bannu of Southern Khyber Pakhtunkhwa, Pakistan. Ethical approval was obtained from ethical review committee of the Kohat University of Science and Technology, Kohat.

A total of 188 blood samples (3ml) were randomly collected in sterilized vacationer from pregnant women of the study area and were transported to Molecular Parasitology and Virology laboratory, department of Zoology, Kohat University of Science and Technology Kohat. Sera was separated and tested for CMV-specific immunoglobulin G (IgG) through ELISA using Cenix diagnostic kit (Dresden Germany) following the manufacturer protocol.

DNA was extracted from blood samples in a biosafety hood type II cabinet using ultra script DNA extraction kit (Anagen, Inc. USA) as per manufacture protocol and stored at -20°C till for further analysis. Regular PCR was performed in a total reaction volume of 50µl by using 10X PCR buffer, 2.5mM magnesium chloride ($MgCl_2$), 10µM of each deoxynucleotide triphosphates (dNTPs), 5 units of Taq DNA polymerase (Fermentas, USA) and 10pmol of each forward and reverse primer: (B1: 5´-CAAGARGTGAACATGTCCGA-3´ and B2: 5´-GTCACGCAGCTGGCCAG-3´). The cycling conditions for PCR were denaturation at 94°C for 2 minutes followed by 35 cycles of 94°C for 30 seconds, 58°C for 40sec, 72°C for 50 sec, followed by final extension at 72°C for 3 min (Shams *et al*., 2011; Revathy *et al*., 2018).

Second round of amplification (nested PCR) was carried out using 5µl of a previous PCR product obtained in regular PCR. Amplification was done with a nested inner sense B3 (5´-TGGAACTGGAACGTTTGGC-3´) and antisense B4(5´-GAAACGCGCGGCAATCGG-3´) primers. The reaction mixture and the optimal conditions for nested PCR were the same as standard for regular PCR. The amplified PCR product was run on 2% agarose gel and visualized under ultraviolet (UV) light (Shams *et al*., 2011; Revathy *et al*., 2018).

Data was analyzed using SPSS version 18 used for data entry and analysis. P value was calculated, where $P< 0.05$ was considered significant at 95% CI (Confidence Interval).

Results and discussion

Among 188 pregnant women, 138(73.40%) and 108(57.44%) were found positive through ELISA and PCR respectively for CMV infection. A similar type of study conducted in India showed 8.5% positivity for CMV infection. In Afghanistan CMV was 99.79 % positive through IgG and 1.24% through IgM antibodies test (Husseini *et al*., 2019). Variation of the present study may be due to the different sample population, vaccination status, climate and geographic conditions from the previous studies.

We also analyzed CMV infection reference to socio-economic status of the study participants (Tables I and II). Results of the present study showed that women of low socio-economic status of both districts; Lakki Marwat (29.79%) and Bannu (17.02%) were at higher risk of getting infection as compared to those from the middle and high socio-economic level. Women of low income and congested family also had a high risk of transmission of CMV infection in the study area.

CMV infection in rural areas was higher in both district Bannu (24.47%) and district Lakki Marwat (30.32%). The high prevalence rate of CMV infection in rural may attribute due to unhygienic and unhealthy environmental condition at homes.

The sensitivity of PCR and ELISA was analyzed (Table III) and ELISA was found more sensitive for CMV infection as compared to PCR which is contrary with the finding of Revathy *et al*. (2018) where PCR was found more sensitive compared to ELISA.

Conclusion

It was concluded from the present study that significant number of the women were positive for CMV infection in the study areas. Education can play a major rule in CMV control and prevention. Individuals with lower socioeconomic status were at higher risk of CMV infection. Overall district Bannu has a low prevalence of CMV infection as compared to Lakki Marwat. In Bannu a low prevalence of CMV infection may be due to high literacy rate compared to district Lakki Marwat. Further studies will be required by taking more number of the women from the study area in order to get a clearer picture of the disease status in the study area and to know better about the sensitivity of diagnostic methods whether it is PCR or ELISA for CMV detection.

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Table I. Prevalence of CMV infection among pregnant women through ELISA.

Variables	Bannu %	Lakki Marwat %	95% Cl		P Value	Odd ratio for Bannu	Odd ratio for Lakki Marwat
			Lower	Upper			
Age group (n)							
12-23 years (53)	11.17	9.57	0.4407	38.559	0.05	0.66	0.51
24-35 years (60)	18.09	13.83	-20.825	80.825	0.08	1.31	0.76
36-47 years (75)	8.51	12.23	-24.972	63.972	0.11	0.27	0.44
Education (n)							
Illiterate (86)	19.68	22.34	7.7345	71.266	0.04	0.76	0.95
Primary (50)	6.91	5.85	-0.7062	24.706	0.05	0.35	0.28
Metric (36)	4.79	5.32	3.1469	15.853	0.03	0.33	0.38
Graduate (16)	3.72	4.79	-4.7062	20.706	0.08	0.78	1.29
Economic position(n)							
Low (126)	17.02	29.79	-108.47	196.47	0.17	0.34	0.80
Middle (37)	5.85	11.17	-47.531	79.531	0.19	0.42	1.31
High (25)	2.66	6.91	-41.825	59.825	0.27	0.25	1.08
Location(n)							
Rural (138)	24.47	30.32	-18.384	121.38	0.07	0.50	0.70
Urban (50)	6.38	12.23	-52.384	87.384	0.19	0.32	0.85

Cl, Confidence interval; one sample t- test; p-value < 0.05.

Table II. Prevalence of CMV infection among pregnant women through PCR.

Variables	Bannu Positive%	Lakki Marwat Positive%	95% Cl		P Value	Odd ratio for Bannu	Odd ratio for Lakki
			Lower	Upper			
Age group (n)							
12-23 years (53)	33.96	24.53	-16.266	47.266	0.10	0.51	0.33
24-35 years (60)	38.33	43.33	5.4407	43.559	0.04	0.62	0.76
36-47 years (75)	22.67	14.67	-24.119	52.119	0.13	0.29	0.17
Education (n)							
Illiterate (86)	19.77	45.35	-111.77	167.77	0.24	0.25	0.83
Primary (50)	20.00	16.00	-3.7062	21.706	0.07	0.25	0.19
Matric (36)	33.33	30.56	5.1469	17.853	0.03	0.50	0.44
Graduate (16)	25.00	43.75	-13.559	24.559	0.17	0.33	0.78
Economic position (n)							
Low (126)	17.46	35.71	-112.62	179.62	0.21	0.21	0.56
Middle (37)	21.62	48.65	-50.531	76.531	0.23	0.28	0.95
High (25)	24.00	36.00	-11.559	26.559	0.13	0.32	0.56
Location (n)							
Rural (138)	26.09	34.06	-162.74	205.74	0.3777	0.35	0.52
Urban (50)	14.00	36.00	-151.74	216.74	0.2672	0.16	0.56

Cl, Confidence interval; one sample t- test; p-value < 0.05.

Table III. Comparative analysis of ELISA and PCR test.

Variables	N %	Bannu		Lakki Marwat	
		IgG + %	PCR + %	IgG + %	PCR + %
Age group (n)					
12-23 years (53)	28.19	11.17	33.96	9.57	24.53
24-35 years (60)	31.91	18.09	38.33	13.83	43.33
36-47 years (75)	39.89	8.51	22.67	12.23	14.67
Education (n)					
Illiterate (86)	45.74	19.68	19.77	22.34	45.35
Primary (50)	26.60	6.91	20.00	5.85	16.00
Matric (36)	19.15	4.79	33.33	5.32	30.56
Graduate (16)	8.51	3.72	25.00	4.79	43.75
Economic level (n)					
Low (126)	67.02	17.02	17.46	29.79	35.71
Middle (37)	19.68	5.85	21.62	11.17	48.65
High (25)	13.30	2.66	24.00	6.91	36.00
Location (n)					
Rural (138)	73.40	24.47	26.09	30.32	34.06
Urban (50)	26.60	6.38	14.00	12.23	36.00

Statement of conflict of interest

The authors have declared no competing interests.

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