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MOLECULAR CLASSIFICATION OF CIRCULATING CMV STRAINS AMONG PREGNANT WOMEN IN PAKISTAN

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ABSTRACT

Background: *CMV impurity during pregnancy is a major concern as it can prime to inborn. CMV is a leading cause of neurodevelopmental disorders, including sensorineural hearing loss, cerebral palsy, and cognitive impairments. CMV is primarily transmitted through bodily fluids, including saliva, urine, blood, and sexual contact.*

Objective: *The objective of this study is to classify the circulating CMV strains based on gB genotype among pregnant women in Pakistan*

Methods: *The experimental cross-sectional study was conducted at the Molecular Diagnostics Laboratory, Riphah International University Lahore. A total of 50 pregnant women aged between 18 to 40 years were recruited for the study. Purposive sampling was used to select participants. Participants were conscripted from multiple antenatal clinics in Pakistan. Collecting blood samples for Polymerase Chain Reaction (PCR) testing requires meticulous attention to detail to prevent contamination and ensure the integrity of the sample. Data were analyzed using SPSS. Phylogenetic analysis was executed to visualize the genetic affairs amongst the identified genotypes.*

Results: *This study was comprised of 50 pregnant womenfolk of ages ranging from 20 to 40 years. Among them, 32 women (64%) reside in rural areas, while 18 (36%) live in urban areas and the majority (37 participants, 74%) belong to the poor socioeconomic category, while a smaller proportion (13 participants, 26%) are classified as rich. The most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%).*

Conclusion: *This study concluded that the most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%) in pregnant women in Pakistan. There is more prevalence in rural areas as compared to urban women and also more prevalence in poor females as compared to rich women when compared to socioeconomic status.*

INTRODUCTION

Cytomegalovirus (CMV) is a double-stranded DNA virus belonging from the Herpesviridae family and the Beta herpesviridae subfamily. It could be an exceedingly predominant pathogen around the world, with seroprevalence rates ranging from 40% to 100

depending on topographical area, socio-economic status, and cleanliness conditions. CMV may be a diligent infection that sets up long-lasting illeness within the have after essential disease, with the potential for intermittent reactivation, especially in immunocompromised people (1).

CMV spreads through sexual contact, congenitally through plancenta and breast feeding, transmitted via infected blood donors and coordinated contact with tainted real liquids such as spit, pee, genital discharges, and transplanted organs(2). Human cytomegalovirus (HCMV), an essential cause of inherent cytomegalovirus (CMV) diseases, could be a concern in both created and created nations. HCMV can be obtained prenatally through Tran's placental procurement of essential or repetitive maternal disease, or amid the perinatal/postnatal stage owing to introduction to contaminated cervical discharges, breast drain, or blood items. Essential disease is more prospective to result in extreme squeal whereas creating realms often have asymptomatic neonates. Cytomegalovirus (CMV) could be a part of an expansive family of herpesviruses that taints people of all ages. Despite being asymptomatic in healthy children and adults, CMV can cause serious diseases in newborns and individuals with compromised immune systems. Scientists have identified 11 species of CMV, with Human beta herpes virus 5 (HHV-5) being the most studied due to its pathogenicity for humans. The virus, which is enveloped by double-standard DNA, has been observed in infants since 1881. In 1957, Thomas Weller and Rowe and Smith isolated the virus, naming it Cytomegalovirus(3).

Normally CMV cause asymptomatic infection or display with gentle flu-like symptom, counting fever, weakness, sore throat, and swollen lymph hubs (mononucleosis-like sickness) (8) On the off chance that essential CMV disease happens amid pregnancy, there's a critical chance of intrauterine transmission, driving to congenital CMV contamination within the hatchling. CMV can cause a range of complications, including hearing loss, intellectual disabilities, vision impairment, microcephaly, hepatosplenomegaly, and intrauterine growth restriction (IUGR). The severity of inbred CMV depends on whether the infection is primary or secondary (reactivation or reinfection) (4).

About 85–90% of infants born with congenital CMV are asymptomatic at birth but may develop late-onset squeal such as hearing loss. The remaining 10–15% present with symptomatic disease, which can include jaundice, petechial, hepatosplenomegaly, and neurological impairments (5).

Despite its significant impact on newborn health, CMV remains an under recognized and underdiagnosed infection, particularly in low- and middle-income countries like Pakistan. There is no licensed CMV vaccine, and antiviral treatments are limited to severe cases. Routine screening for CMV during pregnancy is not widely implemented, highlighting the need for increased awareness, improved diagnostic strategies, and preventive measures such as hygiene education and potential vaccine development (6). High seroprevalence of CMV among pregnant women in Pakistan suggests that a significant majority have been exposed to the virus. However, a subset of women remains susceptible to primary infection during pregnancy, which poses risks for congenital CMV infection in newborns. Given these findings, routine screening for CMV among pregnant women in Pakistan may be beneficial to identify and manage potential risks effectively (7).

When fetus infection is detected, most pregnancies are ended because there is no effective treatment for congenital CMV infection. Congenital CMV can cause physical disabilities in the fetus, such as hearing loss and mental retardation. There is no effective treatment for CMV infection, and pregnancy is usually terminated when a fetus is diagnosed. There is currently no vaccine to prevent CMV, but phase 3 clinical trials in women of reproductive age are ongoing. Furthermore, CMV can be recognized by developing the infection from different example sorts utilizing either a shell vial or conventional culture. Sorts of examples are blood, cerebral liquid, pee, pharyngeal washings, bronchoalveolar lavage liquid, and biopsy examples (8).

The objectives of this study are:

- To detect CMV DNA in serum samples from pregnant women.

- To classify the circulating CMV strains based on gB genotyping.

MATERIAL AND METHODS

The current experimental cross sectional study was conducted at the Molecular Diagnostics Laboratory, Riphah International University Lahore. A total of 50 antenatal women were recruited for the study. Blood samples were collected for Polymerase Chain Reaction (PCR) testing, after applying ternequet, the selected area of the arm was cleaned with 70% alcohol antiseptic and sterile needles and syringes or a vacuum collection system was used to prevent contamination and ensure the integrity of the sample.

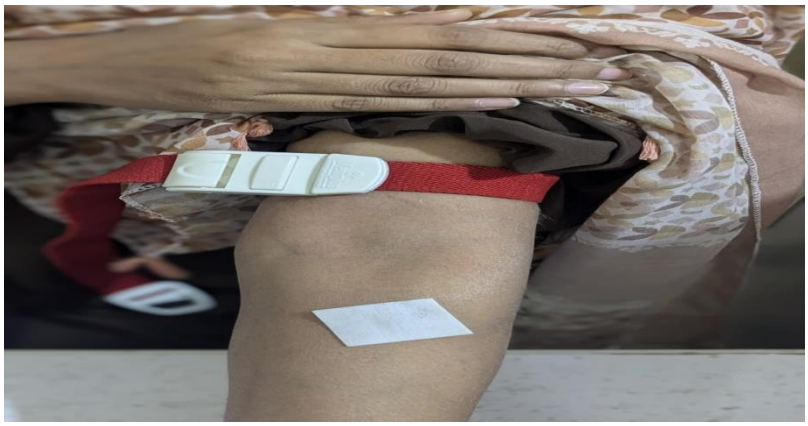


Figure 5: Sample collection procedure

The collected sample was labeled with patient name, lab Id and date accordingly. Immediately dispose of used needles and other sharps in an approved sharps container.

The entire collected sample was prepared for molecular studies to identify CMV in the specimen. First of all, DNA was extracted by suing commercially available DNA extraction kit having Proteinase K enzyme. All the specimens were centrifuged at 10000rpm for 10 minutes and supernatant was collected and 10 µL of proteinase K (20 mg/mL) was added to the sample. Then the mixture was at 56°C for 1 hour. 500 µL of phenolchloroform-isoamyl alcohol mixture was added to the sample and vortex the mixture for 10 seconds. Again centrifuge the mixture at 10,000rpm for 10 minutes and supernatant was collected and transfer it to a new tube. Then 500 µL of chloroform-isoamyl alcohol mixture was added to the sample, vortex the mixture for 15 seconds and centrifuge the mixture to collected supernatant. Added 1/10 volume of 3 M sodium acetate (pH 5.2) to the sample follwed by 2.5 volumes of 95% ethanol to the sample, incubated the mixture at 20°C for 30 minutes and after centrifugation, the pallet was collected and washed with 70% ethanol.

Collected DNA was suspended in TE buffer and glycerol for further process.

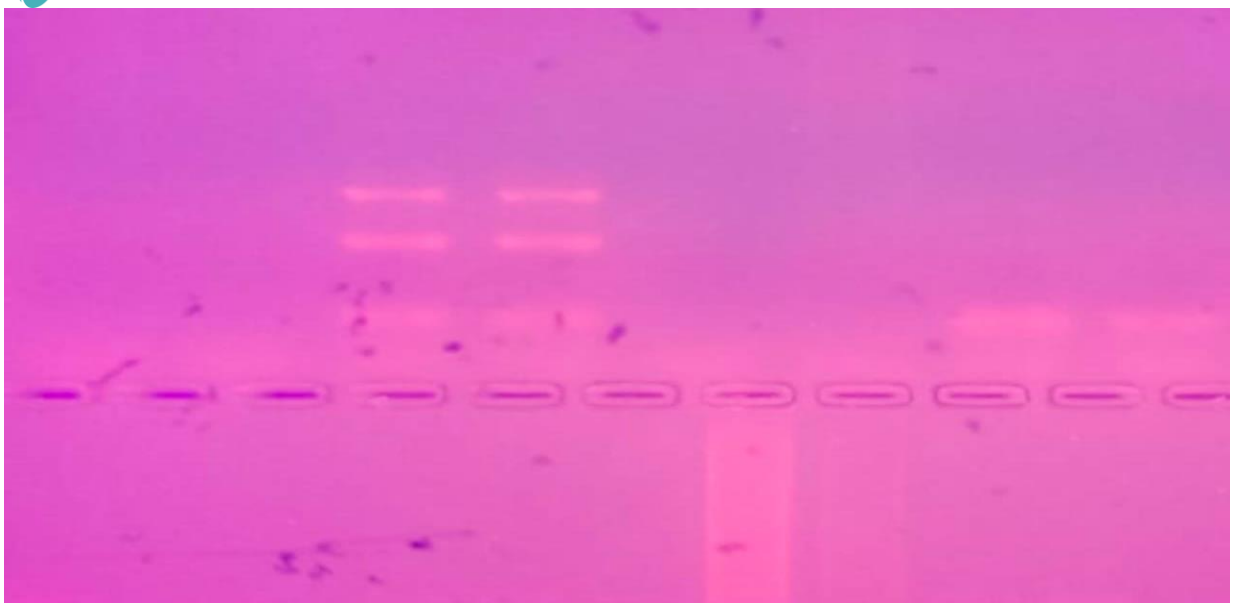
PCR amplification of the gB gene was performed using specific primers:

Forward primer: 5'-GAGTGACATTTTGACTGTTG-3'

Reverse primer: 5'-AGTCCGTTTCTACCCAGTAG-3'

The response blend (25 µL) contained 12.5 µL of ace blend, 1 µL of each ground work (10 µM), 5 µL of DNA format, and nuclease-free water. PCR conditions included an beginning denaturation at 95°C for 5 minutes, taken after by 35 cycles of denaturation (95°C for 30 seconds), strengthening (55°C for 30 seconds), and expansion (72°C for 45 seconds), with a last expansion at 72°C for 10 minutes.

Positive PCR products were purified using a commercial purification kit (Roche) and sequenced using Sanger sequencing. Sequences were aligned and analyzed using BLAST and Clustal W to determine gB genotypes. Phylogenetic analysis was conducted using MEGA software to understand the evolutionary relationships among the identified strains.



RESULTS

This study included a total of 50 pregnant women with ages ranging from 20 to 40 years. Among them, 32 women (64.0%) reside in rural areas, while 18 (36.0%) live in urban areas and the majority (37 participants, 74.0%) belong to the poor socioeconomic category, while a smaller proportion (13 participants, 26.0%) are classified as rich. The most frequently observed genotype is gB2 (38.0%), followed closely by gB3 (36.0%), accounting for 74.0% of the total cases. The gB1 genotype is found in 16.0% of participants, while gB4 is the least common (10.0%).

Table 1: presents data on the residential distribution

Residential Area			
	Frequency	Percent	Cumulative Percent
Rural	32	64.0	64.0
Urban	18	36.0	100.0
Total	50	100.0	

The most common genotype in rural areas is gB3 (15 individuals), followed by gB2 (13), gB1 (3), and gB4 (1). In urban areas, gB2 is the most prevalent (6 individuals), while gB1 (5), gB4 (4), and gB3 (3) are less common. Overall, genotype gB2 appears most frequently (19 occurrences), while gB4 is the least common (5 occurrences)

Crosstab					
Residential Area	Type of Genotype				
	gB1	gB2	gB3	gB4	Total
Rural	3	13	15	1	32
Urban	5	6	3	4	18
Total	8	19	18	5	50

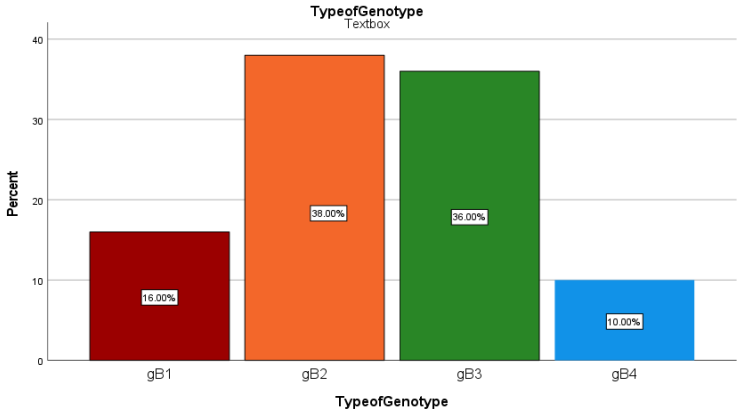
The mean genotype type in rural residents is 2.44, slightly higher than 2.33 in urban residents, indicating a minor variation in genotype distribution. The mean CMV viral load in rural participants (11,141.59 IU/mL) is notably higher than that of urban participants (8,191.39 IU/mL), suggesting a potential increased viral burden in rural areas. Additionally, the standard deviation (SD) for CMV viral load is 3,431.555 IU/mL in rural and 6,093.012 IU/mL in urban areas, indicating greater variability in viral loads among urban participants. Overall, the total mean CMV viral load across all participants is 10,079.52 IU/mL, with an SD of 4,730.343 IU/mL, highlighting a significant range of viral loads within the study population. These findings may reflect differences in healthcare access, environmental factors, or immune status between rural and urban populations.

Report

Residential Area		Type of Genotype	CMV PCR (IU/mL)
Rural	Mean	2.44	11141.59
	Std. Deviation	0.716	3431.555
Urban	Mean	2.33	8191.39
	Std. Deviation	1.138	6093.012
Total	Mean	2.40	10079.52
	Std. Deviation	0.881	4730.343

The table presents the relationship between residential area, CMV genotype type, and CMV viral load (IU/mL) among pregnant women

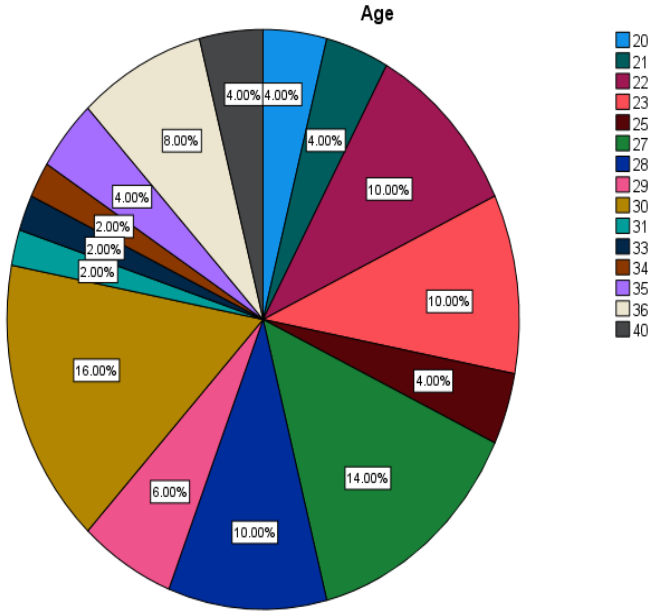
The most frequently observed genotype is gB2 (38.0%), followed closely by gB3 (36.0%), accounting for 74.0% of the total cases. The gB1 genotype is found in 16.0% of participants, while gB4 is the least common (10.0%). The cumulative percentage shows that by including gB2, 54.0% of the study population is covered, and with gB3, it reaches 90.0%, indicating that gB2 and gB3 are the dominant genotypes in this group. The findings suggest a possible regional or population-specific predominance of certain CMV genotypes, which may have implications for disease severity, transmission patterns, and immune response.



The bar chart illustrates the distribution of CMV genotypes

The out cones showed that most frequently occurring age is 30 years (16%), followed by 27 years (14%), indicating that the majority of participants are in their late 20s to early 30s. The cumulative percentage reveals that by age 28, more than half (56%) of the participants are accounted for, suggesting a concentration of cases in younger reproductive-age women.

While younger participants (20-25 years) make up 32%, a smaller proportion (18%) are aged 35 and above, showing a declining frequency with increasing age. These findings suggest that CMV infections or participation in the study may be more common in younger pregnant women, which could have implications for maternal and fetal health outcomes.



The pie chart illustrates the age distribution of the study participants

The chart shows that in rural areas, gB3 is the most prevalent genotype (around 13 cases), followed by gB2 (10 cases), while gB1 and gB4 are almost absent. In urban areas, gB2 remains the most common (around 6 cases), but the distribution is more balanced, with gB1, gB3, and gB4 showing lower but similar frequencies. The findings indicate that rural poor individuals are more likely to have gB2 and gB3 genotypes, while urban poor individuals show a more diverse genotype distribution. This could suggest differences in viral transmission dynamics or environmental factors influencing CMV strain prevalence between rural and urban settings.

Crosstab

Socio Economic status	Residential Area	Type of Genotype				Total
		gB1	gB2	gB3	gB4	
Low	Rural	1	10	13	0	24
	Urban	3	6	2	2	13
	Total	4	16	15	2	37
High	Rural	2	3	2	1	8
	Urban	2	0	1	2	5
	Total	4	3	3	3	13

Total	Rural	3	13	15	1	32
	Urban	5	6	3	4	18
	Total	8	19	18	5	50

Crosstabulation of Type of Genotype with Socioeconomic Statistics

DISCUSSION

The findings of this study reveal a high prevalence of CMV infection among pregnant women in Pakistan, with all 50 participants testing positive for CMV DNA. The predominance of the gB2 genotype aligns with previous studies conducted in South Asia, indicating the possible regional predominance of this genotype. This study included a total of 50 pregnant women with ages ranging from 20 to 40 years. Among them, 32 women (64%) reside in rural areas, while 18 (36%) live in urban areas and the majority (37 participants, 74%) belong to the poor socioeconomic category, while a smaller proportion (13 participants, 26%) are classified as rich. The most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%).

In 2014 Herry Sovereign et al. found that the hazard of intrauterine transmission of cytomegalovirus (CMV) amid pregnancy is much more prominent for ladies who contract essential CMV disease after conception than for ladies with prove of disease (circulating CMV antibodies) sometimes recently conception.

Thinks about conducted over the final 20 a long time convincingly illustrate

that estimation of CMV IgG ardentness is both a delicate and a particular strategy for recognizing pregnant ladies with later essential CMV contamination and hence at expanded chance for vertical CMV transmission. IgG eagerness is characterized as the quality with which IgG ties to antigenic epitopes communicated by a given protein; it develops steadily amid the 6 months taking after essential disease. Moo CMV IgG eagerness is an exact marker of essential contamination inside the going before 3 to 4 months, though tall eagerness avoids essential contamination inside the going before 3 months. In this mini review, we summarize distributed information illustrating the clinical utility of CMV IgG eagerness comes about for assessing time since essential disease in pregnant ladies, portray commercially accessible CMV IgG eagerness measures, and talk about a few of the issues and contentions encompassing CMV IgG ardentness testing amid pregnancy. Similarly in our study gB2 was more common as compared to gB3 and gB1. The most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%) (9).

In 2013, Chakravarti et al. did a think about they found that in asymptomatic pregnant females (n = 505), rubella inspiration was 3.16 % and in ladies with awful obstetric history (BOH) (n = 220), it was 7.72 %, whereas CMV inspiration was 5.9 % in both asymptomatic pregnant ladies and in ladies with BOH. In children (n = 200), the in general inspiration for rubella- and CMV-specific IgM antibodies was 15 and 25 %, separately. A declining drift was watched within the frequency of both rubella and CMV diseases in pregnant ladies and ladies with BOH. In children, the frequency of intrinsic rubella disorder has declined, but the frequency of CMV disease has remained nearly the same in 5 a long time. While in our study we selected only igB strain variants in pregnant women in Pakistan. In our study, the most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%) (10).

CONCLUSION

The findings of this study reveal a high prevalence of CMV infection among pregnant women in Pakistan, with all 50 participants testing positive for CMV DNA. The predominance of gB2 genotype aligns with previous studies conducted in South Asia, indicating the possible regional predominance of

this genotype. This study included a total of 50 pregnant women with ages ranging from 20 to 40 years. Among them, 32 women (64%) reside in rural areas, while 18 (36%) live in urban areas and the majority (37 participants, 74%) belong to the poor socioeconomic category, while a smaller proportion (13 participants, 26%) are classified as rich. The most frequently observed genotype is gB2 (38%), followed closely by gB3 (36%), accounting for 74% of the total cases. The gB1 genotype is found in 16% of participants, while gB4 is the least common (10%).

In conclusion, this study provides a foundational understanding of the molecular classification of CMV strains among pregnant women in Pakistan. The findings underscore the urgent need for continuous surveillance, genotype-specific interventions, and robust public health strategies to manage CMV infections effectively. Significant strides can be made in improving maternal and neonatal health outcomes in Pakistan and beyond by addressing these challenges.

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