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Genotyping of cytomegalovirus glycoprotein n and GB and its relationship with abortion status in an Iraqi female sample

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Abstract
In this study, 120 blood samples was collected from pregnant women attending different hospitals in Anbar which were obtained during the period from December 2021 to June 2022, information was gathered about the pregnant women's ages (16-46) and residences (urban or rural). All samples have been tested for the presence of both IgG and IgM using Rapid Test, ELISA and PCR. The results in the current study showed Rapid test the existence of a high IgG positive (63.3%) rate and an IgM positive (33%) rate. The ELISA-IgG test, the results of the examination showed that the highest positive percentage was in the age group 20-29 years (29.2%), while the lowest percentage (9.17%) was in the < 20 years group. The rural infection percentage (15.8%) was highest than the urban infection rate (3.33%). The results of the current study show a genotyping of isolates taken from pregnant women by comparing the category of aborted and non-abortive women, this heterogeneity may be related to cases of abortion in pregnant women.

Keywords: Abortion, genotyping, glycoprotein, human cytomegalovirus, phylogenetic analysis

1. Introduction
Human cytomegalovirus(HCMV) a large DNA virus, is the most common herpes virus of the planet. CMV is a major medical issue connected to stillbirth and congenital malformations (Sinzger *et al.* 2000) [15]. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) is produce latent infection and spread it to people of every age and socioeconomic class, is highly prevalent with an estimated 83% sero-prevalence in the worldwide populace (Zuhair *et al.* 2019) [16].
HCMV, also known as human herpesvirus-5, is one of the eight human herpesviruses (HHV-5). Infections with congenital cytomegalovirus (cCMV) are frequently caused by HCMV (Cannon 2009) [5]. CMV infection can be contracted before birth (congenitally) through transplacental transmission of a primary or recurrent maternal infection, as well as through contact with infected blood, breast milk, or cervical secretions. HCMV has a very wide tissue tropism, which permits it to infect practically every organ system in the body .Approximality 65% virus was reported of pregnant women during the first trimester and began to diminish dramatically in late pregnancy. In industrialized nations, only 10% of people are infected by this slowly replicating herpes virus, whereas in impoverished nations, IgG positive can reach up to 90% (Pass *et al.* 2006) [13]. Clinical signs can be asymptomatic 88% of cases, and this leads to severe fetal damage and in rare cases to death due to miscarriage, additionally after a few months or even years, 10-15 percent of babies who had no symptoms at birth could develop late consequences, including hearing problems (Buxmann *et al.* 2017) [4]. It is the most genetically complex viral pathogen in humans. HCMV groups are highly diverse, which may allow the virus to evolve in the human host over short periods of time (Renzette *et al.* 2013) [14]. HCMV genotyping is mostly based on variations in surface glycoprotein sequences, which exhibit frequent genetic polymorphism (Pignatelli *et al.* 2010) [12]. HCMV is a physically complicated virus with several envelope glycoproteins, many of which are projected to be heavily glycosylated. gN, a type I glycoprotein that is particularly fascinating for a variety of reasons, is one such highly glycosylated envelope protein, it's part of the gM/gN compound, which is one of the few

envelope proteins shared by all herpes viruses, suggesting that this glycoprotein complex plays a significant role in herpes virus biology (Pellet 2007) [11]. Glycoprotein B is a polymorphic glycoprotein that belongs to the envelope complex gB-I and has four genotypes (gB1-gB4) (Fries *et al.* 1994) [7]. Aims of the Study: There are few studies dealing with the genetic variation of CMV in Iraq. The current study aims to diagnose CMV, its relationship to abortion.

Methods and material

The detection of CMV of blood samples of pregnant women with spontaneous abortion by the following methods.

Detection of (CMV) serological analysis

Raped test OnSite ® CMV IgM/IgG and enzyme Linked Immune Sorbent Assay (ELISA) was performed for both IgG and IgM. Using 3rd generation commercially available ELISA kits, serum samples was examined for the presence of CMV IgG antibodies. (Human/ Germany) for IgG and detection according to the manufacturer instructions.

Principle

A lateral flow chromatographic immunoassay is used in the OnSite® CMV IgG/IgM Rapid Test. The test strip for the cassette device consists of. (1) A colored conjugate pad containing colloidal gold-coated control antibodies and CMV antigens (CMV conjugates). (2) A nitrocellulose membranes strip with 2 test lines (G and M) and a control line (C). Anti-human IgG has been pre-coated on the G line to help it recognize anti-CMV IgG. Anti-human IgM has been pre-coated on the M line to help identify anti-CMV IgM. A control line antibody has been used to pre-coat the C line. A negative test result is indicated by the lack of any test lines (G or M). The colored line of the control antibody immune complex should be visible in the test internal control (C line), which should occur independently of the test lines' color development (M and G). If no control line (C) appears, the test is invalid and the specimen must be retested using other machinery showing in Figure.1.

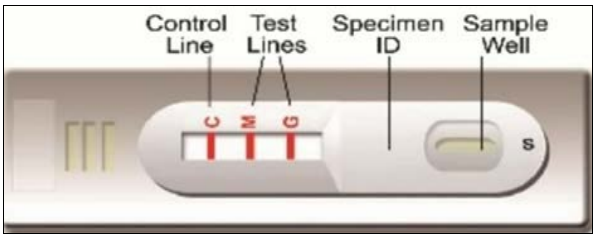

Fig 1: Raped test

Principle

The CMV IgG ELISA Test Kit is a solid phase enzyme immunoassay based on the indirect standard for the detecting of CMV IgG antibodies in serum. CMV antigens are coated on the 96 microwell (12×8) plate. When a pre-diluted specimen is inserted and subsequently incubated If the material includes CMV IgG antibodies, they will attach to the antigens coated on the microwell plate, resulting in immobilized antigen-CMV IgG antibody complexes. Complexes will not form if the material lacks IgG antibodies against CMV. The microwell plate is cleaned after first incubation to eliminate loose elements. The microwell plate is filled with the enzyme-conjugated anti-human IgG antibody, and it is then incubated. Anti-human IgG antibodies that have been enzyme-conjugated will bind to IgG antibody complexes of the inactivated CMV antigen. After the second incubation, the microwell plate is cleansed eliminate loose materials. Substrates A and B are mixed together and incubated to generate a blue hue that indicates the quantity of CMV IgG antibodies present in the samples. To prevent the reaction from changing the microwell plate's hue from blue to yellow, sulfuric acid solution is added. A microplate reader is used to detect the color intensity at 450 nm, which represents the quantity of CMV IgG antibodies present in the samples.

Statistical Analyses

Microsoft Office Excel 2010 was used to extract the average and percentage of the study samples in all its categories, as well as the SPSS program version 26 was used to analyze Chi-Square and Correlation.

Results and Dıscussıons

Results

Distribution of samples according to IgG for (CMV) and age groups by rapid test

The results in the current study show 76 positive samples (63.3%), while the negative samples for the IgG by the rapid test were 44 (36.7%) (Figure 1).

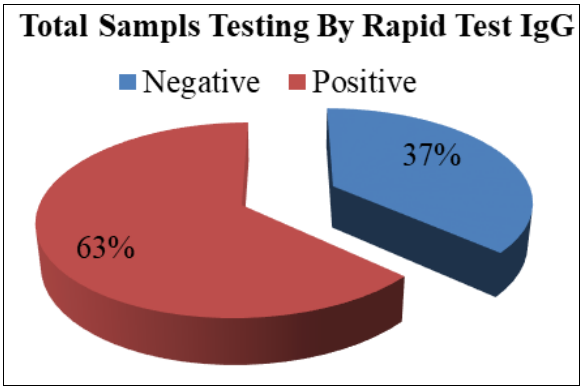

Fig 2: Distribution of samples according to IgG Rapid test for CMV

Table.1, shows the distribution of study samples based on age groups, depending on the Rapid IgG test, the results of the examination showed that the highest positive percentage was in the age group 20-29 years (28.3%), while the lowest percentage (6.67%) was in the < 20 years group. Statistical analysis revealed no significant differences at the ≤ 0.05 level.

Table 1: Distribution of samples according to IgG for CMV and age groups

Chi-Square Tests of Total Samples According to Age Groups and IgG Testing By Raped Test						
IgG Age Groups	Positive		Negative		Total	
	NO.	%	NO.	%	NO.	%
< 20 Years	8.00	6.67	7.00	5.83	15.0	12.5
20-29 Years	34.0	28.3	20.0	16.7	54.0	45.0
30-39 Years	24.0	20	12.0	10	36.0	30.0
> 39 Years	10.0	8.33	5.00	4.17	15.0	12.5
Total	76.0	63.3	44.0	36.7	120	100
Chi-Square P. value	0.827				df:3	

Distribution of samples according to (IgG) for (CMV) and habitation by rapid test

The current findings revealed very high statistically significant differences at the level of significance ≤ 0.05, depending on housing where the highest positive percentage was in the countryside (40%) while the lowest positive percentage was (23.3%) in the city (Table.2).

Table 1: Distribution of samples according to IgG for CMV and habitation

Chi-Square Tests of Total Samples According to Habitation and IgG Testing By Rapid Test						
IgG Habitation	Positive		Negative		Total	
	NO.	%	NO.	%	NO.	%
Rural	48.0	40.0	15.0	12.5	63.0	52.5
Urban	28.0	23.3	29.0	24.2	57.0	47.5
Total	76.0	63.3	44.0	36.7	120	100
Chi-Square P. value	0.002**				df:1	

Distribution of samples according to (IgG) for (CMV) and age groups by (ELISA)

The results in the current study show 76 positive samples (70%), while the negative samples for the IgG by the ELISA were 44(30%) (Figure 4.2). Table.3, shows the distribution of study samples based on age groups, depending on the ELISA-IgG test, the results of the examination showed that the highest positive percentage was in the age group 20-29 years (29.2%), while the lowest percentage (9.17%) was in the < 20 years group. Statistical analysis did not show any significant differences at the level of ≤ 0.05.

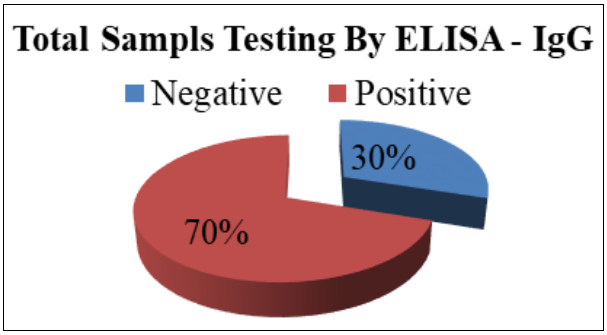

Fig 3: Distribution of samples according to ELISA-IgG for CMV

Table 2: Distribution of samples according to IgG for CMV and age groups

Chi-Square Tests of Total Samples According to Age Groups and IgG Testing By ELISA						
IgG Age Groups	Positive		Negative		Total	
	NO.	%	NO.	%	NO.	%
< 20 Years	11.0	9.17	4.00	3.33	15.0	12.5
20-29 Years	35.0	29.2	19.0	15.8	54.0	45.0
30-39 Years	26.0	21.7	10.0	8.33	36.0	30.0
> 39 Years	12.0	10.0	3.00	2.50	15.0	12.5
Total	84.0	70.0	36.0	30.0	120	100
Chi-Square P. value	0.666				df:3	

Distribution of samples according to (IgG) for (CMV) and habitation by (ELISA-IgG)

The present results showed that there were very high statistically significant differences at the level of significance ≤ 0.05, depending on housing where the highest positive percentage was in the countryside (44.2%) while the lowest positive percentage was (25.8%) in the city (Table.4).

Table 3: Distribution of samples according to IgG for CMV and habitation

Chi-Square Tests of Total Samples According to Habitation and IgG Testing By ELISA						
IgG Habitation	Positive		Negative		Total	
	NO.	%	NO.	%	NO.	%
Rural	53.0	44.2	10.0	8.33	63.0	52.5
Urban	31.0	25.8	26.0	21.7	57.0	47.5
Total	84.0	70.0	36.0	30.0	120	100
Chi-Square P. value	< 0.001**				df:1	

Distribution of samples according to (IgM) for (CMV) and age groups by rapid test

The results in the current study show 39 positive samples (33%), while the negative samples for the IgM by the Rapid test were 67 (67%) (Figure .3).

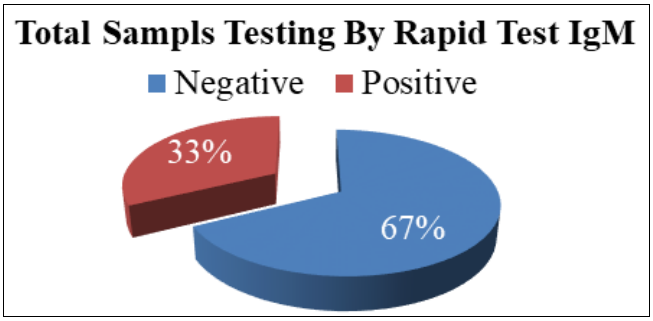

Fig 4: Distribution of samples according to IgM Rapid test for CMV

Table.5, shows the distribution of study samples based on age groups, depending on the Rapid IgM test, the results of the examination showed that the highest positive percentage was in the age group 20-29 years (10%), while the lowest percentage (5%) was in the < 20 years group. The statistical analysis revealed statistically significant differences at the ≤ 0.05 level.

Table 4: Distribution of samples according to IgM for CMV and age groups

Chi-Square Tests of Total Samples According to Age Groups and IgM Testing By Raped Test						
IgM	Positive		Negative		Total	
Age Groups	NO.	%	NO.	%	NO.	%
< 20 Years	6.00	5.00	9.00	7.50	15.0	12.5
20-29 Years	12.0	10.0	42.0	35.0	54.0	45.0
30-39 Years	10.0	8.33	26.0	21.7	36.0	30.0
> 39 Years	11.0	9.17	4.00	3.33	15.0	12.5
Total	39.0	32.5	81.0	67.5	120	100
Chi-Square P. value	0.002**				df:3	

Distribution of samples according to (IgM) for (CMV) and habitation by rapid test

The present results showed that there were very high statistically significant differences at the level of significance ≤ 0.05, depending on housing where the highest positive percentage was in the countryside (21.7%) while the lowest positive percentage was (10.8%) in the city (Table.6).

Table 5: Distribution of samples according to IgM for CMV and habitation

Chi-Square Tests of Total Samples According to Habitation and IgM Testing By Rapid Test						
IgM	Positive		Negative		Total	
Habitation	NO.	%	NO.	%	NO.	%
Rural	26.0	21.7	37.0	30.8	63.0	52.5
Urban	13.0	10.8	44.0	36.7	57.0	47.5
Total	39.0	32.5	81.0	67.5	120	100
Chi-Square P. value	0.031*				df:1	

Discussion

Human cytomegalovirus (HCMV) is the major cause of congenital infections in developing nations. The clinical manifestations of this virus range from asymptomatic forms (in 88%) of cases to severe fetal damage and in rare cases death due to miscarriage (Aljumaili *et al.* 2014) [2]. Furthermore, HCMV is a leading viral cause of fetal infection, which can result in severe clinical complications e.g chorioretinitis, encephalitis, pneumonia, microcephaly, and hearing loss as well as impaired cognitive development (AbdulBasit and Yassir 2018) [3]. Congenital CMV is diagnosed when CMV DNA is detected in any of the infant's bodily fluids within the first 21 days of life. Because CMV infection or reactivation takes advantage of low or compromised immunity, pregnant women are especially vulnerable to CMV reactivation due to immune down-regulation during pregnancy. Pregnancy has been described as an immunological condition that presents a number of difficulties in the diagnosis, prevention, and management of infectious diseases (Mhandire *et al.* 2019) [10].

The current study's results show 76 positive samples (63.3%), while the negative samples for IgG by the Rapid test were 44 (36.7%). Additionally, the results show the distribution of study samples based on age groups, depending on the Rapid IgG test, where the examination revealed that the highest positive percentage was in the age group 20-29 years (28.3%), while the lowest percentage (6.67%) was in the age group 20 years as shown in Figure.1 and Table .1.

According to our study, 39 positive samples (33%) were found, while 67 (67%) were found to be negative for IgM by the Rapid test. Additionally, the results show the distribution of study samples based on age groups, with the highest positive percentage (10%) found in the age group 20-29 years, and the lowest percentage (5 percent) found in the age group 20 years as shown in Figure.3 and Table .5.

The results also revealed that there were very high statistically significant differences at the level of significance 0.05, depending on housing, with the countryside having the highest positive percentage for IgG (40%) and the city having the lowest positive percentage (23.3%). While the results showed that there were statistically significant differences at the level of significance 0.05, the highest positive percentage for IgM was in the countryside (21.7%) and the lowest positive percentage was in the city (10.8%), as shown in (Table .2, .6).

According to IgG for CMV and age groups by ELISA, the current study found 76 positive samples (70%),while 44 negative samples for IgG by ELISA (30%). Furthermore, according to IgG for CMV and Habitation by ELISA-IgG, the current results revealed that there were very high statistically significant differences at the level of significance 0.05, depending on housing, with the countryside having the highest positive percentage (44.2%) and the city having the lowest positive percentage (25.8%), as shown in Figure .2 and Table 4.

Transplacental CMV transmission in women with preexisting seroimmunity may develop during pregnancy as a result of viral reactivation or infection with a different CMV strain (reinfection), although the current study and global reports both show a high seroprevalence of CMV IgG antibodies, preconceptional immunity against CMV only offers partial protection against intrauterine transmission, and adverse outcomes can occur in infected children born to women who were seropositive before pregnancy (Aljumaili *et al.* 2014) [2].

Through the serological detection of CMV IgG antibodies among pregnant Sudanese women (Jalel *et al.* 2017) [8], showed that among the 87 blood samples examined, (73.6%) were CMV positive, while the result was (26.4%) negative, while the result was negative. (71.2%) samples contained anti-CMV IgG antibodies, (28.8%) had anti-CMV IgG antibodies and no sample was negative for both anti-CMV IgG antibodies. The results of our study are in line with the study conducted by (AbdulBasit and Yassir 2018) [3], where they found that the distribution of IgG antibodies increases with age in aborted women and that the highest percentage of IgG was in women aged 26-35 years.

Another study conducted by (Hama and Abdurahman 2013) [6] on the immunoglobulin for human cytomegalovirus (IgG) and immunoglobulin M (IgM) in pregnant women in Baghdad city and their relationship to abortion factors, where found in this study that the prevalence of anti-CMV antibody IgG in primary aborted women (88.5%) of cases are positive and the prevalence of anti-CMV IgG antibodies

in pregnant women (91.2%) of cases are also positive, they found that the seroprevalence of anti-CMV IgG antibodies is higher in pregnant women and aborted women primarily between the age group (26-30) year with an average living situation and survive in an urban residence, and this is consistent with the results of our current study.

For the quantification of anti-HCMV IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA), a study by (Jihad 2015). Women who had miscarried had the greatest rate of CMV seropositivity, with 40% IgG and 25% IgM, whereas infertile women had 20% IgG and 15% IgM. Younger women (20-30) had greater levels of HCMV IgG seropositivity, whereas older women (31-40) had higher levels of IgM, and this is consistent with the results of our current study in terms of seropositivity of HCMV IgG that is higher In younger women. As such, our study is also in agreement with a previous study by (Anwar and Al-Bayati 2018) where 77 (44.5%) of the aborted women were in the age groups between (21-30) years. To estimate the seroprevalence of cytomegalovirus antibodies amongst pregnant women in Nigeria, a total of 97.2% of the pregnant women were anti-CMV IgG positive (Akinbami *et al.* 2011). A previous study on the relationship between CMV infection and miscarriage in Iraqi women found that women with IgG seroreactivity had the highest risk of miscarriage (87.8%) and IgM seropositivity had the lowest miscarriage rate (3 women with a recurrence rate). (4.1%) and 6 (8.1%) tested positive for IgM and IgG, indicating an old and recent infection (Turkey *et al.* 2022) [9].

Conclusions

There is a significant pervasiveness of anti-HCMV IgG and IgM antibodies, as well as a relationship with abortion. Using gN, gB primers, PCR were sensitive, quick, and effective for diagnose CMV infection.

Recommendation

During the current study, it was concluded that there is a genotyping of isolates taken from pregnant women by comparing the category of abortion and non- abortion women, and this heterogeneity may be related to abortion cases. We recommend future researchers to study in depth these variants and mutations.

Conflict of Interest

Not available

Financial Support

Not available

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