IMMUNE ANTIBODIES DIAGNOSIS OF PREGNANT WOMEN IN IRAQ INFECTED WITH HERPES SIMPLEX VIRUS AND HUMAN CYTOMEGALO VIRUS

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Article history:		Abstract:
		The goal of this study was to determine the prevalence of Cytomegalovirus (CMV) infection and Herpes simplex virus(HV) in Iraqi pregnant women based on their age and pregnancy trimester. During the period of January 2018 to January 2020, 300 aborted women's venous blood was obtained from several Iraqi private laboratories. Specific anti-herpes and anti-CMV antibodies were evaluated in serum samples chosen at random (IgM, IgG). A commercial ELISA kit (Human – Germany) was used. The 300 patients were split into two groups: 150 pregnant women with a bad obstetric history and 150 pregnant women with primigravida who aborted at different times (1st, 2nd, and 3rd trimesters of pregnancy). The current findings revealed that anti-herpes IgM levels in group I were significantly higher than in group II, (45.6%) Group I scored 82 out of 150, which was greater than group II (26.6%) 40 out of 150 In addition, IgM in anti-CMV among group I was 80 out of 150 (53%), which is also higher than group II(32.6%) 49 Out of 150. While anti-HV IgG in group I (74%) 111 out of 150 was greater than in group II (61.3%) 92 out of 150, anti-CMV IgG in group II (74.6%) 112 out of 150 was higher than in group I (66%) 99 out of 150. Anti HV-Anti CMV antibodies were found in 43,56 of 150 people in group I and 38, 52 of 150 people in group II. There were 121 women aged 18-28 and 177 women aged 29-44 and 2 ≤ 44 among the 300 women with a mean age of 27.9 ± 5 years and a range of 18-44 years. In the first, second, and third trimesters of pregnancy, group I had a gestational period of (74(49.3%), 42(28%),34(22.6%) and group II had a gestational period of (81(54%), 46(30.6%),23(15.3%)) respectively . This study suggests that because of the high incidence of CMV and HV infection among pregnant women, as well as the high percentage of infection among patients with a high level of illness ignorance, every pregnant woman should be checked for HV and CMV serological status.

INTRODUCTION

Pregnancy is regarded as a one-of-a-kind physiological event in a woman's life. However, in certain circumstances, many twists and turns occur, turning a positive pregnancy outcome into a mortality. Pregnancy can bring up a lot of bad emotions for women who have had a previous disappointing pregnancy[1]. Bad Obstetric History (BOH) refers to a fetal result that has been poor in the past, such as two or more consecutive spontaneous abortions, intrauterine fetal death, intrauterine growth retardation, stillbirth, early neonatal death, and/or congenital abnormalities. Genetic, hormonal, aberrant maternal, immunological response, and maternal infection are all possible causes of BOH. The most common cause of BOH is primary infections caused by cytomegalovirus and herpes simplex virus agents [2, 3]. During pregnancy, viral infections are a leading cause of maternal and fetal morbidity and mortality. Infections can be transplacental, prenatal (through vaginal secretions or blood), or postnatal (by breast milk or other sources) in nature. [3,4]. Members of the Herpesviridae family, HSV and HCMV, are the cause of a wide range of infections. Although they are usually asymptomatic, they might have a negative impact on labor if they are contracted during pregnancy or

delivery. Although there are treatments and preventions for both illnesses, screening exposed women is debatable because the danger of neonatal contamination is not well understood. The human is infected by eight herpesviruses. Herpessimplex virus types 1 (HSV1) and 2 (HSV2) cause labial and genital herpes, respectively, and Herpesvirinae includes varicella and zona virus (VZV),[5]. In HSV, two-thirds of cases, the mother shows no signs or symptoms[6]. The risk of Neonatal Herpes virus in maternal genital at delivery is 75% (no antibodies). risk is 3 to 5% in maternal genital recurrence at delivery (antibodies available),[7] The risk increases by 10% if there is a maternal or paternal genital history. Neonatal Herpes has a 1% chance of occurring in any female patient.[8]. ELISA is used to make the diagnosis. Cell culture or a direct immunofluorescence assay are used to diagnose asymptomatic excretion at the cervix in the context of a previous history of genital herpes This indication is now being studied using gentle PCR amplification. This highly sensitive approach is controversial because it fails to distinguish latency from active infection, which is critical for determining Herpes virus risk[9]. Primary infection during pregnancy is a major public health concern for both viruses because it poses serious dangers to the kid. The viruses never leave the body after primary infection and enter a latency or persistence state, which is crucial to the infection's immunopathogenesis. Human cytomegalovirus is a common pathogen that causes asymptomatic and chronic infections in healthy people[10] It is the most prevalent viral infection that causes congenital CMV infections during the perinatal period, occurring in about 1% of all live births and causing birth abnormalities and childhood impairments[11,12]. In affluent nations, maternal primary CMV infections during pregnancy have been documented to be a major source of severe congenital infection in many prior investigations[13].

MATERIALS AND METHODS
Collection of samples

Samples were gathered from 300 pregnant women between the ages of 16 and 44, all of whom had an abortion. The samples were collected from a number of Baghdad-based private laboratories. Participants were given ten milliliters of venous blood at random. Between January 2018 and January 2020, samples of each woman's blood were centrifuged at 4000 rpm for 5 minutes to separate serum from blood.
Serological analysis

Specific IgG and IgM antibodies to cytomegalo virus and herpes simplex virus were measured by

using Enzyme-linked immunosorbent assay, with commercial ELISA equipment (Human. Germany). The optical density (OD) was read at 450 nm by the spectrophotometer ELISA reader, as per the manufacturer's instructions.
Preparation Of Blood Samples

Each sample prepared in test tube by diluting 10 µL of patients serum and 1 mL of sample diluent, then gently mixing till 5 min. Incubated for 25 minutes after covering with adhesive strips. It was then washed four times at room temperature using washing solution. The conjugate was then added to each well at a volume of 100 µL. 25 minutes at room temperature, covered and incubated. Washing all wells by washing solution five times, then 100 µL of substrate added, after that, incubating for 25 min. After adding 50 µL of stop solution, take a 30-minute absorbance reading at 450 nm.

RESULTS

This study was designed to investigate the prevalence of Cytomegalovirus (CMV) infection and Herpes simplex virus(HV) in pregnant women, as well as their age and trimester of pregnancy. IgG and IgM levels were measured in 300 pregnant women. ELISA is a technique for detecting antibodies. The average age of the patients in the study was 27.9 ± 5 years. varied in age from 18-44 years old. With 121 women aged 18-28 and 177 women aged 29-44 and 2 ≤ 44 years figure1. The age and gestational age of pregnancy were found to have a significantly relationship with CMV and HSV (p <0.01). Table 1 shows the results . The 300 patients were divided into two groups: 150 pregnant women with a bad obstetric history in their first, second and third trimester (49.3%),(28%) and (22.6%)respectively, whereas, the other 150 primigravida women who aborted in first (54%),second(30.6%) and third (15.3%)trimester of pregnancy respectively. As shown in figure2 and table 2 with a high significant correlation. Table 3 shows the seroprevalence of freshly acquired infection of Anti-herpes IgM levels in group I were significantly higher than in group II, according to the current data (45.6%) Group I scored 82 out of 150, which was greater than group II (26.6%) 40 out of 150 as shown in figure 3, In addition, IgM in anti-CMV among group I was 80 out of 150 (53%), which is also higher than group II(32.6%) 49 Out of 150 it shows high significant in figure 4. While, anti-HV IgG in group I (74%) 111 out of 150 was greater than in group II (61.3%) 92 out of 150 with no significant correlation, figure 3. Anti-CMV IgG in group II (74.6%) 112 out of 150 was higher than in group I (66%) 99 out of 150 it

is also no significant correlation shows in figure 4. Anti HV-Anti CMV antibodies were found in 43,56 of 150 people in group I and 38, 52 of 150 people in group II. The positive combination presence of specific anti CMV antibodies IgG and IgM was 17(11.3%) in group I and 29(19.3%) in group II,as shown in figure 4. HSV anti IgM, IgG antibodies was 32(21.3%) and 26(17.3%) for group I and II respectively. statically there is a significant association at (p<0.01). figure 3

Table (1): Distribution the age of pregnancy in relation with (group I) of BOH and (group II) primigravida women

Age	Number tested	Group I(BOH)	Group II (primigravida)	P value
18-28	121	54(44.6%)	106(87.6%)	
29-44	177	94(53%)	44(24.8%)	P= 0.017*
≤ 44	2	2(100%)	0 (0%)	

*significant at (p<0.01)

Table (2): Prevalence of pregnancy according to the abortion time (group I) of BOH and (group II) primigravida women.

Trimester of pregnancy	Group I(BOH)	Group II (primigravida)	P value
First trimester	74(49.3%)	81(54%)	
second trimester	42(28%)	46(30.6%)	P= 0.0112 *
third trimester	34(22.6%)	23(15.3%)	

*significant at (p<0.01)

Table(3): Frequency distribution of Herpes simplex virus and human Cytomegalovirus infection IgM and IgG antibodies using ELISA test in (BOH) and (primigravida)

Factors	Group I(BOH)	Group II (primigravid)	P value
CMV			
Positive (IgM)	80(53%)	49(32.6%)	0.0323 *
Positive(IgG)	99(66%)	112(74.6%)	0.985 NS
positive (IgM,IgG)	17(11.3%)	29(19.3%)	0.0017 *
HSV			
Positive (IgM)	82(45.6%)	40(26.6%)	0.0342 *
Positive(IgG)	111(74%)	92(61.3%)	0.969 NS
positive (IgM,IgG)	32(21.3%)	26(17.3%)	0.0012 *

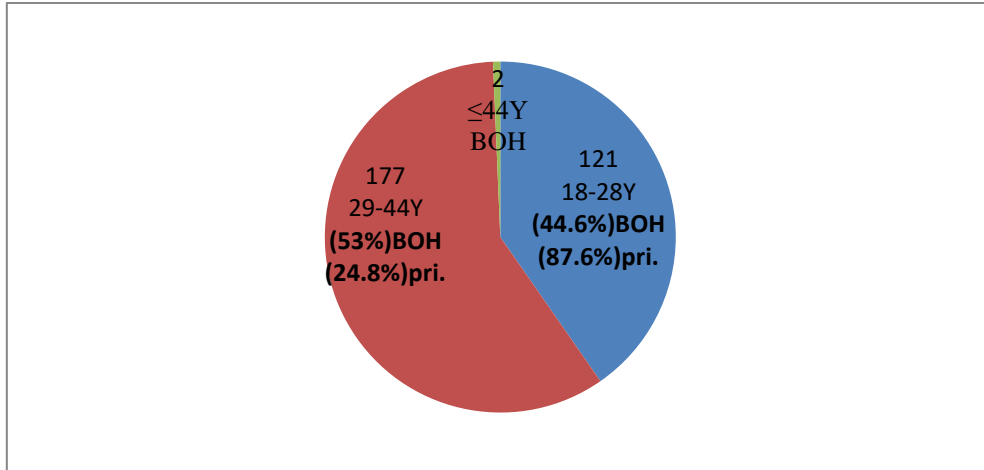*significant at (p<0.01) NS- no significant

Figure (1): Distribution the age of pregnancy in relation with (group I) of BOH and (group II) primigravida women.
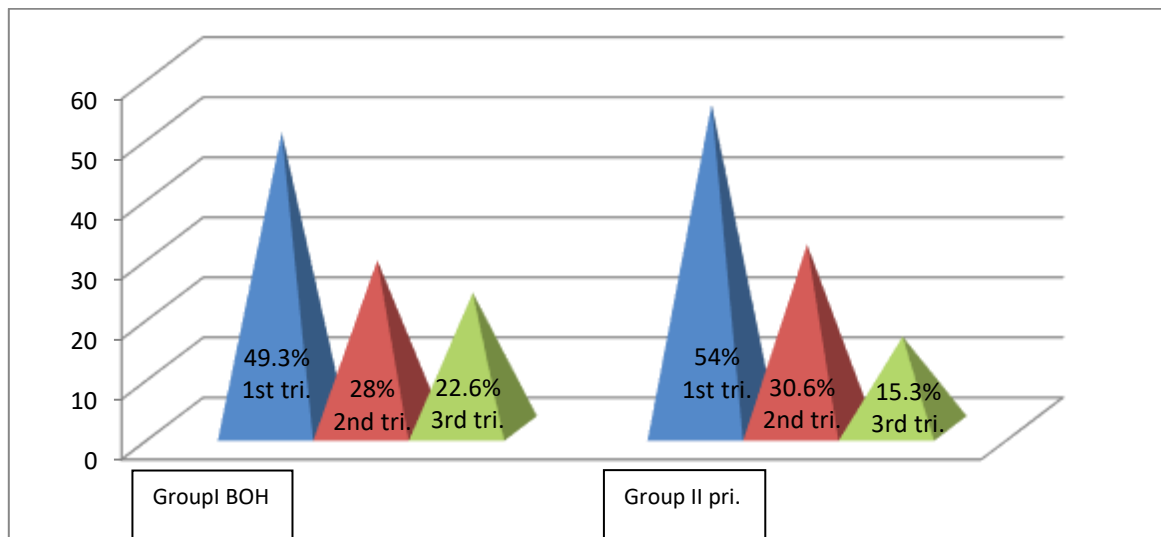

Figure (2): distribution of pregnancy according to the abortion time (group I) of BOH and (group II) primigravida women.
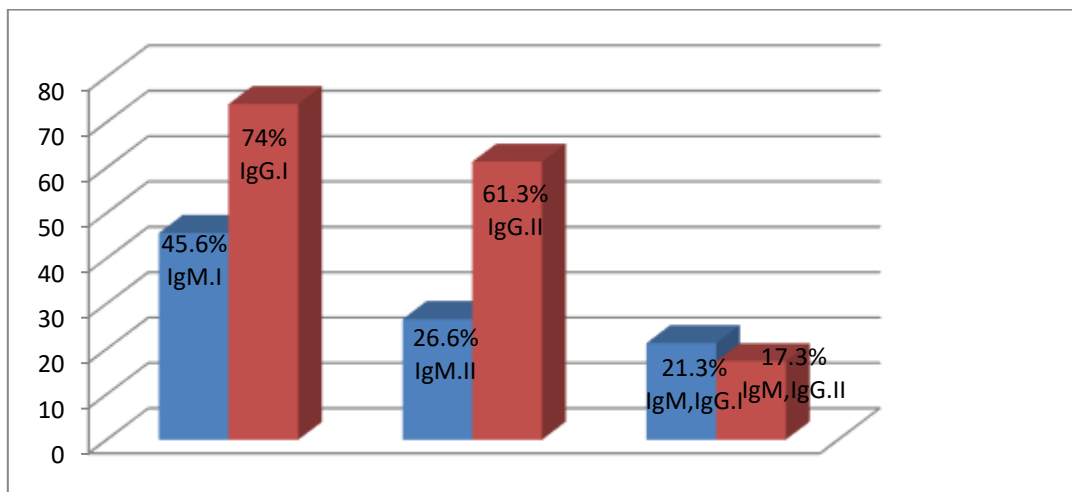

Figure (3): Seroprevalence of HSV IgM, IgG positive results in group I and II.
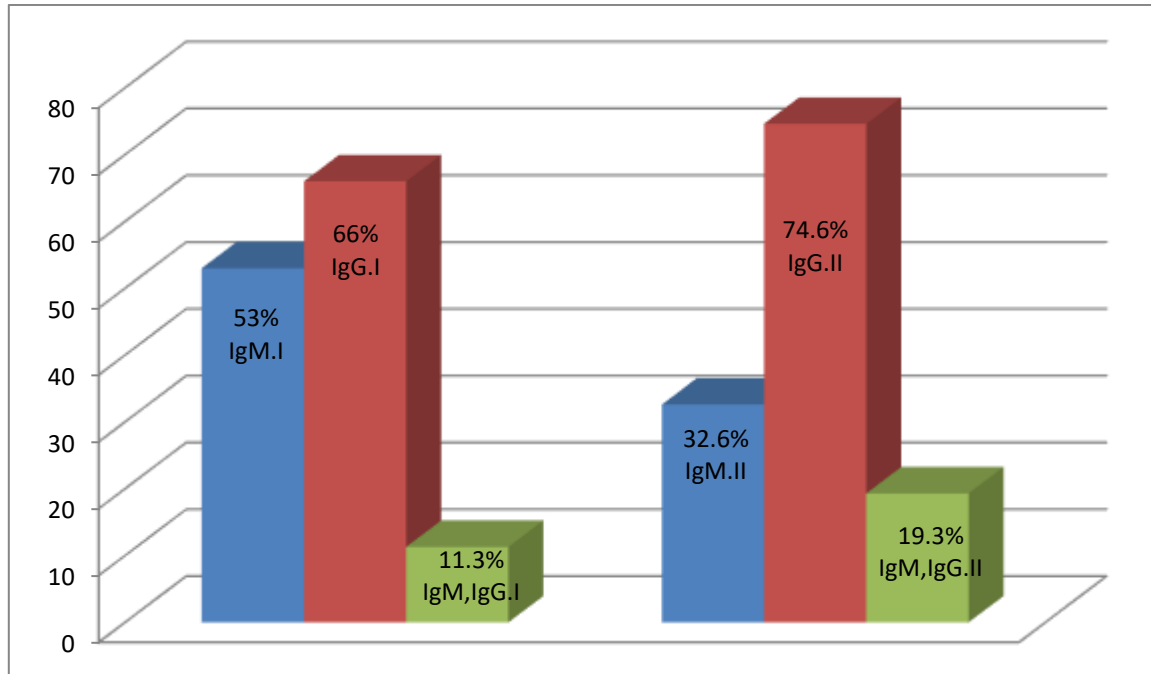
Figure (4): Seroprevalence of CMV, IgM, IgG positive results in group I and II

DISCUSSION

The largest member of the herpes family is cytomegalovirus ,which is spread by close contact and is assumed to be contaminated by blood, urine, saliva, semen, and cervical secretion[14]. Even in the acute stage, the majority of CMV infections seen in pregnant women are asymptomatic. Fewer than 6% of pregnant women with primary infection are reported to have symptoms, and even fewer have mononucleosis syndrome[15]. The best method for making a diagnosis is through laboratory tests (virology and serology). If seroconversion to CMV is found, it is simple to make the diagnosis of primary CMV infection. The discovery of CMV IgM has been utilized as a marker of ongoing or recent CMV infection because confirmation of CMV seroconversion is uncommon and women are not regularly checked for CMV antibodies prior to gestation[16]. Over the past 20 years, both the herpes simplex virus (HSV) and its most dangerous consequence, newborn herpes, have become more common in genital areas.[28,29] When a baby comes into touch with either HSV type 1 (HSV-1) or HSV type 2 (HSV-2) that is present in the birth canal of an asymptomatic mother during labor and delivery, neonatal HSV infection most frequently ensues [30]. Neonatal HSV infection has devastating . As a result of the illness reactivating or contracting genital herpes during pregnancy, HSV may be asymptomatically present in the genital tract at the time of childbirth. [30,31]. Our results showed more than half of

pregnant women who had abortions did so in the first trimester(49.3%) and (54%)for group I , II with highly significant association. The 300 aborted women being split into two groups: BOH and primigravid. This study's findings were in agreement with [17, 18] who reported that the first trimester of pregnancy had the highest prevalence, but they were at odds with [19], who discovered that the second trimester accounted for more than half of all pregnancies (51.2%), and [20], who also found that the second trimester had the highest prevalence of pregnant women. It was hypothesized that because most pregnant women visit hospitals in their second and third trimesters of pregnancy, the first trimester saw the highest incidence of abortions. Our study's patients, whose mean ages ranged from 18 to 44, had a mean age of 27.9 years. They were compared to participants in a previous study,[21] whose mean ages ranged from 15 to 42 and had a mean age of 28.6 years. There was opposition to this in highly significant association [22]. As shown in table (3) the current results for positive CMV IgM in group I and II was 53%, 32.6% with significant correlation which was agree with [17]who reported the IgM CMV for group I was 51%. And disagreed with[23,24] ho found the positive IgM 36.6% but agreed with IgM group II which was 32.5%.Because anti-CMV IgM antibodies are not necessarily linked to primary infection, the diagnosis is uncertain when they are found in a pregnant woman. In reality, during reinfections or reactivations,

pregnant women can produce IgM(25). Additionally, between six and nine months after the end of the acute phase of the original infection, anti-CMV IgM antibodies have been found in certain pregnant women [26]. The most accurate test now available to diagnose primary infection in pregnant women is the anti-CMV IgG avidity test [25,27]. The IgG avidity test is extremely sensitive and 100% specific. The maturity of the immune response is reflected in the gradual and moderate increase in antibody avidity. Low avidity indices show low avidity IgG antibodies in serum resulting from primary CMV infection that is acute or recent. In immunocompetent subjects, low avidity indices are found 16–20 weeks following the onset of symptoms[25]. Present seroprevalence study of HCMV IgG of our was 66%, 74.6% for group I and II with no significant association, that is agreed with [23,27] and disagreed with[17,24]. in case they lack protection from their childhood vaccinations, it is advised that all women of childbearing age be tested for toxoplasma gondii, rubella virus, and HCMV infection. In our results for positive seroprevalence of HSV IgM for group I was 45.6% and 26.6% for group II it was significantly association, The mean HSV IgM seroprevalence across 7 investigations conducted in Iraqi studies was 22%, which is approach to the prevalence seen in this study [32]. The range of HSV IgM seroprevalence in the aforementioned 7 studies was 3.8 % to 74%, hence the prevalence of the current study was within this range. Most cases with positive HSV IgM levels may be positive due to latent virus reactivation rather than the significant seroprevalence observed in some study cohorts. The overall rate of the other 6 studies was 12%, which is more realistic, even with the acutely high prevalence rate of the current study and another Iraqi study that showed a high prevalence rate. In the general population of Iraq, the prevalence of HSV IgG was 30% [33], with no appreciable variations between women who had poor obstetric outcomes and those who had normal pregnancies [33, 34]. Our finding results is in agreement with that, which was 74%, 61.3% in group I and II respectively. HSV-I is typically spread through direct contact with the lesion, whereas HSV-II is typically transferred through sexual contact [35]. However, the epidemiology of HSV-I differs between the first episode and recurrent infection [36].

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