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Comparison between Rapid Test and ELISA in Cytomegalovirus Detection among Pregnant Women in Kirkuk City

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A B S T R A C T

Cytomegalovirus is a member of herpes virus. As CMV affects many living cell, people are at risk of acquiring it especially pregnant women and can transmit it to their fetus. The purpose of this study is to compare two common methods for Cytomegalovirus diagnosis. CMV Rapid Test is performed to detect Abs specific for CMV. Likewise ELISA technique is applied for further validation. Complete blood count is performed to compare (WBC, Hb. Lymphocyte, platelet) count between patients and control groups. By Rapid test, all patients were tested positively for IgG. However only (10.16%) of 128 patients tested positive for CMV IgM. According to ELISA, IgM positivity was only (8.59%). Regarding to CBC test there were significant differences between two groups regarding to WBC count and lymphocyte count with P value (0.0001) and (0.0001) respectively. This study concluded that CMV diagnosis in pregnant women via ELISA test is more accurate and specific than rapid test.

Introduction

Cytomegalovirus is one of the herpes family viruses that affect a wide range population and cause symptom that range between mild to severe especially in immuo-compromised patients since it considered opportunistic. There are numerous methods in which this virus might spread from one person to another, but each one requires direct and close contact with the virus-bearing materials [1]. CMV produces huge cytomegalic inclusion bodies, the name CMV is derived from the Greek words (cyto) and (megalo) which mean "big cell." [2]. Also can be transmit from infected mother to fetus via variety of ways, the virus is typically asymptomatic in people who had primary infection, only a small percentage of transmissions result in mononucleosis-like illness after transmission [3]. CMV develops a persistent infection, remaining dormant in the host and going through productive reactivation cycles that aid in its effective transmission. CMV infects and replicates in a wide range of cells, including epithelial cells of gland and mucosal tissues, smooth muscle cells, fibroblasts, macrophages, dendritic cells, hepatocytes, and vascular endothelial cells [4]. Salivary glands, breast epithelium, prostate, endometrial, kidney tubules, and other organs like bone marrow and the lungs are all affected by the virus. An infection may be latent, lytic (producing virus) , asymptomatic or symptomatic [5].

Infection with other virus such as hepatitis especially hepatitis B, C and D cause chronic infection in neonate more than adult, while CMV infection can causes infection in all age groups especially among immune-compromised [6]. In the vast majority of infections, the host's defense against CMV infection involves cellular and humoral immune responses, which together prevent a serious CMV disease. IgM class antibodies are produced soon after primary infection and may persist for several months. IgG class antibodies also develop shortly after infection and are permanent. CMV can reactivate at specific locations in the body, primarily salivary glands and in mothers who are CMV seropositive before becoming pregnant, or it can infect them again with a different strain [7]. The primary method of diagnosis is serological testing. Pregnant women are typically diagnosed with acute infection through the presence of particular IgM antibodies, which typically decline one to six months after infection and disappear completely within seven months. After the initial increase in IgM level, IgG can be found one to three weeks later [8]. It has been demonstrated that ELISA for CMV-specific IgM antibodies is an effective and superior method for diagnosing active CMV infection. IgM antibodies are linked to active CMV infection, but the presence of CMV-specific IgG antibodies in blood indicates prior exposure to the virus [9].

Materials and methods

Data collection

From October 2022 to March 2023, 180 samples from patients at Azadi Teaching Hospital were collected from women in various stages of pregnancy in order to discover the prevalence of CMV. A complete blood count (CBC) test was then conducted to compare patient group and control about these characteristics. Each participant offered their informed consent.

Sample collection

5 mL of vinous blood from 180 collected. The sample was divided into two tubes: an EDTA tube and a gel tube for the CBC test. One portion of the committed samples was centrifuged at 4000 rpm for 15 minutes after allowing it to coagulate at room temperature. The right patient code was applied after the serum samples were collected, in order to utilize it in rapid test and ELISA IgM kit Bioactive/Germany.

Statistical analysis

The statistical analysis was performed using Graph Pad Prism version 5, and comparisons were made using the t Test, q Square, and spearmen correlation.

Results

This study involved detection of CMV on pregnant women by two common methods and assessment of some hematological parameters in two tested groups include patients and control. Of total 180 participants, (71.11%) were infected with CMV and the rest (28.88%) were control group.

Our data demonstrate that total white blood cell count was significantly elevated (P value < 0.0001) in the patient group with mean (9.837) compare to control group (8.063) when applying Mann Whitney T test as depicted in figure (1) and table (1).

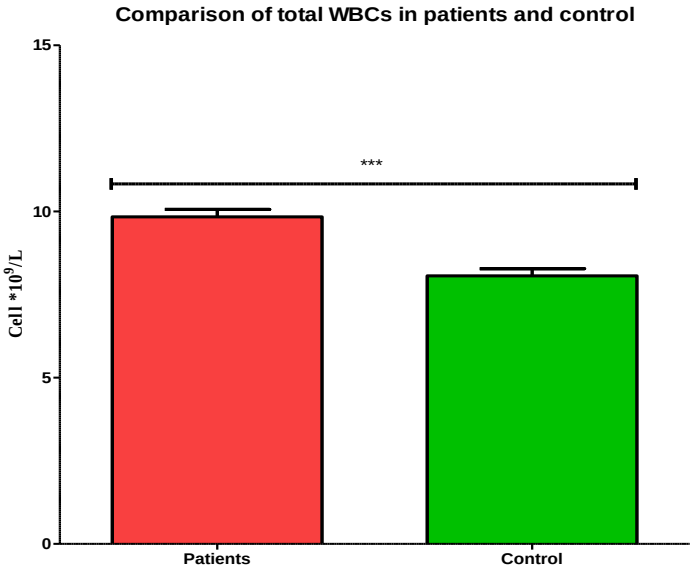

Figure 1: comparison of total white blood cell in patients and control group by using Mann Whitney T test

As far as lymphocyte count is concerned, our research found that the mean level of lymphocyte in patient group (49.23) is significantly higher than in control group (39.16) with (P value < 0.0001) when utilizing Mann Whitney T test, as demonstrated in figure (2) and table (1).

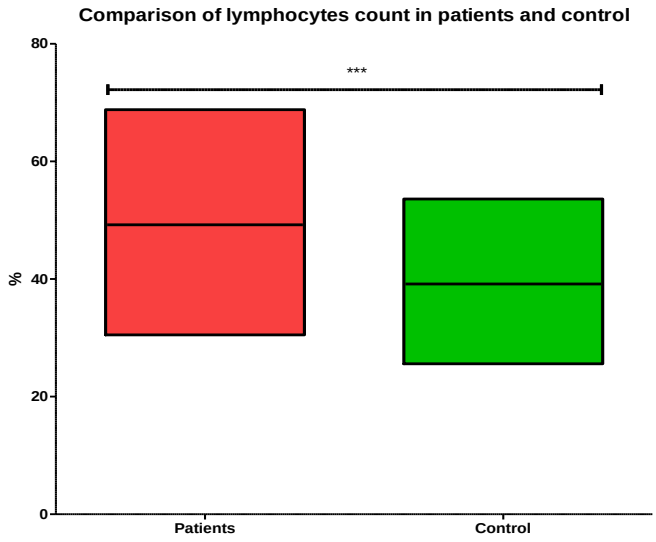

Figure 2: lymphocyte counts in patient group compare to control group utilizing Mann Whitney T test

In term of Hb concentration no significant difference was observed between the two groups with P value =0.3524 using Mann Whitney T test as shown in figure (3) and table (1).

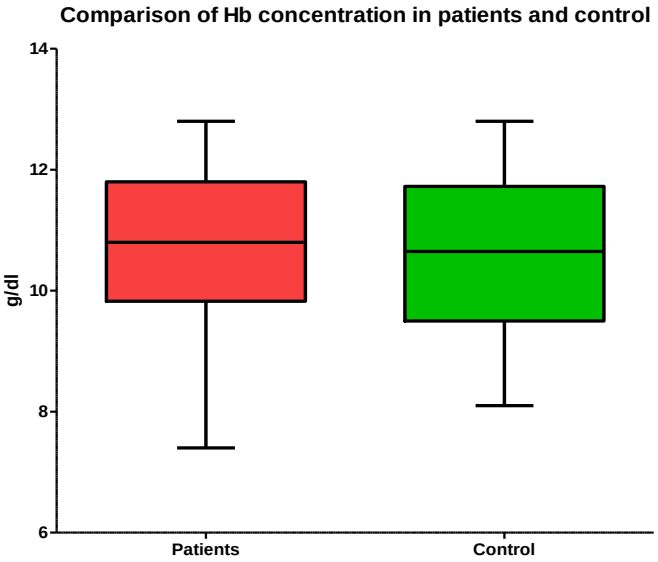

Figure 3: Hb comparisons between patients and control groups using Mann Whitney T test

Regarding platelet level, we found that the count of this parameter in patient group lower than in control group with mean (278.3 and 294.2 $*10^9$) respectively. No significant difference was observed between two groups (P value= 0.0868) through the use of Mann Whitney t Test as demonstrate in figure (4) and table (1)

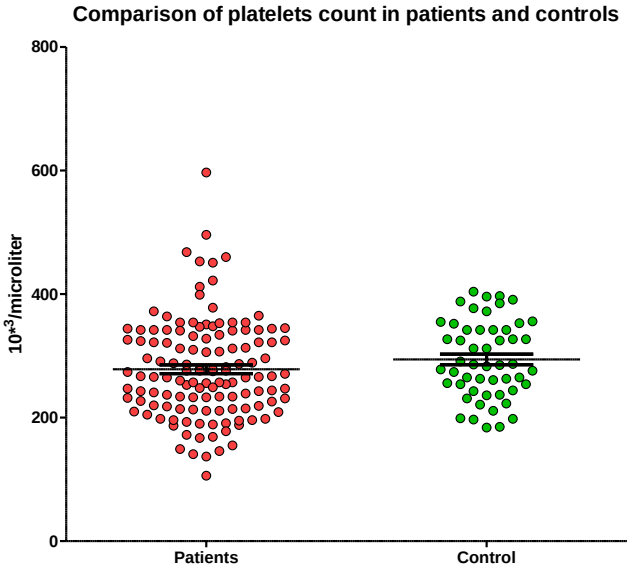

Figure 4: Platelet level in patient group compare to control group utilizing Mann Whitney T test

Table 1: demonstrate differences in hematological parameter between Patients and control

Hematological Marker	Patient		Control		
	Mean	SD	Mean	SD	P value
Total WBC	9.837	2.57	8.063	1.568	0.0001
Lymphocyte	49.23	7.418	39.16	7.254	0.0001
HB	10.77	1.337	10.63	1.209	0.3524
Platelet	278.3	79.9	294.2	62.2	0.0868

Regarding the identification of cytomegalovirus, When applying the Mann-Whitney T test to our data, we found that (10.16%) patients had positive CMV IgM rapid test results, with a P value of (0.0007).In the same way, 100% of the patient group tested positive for CMV IgG via a rapid assay. While in control group neither IgM nor IgG were positive, as showed in Table 2

Table 2: Rapid CMV IgM and IgG result

Rapid CMV test	Patient		Control		P value
	NO.	%.	NO.	%	
IgM+	13	10.16	0	0.0	0.0007
IgM-	115	89.84	100	100.0	
Total	128		52		
IgG+	128	100.00	0	0.0	0.0001
IgG-	0	0.00	100	100.0	
Total	128		52		

The results of the current study showed that the group of patients who were pregnant tested positive for CMV rapid test, was further validated using (ELISA) technique , out of 128 individuals 11 (8.59%) of pregnant females were positive while remaining 117 (91.41%) were negative. While in control group none of the participants were positive. There was no primary infection discovered in any of the participants because all anti-CMV IgM positive samples also tested positive for anti-CMV IgG antibodies. As demonstrates in Table 3

Table 3: Result of ELISA to detect IgM among patients groups

ELISA CMV test	Patient		Control	
	NO.	%.	NO.	%
ELISA IgM+	11	8.59	0	0.0
ELISA IgM-	117	91.41	52	100.0
Total	128		52	

Table 4 compares the detection of CMV by rapid test and ELISA; our findings show that, out of 128 individuals, only 13 (10.16%) tested positive for CMV by rapid test, whereas 11 (8.59%) tested positive for CMV by ELISA IgM. There was no significant distinction between the two tests using Qi square, which had a P value of (0.8307) with 84.61% quick test sensitivity.

Table 4: detection of CMV IgM by both rapid test and ELISA

CMV IgM test	Positive		Negative		Total		P value
	NO.	%.	NO.	%	NO.	%	
ELISA IgM+	13	10.16	115	89.84	128	100.00	0.8307
ELISA IgM-	11	8.59	117	91.41	128	100.00	0.8307
Rapid test sensitivity	84.61						

Discussion

Due to the widespread distribution of cytomegalovirus, particularly among immuno-compromised persons, a more precise detection method, such as an ELISA test, is required for correct diagnosis. ELISA tests are more sensitive and specific because they use an enzyme substrate reaction and washing phases to remove any non-specific antibodies that may be present in patient samples. Contrary to the Rapid test, which is a straightforward screening tool used in the early diagnosis of a variety of diseases, these tests need to be validated using more advanced methods such ELISA techniques that give wide-ranging detection. ELISA is an important technique for scientific research and clinical diagnostics since it provides highly repeatable and quantitative data [10,11]. We observed that 8.59% of the 128 patients tested positive for IgM using the ELISA method. Our results are consistent with other studies conducted in Southern Ethiopia to determine the prevalence of CMV in pregnant women, which found 8.2% [12]. While Sharghi *et al* found that graved women had an IgM positivity rate of (0.06%) by ELISA test [13]. The variation of IgM Abs between our data and other studies could be due to the variation in sample size included in each research or due to bias selection of patients or due to the fact that CMV tend to induced dormant status and reactivation of the virus in pregnant or other immune-compromised individuals is more likely to occur when the immune status is weakened which allow the virus to proliferate and flourish [11].

Regarding hematological finding one study by Canyang Zhan et al. on 257 CMV patients found that the total WBC count was higher in the patient group compared to the control group [14]. While Jaffer Ahmad et al. showed that the total WBC count was lower in the immuno-compromised patients than in the control group [15]. Furthermore in Sultan et al. study on patients infected with parasite observed that total WBC count increased in this groups [16]. The total WBC count in our data was significantly higher than in the control, and this may be because chronic CMV infection can cause immune system chronic alerts that signal the proliferation of specific WBC clones, particularly the lymphocyte group, which serves as the first line of defense against intracellular viruses. Additionally, NK cells and cytotoxic T lymphocytes (CD8) are on top of this [17]. Regarding lymphocyte count, our findings revealed that CMV positive women had a considerable increase compared to non-CMV positive women, which is consistent with earlier research that also found significant lymphocyte elevation in CMV positive participants [18,19,20]. While our findings disagree with Chandra et al., who found a reduction in lymphocyte count in CMV-infected pregnant women [21].

The activation of viral growth, which takes place in specific tissue where the virus remains in hypnotic statues, may be the cause of this increase in lymphocyte count. This may happen, especially in pregnant women, whose immune systems are already impaired [22]. This caused the interferon gamma signaling from infected cells to rise, alerting the essential lymphocyte player to expand and be able to inhibit viral replication [23]. Anemia is a developing cytomegalovirus (CMV) infection side consequence [24]. Alebady et al.'s findings, which demonstrated a substantial decrease in hemoglobin levels among CMV patients, agreed our findings [25].In addition to other study on patients infected with intestinal parasite Ahmed et al. observed that the hemoglobin level decreased among them [26]. On the other hand, our data on platelet count revealed that it was not significantly impacted by CMV infection, and there was a slight reduction among the patient group. This finding is in agreement with those of Echcharii et al. and Shragia et al., who found that platelet count was slightly reduced in people with CMV infection [27,28]. This might be caused by the virus' capacity to infect bone marrow megakaryocytes, hence reducing platelet synthesis [29].

Conclusion

Our investigation concluded that CMV diagnosis among pregnant women by ELISA test was more accurate and precise than Rapid test, since ELISA tests use an enzyme substrate reaction and include washing stages to get rid of any non-specific antibodies present in patient sample, they are more sensitive and specific. Regarding hematological parameter in our study, such as WBC and lymphocyte counts of CMV-infected individuals were considerably higher than those of the control group.

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