

***Detection of some Immunological parameters Related with
Cytomegalovirus(CMV) Infections in aborted women at THI-QAR
province in south of IRAQ**

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SUMMARY The present study aimed to shed light on virus *cytomegalovirus* (CMV) and studying some immunological parameters including anticardiolipids antibodies and antiphospholipids antibodies also measuring blood parameters and phagocytosis. A total of 380 blood samples were collected from aborted women when admitted to Thi Qar hospitals and private laboratories. divide into 4 groups: We found the significant differences ($P \leq 0.05$) between infection rates in aborted women with the highest rate in the group 1 (84.21%) and the lowest rate in group 2 (3.42%), also the results showed significant differences between the values of IgM reached the highest value IgM in group 2 (23.09%) and the lowest value IgM in group 1 (6.25%), while not show significant differences ($P > 0.05$) when measuring IgG and when measuring both IgM and IgG.

The current results showed the decrease significant for hemoglobin Hb, blood volume compact PCV and white blood cells WBC among group 1,2,3 compared with group 4 (control group).

Differential counting leukocytes shows significant reduction in of preparing Neutrophil in first three groups when compared with control at the possibility $P \leq 0.05$, results also show a significant increasing in the percentage of lymphocytes between groups 1 and 2 when compared with the control group. the results showed increase of monocyte in groups 1 and 2 and 3 when compared with the control group. Also the results of differential counting increase the proportion of Eosinophiles in group 1 and a non increase in groups 2 and 3 when compared with the control group. Results didn't show significant differences between the four groups in the proportion of basophiles at a probability level $P > 0.05$.

As for the process of phagocytosis, the results showed a rise significantly in the coefficient of phagocytosis in groups 1 the high is significant in both groups 2 and 3 when compared with the control group. Aim of the study

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Key word: immunological, cytomegalovirus, aborted women

younger than 15 years, and infection with other sexually transmitted diseases. Obstetricians and gynecologists have role in reducing CMV disease by educating patients about preventive measures [6] .

MATERIALS AND METHODS

The current study Conducted on women volunteers suffering from abortion and who admitted to Hussein Teaching Hospital, Shatra Hospital , Rifai Hospital and private laboratories after refer them by a specialist doctor. 380 serum samples were collected from aborted women and doubtful of contracting virus cytomegalovirus where withdrawing(5 ml) of blood venous and divided into:

1. Mode (2 ml) of blood in a test tube container blocker blood clots (EDTA) for the purpose of examining the blood parameters include the percentage of blood hemoglobin Hb and blood volume compact PCV and Total and differential count of leukocytes and the process of phagocytosis.

2. Mode (3 ml) of blood in a test tube and left to clot for half an hour after it separated using a centrifuge and quickly 3000 r / and for 15 minutes so as to obtain serum and Use this serum for the diagnosis of disease *cytomegalovirus* CMV using ELFA test and estimate the level of IgM and IgG and anticardiolipids antibodies and antiphospholipids antibodies.

3.Control group

Collected (10) blood sample to pregnant women healthy does not suffer from abortion

INTRODUCTION

Many viral infections are associated with significant maternal and fetal consequences if acquired during pregnancy and causes abortion . some the most commonly encountered infections with subsequent perinatal effects include *Cytomegalovirus* (CMV) [1]. In general, perinatal infections have more severe fetal consequences when they occur early in gestation, because first-trimester infections may disrupt organogenesis. Second and third trimester infections can cause neurologic impairment or growth restriction [2].

Cytomegalovirus (CMV) is a double-stranded DNA herpes virus that is transmitted by contact with infected blood, saliva, or urine, or by sexual contact. The incubation period of CMV is 28-60 days, with mean of 40 days. Infection induces an immunoglobulin M (IgM) antibody response that disappears within 30-60 days[3] . Primary CMV infection in adults generally is asymptomatic. Occasionally, patients experience mononucleosis like syndrome, with leukocytosis, lymphocytosis, abnormal liver function tests, fever, malaise, myalgias and chills [4]. Viremia can be detected 2-3 weeks following primary infection. After the initial infection, CMV remains latent in host cells; recurrent infection can occur following reactivation of latent virus. In rare cases, recurrent CMV infection can occur by infection with a new strain of virus [5].

Factors associated with an increased risk of CMV infection include history of abnormal cervical cytology, lower socioeconomic status, birth outside North America, first pregnancy at

glacial acetic acid and taking (0.02 ml) of blood using micropipet and then added a solution of dilution to be arrived size to (0.04 ml) and then shake the solution for a minute and left for a period of (10) minutes to break down red blood cells and stained the nuclei of white blood cells.

Then took a drop of it and put on a counting chamber and covered a slide cover and white blood cells were calculated according to the equation:[8]

Rate cells for four boxes $\times 200 = (\text{cell} / \text{mm}^3)$

2. Differential count of leukocytes

Worked (Blood smear) on a glass slide clean, and then left to dry and colored dye to ensure Lishman stain for (2) minutes then flooded slide Buffer dye for (10) minutes then washed slide with running water and left to dry then examined slides using magnification power oil (100x) and calculated at least (200) cell white wildly and then calculated the percentage of these cells either prepare every kind of white blood cells ($\text{cell} / \text{mm}^3$) calculated according to:[9].

3. Phagocytosis

Macrophage portability studied to phagocytic yeast (*Saccharomyces cervisia*) cells slain According stated in [10] with some of modification and in peripheral blood, where mixing 0.025 ml of peripheral blood with 0.05 ml of stuck yeast and 0.025 ml of solution Hank saline balanced (HBSS) in a sterile test tube and then incubated tubes in the incubator (37) C° for half an hour.

and revisions to care of pregnant women department in the Hussein Teaching Hospital.

***Mini VIDAS technique (Vitek Immuno Diagnostic Assay System)**

Used in the current study device Minividas to detect IgM and IgG has been used several test (VIDAS CMV IgM, France) , (VIDAS CMV IgG, France), (VIDAS cardiolipid IgM, France), (VIDAS cardiolipid IgG, France) , (VIDAS phospholipid IgG, France) and (VIDAS phospholipid IgM, France)

The method includes Sandwich two steps enzyme and the basis for action depends on the interaction between immune antibodies in patient serum with antigens that line the pot SPR At the end of the steps is to measure the amount of enzyme linked fluorescent commensurate with the concentration of antibodies in the sample.

***Hemoglobin level determination:**

Using Microhaematocrite been measuring the level of hemoglobin in a blood Capillary tubes container material heparin and blocked one of extremes and then blood separation by centrifugation speeds 6500 r / min for 5 minutes and then depending on the size of blood cells compressed using a ruler private [7] .

***Total and differential count of leukocytes**

1.Total count of leukocytes:

Used Haemocytometer or called counting chamber in the calculation of white blood cells, where a dilution solution, which is a 2%

running water and left to dry and examined using optical microscopy on the power zoom (100 x) and by phagocytosis coefficient according to the following equation:

Attended Blood smear on a glass slide and left to dry colored dye Wright (Wright's stain) and used the dye ready produced by CRESCENT in Saudi Arabia, left for (2) minutes and then flooded slide Buffer dye for (10 - 20) minutes then washed slides with

cells phagocytic number

$$\text{Phagocytosis coefficient (\%)} = \frac{\text{cells phagocytic number}}{\text{The total number of phagocytic cells}} \times 100$$

The total number of phagocytic cells

anticardiolipids antibodies
and measuring antiphospholipids antibodies
Aten control sample of non infected women
were collected

*Statistical analysis

the results of the current study were analyzed by Chi – square(X^2), (Mean±S.E.) and Revised Least Square Difference (RLSD) and below the level of probability 0.05 using SPSS.[11] .

Table No. (1) the existence of significant differences ($P \leq 0.05$) between infection rates among aborted women and reached the highest rate in the Group 1 (84.21%) and lowest in Group 2(3.42%). Also the current study showed significant differences between the values of IgM the highest value of IgM in group 2 (23.09%) and the lowest value IgM in group 1 (23.09%), while no significant differences when measuring IgG and when measuring both IgM and IgG.

RESULTS

The current study was conducted on 380 blood samples from aborted women and doubtful infected their when application hospitals in the province of Thi Qar and private laboratories and divided into 4 groups: A total of 380 sample of blood for aborted women were diagnosis with CMV, measuring

Table (1) the incidence of immune antibodies between groups studied using MiniVIDAS

****IgM+ IgG %	***IgG %	**IgM %	*Infected samples%	Examined samples	studied groups
1.25	92.5	6.25	84.21	380	Groubs 1 (CMV)
0	76.92	23.09	3.42	380	Groubs 2 Cardiolipid
0	88.89	11.11	4.74	380	Groubs 3 Phospholipid

high founded in WBC both groups 1 when compared with groups 2 and 3, as well as when compared with group 4 (control sample) at a probability level $P \leq 0.05$.

The present study did not show significant differences in groups 2 and 3 and the control group also did not show significant differences in the preparation of WBC in groups 2 and 3 when compared with Group 4 (control sample).

Table No. (2) blood parameters obtained a significant reduction in the concentration of HB and PCV in groups 1 when compared with Group 4 (control sample) at a probability level $P \leq 0.05$, current study also showed a significant decline in concentration of HB and PCV in groups 1 When the comparison with groups 2 and 3, as well as when compared with group 4 (sample control) at the possibility $P \leq 0.05$. also explained the current results for the

Table (2) blood parameters of the groups studied in the province of Thi Qar

Groups	NO.	Hb	PCV	WBC
1	40	b11.34 ± 0.72	b34.88 ± 2.24	a9.14 ± 1.65
2	10	a11.96 ± 0.47	a36.80 ± 1.39	b7.77 ± 1.41
3	10	a12.21 ± 0.66	a37.60 ± 2.01	b7.34 ± 2.15
4 Control	10	a12.08 ± 0.70	a37.40 ± 2.17	b6.55 ± 1.42
RLSD*		0.60	1.90	1.37

a, b: Means of each variable with different letter differ significantly at 5%. when compared with Group 3 at a probability level $P \leq 0.05$ and the results showed the difference was not significant in Group 4 when compared with the control group.

On the other hand the results showed a rise significantly in the proportion of monocyte in groups 1 and 2 and 3 when compared with the control group, as well as when compared with group 1 as well as increased significantly in both groups 2 and 3 when compared with Group 4 at the possibility $P \leq 0.05$. Also explained the results of counting differential rise significantly in the proportion of eosinophiles in group 1 and a non significant increase in groups 2 and 3 when compared

***RLSD=Revised Least Square Difference**

Table No. (3) differential counting leukocytes showed significant reduction in the proportion of preparing neutrophil in first three groups when compared with Group 4 (control) at the possibility $P \leq 0.05$, as well as a significant reduction in Group 2 when compared with the rest of groups when probability level $P \leq 0.05$.

The current of results also showed a significant increasing in the percentage of lymphocytes between groups 1 and 2 when compared with the control group as well as

coefficient of phagocytosis in groups 1 and 2 and the high is significant in both groups 3 when compared with the control group as well as founded a rise significantly in both groups 1 when compared with groups 3 and 4 at the possibility $P \leq 0.05$.

with the control group. Results did not show significant differences between the four groups in the proportion of basiophiles at a probability level $P > 0.05$.

As for the process of phagocytosis, the results showed a rise significantly in the

Table (3) differential count of leukocytes and phagocytosis in blood samples studied in the province of Thi Qar

Groups	No.	N	L	M	E	B	P
1	40	b 58.63 ±4.63	a35.30 ±9.74	a8.18 ±3.889	b3.08 ±1.11	0.73 ±0.784	a61.78 ±9.770
2	10	c53.70 ±4.11	a34.20 ±3.43	a10.10 ±2.470	b2.30 ±0.67	0.50 ±0.527	b54.40 ±5.060
3	10	b59.30 ±5.46	b31.60 ±3.92	b6.40 ±2.366	b2.20 ±0.42	0.60 ±0.52	b52.50 ±3.866
4	10	a 65.20 ±4.83	b29.83 ±4.43	c3.20 ±2.39	b2.00 ±0.66	0.70 ±0.82	b49.80 ±1.751
RLSD		4.9	3.7	2.7	1.8	NS	6.3

a, b: Means of each variable with different letter differ significantly at 5%.

***RLSD=Revised Least Square Difference**

may be due to environmental factors and climate and health conditions, race and socio-economic factors[16].

The results of immunoglobulines incidence of chronic IgG is the highest reached 92.5% either incidence in IgG and IgM were 1.25% and this is consistent with the study [17] in the province of Thi Qar and study [18] in Hilla and study [19] in Italy and study[20] in Malaysia, where recorded these studies rate infection IgG ranges between 40-100% and IgM between 0-10%, while study found [21]in Iraq, found the incidence of acute IgM was higher than the chronic 45.9% while the proportion IgG at 20.7%.

DISCUSSION

The current study recorded highest infection rate in the CMV and reached 84.21%, which is as high as in the studies conducted in the province of Thi Qar recorded incidence of 100% done by researchers Al-Kafaji and Al-Zubaidi[12], also recorded study Al-Ghazi[13] in Saudi Arabia increased infection amounted to 92.1% and study Ohland *et al*[14] in Russia 93.1% and there are studies that recorded the rate of infection less as in the study Alanen *et al.*, [15] in southwestern Finland amounted to 56.3% note of Results a disparity in the rates of infection in different regions and countries

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20.Saraswathy, T.S.; Az-Ulhusna, A.; Nurul Asshikin, R.; Suriani S and Zainah,

التحري عن بعض المعايير المناعية لدى النساء المجهضات والمصابات بفيروس *Cytomegalovirus* في محافظة ذي قار

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الخلاصة

اجريت الدراسة الحالية على النساء المجهضات المحالات من قبل الاطباء المختصين الى كل من مستشفى الحسين والشرطة والرفاعي والمختبرات الخاصة ، جمعت 380 عينة دم للكشف عن الضداد المناعية IgG و IgM للفيروس CMV والدهون القلبية Cardiolipids والدهون الفوسفاتية Phospholipids بأستعمال تقنية (mini VIDAS) كما تم فحص المعايير الدموية Blood parameters وعملية البلعمة Phagocytosis سجلت الدراسة الحالية اعلى نسبة اصابة في المجموعة المصابة بالفيروس المضخم للخلايا CMV حيث بلغت 84.21 % وكانت نسبة الضد المناعي IgG اعلى من IgM حيث بلغت 92.5 % كما سجلت الاضداد المناعية للدهون القلبية نسبة اصابة بلغت 3.42 % وكانت نسبة الضد المناعي IgG اعلى من IgM حيث بلغت 76.92 % اما الاجسام المضادة للدهون الفوسفاتية فبلغت نسبة الاصابة بها 4.74 % وكانت نسبة IgG اعلى من IgM فبلغت 88.89 % .

اظهرت نتائج المعايير الدموية فروقا معنوية بين المجاميع المفعولة حيث حصل انخفاضاً بسيطاً بنسبة Hb لدى المجموعة المصابة بفيروس CMV والدهون القلبية مقارنة مع مجموعة السيطرة كما اظهرت نتائج العد التفرقي لكريات الدم البيض انخفاضاً في نسبة الخلايا العدلة Neutrophiles وارتفاعاً في نسبة الخلايا اللمفية Lymphocytes في المجاميع 1 و 2 و 3 بالمقارنة مع مجموعة السيطرة كما سجلت نتائج عملية البلعمة Phagocytosis ارتفاعاً ملحوظاً لدى المجاميع المصابة 1 و 2 و 3 بالمقارنة مع مجموعة السيطرة.

الكلمات المفتاحية : دراسة مناعية ، الفيروس المضخم للخلايا ، النساء المجهضات