Study The Effect of Cytomegalovirus and Luteinizing Hormone on Pregnancy and Spontaneously Abortion Assay by ELISA Technique

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Abstract

In this study indirect ELISA test was conducted on 118 serum samples for Cytomegalovirus collected from women in different ages and cases in Bent AL-Huda hospital. Indirect ELISA was applied on all these samples depending on the optical density (OD) value of sera previously prepared from blood of well know healthy and non-vaccinated women. According to ages groups the higher ratio are (56.5%) was observed in first age group. A highly significant differences were observed in the seropositivity concerning to age groups (**P< 0.01).** According to Cases groups the higher ratio of seropositivity were found in women (75.61%) was observed in Recurrent abortion and then in Bleeding (75 %). The Quantitative ELISA test was used to detect the Luteinizing hormone as antigens in same serum samples. the higher rate of Luteinizing hormone positivity was observed in the last age groups (83.33%). there was a highly significant difference between the ages groups. Depending on Cases the higher rate of Luteinizing hormone positivity was (87.50%) in bleeding case , there was highly significant difference in the Luteinizing hormone in Cases groups of Quantitative ELISA.

Introduction

with young children or through sexual transmission Cytomegalovirus infection tends to cause few symptoms in Human Cytomegalovirus (CMV) is a member of Herpesviridae family , and therefore has the ability to establish latency following primary infection(1). Primary infection occurs in seronegative, susceptible host. Recurrent infection represents reactivation of latent infection or reinfection of seropositive immune host. Disease may result from primary or recurrent infections, Cytomegalovirus the former is more commonly associated with sever disease(2). Primary infection often produces no symptoms or mild non-specific symptoms(3). Cytomegalovirus is spread through contact with infected body fluids, such as urine or saliva. Infection among pregnant women most frequently occur through close contact immuncompetent individuals, but can serious disease among HIV-infected persons, organ transplant recipients on immunosuprresive therapy, and fetuses(4). Cytomegalovirus is the most common congenital infection and the leading cause of sensorineural hearing loss, mental retardation, retinal disease, and cerebral palsy(5).Cytomegalovirus is the largest of the herpesviruses, and has a diameter of 200 nm with douple-stranded DNA viral genome of 240 kb in a 64 nm core enclosed by an icoshedral capsid composed of 162 capsomers. The core is assembled in

nucleus of the host cells. The capsid is surrounded by a poorly defined amorphous tegmument, which itself surrounded by a loosely applied lipid-containing envelope. Mature viruses exit the cells by reverse pinocytosis (2).The prevalence of infection is quite variable as it varies in different geographic regions, different ethinic populations, and different socioeconomic level communities(6). The prevalence increase with age, and is higher in developing countries and among lower socioeconomic strata of the more developed nations(2). Transmission sources of Cytomegalovirus include saliva, breast milk, cervical and vaginal secretions, urine, semen, stools, blood and tissue or transplants. The spread of Cytomegalovirus requires very close or intimate contact because it is very labile. Transmission occurs by direct person-to-person contact, but indirect transmission is possible via contaminated fomites (2). Transmission can be horizontal via direct human-human contact and with sexual activity. Vertical transmission can occur during pregnancy, delivery, and breast feeding. The virus shedding from cervix increases as the pregnancy advances in gestation(7).

The incidence of infection in pregnancy is estimated to be as high as 1 to 200 pregnancies, of which around 40% will result in fetal infections(3). Congenital Cytomegalovirus occurs in 0.05%-1.5% of births[4]. Higher rate of infection among populations with lower economic standard of living. The risk of fetal infection is greatest with maternal primary Cytomegalovirus infection 30% and much less likely with recurrent infection <1% (5).

Rate of transmission of Cytomegalovirus infection are 3-7 times greater in infant born to adolescent mothers(5). Perinatal transmission is common, accounting for an incidence of 10-60% through the 1st 6 moths of life. The most important perinatal sources of virus are genital tract secretions at delivery and breast milk. Among Cytomegalovirus seropositive mothers, virus is detectable in breast milk in 96%, with postnatal transmission occurring in approximately 38% of infant(8). About 90% of infected infants are asymptomatic(3).

Materials and MethodsDiagnostic Kits

The BioCheck Cytomegalovirus (CMV) IgM ELISA kit: designed to detect CMV specific antibodies in human serum samples / BioCheck / Inc.The Veda. Lab. Luteinizing hormone (LH) glycoprotein ELISA kit: designed to detect LH hormone glycoprotein in human serum samples/ Veda. Lab / France.Blood Samples   The blood samples were collected from the women in Bint-Al huda hospital into 10 ml in sterile tubes without anticoagulant and were stored overnight at room temperature(18-25)C° for serum separation . Ten blood samples were collected from each well known healthy non-vaccinated women and considered as control. The samples were then centrifuged at high speed 3000rpm for 5 minutes . The serum was transferred into a single clean, sterile cryovial to conserve at -20C° until assayed (9) .

The BioCheck, Cytomegalovirus (CMV) IgM kit procedure

The BioCheck Cytomegalovirus (CMV) IgM ELISA kit procedure was done according to the instructions of BioCheck Company kit, starting with dilution till a blue-green color develops, which was due to the conversion of the substrate by the conjugate and read microtitre plate by microphometer at measured optical density (OD) 450 nm. The corrected OD values(ODcorr) as to the following equationOD $_{CMV}$ $_{IgM}$ __OD $_{control}$ = OD $_{corr}$While the percent positivity Value (PP) as in PP=$\frac{Test\ Sample\ or\ Neg(ODcorr)}{Positive\ Control(ODcorr)}$ x 100   Interpretation of the results: Interpretation of test sample result as below:

Serum	PP	Interpretation
	< 50	Negative
	≥ 50	Positive

The Veda. Lab. Luteinizing hormone (LH) glycoprotein kit procedure
The Veda. Lab. Luteinizing hormone (LH) glycoprotein kit procedure was done according to the instructions of Veda. Lab. Company kit, starting with dilution till a blue-green color develops, which was due to the conversion of the substrate by the conjugate and read microtitre plate by microphometer at measured optical density (OD) 450 nm.

The corrected OD values(Y) as to the following equation
 Y= -1.4+ 1.08$_{(x)}$
X=OD of Control
The values ranged from 0.1 to 150 mIU/mL. the minimum detectable concentration of LH by this assay is 0.3 mIU/mL.
 Interpretation of the results: Interpretation of test sample result as below:

Serum	PP	Interpretation
	1.68 - 15	Normal
	> 15	High

Statistical analysis
   The statistical analysis of the data was calculated for ELISA test by using (SPSS Version 15).
Results and Discussion
Analysis of serum samples by Indirect & Quantitative ELISA

From the 118 total serum samples examined by indirect ELISA for the presence of antibodies to the proteins of Cytomegalovirus, positive sera was observed in 10-20 years group with 13(56.5%) and in Quantitative ELISA were highest positive age group was observed in 41-45 years group with 5(83.33%).

CMV seropositively according to age group of women

The seropositively results in all age group is highly significant levels indicate by ELISA technique as well as this results (10) . Discussed presence of antibodies in the blood refers to increase of percentage in age group (10-20) and (21-30) with a percentages of (56.50%) and (47.50) , respectively(11).

In comparative study there is increase where due to a highly tolerance levels in group ELISA positive compare with age group of ELISA negative(12) (table 1) .

Table(1): The seropositively against CMV according to Age group of women

Women		ELISA Positive(+)		ELISA Negative(-)	
Age(years)	number	Number	percentage	number	Percentage
10-20	23	13	(56.5%)	10	(43.5%)
21-30	61	29	(47.5%)	32	(52.5%)
31-40	28	12	(42.9%)	16	(57.1%)
41-45	6	2	(33.3%)	4	(66.7%)
The total	118	56	(47.5%)	62	(52.5%)
P < 0.01					

P= Proportion values in Chi-square .
hormone seropositivity according to Age   group of women

The result show in table (2) that in the increase levels of LH hormone (13) (induction of ovulation hormone) in age group (41-45) with percentage of (83.33%) and age group (31-40) with percentage of (71.43%) respectively (14).

This variation in percentage of highly significant increase of hormone in larger age group compared with smaller age group (15).

The positive strong effect of this advance age significantly reflected the percentage of increase hormone which is influence directly on the hormonal balance in blood serum and refer to the presence of CMV in results of ELISA positive compare to E LISA negative in this study (16).

This correlation refer to positive results in significantly in positive compare to no significantly (17).

Table(2): The seropositively against LH hormone according to Age group of women

Women		ELISA Positive(+)		ELISA Negative(-)	
Age(years)	number	Number	percentage	number	Percentage
10-20	23	16	(69.57%)	7	(30.43%)
21-30	61	25	(47.5%)	36	(52.5%)
31-40	28	20	(71.43%)	8	(28.57%)

41-45	6	5	(83.33%)	1	(16.67%)
The total	118	66	(55.93%)	52	(44.07%)
P < 0.01					

P= Proportion values in Chi-square .

Table(3): ELISA CMV and LH SE Values in relation to Age group.

Women		CMV	LH
Age(years)	number		
10-20	23	11.3	19.57
21-30	61	2.6	2.1
31-40	28	7.1	21.34
41-45	6	16.7	33.34
The total	118	4.83	8.37
P < 0.01			

P= Proportion values in Chi-square .
SE value = Stander error

   Also in table (3) showed different SE value obtained by ELISA against CMV and LH in age group women.

CMV seropositivity according to Case group of women

   In table (4) the seropositively of ELISA refers to presence of recurrent abortion percentage (75.61%) may be due to either corpus luteum immaturation or chromosomal sex determination abnormalities (18) or due to Toxoplasmosis which is occurs in first Trimester (the first the month of pregnancy) (19) . Congenital anomalies may be due to administration of drug for folic acid antagonists with percentage (61.90%) with ELISA positive compare with (24.39 %) which is significantly results and highly strong positive correlation (20).

   Pseudopregnancy may occurs in abnormal positive of pregnancy which may happened in ampoullary regions of fallopian tube, the Pseudo -pregnancy refers to HCG hormone in blood serum (10 days of pregnancy) and (8 days) in urine (21) .

   Ectopic pregnancy occurs in external, peritoneum of abdominal cavity (22) . This case lead to fetal mistake and death (23) .

   Ectopic pregnancy present with abnormal uterine bleeding and unilateral pelvic pain, which must be differentially diagnosis from appendicitis , an a benting intrauterine pregnancy or a bleeding corpus luteum of a normal intrauterine pregnancy (24) .

Fatel death occurs in pregnancy women due to disturbance and disorder mistake in maternal-fetal circulation and prevention of reaching of adequate amount of blood to the fetus form placenta and umberical card (16) . in this study the high levels percentage due to occurs of this case in almost pregnancy women (26) .

Table(4): The seropositively against CMV according to Case group

Women		ELISA Positive(+)		ELISA Negative(-)	
Case	Number	number	percentage	number	percentage
Recurrent abortion	41	31	(75.61%)	10	(24.39 %)
Congenital anomalies	21	13	(61.90%)	8	(38.10 %)
Non pregnancy	25	14	(56%)	11	(44%)
Ectopic pregnancy	16	5	(31.25 %)	11	(68.75 %)
Bleeding (unilateral pelivic)	8	6	(75 %)	2	(25 %)
Fetal death	7	5	(71.43%)	2	(28.57 %)
The Total	118	74	(62.71 %)	44	(37.29 %)
$P < 0.01$					

P= Proportion values in Chi-square .

LH hormone seropositively according to Case group of women

There is a highly significant correlation in seropositivity against LH hormone increase in recurrent and spontaneously abortion due to no secretion of HCG hormone as a test of pregnancy a s results of low value levels in this case as compare with normal pregnancy in uterine tube and region of fallopian canal (27) .

Unilateral pelvic pain due to prevention of continuous gestation as a result as a result of abnormal uterine bleeding of corpus luteum of abnormal intrauterine pregnancy (28) .

In this study the low levels of LH hormone in this assay (fetal death, pseudo pregnancy, no pregnancy and congenital anomalies due to no presence and secretion as stimulation of anterior lobe of pituitary gland due to stimulation of GNRH and secretion of LH and FSH lead to highly associated with hormone inhibition and activity (the feed back control) (29)(30) .

Also in table (6) showed different SE value obtained by ELISA against CMV and LH in age group women.

Table(5): Seropositively against LH hormone according to case group of women

Women		ELISA Positive(+)		ELISA Negative(-)	
Case	number	number	percentage	number	Percentage
Recurrent abortion	41	34	(82.93%)	7	(17.07 %)
Congenital anomalies	21	15	(71.43%)	6	(28.57 %)

Non pregnancy	25	12	(56%)	13	(44%)
Ectopic pregnancy	16	6	(37.50 %)	10	(62.50 %)
Bleeding (unilateral pelvic)	8	7	(87.50 %)	1	(12.50 %)
Fetal death	7	4	(57.14%)	3	(42.86 %)
The Total	118	78	(66.10 %)	33.90	(37.29 %)
P < 0.01					

Table(6): ELISA CMV and LH SE Values in relation to Case group of women.

Women		CMV	LH
Case	NO		
Recurrent abortion	41	25.61	32.93
Congenital anomalies	21	11.9	21.43
Non pregnancy	25	6.1	6.1
Ectopic pregnancy	16	18.75	12.5
Bleeding (unilateral pelvic)	8	25.1	37.5
Fetal death	7	21.43	7.14
The total	118	5.86	6.96
P < 0.01			

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دراسة تاثير الفيروس المضخم للخلايا والهرمون اللوتيني على الحمل وحالات الاجهاض الذاتي بواسطة تقتنيه الاليزا

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الخلاصة

في هذه الدراسة اجري اختبار Indirect ELISA للفيروس المضخم للخلايا على 118 عينه مصل جمعت من نساء بأعمار وحالات مختلفة في مستشفى بنت الهدى . اعتمادا على قيمة المصل المحضر سابقا من دم جمع من نساء سليمة وغير ممنعة. النسبة المصلية الموجبة في اختبار Indirect ELISA في حاله تأثير العمر, قد أظهرت الفئه العمريه الاولى اعلى نسبه مصليه موجبه   (56.5%). وقد لوحظ فرق إحصائي محسوس فيما يتعلق بتأثير العمر   (0.01 >P). اعتمادا على الحالات المرضيه للنساء التي خضعت للدراسة كانت أعلى نسبه مصلية موجبة (75.61%) في حاله الاجهاض المتكرر تلتها حاله النزيف (75 %). فقد سجل فرقا إحصائيا محسوسا   < (0.01) للنسبة المصلية الموجبه للنساء و في جميع الحالات المرضيه. أستعمل اختبار Quantitative ELISA لقياس الهرمون اللوتيني في عينات المصل , وقد شكلت أعلى نسبه لها في الفئة العمرية الاخيره (83.33%) كما لوحظ وجود فرق إحصائي محسوس (0.01 >) . اعتمادا على الحالات المرضيه للنساء التي خضعت للدراسة كانت أعلى نسبه مصلية موجبة (87.50%)في حاله النزيف وقد سجل فرقا إحصائيا محسوسا (0.01 >) للنسبة المصلية الموجبه للنساء و في جميع الحالات المرضيه.