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SERLOGICAL DIAGNOSIS OF ANTIRUBELLA AND ANTICYTOMEGALOVIRUS (IGM AND IGG) IN IRAQI WOMEN SERA USING THE ENZYME LINKED FLUORESCENT ASSAY (ELFA)

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ABSTRACT
A serological diagnosis of anti Rubella and anti Cytomegalovirus (IgM & IgG) in women specimens 178 (72 pregnant and 106 non pregnant , 19-39 years old) using enzyme linked fluorescent assay (ELFA) .The results were 3 samples (1.6%) positive for anti Rubella Igm , antibodies and 18 samples (10.1%) positive for anti Rubella IgG, and only 5 samples (2.8%) positive for anti cytomegalovirus IgM antibodies and 24 samples (13.4%) positive for anticytomegolo virus IgG antibodies. In Conclusion**,** all the women with positive anti Rubella IgM and anti CMV IgM antibodies were correlated with recent and primary infection by each of two viruses. Also women with positive anti Rubella IgG and anti CMVIgG were correlated with current or convalescent infection by each two viruses; this infection may be confirmed by IgM and IgG avidity test or viral culture.

KEYWORDS: Serodiagnosis, antiRubella and antiCytomegalovirus, IgM and IgG,WomenELFA.

INTRODUCTION

Rubella viruses is RNA virus belong to togaviridae family cause mild self-limiting disease of short duration. It causes severe congenital defects in the fetus especially when infections occur during the first trimester of pregnancy [1]. It is important to determine the immune status of women of childbearing age, pregnant or individuals such as health care workers who may have close contact with contaminated individuals [2]. The diagnosis of Rubella infection is based on serology and in particular on the detection of specific IgM and IgG[3,4]. Detection of the anti rubella IgM is useful in the diagnosis of recent infection particularly in pregnant women, whereas, a significant rise in IgG level through 3 weeks seroconverson in same women is evidence of evolving Rubella infection. Cytomegaloviruse is a herpes virus (DNA virus) can cause serious disease in infants and adult. The virus can persist in human body for several years and can cause recurrent infections or to be transmitted to other individuals, CMV infections are very common 60-85% of population have been infected symptomatically but in 5% of infected cases with hepatosplenomegally, hydrocephalus, micro-cephalism, prematurity deafness or blindness and fetal death[5,6].The detection of IgM and IgG antibodies toRubella and CMv can be useful in diagnosis of primary infection in pregnant women[7,8] and it is useful in assessing the immune status of patients for this reason, this study aimed at:

1- Detection level of Rubella IgM and IgG antibodies in serum of pregnant and non pregnant women using - minividas – enzyme linked fluorescent assay (ELFA).
2- Detection level of CMV IgM and IgG antibodies in serum of pregnant and non pregnant using minividas – enzyme linked fluorescent assay (ELFA).

MATERIALS & METHODS

Pregnant (72) and non pregnant (106) women 19-39 years old are send from different Gynecology private clinics in Baghdad city to Al- Razi clinical laboratory for defection level of Rubella IgM and IgG and cytomegalovirus IgM and IgG antibodies , using enzyme linked fluorescent assay (ELFA).

Minividas – Rubella IgM and CMV IgM tests are a qualitative automated enzyme immunoassay for detection IgM in human serum using the enzyme linked fluorescent assay (ELFA).

Minividas – Rubella IgG and CMV IgG are on automated quantitative tests for measuring IgG directed against Rubella and CMV in human serum using the enzyme linked fluorescent assay (ELFA).

Contents of the ELFA kits:

1- STR strips
2- SPR solid phase Respectacle coated with Rubella or CMV Ag; (in case of Rubella IgM senitized with antihumanμ chain antibodies (goat) purified by affinity).
3- C1 Rubella or CMV control positive
4- C2 Rubella or CMV control negative .
5- S1 standard
6- MLE cord (master lot entry)

Principles

In case of Rubella IgM the assay principle combine the enzyme immunoassay Rubella IgM captured by polyclonal antibodies coating the solid phase receptacle (SPR) and anti Rubella IgM are specifically detected by inactivated Rubella Ag which itself revealed by an alkaline phosphates labeled anti Rubella monoclonal antibodies (conjugate).

During the final defection the hydrolysis of substrate (4-methy umbelliferyl phosphates) were catalyzed by

conjugate enzyme into fluorescent product measured at 450 nm and the results were automatically calculated by minividas instrument.

In case of Rubella IgG, CMV IgM and IgG; the assay principle combine a two – step enzyme immunoassay sandwich method with a final fluorescent detection. After the dilution the sample is incubated with the solid phase respectable (SPR) and anti Rubella IgG, CMV IgM & IgG antibodies present in specimen combine with Rubella Ag or CMV Ag coated the SPR, unbound component were eliminated during washing steps.

During the final dilution step, the hydrolysis of substrate (4-methyl umbelliferyl phosphates, were catalyzed by conjugate enzyme (Alkaline phosphates labeled mouse monoclonal antihuman Rubella IgG or CMV IgM and IgG), the conjugate enzyme is binding to human anti Rubella IgG and anti CMV and IgG coated on the SPR depending on the type of test done. following catalyzes the substrate product measured at 450nm and the results are automatically calculated by the minividas instrument.

1- All the required reagent will allow them to come to room temperature for 30 minutes.
2- Use one strip and solid phase Respectacle (SPR) for each of Rubella IgM, IgG and CMV IgM, IgG for each sample standard S1, and control C1, C2 to be tested; also the standard S1, C1, C2 should be identified by S1, C1, C2 respectively.
3- Mix the standard, control and sample using vortex mixer.
4- Pipette 100 μ of standard, control C1, C2 and sample into samples wells.
5- Insert the SPR and reagent strips into the minividas instrument.
6- Initiate the assay as directed in the operator's manual, all the assay steps were performed automatically by the instruments.

The all assays were completed within 40 minutes for Rubella IgG and CMV IgG and within 60 minutes for Rubella IgM and CMV IgM.

7. Dispose of the used SPR and strips into an appropriate recipient.

Procedures

Interpretation of the Results

Rubella IgM Index	Rubella IgG Titer i-u/ml	CMV IgM Index	CMV IgG Titer i.u/ml
< 0.80 negative	< 10 Negative	< 0.70 negative	< 4 negative
0.80 to < 1.20 equivocal	10 to ≤ 15 equivocal	0.70 -< 90 equivocal	4 to ≤ 6 equivocal
> 1.20 positive	≥ 15 positive	≥ 90 positive	≥6 positive

RESULTS & DISCUSSION

The present study revealed that out of 178 serum samples from pregnant and non pregnant women, only 3 samples (1.6% indox> 1.20) were positive for anti Rubella IgM antibodies and (18 samples (10.1% titer > 15 i.u / ml were positive for anti Rubella IgG antibodies and out of 178 serum samples from pregnant and non pregnant women only 5 samples (2.8% index > 0.90) were positive for anti CMV IgM antibodies and 24 samples (13.4% titer >6 i.u/ml) were positive for anti CMV IgG antibodies .

The presence of anti Rubella IgM antibodies in women serum was correlated with primary infection[4] and also it is useful in the diagnosis of this primary infection.

Regarding the anti Rubella IgG antibodies detection in the women serum aids in the diagnosis of Rubella infection and determine the immune status [3] of patients with regard to this virus so if the result of the anti Rubella IgG assay is positive, the diagnosis of current or convalescent infection must be confirmed using a second specimen collected 3 weeks later by testing for increase in or stabilization of the IgG titer. It is recommended to test the initial specimen in the same run as the second specimen using the same lot, a significant rise of IgG was evidence for an evolving Rubella infection but no increase of IgG level does not exclude the active Rubella infection, other biological tests such as Rubella IgM, IgG avidity, viral culture can confirm the diagnosis of Rubella infection.

The detection of anti CMV IgM antibodies in women serum can be useful in the diagnosis of current primary infection especially in pregnant women [7,8] CMV infection also can be severe with positive index of IgM antibodies for CMV in the immunocompromised patient (HIV positive or argan transplanted) (9) the anti CMV IgM present in 70% of patient with primary infection will persist for 16-20 weeks after infection and reappear irregularly during reactivation (10).

The detection of anti CMV IgG in women serum is useful for assessing immune state of patient and for the diagnosis of CMV infection[7,8], similarly in immune compromised patient (HIV positive and organ transplanted), CMV infection also sever with positive titer level of anti CMV 1gG [9] .

The presence of positive titer level of anti Rubella IgM and IgG in women serum in this study were 1.6% and 10.1% respectively, this result were varied from other studies in Iraq[11] reported that presence of anti Rubella IgM and IgG (4% and 86% respectively) in women serum, whereas[12] reported that the presence of anti Rubella IgM and IgG (3.3% and 86.7% respectively) in women serum and in other countries [13] reported presence of anti Rubella IgM and IgG (8.42% and 91.05% respectively) these variation in the presence of antiRubella IgM IgG level were related to variation in number of women specimens examined, geographical distribution and variation in the sensitivity of different immunoassays in detection of anti Rubella IgM and IgG levels in women sera .

The presence of positive titer level of anti CMV IgM and IgG (2.8% and 13.4% respectively) in women serum in this study were varied from other studies in Iraq[12] reported presence of anti CMV IgM and IgG (18.3% and 93.3% respectively) in women serum, whereas [14] reported the presence of anti CMV IgM (4.83%) and [15] reported the presence of anti CMV IgM (16.8%) in women serum.

In other countries [13, 16] reported the presence of anti CMV IgM and IgG (8.42% and 33.58%, 5.5% and 56.8% respectively) this variation in the presence of anti CMV IgM and IgG positive level in women sera were related to the number of patient sera examined, geographical distribution and also sensitivity of different immunoassays for defection of level of anti CMV IgM and IgG .

TABLE 1: Seronegative and seropositive of anti Rubella and anti CMV (IgM and IgG antibodies in women specimens.

	IgM	IgG
Rubella	Negative 175(98.4%)	Negative 160 (89.9%)
	Positive 3 (1.6%)	Positive 18 (10.1%)
CMV	Negative 173 (97.2%)	Negative 154(86.6%)
	[psotove 5(2.8%)	Positive 24 (13.4%)

- Women ages 19-39 years.

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