

Comparative assessment between immunological and molecular diagnostic methods to Rubella virus and Cytomegalovirus among Iraqi women with spontaneous abortion

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Abstract

Objectives: The aim of this research was to compare between the results of immunological assays by ELISA test and molecular methods by RT-PCR technique in the diagnosis of Rubella virus and Cytomegalovirus as a direct cause of spontaneous abortion in Iraqi pregnant women especially in the first trimester.

Methods: The study was carried out in three provinces Baghdad, Babylon and Al-Najaf at teaching hospitals, the study involved 90 aborted women between first trimester and second trimester, who attended the hospitals during the period from June 2017 to January 2018.

Results: The results of Rubella virus showed that 20 (22.2%) were seropositive for IgM and 37 (41.1%) were seropositive for IgG, in addition 17 (18.9%) showed seropositive for both IgM and IgG. Regarding to Cytomegalovirus infection, the results showed that 37 (41.1%) were seropositive for IgM and 55 (61.1%) were seropositive for IgG, while 33 (36.7%) were showed seropositive for both IgM and IgG. Serum results of IgM for both Rubella virus and Cytomegalovirus on aborted women were positive 4(4.4%), and serum patients showed seropositive for IgG of both Rubella virus and Cytomegalovirus 13 (14.4%), while 14 (15.5%) positive for both IgM and IgG of Rubella virus and Cytomegalovirus together.

The results of real time-PCR (RT-PCR) test for aborted women showed that only 12 (13.3%) cases were positive for Rubella virus and 6 (6.7%) cases positive for Cytomegalovirus infection.

Conclusion:

ELISA test should be used as screening test only and not based on for the diagnosis of Rubella virus and Cytomegalovirus infections, so the accurate diagnosis should be done by molecular methods such as RT-PCR.

Recommendation:

It was recommended to screening for Rubella virus and Cytomegalovirus infections to be practising routinely, this will protect the fetus from infected by these viruses.

Keywords: abortion, ELISA, Real time-polymerase chain reaction, Rubella virus Cytomegalovirus, Immunoglobulin.

INTRODUCTION

Abortion is an important reason of morbidity and mortality in mothers during reproductive period particularly in developing countries⁽¹⁾, and it can be defined as the expulsion of the concept product from uterus before 20th weeks of gestation period because fetus not viable. Spontaneous abortion is one of commonly complication occurs in early pregnancy especially in first trimester with percentage 80% from total of spontaneous abortion cases⁽²⁾. World Health Organization estimate that one to third of pregnancies end with spontaneous abortion⁽³⁾, which can be diagnosing by spontaneous removing of fetus from the uterus or fetus die in uterus but not be expelled physically⁽⁴⁾. Rubella virus or German measles virus infection during pregnancy may cause abortion, congenital rubella syndrome or congenital anomalies by infecting fetus via placenta⁽⁵⁾, fetal damage happens through destructions of cells as far as mitotic arrest⁽⁶⁾. Laboratory serological diagnosis of specific rubella antibodies remains crucial in detecting rubella virus infections, although clinical diagnosis is difficult due to non-specific symptoms. Despite a self-limited disease in childhood; if infected pregnant women during first trimester of pregnancy one of the most important sequels is spontaneous abortion⁽⁷⁾. Human Cytomegalovirus or Human Herpes virus 5⁽⁸⁾ is one from different causes of severe fetal damage leading to abortion⁽⁹⁾ and congenital malformation⁽¹⁰⁾ resulting from intrauterine viral infection and is frequently widespread in industrialized countries⁽¹¹⁾, transmission could occur during gestation period or after birth from breast milk, urine, saliva and from fomites⁽¹²⁾, other infants also may be infected during delivery causing different complications and injuries among neonates.⁽¹³⁾

Real Time-Polymerase Chain Reaction (RT-PCR) technology is a widespread using test to quantification nucleic acids simply, has many advantages rather than older forms of quantitative PCR, like

comparable sensitivity, superior precision and reproducibility as compared with previous methods⁽¹⁴⁾. Real-Time PCR can be used to evaluate cancer status, in addition to measuring viral and bacterial loads.⁽¹⁵⁾

MATERIALS AND METHODS

Patients and Study design: ninety samples of blood were obtained from women with spontaneous abortion, their ages were between (16-46) years, time of their abortion between first trimester and second trimester. Aborted women were attending at teaching hospitals in Baghdad, Babylon and Al-Najaf provinces from June 2017 to January 2018.

Sample collection: 5 ml of venous blood were obtained from each women with spontaneous abortion by vein puncture using a disposable syringe, then divided the blood into two groups : Group one-3ml of blood grouped in sterile serum tube for serum collection and stored at -20^oC to diagnosed by ELISA test for Rubella virus (IgM and IgG), and Cytomegalovirus (IgM and IgG). Group two-2ml of blood collection in EDTA tube then extraction of DNA and RNA from blood for each sample in the Eppendorf tubes (1-2) ml immediately for RT-PCR testing.

Serological diagnosis

Enzyme linked immune sorbent assay for quantities and qualitative detection of serum antibodies IgM and IgG for Rubella virus and Cytomegalovirus. ELISA Procedure was done according to manufacturing company protocol (BioCheck, USA).

Molecular detection by RT-PCR

-DNA and RNA extraction using DNA and RNA extraction kit supplied by Geneaid Company (USA).

-Real Time-PCR

RT-PCR test for qualitative detection of Rubella virus and Cytomegalovirus in women with spontaneous abortion were measured by RT-PCR kits (Sacace Biotechnologies-company-Italy), programming RT-PCR thermo cycler conditions (amplification), RT-PCR thermo cycler condition was set according to kit instructions.

RESULTS

From ninety women with spontaneous abortion , 20 patients (22.2 %) were seropositive for IgM of Rubella virus, while 37 patients (41.1 %) were seropositive for IgG of the same virus, and only 17 (18.9 %) patients were showed seropositive for both IgM and IgG of Rubella virus at the same time (table 1).

Table (1): ELISA results of aborted women with positive Rubella virus antibodies.

Rubella virus antibody	Patient number (90)	Percentage(%)
IgM	20	22.2
IgG	37	41.1
IgM & IgG	17	18.9

The results showed that 37patients (41.1 %) were seropositive for IgM of Cytomegalovirus and 55 (61.1 %) were seropositive for IgG of the same virus, and only 33 (36.7 %) patients were seropositive for IgM and IgG of Cytomegalovirus at the same time (table 2).

Table (2): ELISA results of aborted women with positive Cytomegalovirus antibodies.

Cytomegalovirus antibodies	Patient number (90)	Percentage (%)
IgM	37	41.1
IgG	55	61.1
IgM & IgG	33	36.7

Aborted women results of real time- PCR (RT-PCR) test showed that only 12 (13.3%) cases were positive for Rubella virus and 6 (6.7%) cases positive for Cytomegalovirus (table 3).

Table (3): ELISA results of aborted women with positive IgM, IgG, and IgM + IgG of Rubella virus and Cytomegalovirus antibodies in comparison with RT-PCR positivity.

Virus type Total number (90)	IgM number (%)	IgG number (%)	IgM+IgG number (%)	RT-PCR number (%)
Rubella virus	20 (22.2)	37 (41.1)	17 (18.9)	12 (13.3)
Cytomegalovirus	37 (41.1)	55 (61.1)	33 (36.7)	6 (6.7)

$X^2= 5.395$ $df= 7$ $p= 0.497$

ELISA serum results of IgM for both Rubella virus and Cytomegalovirus on aborted women were positive 4(4.4%) , and serum patients showed seropositive for IgG of both Rubella virus and Cytomegalovirus 13 (14.4%), while 14 (15.5%) positive for both IgM and IgG of Rubella virus and Cytomegalovirus together.

Table (4): ELISA serum results of aborted women mixed infection with Rubella virus and Cytomegalovirus IgM, IgG and IgM+IgG antibodies.

Antibody	Positive Rubella virus number (%)	Positive Cytomegalovirus number (%)	Positive Rubella virus and Cytomegalovirus number (%)
IgM	20 (22.2)	37 (41.1)	4 (4.4)
IgG	37 (41.1)	55 (61.1)	13 (14.4)
IgM & IgG	17 (18.9)	33 (36.7)	14 (15.5)

$X^2= 10.33$ $df= 2$ $p= 0.0177$

ELISA results showed coexistence of Rubella virus and Cytomegalovirus for IgG antibody in numbers 14 (15.5%) due to previous infection which play a role in cross reaction(table 5).

Table (5): ELISA results seropositive for IgG of both Rubella virus and Cytomegalovirus

Antibody results	Number (%)	Cytomegalovirus IgG Negative number(%)	Cytomegalovirus IgG Positive Number(%)
Rubella virus IgG negative	53 (58.9)	12 (13.3)	41 (45.5)
Rubella virus IgG positive	37 (41.1)	23 (25.5)	14 (15.5)
Total patients	90 (100)	35 (38.9)	55 (61.1)

$X^2= 0.753$ $df= 1$ $p= 0.282$

Very interesting results of RT-PCR because it appearance only in one disease, and there is no role for cross reaction that is why the results of positive Rubella virus consists of 12(13.3%), and for Cytomegalovirus 6(6.7%) only from the total aborted women (table 6).

Table (6): RT-PCR results of aborted women infected with Rubella virus and Cytomegalovirus

Antibody results	Number(%)	Cytomegalovirus RT-PCR Negative number(%)	Cytomegalovirus RT-PCR Positive Number(%)
Rubella virus RT-PCR negative	78 (86.7)	72 (80)	6 (6.7)
Rubella virus RT-PCR positive	12 (13.3)	12 (13.3)	0 (0)
Total patients	90 (100)	84 (93.3)	6 (6.7)

$X^2= 0.392$ $df= 1$ $p= 0.781$

DISCUSSION

This research was design to study the association between spontaneous abortion and specific serum IgM and IgG of Rubella Virus and Cytomegalovirus, in addition to prove this relation by molecular method (RT-CR).The present study showed that 22.2% of aborted women were positive for Rubella virus IgM antibody, so this result reveal decrease in percentages as compared with the

results of Hamdan *et al.*, (2011)⁽¹⁶⁾, who reported that Rubella virus was detected in 65.3% , and the results of Adam *et al.*, (2013)⁽¹⁷⁾, who recorded also the percentage of infection in 95.1% among Sudanese women. Our results showed that the seropositive of aborted women for Rubella virus IgG antibody (41.1%), which was less than the result reported by Lenochova in Turkey⁽¹⁸⁾, this indicate either previous vaccination with Rubella vaccine during adolescent age and before pregnancy or may be due to a previous infections with Rubella virus, another study in Baghdad area reported by Abdul-karrim *et al.*, (2009)⁽¹⁹⁾, who found that the result of the seroprevalence of Rubella virus infection in women with spontaneous abortion was 34.2%, another investigation in Palestine demonstrated that Rubella virus was considered as an important etiologic agent of abortion⁽²⁰⁾. In present study the percentage of Cytomegalovirus IgM antibody among aborted women was 41.1%, diagnosis of IgM antibody of Cytomegalovirus is the best suitable index for screening of pregnant women, Cytomegalovirus IgM antibody test can be used to detect recent or active infection and could be the best parameter to diagnosis acute infections⁽²¹⁾. Primary Cytomegalovirus infection in pregnancy has a higher incidence of congenital infection such as hearing loss, vision loss, mental retardation⁽²²⁾, and fetal loss⁽²³⁾. This infection is asymptomatic in adults and is difficult to diagnosis clinically⁽²⁴⁾. as well as, this result is supported by Mimi(2009), who reported that the prevalence of Cytomegalovirus IgG antibody among aborted women in Turkey was between (84.5 – 95 %)⁽²⁵⁾, and such result was higher than our result (61.1%), hence Cytomegalovirus infections in pregnancy has higher incidence in fetal loss⁽²⁶⁾. Similar to the previous studies, our results demonstrate that, about 1/3 of the seropositive cases by ELISA were diagnosed by PCR , this means that the seropositive results of ELISA were not specific or less significant because the eventuality of false positive that could be as a result of infections with other microorganisms⁽²⁷⁾. infections with Rubella virus and Cytomegalovirus have a significant risk in infants at birth although the risk of late manifestation is unclear, whereas such data are fairly known for Rubella virus⁽²⁸⁾. Appears of IgM antibody during the first week may be inadequate to expect the presence of the pathogens by RT-PCR test⁽²⁹⁾. The study showed that out of ninety patients only 17(18.9%) aborted women were seropositive for IgM and IgG antibodies of Rubella virus and only 12 patients were positive by RT-PCR technique . Similar to the previous studies, our results agreed with the results of Nolan (2006)⁽³⁰⁾, who demonstrated that out of 17 positive cases of Rubella virus by ELISA test only 6 cases were positive by RT-PCR technique . The results showed that the percentage of seropositive IgG antibody for Rubella virus and Cytomegalovirus were (41.1%) and (61.1%) respectively, RT-PCR technique will reveals that the percentage was decline to (13.3%) for Rubella virus and (6.7%) for Cytomegalovirus , which give the real incidence of infection of these microorganisms and reflect the false positive results that will be given by routine ELISA test, therefor RT-PCR technique was effective, highly sensitive, and more specific than ELISA test on the diagnosis of Rubella virus and Cytomegalovirus infections.⁽³¹⁾

CONCLUSIONS

1- Low prevalence of Rubella virus IgG antibodies in Iraqi women is mainly due to default of vaccination (MMR) programs that is concentrated only on secondary school girls.
2- The results of RT-PCR test showed that there is no mixed infection of Rubella virus and Cytomegalovirus in contrary to ELISA test results, which reflect the sensitivity and specificity of RT-PCR test , in addition, reflect the false results and cross reaction

of ELISA technique , hence there is no reason for treatment of mixed infections with Rubella virus and Cytomegalovirus in pregnant women.

3- Diagnosis of Rubella virus and Cytomegalovirus infections should be done by molecular method and not based on ELISA test which should be used as screening test .

4-Recently, RT-PCR technology is a robust and broadly used methodology for biological investigations because, it can be detect and quantify very small amounts of specified nucleic acid sequence, as a research tool, major application of this technology is rapid and accurate assessment for changes in gene expression as outcome of physiology, pathophysiology or development. Clinical molecular diagnosis present applications and future perspectives for this technology in biomedical sciences and in life science education.

RECOMMENDATIONS

1-Rubella virus IgG antibodies should be consisted in the listing tests for pre married girls in order to give vaccines to negative one, and it is must recommended routine screening of Rubella virus for pregnant women in Iraq to reduce the abortions caused by this virus.
2-The incidence of Cytomegalovirus infection found in our country, therefore, support using of serological screening test, especially in the first trimester of pregnancy, and in the case of seronegative, possibly screening once in the second and third trimester.

3- Before giving management for pregnant women by the gynecologist, must be dependant on the diagnosis of mixed infection on the molecular methods like RT-PCR technique, because it is more specific and sensitive technique than serological methods like ELISA test in the diagnosis of viral infections.

4- Prior of pregnancy, screening for Rubella virus and Cytomegalovirus antibodies are recommended to be practicing routinely, will prevent fetus from infected by Rubella virus and Cytomegalovirus in uterus.

5-there is a need to modifies vaccine strategies for immunization all adolescent girls and women in reproductive age before conception to decrease the incidence of congenital rubella syndrome infection and bad obstetrical outcome.

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