MOLECULAR AND IMMUNOLOGICAL INVESTIGATION OF RUBELLA VIRUS ISOLATED FROM ABORTED WOMEN

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Abstract

The rubella virus is a potent, highly infectious, and teratogenic agent that may cross the placenta and cause fetal infection; such infection in the first trimester of pregnancy can result in miscarriage. The present study comprised of 85 female patients suffered from recent abortion. The blood sample was collected from the female patients who attended to the Hospitals in Baghdad City. The samples collection was carried out in the period between the beginning of November 2018 and the end of January 2019. The age of female patients ranged from 25-45 years, The results showed that the anti-rubella IgG antibodies concentration can be divided into three groups; the first group contains 59 aborted females with anti-rubella IgG antibodies concentrations range from 0.19-200 IU/L, the second group contains 13 aborted women with anti-rubella IgG antibodies range from 200-400 IU/L, whereas the third group contains 13 aborted women with anti-rubella IgG antibodies >400 IU/L. A total of 40 samples were selected and tested for rubella virus using RT-PCR. Rubella virus was detected in 30 out of 40 samples, selected samples were assayed to determine the rubella virus. RT-PCR was a complementary assay performed

Keywords : Rubella antibodies, pregnant women, Baghdad, Iraq.

Introduction

Rubella infection usually presents as a mild or asymptomatic infection in children and adults. However, the rubella virus is a potent, highly infectious, and teratogenic agent that may cross the placenta and cause fetal infection; such infection in the first trimester of pregnancy can result in miscarriage, fetal death, or an infant born with serious birth defects including hearing impairment, cataracts, and cardiac defects, collectively known as congenital rubella syndrome (Grimes and Stuart, 2010).

Recurrent pregnancy loss is secondary to multiple illnesses. An important cause sometimes undiagnosed is the antiphospholipid syndrome, an autoimmune disease with various clinical alterations (Kutteh and Hinote, 2014). Recurrent pregnancy loss is secondary to multiple illnesses. An important cause sometimes undiagnosed is the antiphospholipid syndrome, an autoimmune disease with various clinical alterations (Kutteh and Hinote, 2014). Abortion is the ending of pregnancy due to removing an embryo or fetus before it can survive outside the uterus. An abortion that occurs spontaneously is also known as a miscarriage. When deliberate steps are taken to end a pregnancy, it is called an induced abortion, or less frequently an "induced miscarriage (Grimes and Stuart, 2010). Therefore, promoting the level of awareness among the Iraqi pregnant women through screening is mandatory to control the abortion cases, which means there is a considerable need for early detection of viral infections that can be attributed in abortion incidence,

The aim of this study is to a determination of Immunological investigation of rubella virus in aborted women by estimation of anti-rubella virus antibodies as immunological markers for this viral infection, Determination of abortion risk factors includes anti-phospholipid and anti-cardiolipin antibodeis, Molecular detection of rubella virus in aborted women by using reverse transcriptase PCR (RT-PCR).

Materials and Methods

Patients and samples collection

During the period of current study, 85 of aborted female who attended to the Al-Zafaraniyah General Hospital, Fatima-Alzahra Hospital and Teaching Al-Yarmook Hospital in Baghdad City, were subjected to detect the rubella virus infection and estimation of other factors associated with abortion incidence include anti-phospholipid IgG and IgM antibodies and ant-cardiolipin IgG and IgM antibodies.

Chemolumi immunoassay method using Cobas E411.

The anti-rubella IgG antibodies was detected in serum samples by chemolumi immunoassay method using Rubella IgG Cobas E411kit.

Molecular detection of rubella virus by RT- PCR

The rubella virus was detected in the plasma samples that collected from aborted women by RT- PCR using Rubella Real-TM Qual kit (Sacace/ Italy). Rubella virus detection by the PCR is based on the amplification of the pathogen genome specific region by using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without reopening of the reaction tubes after the PCR run. RUBELLA Real-TM Qual PCR kit is a qualitative test that contains the Internal Control (IC), which must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. RUBELLA Real-TM Qual PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF), which is activated by heating at 95 °C for 15 min**.**

Statistical analysis:

The Statistical analysis system-SAS (2012) programme was used to effect of difference factors in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

Results and Discussion

During the period of current study, 85 of aborted female who attended to the Al-Zafaraniyah General Hospital, Fatima-Alzahra Hospital and Teaching Al-Yarmook Hospital in Baghdad City, were subjected to detect the rubella virus infection and estimation of other factors associated with abortion incidence include anti-phospholipid IgG and IgM antibodies and ant-cardiolipin IgG and IgM antibodies.

The serum of the aborted females of current study were subjected to detect the anti-rubella IgG antibodies by chemolumi immunoassay method using Rubella IgG Cobas E411kit. The results showed that the anti-rubella IgG antibodies concentration can be divided into three groups; the first group contains 59 aborted females with anti-rubella IgG antibodies concentrations range from 0.19-200 IU/L, the second group contains 13 aborted women with anti-rubella IgG antibodies range from 200-400 IU/L, where as the third group contains 13 aborted women with anti-rubella IgG antibodies >400 IU/L.

Also the results of statistical analysis revealed that the 51% of women had one abortion, 35.2% of aborted women with two abortions, and 12.8% of aborted women with three abortions. In addition, the results showed that there was no significant differences found between the number of abortions and concentration of anti-rubella IgG antibodies in the aborted women. In addition, the results revealed that the IgG seroprevalence was the highest in pregnant women who had one abortion in comparison with multiple abortions which means that IgG antibodies had a very important role in decreasing the rate of abortion in pregnant women as shown in (Table 1).

Table 1 : Relationship between abortion numbers and rubella IgG.

Abortion numbers	Rubella IgG			Chi-Square (χ^2)
	Low level (0.19-200)	Moderate level (200-400)	High level (>400)	
1	38 (44.71%)	3 (3.53%)	3 (3.53%)	10.945 **
2	17 (20.00%)	8 (9.41%)	5 (5.88%)	5.315 *
3	4 (4.71%)	2 (2.35%)	5 (5.88%)	0.791 NS
Chi-Square (χ^2)	11.503 **	3.566 NS	0.671 NS	----
* (P<0.05), ** (P<0.01).				

Total No. = 85.

The results of present study agree with results of other studies showed that the pregnant women who had three abortions were constitute only 12.8% of the studied women (Rager *et al.*, 2003; Alboa and Cort, 2010).

Table 2 : Relationship between age groups and rubella IgG.

Age group (year)	Rubella IgG			Chi-Square (χ^2)
	Low level (0.19-200)	Moderate (200-400)	High (>400)	
Less than 30	9 (10.59%)	4 (4.71%)	3 (3.53%)	3.263 NS
30-40	43(50.59%)	9 (10.59%)	8 (9.41%)	9.872 **
More than 40	7 (8.23%)	0 (0.00%)	2 (2.35%)	4.612 *
Chi-Square (χ^2)	10.437 **	4.522 *	3.084 NS	----
* (P<0.05), ** (P<0.01).				

Total No. = 85.

The results revealed that 16 patients (18.7%) were in the age group less than 30 years and 60(70.4%) in the age groups 30-40 years then 9(10.4) in the age groups more than 40 (Table 1). The result of the present study is in agreement with that reported by (Nada *et al.*, 2014) who found the highest percent seroposivity rate (52.2%) in pregnant women who were tested for anti-rubella IgG aged between 20 and 29 years.

The present study disagree with the results of the study reported by (Hindi *et al.,* 2010) who found the highest rate of rubella IgG (94,1%) in the age group 35-44 years. On the other hand, (Aysegul and Fazilet, 2012) found the highest rate of anti-rubella- IgG (84.6%) among the pregnant women of the age group 18-25 years.

A total of 40 samples were selected and tested for rubella virus using RT-PCR. Rubella virus was detected in 30 out of 40 samples, selected samples were assayed to determine the rubella virus. RT-PCR was a complementary assay performed.

Molecular detection of rubella virus by RT- PCR findings that more than half (60%) of pregnant women were seropositive for rubella virus, The results of present study agree with results of other studies showed that the pregnant women who had abortions which showed a seroprevalence of 65-68% (Hamdan *et al.*, 2011).

And, the results of current study go together with different studies they concluded higher rubella seroprevalence (90.1 and 96.2%) among aborted pregnant women (Tahita *et al.,* 2010).

Other study, show85% of rubella seroprevalence (Jonas *et al.*, 2010) Also the result of (Zanga *et al.,* 2017) in which viral genome was detected in 60% of pregnant aborted women, this fact agreed with our results.
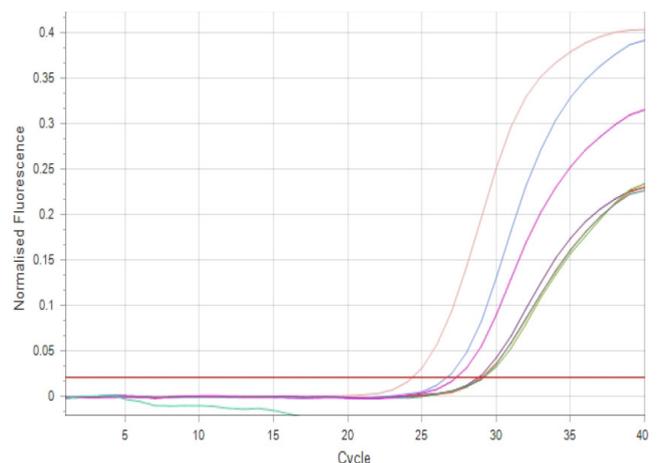

Fig. 4.1 : RT- PCR curve of rubella virus detection.

Immunological detection of anti-cardiolipin antibodies IgM and IgG

The results revealed that the anti-cardiolipin IgG seroprevalence was the highest in pregnant women who had one and two abortion in comparison with three abortions which means that IgG antibodies had a very important role in increasing the rate of abortion in pregnant women as shown in (Table 3).

Table 3 : Relationship between Abortion numbers and Anti-Cardiolipin IgG

Abortion numbers	Anti Cardiolipin IgG			Chi-Square (χ^2)
	Equvocal border line 12-18	Positive >18	Negative <12	
1	1 (2.50%)	4 (10.00%)	9 (22.50%)	9.016 **
2	1 (2.50%)	12 (30.00%)	6 (15.00%)	9.863 **
3	0 (0.00%)	4 (10.00%)	3 (7.50%)	4.6719 *
Chi-Square (χ^2)	0.849 NS	7.250 **	5.692 *	----
* (P<0.05), ** (P<0.01).				

Total No. =40 .

The results of present study agree with results of other studies showed that the pregnant women who had abortions (Godoy *et al*., 2006)

And, the results of current study go together with different studies they concluded that anticardiolipin antibody is found to be the most important factor for recurrent abortion (Velayuthaprabhu and Archunan, 2005)

Other study mentioned that the concentration of Anti-cardiolipin antibodies was observed that anti-cardiolipin is a major cause of recurrent fetal loss (Sheth and Sheth, 2001), this fact agreed with our results

In addition, the results showed that there was significant differences found between the number of abortions and concentration of anti-cardiolipin IgM antibodies in the aborted women. In addition, the results revealed that the IgM seroprevalence was the highest in pregnant women who had one abortion in comparison with multiple abortions which means that IgM antibodies had a very important role in increasing the rate of abortion in pregnant women as shown in

Table 4: Relationship between Abortion numbers and Anti-Cardiolipin IgM

Abortion numbers	Anti-Cardiolipin IgM			Chi-Square (χ^2)
	Equvocal border line 12-18	Positive >18	Negative <12	
1	1 (2.50%)	1 (2.50%)	12 (30.00%)	9.064 *
2	3 (7.50%)	5(12.50%)	10 (25.00%)	6.589 **
3	0 (0.00%)	3 (7.50%)	5 (12.50%)	5.138 *
Chi-Square (χ^2)	2.641 NS	4.502 *	6.935 **	----
* (P<0.05), ** (P<0.01).				

Total No. =40.

The results of present study agree with results of other studies showed that the pregnant women who had abortions (Godoy *et al*., 2006)

And, the results of current study go together with different studies they concluded that anticardiolipin antibody is found to be the most important factor for recurrent abortion (Velayuthaprabhu and Archunan, 2005)

Immunological detection of anti-phospholipid antibodies IgM and IgG

The IgM concentrations could be divided into three groups; the first group contains females patients with IgM concentrations (0.2 border line)and the second group patients with IgM concentrations Negative (<0.2) where as the third group contains patients with IgM concentration Positive (>0.21) the results of statistical analysis revealed that the 15% of women had one abortion, 15% of aborted women with two abortions, and 7.5 % of aborted women with three abortions. In addition, the results showed that there was high significant differences found between the number of abortions and concentration of anti-phospholipid antibodies IgM in the aborted women as shown in (Table 5).

Table 5 : Relationship between Abortion numbers and Anti phosphor- lipid Igm

Abortion numbers	Anti phosphor-lipid IgM			Chi-Square (χ^2)
	IgM =0.2 border line	Positive >0.21	Negative <0.2	
1	1 (2.50%)	5(12.50%)	8(20.00%)	6.839 **
2	2 (5.00%)	4(10.00%)	12(30.00%)	8.465 **
3	0 (0.00%)	3(7.50%)	5(12.50%)	5.173 *
Chi-Square (χ^2)	1.276 NS	1.364 NS	6.702 **	----
* (P<0.05), ** (P<0.01).				

Total No. =40 .

The present study showed that the most important factor for recurrent abortion anti-phospholipid antibodies and this result agree with results other studies show Recurrent pregnancy loss (Adelowo and Adetoro, 2010) In addition, several studies conducted in different countries agree with this study found a relationship between anti-phospholipid antibodies and Recurrent pregnancy loss (Chen *et al.,* 2012), these results goes with results of different study reported Association between anti-phospholipid antibodies and recurrent miscarriage (Roye-Green *et al.,* 2011).

The anti-phospholipid antibodies IgG concentrations could be divided into three groups; the first group contains females patients with IgG concentrations (Moderate =2.4 border line)and the second group patients with IgG concentrations Negative (<2.4) whereas the third group contains patients with IgG concentration Positive, the results of statistical analysis revealed that the 12.5% of women had one abortion, 12.5% of aborted women with two abortions, and 10% of aborted women with three abortions. In addition, the results showed that there was significant differences found between the number of abortions and concentration of anti-phospholipid antibodies IgG in the aborted women as shown in (Table 6).

Table 6 : Relationship between Abortion numbers and Anti phospho-lipid IgG

Abortion numbers	Anti phospho-lipid IgG			Chi-Square (χ^2)
	Moderate =2.4 border line	Positive 2.6-3.1	Negative <2.4	
1	2 (5.00%)	3 (7.50%)	9(22.50%)	6.852 **
2	2 (5.00%)	3 (7.50%)	12(30.00%)	8.134 **
3	0 (0.00%)	4 (10.00%)	5(12.50%)	4.766 *
Chi-Square (χ^2)	1.362 NS	0.849 NS	6.182 **	----
* (P<0.05), ** (P<0.01).				

Total No. =40 .

The present study showed that the most important factor for recurrent abortion anti-phospholipid antibodies and this result agree with results other studies show Recurrent pregnancy loss (Adelowo and Adetoro, 2010) In addition, several studies conducted in different countries agree with this study found a relationship between anti-phospholipid antibodies and Recurrent pregnancy loss (Chen *et al.,* 2012), these results goes with results of different study reported Association between anti-phospholipid antibodies and recurrent miscarriage (Roye-Green *et al.,* 2011).

Conclusions

This study exhibits a remarkable association of rubella virus as an etiologic factor for abortion in Iraq, Diagnosis of Rubella virus by immunological methods are considered as essential steps, but not absolutely accurate methods in the detection of these virus in comparison with molecular methods like RT-PCR technique. Women were antibodies positive against rubella virus infection suggestive of placing many unborn babies to high risk for acquiring rubella infection. The increase in the levels of IgG, IgM, anti-phospholipid and anti-cardiolipin antibody of patients with recurrent abortions refer to their role in abortion.

References

Adelowo, O.O. and Adetoro, O.O. (2010). Recurrent pregnancy loss and antiphospholipid syndrome: an overlooked association. Afr J Med Med Sci., 39(3): 227-31.

Alboa and Cort. (2010). World Health Organization (2000) Rubella vaccines: WHO position paper. Weekly Epidemiological Record, 75: 161-169.

Aysegul, Co. and Fazilet, Du., (2012). Investigation of Rubella antibodies among women of childbearing age in Sanliurfa province: An evaluation of three years period. Journal of Dicle Medical, 39: 174-178.

Chen, L.; Quan, S.; Ou, X.H. and Kong, L. (2012). Decreased endometrial vascularity in patients with antiphospholipid antibodies-associated recurrent miscarriage during midluteal phase. Fertil Steril. 98: 1495–1502.

Chen, M. and Icenogle, J. (2007). Molecular virology of rubella virus, p1-18. In Rubella virus. Edited by Banatvala J, Peckham C. Oxford, United Kindom: Elsevier

Godoy, J.M.P.; Spegiorin, L.C.J.F.; Dallaqua, J.; Carvalho, C.F.C. and Costa, I.L. (2006). Gestation in patients with high levels of anticardiolipin antibodies, a history of deep venous thrombosis and miscarriages. Arch Med Sci., 23: 205-7

Hamdan, Z.H.; Ismail, E.; Abdelbagi, I.E.; Nasser, M.N. and Adam, I. (2011). Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. Virol J., 8: 217.

Hindi, A.; Al-Helou, T. and Al-Helou, Y. (2010). Seroprevalence of *Toxoplasma gondii*, cytomegalovirus, rubella virus and *Chlamydia trachomatis* among infertile women attending *in vitro* fertilization center, Gaza strip, Palestine. Journal of Egypt Society parasitology, 40: 451-458.

Jonas, A.; Cardemil, C.V.; Beukes, A.; Anderson, R.; Rota, P.A.; and Bankamp, B. Rubella immunity among pregnant women aged 15-44 years,

Nada, M.; Al-Tawalah, H.; Abdul Khalik, D. and Al-Nakib, W. (2014). A Relatively High Number of Pregnant Women in Kuwait Remain Susceptible to Rubella: A Need for an Alternative Vaccination Policy. Journal of Medical Principle and Practical, 23: 145-148.

Rager-Zisman, B.; Bazarsky, E.; Skibin, A.; Chamnwy, S.; Belmaker, I. And Shai, I. (2003). The effect of measles-mumps - rubella (MMR) immunization on the immune responses of previously immunized primary school children. Vaccine, 21: 2580- 2588 research, 44: 69-160.

Roye-Green, K.; Frederick, J.; Wharfe, G.; Choo-Kang, E.; Dacosta, V.; Fletcher, H. and Smikle, M. (2011). Antiphospholipid and other autoantibodies in a cohort of habitual aborters and healthy multiparous women in Jamaica. Hum. Antibodies 20: 1–5.

Sheth, J. and Sheth, F.J. (2001). Study of anticardiolipin antibodies in repeated abortions-an institutional experience. Indian J Pathol Microbiol. 44(2): 117-21 Namibia, 2010. Int J Infect Dis. 49: 196–201.

Tahita, M.C.; Hubschen, J.; Tarnagda, Z.; Ernest, D.; Charpentier, E. and Kremer, J.R. (2013). Rubella seroprevalence among pregnant women in Burkina Faso. BMC Infect Dis. 13: 164.

Velayuthaprabhu, S. and Archunan, G. (2005). Evaluation of anticardiolipin antibodies and antiphosphatidylserine antibodies in women with recurrent abortion. Indian J Med Sci.*,* 59(8): 347-52.

Zhang, J., Guan, Z., Murphy, A.N., Wiley, S.E., Perkins, G.A., Worby, C.A. and Raetz, C.R. (2011). Mitochondrial phosphatase PTPMT1 is essential for cardiolipin biosynthesis. Cell metabolism, 13(6): 690-700.