



FACTORS AFFECTING IMMUNE RESPONSE DURING MISCARRIAGE DUE TO RUBELLA VIRUS, IRAQI WOMEN

Suhailah Ismaeel Aajb* and Musa Nima Mezher Al_Jaifry

College of Science, University of Kufa, Iraq.

Abstract

Abortion because one of more problems that face women in the world. Because rubella virus part of this problem with complications that result from this infection. Rubella virus beyond to toga virus family and is composed RNA nucleic acid. Immune system have important roles in save and continuous pregnancy and protection against different infection. Hormones that estradiol and progesteron are working consistently to implantation fertility egg and development of fetus through pregnancy period. The study focused on relationship between sex hormones effect and immune response in pregnant women that infected by rubella virus. And study of effect sex hormones in immune response, Collection samples from blood 60 aborted women who attended hospitals from Al-Zahra Teaching Hospital Maternity and Children and Al-Sajad hospital in Al-Najaf a period from September 2019 to January 2020. Detection of IgM Antibodies to Rubella virus in patients serum by Minividas technology. Estimation the Estradiol 2 and Progesteron hormones by Minividas technology. The TNF- α and IL-10 serum level of was evaluated by ELISA technique. The ratio 83.33% of aborted women were positive for rubella virus while 16.66 were negative and the percentages of immunoglobulin G were more than for immunoglobulin M. Aborted women in recurrent study divided in three groups according to number of abortion Women aborted once, two and the three times. Distribution of IgG and IgM serum antibodies to Rubella virus in age groups, distribution of IgG and IgM serum antibodies to Rubella virus according to residence groups Estimation of estrogen and Progesterone concentration in groups of Rubella -positive and negative for IgG and IgM antibodies in abortion patients, The results of the statistical analysis showed a significant increase in the mean concentrations of Estrogen hormone in patients with IgG positive at a mean concentration (328 Pg./ml) compared with negative IgG and control groups (81.9 pg./ml and 132.8 pg./ml) respectively and also increased in mean concentration of progesterone hormones (34.8 ng/ml) compares with negative IgG and control groups (7.2 ng/ml and 13.5ng/ml) respectively. Distribution of estrogen and progesterone concentration in groups of Rubella according to number of abortions in patients, Distribution of estrogen and progesterone concentration in groups of Rubella according to age groups of abortions patients. Distribution of estrogen and progesterone concentration in groups of Rubella in Urban and rural groups of abortions patients. Estimation of IL10 and TNF- α concentration in groups of Rubella -positive and negative for IgG antibodies in abortion women. The results of the statistical analysis showed a high significant increase in the mean concentrations of IL-10 in patients with IgG positive at a mean concentration (43.6 Pg./ml) compared with negative IgG and control groups (16.9 and 16.8) pg/ml respectively and also statically increased in the mean concentration of TNF- α (33.5 ng/ml) compares with negative IgG and control groups (14 and 15.5) ng/ml respectively and estimated to IgM antibody. Estimation of IL10 and TNF- α concentration in groups of abortions patients according to residence, age and number of times of abortions patients.

Key words: Abortion, miscarriage, Rubella virus, congenital syndrome

Introduction

Rubella or German measles is disease caused by rubella virus infects childhood with mild symptom self - limited. Unlike symptoms in childhood, the on sets of the virus more serious during pregnancy and the virus has devastating effects on fetus developing ,and cause serious consequences as well as congenital malformation

**Author for correspondence* : E-mail : suhailaism@gmail.com

(MMWR, 2001). Rubella virus is sole member in genus RNA strand. In addition it can transmission person ,human are the only reservoir (MMWR 2010). The fetus damage happens through destruction the cell as much as mitotic arrest (2013).

Maternal infection for the duration of the first trimester is often associated with stillbirth, spontaneous abortion, and adverse neonatal outcome including heart problem,

cataract, and deathless which is known congenital Rubella syndrome, which has a major neonatal morbidity along with problem to family. Furthermore, some defect have been stated later following maternal infection in the second trimester (Ojata *et al.*, 1973).

Pregnancy state represents an extreme challenge to immune system. To support successful pregnancy, it is evolutionarily advantageous for female immune responses to shift away from inflammatory response that contribute in fetal rejection and toward to anti-inflammatory immune responses that aid to passive transfer of antibodies to developing fetus (Raghupathy, 1997). Hormones contribute significantly to the shift in immune function that occur over three trimester of pregnancy. Pregnant women are not immunosuppressed, but rather their immune responses are biased toward an anti-inflammatory phenotype that influences not only the outcome pregnancy, but disease pathogenesis as well.

Pregnancy is associates with changes in concentration of several hormones include estradiol (E2), estriol (E3), progesterone, corti-costeroid ,prolactin, these hormonal changes contribute to immunological shift during pregnancy. Altered activity of innate immune cells contributes to the differential induction of cell-mediated and humoral responses during pregnancy. We will focus in sex hormones E2, Progesteron in this study (Raghupathy, 1997).

Hormonal changes that occur during pregnancy underlie some of the distinct immunological changes associated with pregnancy. High levels of progesteron-4 stimulate synthesis of progesterone-induced binding factor (PIBF) by lymphocytes (Szerkeres-Bartho J. and Polgar B, 2010). High concentrations of PIBF promote differentiation of CD4⁺ T cells into helper T cell type 2 (Th2) cells that secrete high concentrations of anti-inflammatory cytokines, including IL-4, IL-5 and IL-10 (Szekers-Bartho *et al.*, 1996). Inflammatory directly cytokines, like IFN- λ and TNF- α , can damage the placenta and developing fetus either or by activating cytotoxic cells, including natural killer (NK) or T cells (Raghupathy, 1997). Successful pregnancies in humans are associated with elevated IL-4 and IL-10 and reduced IL-2 and IFN- λ production by peripheral blood mononuclear cells (PBMCs), with differences in cytokine production being greatest during the third trimester of pregnancy (Marzi *et al.*, 1996). Two specific antibodies are influence to Rubella virus, the first to appears Immunoglobulin (Ig) M antibody which rises and peaks 7-10 days of infection and then reduces after several weeks. The second specific antibodies are Immunoglobulin G (IgG), that develops very slowly, but

remains positive for lifetime ,hence conferring immunity against repeat infection. Therefore, in women patients the presence of IgM antibody indicates a recent infection, while IgG antibody indicates an old infection and immunity (Lombardo P.C., 2011).

Materials and methods

The Samples collection

Five ml of venous blood were collected from each aborted women (60) samples and (20) samples as control ,the blood samples were obtained by vein puncture from all patients after cleaning the skin with 70% of alcohol ,and placed in serum tube. After coagulation of blood samples centrifugation for 5 minutes at 3000 (rpm) to separate serum, withdrawal it and divided to three divisions in plan tube, each sample was labeled and given a serial number. Keep these tubes that contain serum in deep freeze (-20^oc) until samples processing.

Detection of IgM Antibodies to Rubella virus in patient's serum by Minividas test

The Principle of Minividas technique that uses in detection of Rubella virus IgM and IgG antibodies according to (Enders M *et al.*, 2013).

Determination of IgM antibody

Reagents for the assay are ready –to- use and predisposed in the sealed reagent strips. SPR is coated during production with anti –IgM monoclonal antibodies (sheep). Each SPRs is identified by the prog. code. Only remove the required number of SPRs from the pouch and carefully reseal the pouch after opening. The strip consists of 10 wells converted. with a labeled seal. The foil of the first well is perforated to facilitate the introduction of the sample 200 micro-liter. The VIDAS uses a solid-phase receptacle coated with rubella virus as the reaction vessel. It is also a fluorescence-based enzyme immunoassay that uses an anti-human IgM conjugate and 4-methylumbelliferyl phosphate as substrate (smith J. *et al.*, 1993).

Detection of IgG Antibodies to Rubella virus in patient's serum by Minividas test

Determination of IgG antibody

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid Phase. Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. SPR is coated during production with anti-IgG monoclonal antibodies (sheep). Each SPR is identified by the IgG

code. Only remove the required number of SPRs from the pouch and carefully reseal the pouch after opening. The strip consists of 10 wells converted with a labeled seal. The foil of the first well is perforated to facilitate the introduction of the sample 200 μ L.

Estimation of Interleukin-10 by ELISA techniques

A Reagents preparation

Procedure

1- Addition 100 μ l from each control, specimen, and standard to the whole wells, also use a sealer to the covered plate and finally place the plate in the incubator at 37°C to ninety minutes.

2- Liquid was excluded from whole wells, was added 100 μ l from detection Ab working solution to every well, then was covered plate by sealer and Incubated for one hour at 37°C.

3- Solution was decanted from wells and was added 350 μ l from buffer solution, then wash was repeated three times in total.

4- One hundred (100 μ l) from working solution (HRP conjugate) has been applied to the wells, the sealer was covered plate, under 37°C and for 30 minutes was Incubated.

5- Removed the solution from the wells and the washing method was acted 5 times.

6- Adding of 90 μ l from reagent of the substrate, and another sealer covered the plate, then was incubated for fifty minutes under 37°C.

7- The stop solution, 50 μ l was similarly added to all wells was achieved in the step of substrate solution.

8- Determined of optical density by use spectrophotometer at wave length 450 nm.

Estimation of human TNF-Alpha in serum

Reagents preparation

Procedure :-

- The same step remembered in the first point in the previous procedure of IL-10.

Estimation the hormones concentration

Progesterone detection :

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to-use and pre-dispensed in the sealed reagent strips. SPR is coated during production with anti-PRG monoclonal antibodies (sheep). Each SPR is identified by the progesterone code. Only remove the required number of SPRs from the pouch after opening. The strip consists of 10 wells converted with a labeled seal. The foil of the first well is perforated to facilitate the introduction of the sample 100 μ l.

Estrogen detection:

The same step remembered in the previous procedure of progesterone determination.

Result and Discussion

Distribution of Aborted women to Rubella virus

A total of (60) clinical specimens were collected from patients with aborted women who attended hospitals from Al-Zahra Teaching Hospital Maternity and Children and Al-Sajad hospital in Al-Najaf Province over a period from September 2019 to January 2020. As shown in Fig. 1 and table (4-1) the ratio 83.33% of aborted women were positive for rubella virus while 16.66 were negative and the percentages of immunoglobulin G were more than for immunoglobulin M. The emergence of cases of infection with a virus may be due to the fact that there is no permanent immunity to the virus and not taking the vaccine during pregnancy so the disease is occurred. This results aggregate with study by (Crooke, S.N., *et al.*, 2019). That suggested 2.2% of subjects were found 2.2% of subjects were seronegative and, 97.8% of subjects were seropositive for rubella specific IgG antibodies. While realved other study that only 39% of patient gave positive results to IgG anti-rubella virus. (Khudhair M.K. and Ahmed R.I. 2015).

Table 1: Dilution of standard solutions suggested are as follows.

800 pg/ml	Standard NO 5	120 original standard + 120 standard diluent			
400 pg/ml	Standard No4	120 standard No 5+120 MI standard diluent			
200 pg/ml	Standard No 3	120 Standard No4 + 120 M standard diluent			
100 pg/ml	Standard No 2	120 Standard No3 + 120 M standard diluent			
50 pg/ml	Standard No 1	120 Standard No2 + 120 M standard diluent			
Standard concentration	Standard No 5	Standard No 4	Standard No3	Standard No2	Standard No 1
1600 pg/ml	800 pg/ml	400 pg/ml	200 pg/ml	100 pg/ml	50 pg/ml

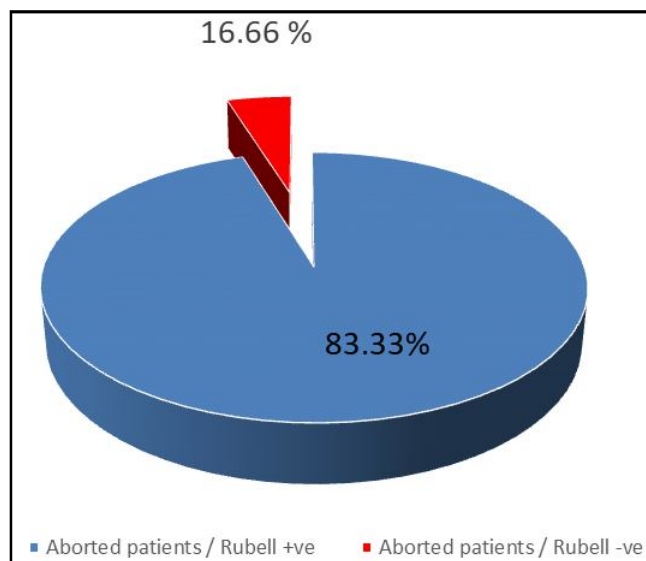


Fig. 1: Distribution of aborted women to Rubella virus.

Detection of IgG and IgM serum antibodies to Rubella virus in aborted women

Aborted women in recurrent study divided in three groups according to number of abortion Women aborted once, two and the three times.

As shown in table 2 the IgG antibody was detected in 50 out of 60 patients with ratio 83.33% and these were distributed to 29 (48.33%) with the case of aborting one and 15 (25%) with two case and 6 (10%) with three case, while in other hand the detection of IgM antibodies is 7 out 60 aborted women with ratio of (11.66%) and these were distributed to 5 (8.33%), 1 (1.66%) and 1(1.66%) for one aborted case and two and three respectively. Increased availability of IgG may due to The development of IgG antibody is an effort made by the immune system to help neutralize the virus. This antibody prolongs life and confers immunity against reinfection. Therefore, it is correct to assume that the pregnant women that had IgG antibodies are immune.

While Fig. 3 shows astatically differences of mean concentration of IgM antibodies between three groups and control, where we found a high mean concentration of patient groups with one case of abortion registered (7.26 IU/ml) compare with other and control groups, may

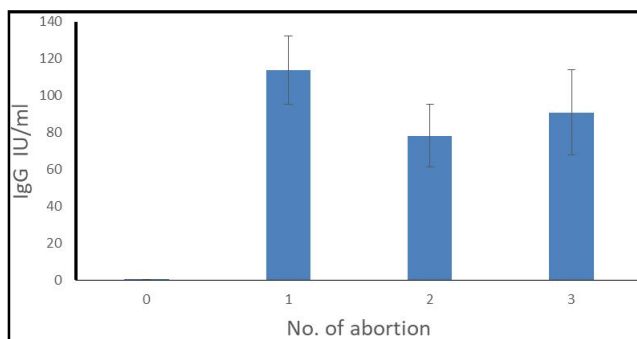


Fig. 2: Mean concentration of IgG antibodies according to number of abortions.

be because most infections are chronic not acute and new infections are often rare, so it appears the IgM less than from IgG.

Distribution of IgG and IgM serum antibodies to Rubella virus in age groups

In this study, the age groups were divided into five categories, the results of this study showed that the age group 21-25 revealed the highest percentage (30%) of high number of patients with antibody compared to other age groups, Where the ratios reached 15%, 12.66%, 10% and 6.66% for age groups (25-30), more than 36, 31-35 and less than 20 respectively. While it showed the highest positive cases of IgM in the age group (21-25) while no presence of the positive antibody was recorded in age groups (31-35) and more than 36). Our results showed the most affected range of patient’s ages with abortions was mainly in age groups (26- 30) years, this is shown by the Fig. (4-4) with a significant increase (p value <0.005)

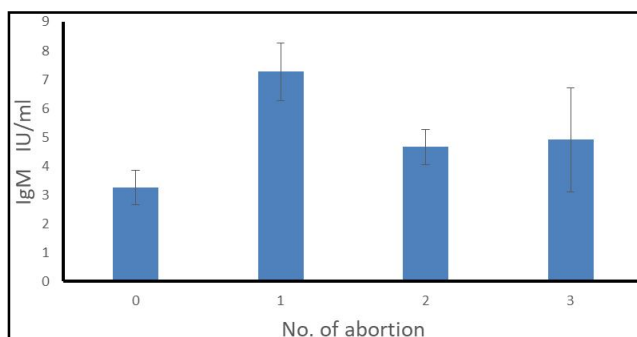


Fig. 3: Mean concentration of IgM antibodies according to number of abortions.

Table 2: Dilution of standard solutions suggested are as follows.

480 pg/ml	Standard NO 5	120 original standard + 120 standard diluent			
240 pg/ml	Standard No4	120 standard No 5+120 Ml standard diluent			
120pg/ml	Standard No 3	120 Standard No4 + 120 M standard diluent			
60 pg/ml	Standard No 2	120 Standard No3 + 120 M standard diluent			
30pg/ml	Standard No 1	120 Standard No2 + 120 M standard diluent			
Standard concentration	Standard No 5	Standard No 4	Standard No3	Standard No2	Standard No 1
960 pg/ml	480 pg/ml	240 pg/ml	120 pg/ml	60 pg/ml	30 pg/ml

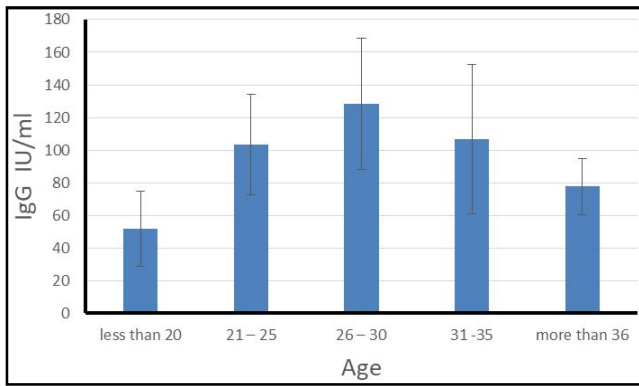


Fig. 4: Mean concentration of IgG antibodies according to age groups.

in the concentration rates of IgG with mean (128.2 IU/ml) while the age group (more than 36) recorded the lowest concentration rate at the rate of (77.6 IU/ml) compared to other groups.

On the other hand, we notice in Fig. 5 the concentration rates of IgM antibodies, which were distributed among age groups, where we note that the age group (26-30) recorded the highest concentration rate (8.33 IU/ml), while the lowest concentration was the age group (31-35) years old, it was (3.7 IU/ml) while there were no significant differences between the groups themselves.

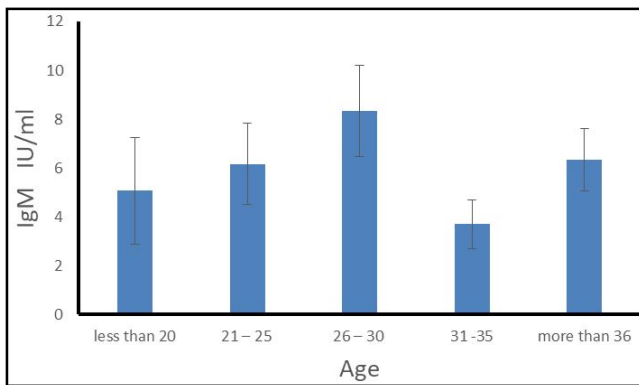


Fig. 5: Mean concentration of IgM antibodies according to age groups.

Distribution of IgG and IgM serum antibodies to Rubella virus according to residence groups

In our current study to compare the numbers abortions among patients that was infected with Rubella virus between two groups, the first group for patients from Urban residents and the other from rural residents, we have noticed from the table 4 that there are no differences in virus infection with regard to rural and Urban residents where was the percentage (45% and 3.33%) respectively for IgG and IgM in Rural while (38%, 8.33 %) in Urban for both antibodies. The explanation for the result in our study may be there are no significant differences for the

presence of the virus between patients from the rural population and the same from the residents of the urban, because the study samples are not in sufficient number in addition that the study area is almost similar in terms of social services and health care.

Estimation of estrogen and Progesterone concentration in groups of Rubella -positive and negative for IgG and IgM antibodies in abortion patients

The results of the statistical analysis showed a significant increase in the mean concentrations of Estrogen hormone in patients with IgG positive at a mean concentration (328 Pg./ml) compared with negative IgG and control groups (81.9 pg./ml and 132.8 pg./ml) respectively and also increased in mean concentration of progesterone hormones (34.8 ng/ml) compares with negative IgG and control groups (7.2 ng/ml and 13.5ng/ml) respectively as shown in Fig. 8. Pregnancy hormones play a role in coordinating immune system at the local of infection and this hormone may play a role for activation of immunity especially activation of B cell.

On the other hand, we noticed a significant increase in the hormones concentrations in patients with IgM positive at the mean concentrations (337pg/ml) for estrogen and (36.3ng/ml) for progesterone compared to

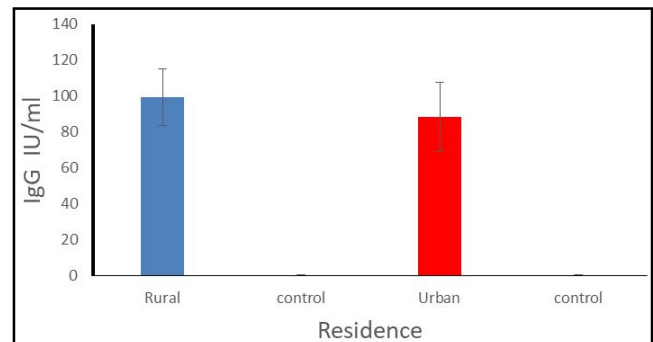


Fig. 6: Mean concentration of IgG in residence groups of abortion patients.

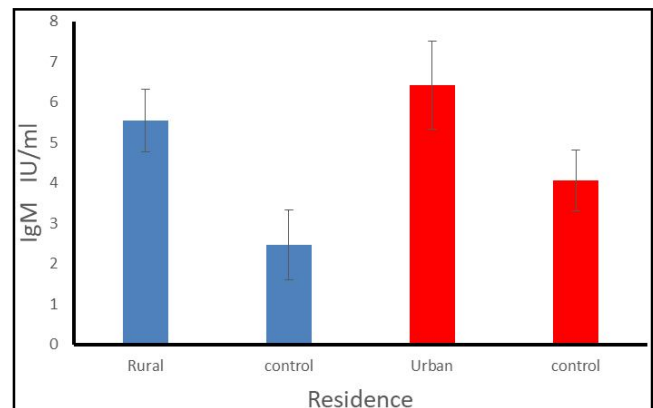


Fig. 7: Mean concentration of IgM in residence groups of abortion patients.

negative patient IgM and control groups at concentrations (282.2pg/ml and 132.8pg/ml) respectively for estrogen and (21.3ng/ml and 13.5ng/ml) respectively for progesterone as it is shown in Fig. 8.

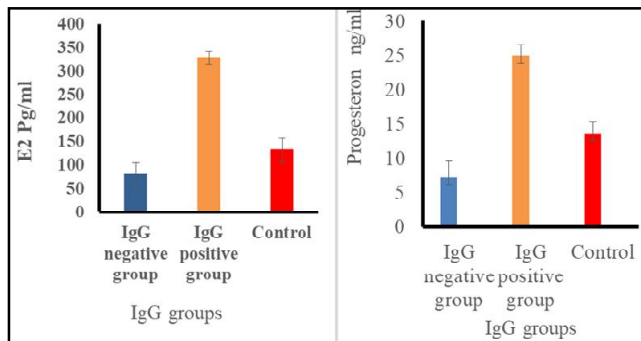


Fig. 8: Mean concentration Estrogen and progesterone hormones of positive and negative IgG antibodies in abortion patients.

Conclusions

It can be concluded from this research that

- 1- The results revealed that the Rubella Virus has a relationship with abortion and can affect pregnant women.
- 2- The detection of IgG and IgM antibody in the aborted women's serum was higher than in pregnant women's control and this increases the possibility of this virus as one of the major viral factors causing miscarriage.
- 3- The percentage of IgG is more than IgM that mean the chronic infection is more effective than acute infection.
- 4- The most common of the IgG antibody was found in women with a one-time miscarriage compared to the number of other abortions and the 21-25 age group is most infected with the virus due to high antibody levels and There is no difference in infection between women who live in the Urban and Rural.
- 5- Increase the levels of Estrogen and progesterone hormones concentration in women with Rubella virus who have miscarriages compared to control group.
- 6- Increase the concentration of IL10 and TNF- α were higher than normal for aborted Rubella-positive women compared to aborted women non-infected with Rubella.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest

Non

Funding: Self-funding

References

- Crooke, S., I. Haralambieva, D. Grill, I. Ovsyannikova, R. Kennedy and G. Poland (2019). Hide details Seroprevalence and durability of rubella virus antibodies in a highly immunized population *Vaccine*, **37(29)**: 27.
- Enders, M., U. Bartelt, F. Knotek, K. Bunn, S. Strobel, K. Dietz and G. Enders (2013). Erformance of the Elecsys Rubella IgG Assay in the Diagnostic Laboratory Setting for Assessment of Immune Status, *Clin Vaccine Immunol.*, **20(3)**: 420-426. doi: 10.1128/CVI.00688-12 PMID: PMC3592356 PMID: 23345585.
- Khudhair, M.K. and R.I. Ahmed (2015). Effect of anti-Rubella (IgG) on a number of abortionin pregnant women of living in Diyala Province. *Journal of Dental and Medical Sciences*, **14**: 60-63.
- Lombardo, P.C. (2011). Dermatological manifestations of rubella. [Accessed March 22 2012]. Available from: <http://emedicine.medscape.com/article/1133108-overview>.
- Morbidity and Mortality weekly Reporte (2001). Control and prevention of Rubella : evaluation and management of suspected outbreak, Rubella infection in pregnant women and surveillance congenital Rubella syndrom *Mob. Morta. Wkly. Rep.*, **50(RR-12)**: 1-23, <http://www.manitoba.ca/health/publichealth/cdc/protocol/rubella.pdf>.
- Morbidity and Mortality weekly Reporte (2010). Progress toward and controll of Rubella and prevention Rubella congenital syndrom worldwide, 2009. *Mob. Morta. Wkly. Rep.*, **59(40)**: 1307-1310.
- Morbidity and Mortality weekly Reporte (2013). Rubella and congenital rubella control and elimentation -globale progress 2000-2012. *Mob. Morta. Wkly. Rep.*, **62(48)**: 983-986.
- Ojata, P., T. Vesikari and O. Elo (1973). Rubella during pregnancy as a cause of congenital hearing loss. *Am. J. Epidemiol.*, **98**: 395-401.
- Raghupathy, R. (1997). Th1-type immunity is incompatible with successful pregnancy. *Immunol Today*, **18**: 478-482. [PubMed] [Google Scholar].
- Smith, J., G. Osikowicz and C. Chem (1993). Abbott AxSYM random and continuous access immunoassay system for improved workflow in the clinical laboratory, DOI: 10.1093/clinchem/39.10.2063 Corpus ID: 161373.
- Szekeres-Bartho, J. and B. Polgar (2010). PIBF: the double edged sword. Pregnancy and tumor. *Am. J. Reprod. Immunol.*, **64**: 77-86. [PubMed] [Google Scholar].