SEROPREVALENCE OF IgM AND IgG ANTIBODIES AGAINST RUBELLA IN PREMARITAL WOMEN

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Abstract

Congenital Rubella is a clinically serious problem, which is due to maternal infection with Rubella virus during the first trimester of pregnancy. It may result in spontaneous abortion or in fetal infection leading to fetal birth defect.

The prevalence of Rubella antibodies and age of exposure to Rubella among premarital women's, the sera samples of 91 premarital women's were collected. All samples were screened for Rubella IgG antibodies using Enzyme linked immunosorbent assay. Off 91 sera, 77 were positive for Rubella IgG. All IgG negative sera were also IgM negative. These people are susceptible to reinfection of Rubella virus until they arte vaccinated .Thus; the individuals were advised to get immunized against Rubella virus.

Key words – Rubella, Young adults, Vaccination, Antibodies, ELIZA.

I. INTRODUCTION

Rubella virus is the only member of the Genus of Rubivirus and belongs to the family Togaviridae whose members commonly have a genome of single -stranded RNA of positive polarity which is enclosed by an icosahedral capsid.

Rubella was first described in the mid- eighteenth century. Friedrich Hoffmann made the first clinical description of Rubella in 1740.Henry Veale, coined the name "Rubella "in 1866.

Rubella is transmitted by the respiratory route. The incubation period is 13 to 20 days, during which a viraemia occurs and virus disseminates throughout the body. In children the onset is abrupt with the appearance of the rash. In adults a prodormal phase may be present with fever and malaise for a day or two before the rash develops.

The rash is typically a maculopapular rash, which first appears on the face and then spreads to the trunk and the limbs. Joint involvement is the commonest complication and occurs upto 60% of adult females. The fingers, wrists knees and ankles are most frequently affected. Thrombocytopenic purpura may occur which may present as Purpuric rash, Epitaxis, Haematuria and GI bleeding.

Virus can be isolated from Nasopharyngeal secretions 7 days before and up to 7 days after the appearance of the rash. Virus in stools and Urine do not play an important role in the transmission of the virus. Viraemia is present for a week before the onset of the rash but as Rubella antibodies develop the Viraemia cases.

Rubella virus enters the fetus during the maternal viraemic phase through the placenta. The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others. Chromosomal aberrations and reduced cell division takes place. The fetus is almost invariably infected if the mother is infected during the first trimester.

After the first trimester, the virus is isolated infrequently from the neonates, probably because fetal immune mechanisms can be activated and infection can be terminated. Following intrauterine infection in early pregnancy the virus persists throughout the gestation and can be isolated from most organs at autopsy. Virus can also recover from nasopharyngeal secretions, urine, stool and CSF from survivors.

The congenital Rubella syndromes are Thrombocytopenia purpura, Hepatoslenomegaly, Hemolytic anaemia, Sensourinal deafness, Mental retardation, Insulin-dependent diabetes, Heart defect (Patent Ductus, VSD, Pulmonary valve stenosis), Eye defect (Retinopathy, Cataract, Microopthalmia, Glaucoma, Severe Myopia), CNS defects

(Microcephaly, Psychomotor retardation) are the permanent congenital Rubella syndrome.

The presence of Rubella specific IgG in an unvaccinated population is a long term marker of previous Rubella infection. The antibodies persists life long and said to protect the individual from Rubella infection.

The percentage of infection in the Fetuses of mother infected by Rubella during the first trimester of pregnancy is greater than 80%. As a result, the target group for the vaccination is women containing the Rubella antigen are to prevent congenital Rubella syndrome. Successful Rubella vaccination policies have been implemented in most western developed countries and high seropositivity rates have been obtained through vaccination.

Serology is the mainstay of diagnosis of Rubella infection. A recent Rubella infection can be diagnosed by (1) detection of Rubella –specific IgM, (2) Rising titers of antibody in HAI and ELISA tests and (3) Seroconversion. It is essential to obtain accurate information relating to the date and time of exposure, the date of onset of illness.

An antibody titer of equal or greater than 15 IU/ml is regarded as being immune to Rubella. It is quite clear that lower levels of antibody, such as 10 IU/ml would probably be protective as well.

The diagnosis of congenitally acquired Rubella is identified by 1. Presence of Rubella IgM in cord blood or Serum samples taken in infancy. 2. Detection of Rubella antibodies at a time when maternal antibodies should have disappeared. 3. Isolation of Rubella virus from infected infants in the first few months of life.

The detection of Rubella-specific IgM in cord blood or infant sera is the method of choice for the diagnosis of congenital Rubella. Specific IgM has been demonstrated in all confirmed cases to the age of 3 months. If the IgM result is negative or equivocal and where has been a history of Rubella in pregnancy, a serum can be taken at 9-12 months to look for the presence of specific IgG.

The detection of specific IgG may be of value where test for IgM have not been conducted in early infancy. Since Rubella is uncommon under the age of 2, IgG detected between 1 and 2 may been indicative of congenital infection. However each case must be assessed individually, taking into account factors such as age, maternal history, and presence of clinical findings.

The women who are not immune to Rubella can be detected by performing ELISA test for the presence of IgG antibodies. IgM antibodies detection using ELISA is done to ensure the recent infection. The presence of IgG antibodies indicates that immune against Rubella infection. By detecting the susceptibility in premarital women, we can prevent the future infection by proper vaccination.

II. MATERIALS AND METHODS

A. Colletion of Blood:

There are two ways of obtaining the blood sample:

1. From the vein
2. From the capillaries.

B. Venous Blood Collection:

Venous blood is obtained by vein puncture. Ideally the patient should be lying down. In the adult one of the veins in the anticubital forsa is chosen. Make the vein prominent by applying a tourniquet or a blood pressure cuff kept at the diastolic pressure.

Select the vein that is both visible and palpable and well fixed to surrounding tissue check all the equipment, the needle should be sharp and then patient and right fitting. The syringe should be dry and with all the air expelled from it and the bottle or the tube to receive the sample must be ready in hand. The patient is reassured the part cleaned with cotton moistures with rectified spirit.

The patient forearm is grasped with left hand and to study the vein, the thumb retracts downward soft tissue below the site of puncture. The needle is brought to the skin over the vein.

If the vein is large and well fixes the skin and the vein may be punctured with the single short thrust. When the blood start flowing into the syringe the tourniquet site is gently pressed with cotton. Collected blood is transferred into a sterile test tube and kept in a slanting position for 30 minutes.

C. Separation of Serum:

In blood, the serum is the component that is neither a blood cell nor a clotting factor.

Serum includes all protein not used in blood clotting and all the electrolytes, antibodies, antigens, hormones and any exogenous substances.

The study of serum is serology and may also include proteomics. Serum is used in numerous diagnostic testing, as well as in blood typing.

The blood sample was centrifuged at 2500rpm for 10 minutes. The supernatant was transformed into a clean dry sterile storage vial. The serum sample is then stored in deep freezer at -20'C.

D. Enzyme Linked Immunosorbent Assay (ELISA):

The Enzyme linked immunosorbent assay has become one of the most widely used serological test for antibody or antigen detection. This test involves the linking of various label enzymes to either antigen or antibodies.

Principle of the Assay:

The Rubella IgG is a quantitative enzyme-linked immunosorbent assay for the detection of specific IgG antibodies to Rubella virus, in human serum.

During the first incubation, only anti-Rubella specific antibodies present in serum bind to the inner surface of the wells coated with the Rubella antigen. After the first incubation the wells are washed to remove non-reactive serum components.

During the second incubation a monoclonal antibody anti-human IgG conjugated with horse raddish peroxidase is added. After a second washing cycle, a substrate – TMB solution is dispensed into the wells in order to detect specific antibodies during a subsequent incubation.

The enzyme reaction is then stopped by adding a stop solution which changes to yellow the blue color developed into the wells. The amount of color is directly proportional to the specific IgG ANTI-Rubella concentration in the patient's samples. The test samples are quantified by use of ELISA reader at 450/620 nm.

E. Rubella IgM– Semiquantitation

PREPARATION OF REAGENT:

Prepare the washing buffer by adding the entire contents of the wash buffer concentrate to 950 ml distilled water in a clean plastic wash bottle. Mix gently to dissolve and store at 2-8'C.

Reconstitute the lyophilized vial with 3 ml of Complex Diluents. It's recommended to add the volume of Complex diluents into the vial, close the cap, wait 15 minutes at room temperature and gently mix in order to avoid foaming. Add 50 %l of Conjugate to the reconstitute vial and gently mix. The complex of Ag-Mab-Conjugate must be prepared 1 hour before being dispensed into wells.

Assay Procedure:

1. The samples were diluted to be assayed with the sample diluents BEIA System.
2. The numbers of strips required for the assay are selected according to the number of samples to be assayed and the calibrators and the pouch was resealed.
3. 100%l of each calibrator and the diluted samples were dispensed into the selected wells.
4. The plates were incubated at Room temperature (18-25'C) for 60 minutes.
5. The washing cycles were performed by washing the wells three times with the wash buffer.
6. 100%l of Complex Ag-Mab-Conjugate were dispensed into each well.
7. The plates were incubated at room temperature (18-25'C) for 60 minutes.
8. The washing cycles were performed by washing the wells three times with the wash buffer.
9. 100%l of Substrate-TMB were dispensed into each well.
10. The plates were incubated at room temperature (18-25'C) for 30 minutes.
11. 100%l of stop solution were dispensed into each well to stop the chromogenic reaction.
12. The optical density (O.D) in bi-chromatism at 450/620nm was read in ELISA reader.

D. Rubella IgG-Semiquantitation

PREPARATION OF REAGENT:

Prepare the washing buffer by adding the entire contents of the wash buffer concentrate to 950 ml distilled water in a clean plastic wash bottle. Mix gently to dissolve and store at 2-8'C.

ASSAY PROCEDURE:

1. The samples were diluted to be assayed with the sample diluent BEIA system.
2. The number of strips required for the assay is selected according to the number of samples to be assayed and the calibrators and the pouch was released.
3. 100%l of each calibrator and the diluted samples were dispensed into the selected wells.
4. The plates were incubated at room temperature for 30 minutes.
5. The washing cycles were performed by washing the wells three times with the wash buffer.
6. 100%l of Conjugate were dispensed into each well.
7. The plates were incubated at room temperature for 30 minutes.
8. The washing cycles were performed by washing the wells three times with the wash buffer.
9. 100%l of Substrate –TMB were dispensed into each well.
10. The plates were incubated at room temperature for 15 minutes.
11. 100%l of stock solution were dispensed into each well to stop the chromogenic reaction.
12. Optical density of Bichromatism at 450/560nm was read in ELISA reader.

III. RESULT AND DISCUSSION

91 samples were collected from unmarried women age between 18-23 years. The IgM and IgG antibody were read at 450/620nm.

RUBELLA SPECIFIC IgM ANTIBODY DETECTION BY ELISA

An antibody titer greater than 11.5 AU/ml is an indicative of acute Rubella infection. A negative result less than 8.5AU/ml indicates probably excludes a recent Rubella.

Of 91 samples tested for IgM antibody (refer Fig:1), 42 samples found to have less than 8.5 AU/ml which indicates the presence of Recent Rubella infection of the individuals.

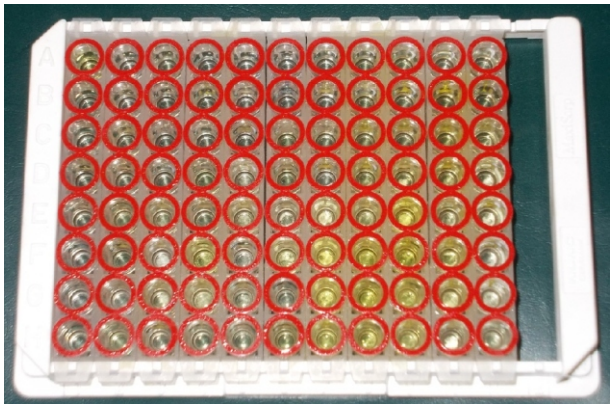

Fig. 1

KEY:
A1,B1,C1,D1,E1 – Calibrators 0,1,2,3,4
F1 to H11 -- Samples

RUBELLA SPECIFIC IgG ANTIBODY QUANTITATION BY ELISA

An antibody titer greater than 15 IU/ml is generally considered to be protective against reinfection and antibody titer less than 8IU/ml indicates probably no protective against Rubella infection.

Of 91 samples tested, 77 individuals had an IgG antibody titer greater than 15 IU/ml and are considered to be protective against reinfection. 14 samples have IgG antibody titer than 8 IU/ml are considered to be seronegative. These people are susceptible to reinfection of Rubella virus until they are vaccinated.

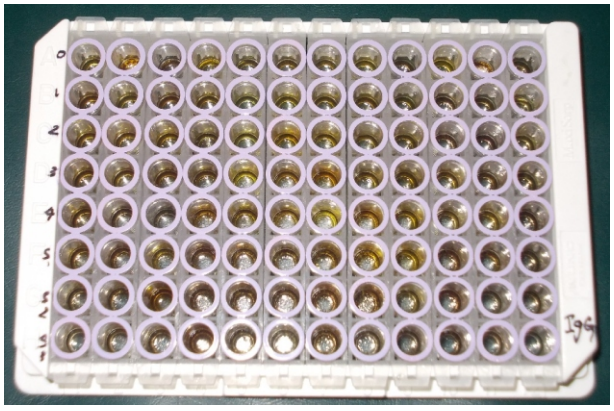

Fig. 2

KEY:

A1,B1,C1,D1,E1 - Calibrators0,1,2,3,4.

F1 to H12 - Samples

Rubella serosurveys are useful particularly to determine the proportion of women of childbearing age who are susceptible to Rubella. Regarding Rubella susceptibility in women, a recent review of 45 low-income countries prior to introduction of Rubella vaccine reported proportions of susceptible women of 10% in 13 countries, 10-24% in 20 countries and 25% in 12 countries (Cutts et al ., 1997).

Serosurveillance of women of childbearing age should be continued and immunization policy needs to be developed for adolescent girls and women of childbearing age group before conception to control Congenital Rubella syndrome. Vaccination of childbearing age can achieve the goal of reduction to complete prevention of Congenial Rubella Syndrome.

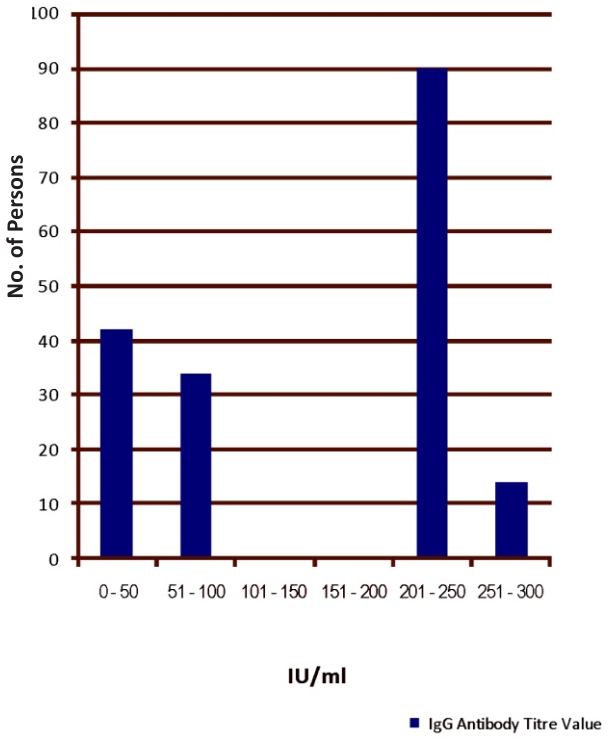

Fig. 3 IgG Antibody Titer Value

IV. CONCLUSION

Rubella is essentially a benign infection of childhood. However if there is an occurrence during the first month of Pregnancy, it can be serious threat to the fetus. Therefore, an accurate diagnosis of the infection is essential. Women for IgG and IgM seroprevalence of childbearing age are susceptible to Rubella may lead to increased cause of Congenital Rubella Syndrome in future. Therefore, Seroprevalence of IgM and IgG antibodies against Rubella must studied and proper vaccination programs must be introduced to enhance the immune status of the susceptible person.

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