Sero-Detection of Rubella Antibodies among Women with Spontaneous Recurrent Miscarriage in Gezira State (Sudan): Case Control Study

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Abstract

Background: Recurrent miscarriage is a critical problem and it is increasing during the current decade. Rubella virus is associated with recurrent miscarriage cases according to previous reports previously published in many countries.

Objective: To sero-detect rubella virus antibodies among women with spontaneous recurrent miscarriage in Gezira State (Sudan).

Materials and methods: In this analytic, case control study, 45 women presenting with spontaneous recurrent miscarriage (test group) attending Wad Madani Teaching Hospital, Al Gezira State (Sudan), and another 45 healthy pregnant women (control group) were investigated. Rubella IgG and IgM antibodies were analyzed in sera collected from all participants using ELISA and VIDAS techniques.

Results: By ELISA technique, 3 rubella IgM (6.7%) cases were found positive among the test group patients and by VIDAS technique, one IgM rubella (2.2%) case was found positive among the control group participants. Also, by ELISA technique, 41rubella IgG (91.1%) cases were found positive among the test group patients, and by VIDAS technique, 38 rubella IgG (84.4%) cases were found positive among the test group patients. Also, by ELISA technique, 43 rubella IgG (95.6%) cases were found positive among the control group, and by VIDAS technique, 32 rubella IgG (71.1%) cases were found positive among the control group patients.

Conclusion: There was an association of sero-positive rubella IgG with spontaneous recurrent miscarriage in Gezira State (Sudan).

Key words: Rubella antibodies, Spontaneous recurrent miscarriage, ELISA, VIDAS.

Introduction

Miscarriage, also known as "spontaneous abortion" and "pregnancy loss", is the natural death of an embryo or fetus before it is able to survive independently. Some use the cutoff of 20 weeks of gestation after which fetal death is known as a stillbirth. The most common symptoms of a miscarriage are vaginal bleeding with or without pain. About 80% of miscarriages occur in the...
first 12 weeks of pregnancy (the first trimester), and the underlying cause in about half of cases involves chromosomal abnormalities. Recurrent miscarriage is a critical problem in which many factors play a crucial role such as anti phospholipid antibodies (APA) and anti cardiolipin antibodies (ACA). Recent studies suggested celiac disease as a possible cause of recurrent miscarriage (RM). A number of infections had been linked to recurrent miscarriage such as Rubella virus, Toxoplasma, Cytomegalovirus, etc. It affects about 5-15% of all pregnancies worldwide.

Rubella virus, a member of the *Togaviridae* family is the sole member of the genus Rubella virus. It has an envelope and a single stranded-ribonucleic acid genome. Infection with Rubella virus during pregnancy, especially during the first trimester, can result in congenital rubella syndrome (CRS). The burden of rubella infection in most developing countries is however not well documented because of limited epidemiological data. The symptoms of rubella infection include: skin rash, low-grade fever, arthralgia, and lymphadenopathy. In most cases, the disease is self-limited and rarely causes complications. Complications of CRS may include: miscarriage and severe congenital abnormalities of the fetus such as: cataracts, retinopathy, heart defects, neurological deficits, and deafness.

Rubella is mild disease that typically occurs in childhood. The risks of congenital infection and defects depend on the gestation age of infection. Immunity to Rubella virus (RV) is commonly determined by measuring the specific immunoglobulin G (RV IgG). However, RV IgG results and their interpretation may vary depending on the immunoassay, even though most commercial immunoassay’s (CIAs) have been calibrated against an international unit per milliliter. Selective or universal vaccination programs adopted by some countries led to a tremendous improvement in the control of congenital rubella in the ensuing 50 years, including elimination in the Americas. The target of one case of congenital rubella syndrome per 100,000 live births by 2015 had to be renewed by the World Health Organization (WHO) regional office for Europe. The Global Measles and Rubella Strategic plan for 2012-2020 aims to eliminate measles and rubella in at least five WHO regions by the end of 2020.

In developed countries, women at the childbearing age are routinely screened for rubella antibodies to identify and vaccinate susceptible candidates. In a cross-sectional sero-survey of rubella IgG antibody among pregnant women attending the antenatal clinic of Ladoke Akintola University of Technology Teaching Hospital (Osogbo, Nigeria), 200 samples were evaluated for the rubella immunoglobulin G antibody. In this study, 175 participants (87.5%) were found positive and 25 participants (12.5%) were found negative. This finding indicated a prevalence rate of 85.7% in 15-19-year age group, 86.8% in 20-24-year age group, 89.6% in 25-29-year age group, and 100% in greater than 40-year age group. As the immunity gap in the studied population was high, rubella vaccination should be conducted on all women of the childbearing age and their children.

The sero-prevalence rate of rubella among pregnant women in Khartoum State (Sudan) was studied. A total of 500 pregnant women who visited 7 antenatal clinics from November 2008 to March 2009 were examined for the presence of rubella IgG antibodies using ELISA, rubella IgG antibodies were detected in 95.1% of women.

In this study the immunity gap was high, and there were no Rubella vaccines applied to the

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studied population. The other gap was the poor of routine screen for Rubella, CMV, and HSV for the pregnant women, and the investigating association between the infections and preeclampsia.

**Materials and methods**

This was a case- control study conducted at Wad Madani Teaching Hospital (Department of Obstetrics and gynecology), Al-Gezira State (Sudan), during the period from July-October 2018. The study was approved by the Ethics Committee of Wad Madani Teaching Hospital (Wad Madani, Sudan). Permission to collect the specimens was taken from authorities of Wad Madani Teaching Hospital. Informed consent was obtained from each patient for the purposes of the current study. The specimens and information collected from all participants had not been used for any purpose other than this study.

Data were collected through an interview using a self administered questionnaire. 45 women presenting with spontaneous recurrent miscarriage (test group) attending Wad Madani Teaching Hospital, Al Gezira State (Sudan), and another 45 healthy pregnant women (control group) were investigated. 90 women were investigated in the two groups to obtain an over 80% power to detect a difference of 5% at \( p = 0.05 \). It was assumed that 10% of the women might have incomplete data.

A volume of 5 ml blood specimens were collected from each patient through venipuncture technique, displaced into plain containers, allowed to clot, centrifuged, and then stored at -20°C until serological analysis is performed at the Central Research Laboratory.

Complete blood counts were conducted using the hematological analyzer Sysmex-XP 300. The three physical technology means used were direct current impedance, advanced optical light scatter technology, and flour-scent flow cytometry and spectrophotometry. These technology means were used in combination with chemical reagents that lyse or alter blood cell composition to extend the measurable parameters to a wide range of tests.

Biomass index was measured. ABO blood groups and Rhesus typing were determined for all participant women by the direct slide method. This was performed by using clean, dry slides and adding anti A, anti B, and anti D antibodies separately to the blood sample. If there is agglutination, the test is positive.

The blood specimens were also analyzed for detection of rubella IgG and IgM antibodies by the commercially enzyme-linked immunosorbent assay technique, using the kit of Chemux Bioscience Company (USA) following instructions of manufacturer. The reagents used have positive and negative controls. Positive results were read by a cut-off of rubella index more than 1.0 IU/ml. Negative results were read by a cut-off of rubella index less than 1.0 IU/ml.

The blood specimens were also analyzed for detection of rubella IgG and IgM antibodies by the VIDAS assay technique. The mini VIDAS instrument, which is a compact automated immunoassay system based on the enzyme linked fluorescent assay (ELFA) technique.

The reagent strips consists of 10 wells covered with a labeled antigen. The foil seal the label comprises a bar code which mainly indicate the assay code, kit lot number, and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample; and the last well of each strip is a cuvette in which the fluorometric reading is shown. The wells in the center of the strip contain the various reagents required for the assay. Assay reagents were dispensed in the
sealed reagent strips. All of the assay steps were performed automatically by the instrument. The collected data were analyzed using the Statistical Package for Social Science (SPSS) program and double checked prior to analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated for rubella sero-positive groups. Univariate and multivariate analyses were used for rubella IgG and IgM sero-positive groups as dependent variables, and socio-demographic and obstetrics data as independent variables. Odds ratio or with 95% confidence interval was calculated and statistical significance was defined as p-value < 0.05.

**Results**

Forty five women were enrolled in this study. Sero detection of rubella IgG and IgM antibodies were conducted by two comparative techniques: ELISA and VIDAS techniques. By ELISA technique, 3 rubella IgM (6.7%) cases were found positive among the test group patients and by VIDAS technique, one IgM rubella (2.2%) case was found positive among the control group participants. Also, by ELISA technique, 41 rubella IgG (91.1%) cases were found positive among the test group patients, and by VIDAS technique, 38 rubella IgG (84.4%) cases were found positive among the test group patients. Also, by ELISA technique, 43 rubella IgG (95.6%) cases were found positive among the control group, and by VIDAS technique, 32 rubella IgG (71.1%) cases were found positive among the control group patients participants (Table 1).

**Table (1): Detection of rubella IgM and IgG antibodies using ELISA and VIDAS techniques**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No. tested</th>
<th>Positive IgM</th>
<th>Positive IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ELISA</td>
<td>VIDAS</td>
</tr>
<tr>
<td>Test group</td>
<td>45</td>
<td>3 (6.7%)</td>
<td>1 (2.2%)</td>
</tr>
<tr>
<td>Control group</td>
<td>45</td>
<td>3 (6.7%)</td>
<td>2 (4.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>6 (6.7%)</td>
<td>3 (3.3%)</td>
</tr>
</tbody>
</table>

Socio-demographical and clinical characteristics of test group patients and control group participants studied were: age, biomass index, RBCs, Hb, TWBCs, platelets, PCV, MCV, MCH, MCHC, MPV, PCT, RDWCV, RWDSD, neutrophils, lymphocytes, monocytes, eosinophils, basophils, rubella IgG antibody, and rubella IgM antibody. Significant correlation with miscarriage was found with the following socio-demographical and clinical characteristics: age (p = 0.0003), biomass index (p = 0.0104), MCV (p = 0.0001), MCHC (p = 0.0125), MPV (p = 0.0006), RDWCV (p = 0.0044), and RWDSD (p = 0.0001).

On performing miscarriage univariate and multivariate analyses, the predictor factors exhibited were preeclampsia, microcytic hypo-chromic anemia, vaginal bleeding, sero-positivity of anti-rubella IgG (by VIDAS technique), menstruation cycle, and biomass index. By the univariate analysis, significant correlation with miscarriage was found in the following predictor factors: diabetes mellitus (p = 0.000), age (p = 0.001), family history (p = 0.000), vaginal bleeding (p = 0.001), microcytic hypochromic anemia (p = 0.04), sero-positivity of rubella IgG antibody (p = 0.000), thyroid disease (p = 0.000), and preeclampsia (p = 0.01).

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By the multivariate analysis, significant correlation with miscarriage was found in the menstruation cycle predictor factors (p = 0.028).

**Discussion**

In this study, a high prevalence rate of rubella IgG antibody sero-positivity in miscarriage cases on using the two techniques: ELISA (91.1%) and VIDAS (84.4%). While there was a low prevalence rate of rubella IgM antibody sero-positivity in miscarriage cases on using the two techniques: ELISA (6.7%) and VIDAS (2.2%). The data interpretation of Bland-Altman assay showed 0.760143 Bias and SD of Bias 0.758002 with 95% limits of agreement from 0.725541 to -2.24583 for IgM antibody and for IgG antibody 0.149598 Bias and SD of Bias 0.286381 with 95% limits of agreement from 0.411710 to -0.710905.

200 samples were investigated for rubella IgG antibody among pregnant women attending the antenatal clinic of Ladoke Akintola at University of Technology Teaching Hospital (Oshogbo, Nigeria). The study found 175 pregnant women (87.5%) positive and 25 pregnant women (12.5%) negative.

Another study was conducted to study the sero-prevalence rate of rubella infection among pregnant women in Khartoum State (Sudan). The study investigated 500 pregnant women visiting antenatal clinics from November 2008 to March 2009 for the presence of rubella IgG antibodies using ELISA, and rubella IgG antibody was detected in 95.1% (95% CI: 93.2% - 97.0%) of women.

Likewise Schwartztenburg and colleagues reported that a significantly higher prevalence rate of preeclampsia (50%) was detected in women with rubella non-immune patients compared with low rate of preeclampsia among immune women (3.5%).

Also, a case-control study was conducted to investigate the association of rubella, toxoplasma and cytomegalovirus infection. With recurrent miscarriages in Bonb-Iran, blood samples from 100 women with recurrent miscarriage and 100 healthy women aged 20-35 years were taken and their sera were tested for the antibodies of rubella, CMV and toxoplasma. A total of 29 cases and 11 controls were found positive for rubella IgG antibody.

In another report from Iran, the sero-prevalence rate of rubella virus in women with spontaneous abortion showed that there were significant differences in the sero-prevalence rate of anti-rubella IgG and IgM in the test group than in the control group.

In the current study the predictors for miscarriage were preeclampsia, and microcytic hypochromic anemia. Sero-positivity of anti-rubella IgG had a high risk for miscarriage as per univariate and multivariate factor significant effects. While women with thyroid disease, diabetes mellitus, vaginal bleeding, menstruation cycle, and family history have a high risk for miscarriage as per univariate analysis.

In this study, there is an association with miscarriage with age (p = 0.0003), biomass index (p = 0.0104), PCV (p = 0.098), MCV (p = 0.0001), MPV (p = 0.0006), RDW CV (p = 0.0044), and RDW-SD (p = 0.0001). Rubella vaccine is recommended for childbearing age of women.

**Conclusion:** There was an association of sero-positive rubella IgG with spontaneous recurrent miscarriage in Gezira State (Sudan).

**Competing interests:** Authors declare that no competing interests exist.

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