Sero-screening for Toxoplasma, Rubella, Herpes simplex, and Cytomegalovirus (TORCH) Infections in Sudanese Pregnant Women

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Abstract

Background: Infections acquired in utero or during the birth process are a significant cause of fetal and neonatal mortality and an important contributor to early and later childhood morbidity. Careful hygiene is the best prevention tool against these infections. Intrauterine transmission from transplacental or ascending infection may occur as evidenced by the presence of early neonatal infection despite delivery by cesarean means before labor and rupture of fetal membranes.

Objective: To sero-screen for Toxoplasma, Rubella, Herpes simplex, and Cytomegalovirus (TORCH) infections in Sudanese pregnant women.

Materials and methods: The study analyzed qualitatively the frequency rate of TORCH infections among 71 pregnant women attending the Maternity Department at Khartoum North Teaching Hospital, during the period from February to June, 2012. Serum samples were collected and investigated for the presence of TORCH IgM antibodies using the enzyme-linked immunosorbent assay (ELISA) technique.

Results: Five patients (7.0%) were found TORCH positive, and 14 patients (19.7%) had a history of contact with animal pets. Also 39 patients (54.9%) had a past history of abortion; and 26 patients (36.6%) were in the first trimester of pregnancy.

Conclusion: The frequency rate of TORCH antibodies was higher among aborting pregnant women due not only to their contact with animal pets but also due to their contact with fertile soil especially in rural areas.

Key words: TORCH IgM antibodies, ELISA technique, Sudanese pregnant women

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Introduction

The original concept of the TORCH perinatal infections was to group four infections with similar presentations, including rash and ocular findings. These are four infections: Toxoplasma, Rubella, Herpes simplex virus (HSV), and Cytomegalovirus (CMV). This acronym is well recognized in the field of neonatal/perinatal medicine. However, there are other well-described causes of in utero infection, including enteroviruses, varicella zoster virus, and parvovirus. Routine screening of pregnant women at the first prenatal visit for TORCH titers is commonplace in many parts of the world. However, the value of this testing has been questioned in numerous quarters. Recognition of in utero and perinatal infection is critical, but the validity of the TORCH designation and widespread screening for these conditions has been questioned. Alternatives proposed have been to broaden the TORCH designation to include more pathogens in the "other" category or to screen infants with suspected congenital infections for a wider range of organisms.

Perinatal infections account for 2% to 3% of all congenital anomalies. Most of the TORCH infections cause mild maternal morbidity, but have serious fetal consequences, and treatment of maternal infection frequently has no impact on fetal outcome. Therefore, recognition of maternal disease and fetal monitoring once disease is recognized are important for all clinicians. Knowledge of these diseases will help the clinician appropriately counsel mothers on preventive measures to avoid these infections, and will aid in counseling parents on the potential for adverse fetal outcomes when these infections are present.

The primary infection of TORCH remains a major problem in pregnant women today. Pregnancy loss has been attributed to several factors involved in human reproduction. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, infectious agents, environmental pollutants, psychogenetic factors and endometriosis are most important causes of spontaneous abortion.

Spontaneous abortion has social and economic impact. After the age of 30-35 years, potential fertility declines and the rate of spontaneous abortion increases. But on the other hand, teenage pregnancy is a fairly common occurrence in countries like Sudan. Some maternal infections, especially during the early gestation, can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the insemination of infectious organisms to various tissues. The fetus and/or neonate are infected predominantly by viral but also by bacterial and protozoal pathogens. Infections with various pathogens cause miscarriage or may lead to entail anomalies in the fetus. The incidence of maternal infection during pregnancy ranges from 1 to 8 per 1000 susceptible pregnancies, with the highest reported rates in France. The risk of transmitting infection to the fetus increases steeply with the gestational age at seroconversion.

This study was conducted among pregnancy cases to assess the role of TORCH pathogens (T. gondii, Rubella, Cytomegalovirus and H. simplex) infections in pregnancy related complications, especially miscarriage. Recognizing the prevalence infections with intrauterine and other pathogens in both mother and fetus is an important part of prenatal care. There is no published data concerning TORCH seroprevalence in pregnant women in Sudan. The basic data concerning TORCH infections during pregnancy is important for health planners and care providers.

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Thus, the aim of the current study was to investigate the associated possible risk factors for TORCH infections among pregnant women in Sudan.

Materials and methods

This was a qualitative, descriptive, facility base, case study, conducted during the period from February to June, 2012, at the Maternity Department of Khartoum North Teaching Hospital (Sudan). The population investigated was pregnant women attending this hospital. Approval to conduct the study was taken from Al Neelain University (Khartoum, Sudan). Permission to collect the specimens was granted by the authorities of Khartoum North Teaching Hospital (Sudan). Verbal consent was obtained from all patients of the study. Positive and negative results were handed to all patients included in this study. Treatment was given by hospital consultants to all positive patients. The collected data was analyzed using master sheets and the SPSS software program. Pre-testing (pilot study) was performed by evaluation of the laboratory procedures, using a small number of true positives, true negatives, and controls. Validity of all reagents, kits and equipment were pre-tested to check their validity.

The sampling technique adopted in this study was a non-probability, convenience sampling type. Sample size was 71 specimens collected from pregnant women. Data was collected as per a structural interview questionnaire specially designed to collect and maintain all information obtained from each patient examined.

The patients’ demographic data and medical history were collected from each patient: age, gravida, parity, previous miscarriages, and pregnancy. Serum samples were collected and stored in small screw caped vials at –20°C until serological analysis was performed. Samples were investigated for the presence of TORCH IgM antibodies that include antibodies of T. gondii, Rubella virus, Cytomegalovirus and H. simplex virus using ELISA kits of Equipar Diagnostics (Italy). The tests were done as per the directions supplied along with the kits. The results were read at 450 nm in an ELISA reader (DiaPro.Diagnostic, Milano, Italy). The IgM levels were interpreted as negative, low positive or high positive. A positive IgM is an indication of recent infection.

Principle of the test is based on “1gM capture” where 1gM class antibodies in the sample are first captured by the solid phase coated with goat anti 1gM antibody. After washing out all the other component of the sample and in particular 1gM antibodies in the second incubation bound anti TORCH are detected by the addition of a complex composed of TORCH antigens and TORCH conjugate labeled with peroxidase. This is washed to remove unbound conjugate, a substrate is added, and optical density is read.

The procedure passes over the following steps:
1) The required number of micro-wells was placed in the micro well holder. The first well was left empty for the operation of blanking.
2) 200 µl of negative control and positive control were dispensed in proper wells which were pre-diluted and made ready to use.
3) 200 µl of sample diluent (DiL SPE) was added to all the sample wells, then 10 µl sample was dispensed to each well. The plate was mixed gently.
4) The micro-plate was incubated for 45 min. at 37°C.
5) The micro-plate was washed with an automatic washer and diluted 350 µl/ well was aspirated.

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6) 100 µl of immune complex was pipetted into each well except the first blank well, and plate was sealed. The micro-plate was incubated for 45 min at 37°C.
7) The micro-wells were washed. 100 µl chromogen/substrate mixture was pipetted into each well including the blank well. Then the micro-plate was incubated at room temperature (18-24°C) for 15 minutes.
8) 100 µl sulphuric acid was pipetted into all wells to stop the enzymatic reaction. This changes positive control and positive samples from blue to yellow.
9) The color intensity of dilution was read using the 450 nm filter.

Results

A total number of 71 serum specimens were included in this study. The age range of patients studied was 16-43 years. Most of the patients investigated (25/35.2%) were in the age range 23-29 years; and the least investigated (3/4.2%) were in the age range 37-43 years. From the patients examined, 5 patients (7.0%) were found TORCH positive, and 66 patients (93%) were found TORCH negative. Also 14 patients (19.7%) had a history of contact with animal pets; and 39 patients (54.9%) had a past history of abortion. On the other hand, 15 patients (21.1%) had a past history of fever during pregnancy. As regard infection during pregnancy, 32 patients (54.1%) were in the third trimester, and 26 patients (36.6%) were in the first trimester (Table I).

Table (I) Distribution of patients investigated according to contact with animal pets, past history of abortion, past history of fever, and trimester of pregnancy

<table>
<thead>
<tr>
<th>Criteria</th>
<th>No. investigated</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with animal pets</td>
<td>14</td>
<td>19.7</td>
</tr>
<tr>
<td>Past history of abortion</td>
<td>39</td>
<td>54.9</td>
</tr>
<tr>
<td>Past history of fever during pregnancy</td>
<td>15</td>
<td>21.1</td>
</tr>
<tr>
<td>First trimester pregnancy</td>
<td>26</td>
<td>36.6</td>
</tr>
<tr>
<td>Second trimester pregnancy</td>
<td>13</td>
<td>18.3</td>
</tr>
<tr>
<td>Third trimester pregnancy</td>
<td>32</td>
<td>45.1</td>
</tr>
</tbody>
</table>

Discussion:

The primary infection of *Toxoplasma gondii, Rubella virus, Cytomegalovirus, or Herpes simplex* virus (abbreviated as TORCH) remains a major problem in pregnant women in Sudan. The teenage pregnancy rates reported from various parts of the world ranged from 8-14%\(^5\). In this study the teenage pregnancy rate was 29.6 %.
TORCH infections observed by some workers was 20% among pregnant ladies \(^6\). Also the infective rate of TORCH reported elsewhere was 17.2% \(^7\). As per our study the infective rate of TORCH was 7%.
The incidence of abortion among the pregnant patients investigated in the current study was 54.9% (Table I). This rate was higher than the 5.5% abortion rate observed by Rodier and his colleagues \(^8\).

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The frequency rate of patients in contact with animal pets in this study was 19.7% (Table I). The high rate of TORCH infections may depend on the environment and life style of the people infected. The frequency rate of TORCH antibodies was found to be higher among aborters due to contact with soil, regardless of whether cats are kept as pets or not. Bhalerao and his co-workers reported a high infectivity rate of TORCH agents among women in the first trimester stage of pregnancy. In the present context, 26 pregnant patients (36.6%) were in the first trimester (Table I).

From this study it may be recommended that epidemiological surveys should be carried out to determine the magnitude and distribution of TORCH infections among pregnant ladies. It is also essential to conduct health educational programmes to make families aware of the modes of transmission, prevention and control of TORCH infections.

Conclusion: This study had pointed out to the negative effects of TORCH pathogens on the success of gestation among pregnant women. The frequency rate of TORCH antibodies was higher among aborting pregnant women due not only to their contact with animal pets but also due to their contact with fertile soil especially in rural areas.

References


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