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Association between TORCH infection and lupus anticoagulant antibody in pregnant women with recurrent abortion

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ABSTRACT

Abortion is a medical condition that describes pregnancy loss due to various causes including TORCH infections (toxoplasmosis, others (syphilis and hepatitis B), rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) infection). TORCH infections may pass to the fetus that has low immunity to fight the disease, besides; the complications are much higher during the early stages of pregnancy which may involve abortion. Therefore, investigating the possible factors that could be associated with TORCH infection including the clotting factor lupus anticoagulant antibody is vital. In this study, TORCH antibodies in 33 pregnant women were assessed and then lupus anticoagulant antibodies were tested using Diagnostica Stago STart 4 Hemostasis Analyzer. The serum concentration of immunoglobulin M (IgM) and immunoglobulin G (IgG) for toxoplasma, rubella and CMV were quantitatively determined with Cobas e411. The serum concentration of IgG for rubella and CMV increased in the blood were 90.9% and 66.6% of pregnant women respectively, while toxoplasma results showed an increasing level of IgG in 24.4% of patients. IgM levels for toxoplasma, rubella and CMV are elevated in 6.1%, 3.03% and 24.2% of patients respectively. Lupus anticoagulant antibody is increased in the blood of 72.7% of pregnant women. It was concluded that only rubella and CMV infections cause the increase of lupus anticoagulant antibodies in the tested pregnant women, not toxoplasmosis.

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Introduction

TORCH co-infection is the major cause of pregnancy loss in women and preventing TORCH infection is an important target in reducing the incidence of fetal abnormalities and abortion worldwide (1, 2). This group of microorganisms affects women and is transmitted to the fetus through the placenta producing congenital malformations, intrauterine growth restriction or even fetal death (3-5). Birth defects and fetal abnormalities are mainly due to the less developed immune system of the fetus which cannot resist and retarding pathogenic microorganisms such as TORCH (2, 6). Thus TORCH infection which is composed of toxoplasmosis, others (syphilis and hepatitis B), rubella, and cytomegalovirus is effective in the first trimester or early stage of pregnancy and may lead to fetal loss (6-8). While fetus abnormalities due to TORCH infection may appear in the late stage or even after birth (4).

Pregnancy loss due to TORCH infection requires more investigations as these pathogens may have adverse effects on the fetus through other factors than the role of the immune system which has been reported in several studies. It is well documented that recurrent abortion and fetal death are significantly associated with autoimmune diseases including phospholipid antibodies (APAs) such as lupus anticoagulant (LA) and anticardiolipin antibodies (ACAs) (9). Among these antibodies, LA appears to be more related to arterial or venous thrombosis and pregnancy loss in women with recurrent fetal loss (10). It is not clear whether the increased level of LA reported in pregnant women is due to TORCH infection or other undetermined factors. Therefore, this study aims to investigate the relationship between TORCH co-infection and the level of LA in pregnant women with recurrent fetal loss.

Materials and Methods

Patients

Blood samples were obtained from 33 pregnant women aged 21 or over. Patient details are shown in Table 1.

Sample preparation

Five millilitres of blood were collected from each pregnant woman and then transferred into a gel containing and sodium citrate tubes for separating serum and plasma. Samples were allowed to clot at room temperature in the gel tubes and then they were centrifuged for 5 minutes at 4000 rpm to prepare serum which was later used to determine the blood concentration of IgG and IgM antibodies for toxoplasma, rubella and CMV following the instructions of the fully automated ElectroChemiLuminescence (ECL) analyzer (Cobas-e411). Results below 1.0 COI was considered negative for CMV IgM and IgG antibody, and above 1.0 COI was considered positive for these antibodies (Table 3). Toxoplasma and rubella IgG and IgM results were also negative below 1.0 IU/mL and positive above this number and unit (Table 3).

Samples in the sodium citrate tubes of a ratio of 9:1 were centrifuged for 15 minutes at 3000 rpm to prepare plasma. Separated plasma samples were then transferred into clean plastic tubes and re-centrifuged for another 15 minutes at the same speed to obtain platelet-poor plasma.

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Table 1. Characteristics of Pregnant Women.

Patient Characteristics	Number	Percentage
Age (Years)		
21-25	16	48.4 %
25-28	10	30.3%
>28	7	21.2 %
Previous history of abortion		
One	21	63.6 %
Tow	9	27.2 %
Three	3	9.1 %
Time of abortion		
1st Trimester	19	57.5 %
2nd Trimester	8	24.2 %
3rd Trimester	6	18.1 %
Vaccination		
CMV	No	
Rubella	No	
Toxoplasma	No	

Prepared plasma samples were subjected to a lupus-sensitive activated partial thromboplastin time (aPTT-LA) analysis according to the manufactures instructions. A hundred microliter (µL) of plasma was mixed in a clean cuvette with 100 µL of PTT-LA and incubated at 37 °C for 5 minutes. Then 100 µL of CaCl2 (0.025 M) was added to the mixture and mixed thoroughly then lupus anticoagulant was determined using Diagnostica Stago STart 4 Hemostasis Analyzer. Results in the range of 31 to 43 seconds were considered negative for lupus antibody, while Stago results above 43 were considered positive (Table 3).

Results

The association between TORCH infection and lupus anticoagulants in the blood of pregnant women was studied. Out of 33 pregnant women involved in this study, 22 (66.6%) of them showed a positive increase in IgG for CMV in their blood, whereas only 8 (24.2 %) of them were positive for anti-CMV IgM. This was true for anti-rubella IgG as 30 (90.9 %) out of 33 pregnant women showed a high concentration of this antibody and only 3 participants (9.09 %) were positive for anti-rubella IgM antibody. The results of toxoplasmosis were different as a low number of pregnant women 8 (24.4 %) showed a positive test for IgG antibody and only 2 (6.06%) of them had a high concentration of IgM for toxoplasma in their blood (Table 2, 3 and Figure 1).

To determine the presence of blood clots, plasma was

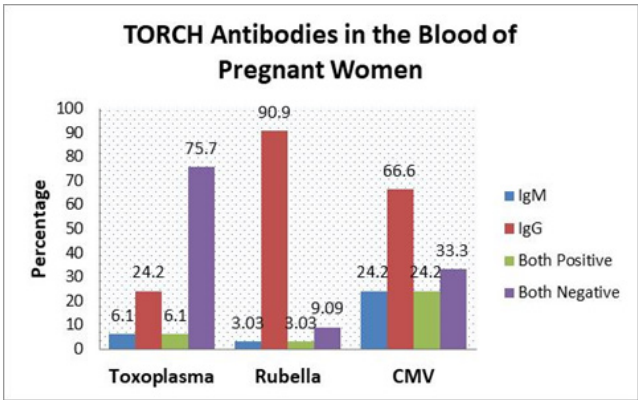

Figure 1. Serum concentration of IgM and IgG antibody for TORCH in pregnant women.

Table 2. Percentage of TORCH Infection in Pregnant Women.

Variable	Result	Patients (n=33) No (%)
CMV IgG	Positive	22 (66.6)
	Negative	11 (33.3)
CMV IgM	Positive	8 (24.2)
	Negative	25 (75.7)
Rubella IgG	Positive	30 (90.9)
	Negative	3 (9.1)
Rubella IgM	Positive	1 (3.03)
	Negative	32 (96.96)
Toxoplasma gondii IgG	Positive	8 (24.2)
	Negative	25 (75.7)
Toxoplasma gondii IgM	Positive	2 (6.1)
	Negative	31 (93.9)

Table 3. The serum concentration of IgM and IgG for CMV, Rubella, Toxoplasma, and Lupus Antibodies in the blood of pregnant women.

No.	Toxoplasma IgM IU/mL	Toxoplasma IgG IU/mL	Rubella IgM IU/mL	Rubella IgG IU/mL	CMV IgM COI	CMV IgG COI	Lupus Seconds
Case #1	0.512	0.473	0.347	312.99	0.311	0.422	31.2
Case #2	0.318	0.555	0.411	483.95	0.395	231.78	38.6
Case #3	0.503	0.479	0.361	485.3	0.201	0.235	35.9
Case #4	0.429	0.511	0.395	5.25	0.437	484.5	35.2
Case #5	58.77	311.35	22.83	273.22	13.95	468.15	50.3
Case #6	0.311	0.295	0.263	143.2	0.385	78.95	46.2
Case #7	0.299	0.374	0.436	205.77	4.08	88.45	55.3
Case #8	0.308	0.244	0.379	97.33	0.406	22.12	48.1
Case #9	0.505	488.31	0.393	498.12	0.475	0.525	37.7
Case #10	0.588	0.673	0.299	270.13	0.432	112.3	48.5
Case #11	0.305	0.311	0.397	311.55	0.455	228.33	46.1
Case #12	0.575	537.44	0.673	438.55	1.08	118.3	45.8
Case #13	0.538	68.15	0.235	39.74	0.211	206	45.1
Case #14	0.27	0.344	0.599	0.463	0.242	0.283	36.3
Case #15	0.516	0.533	0.374	0.288	0.405	0.311	45.1
Case #16	0.465	0.503	0.377	0.395	0.388	0.443	38.6
Case #17	0.517	0.505	0.397	380	0.428	0.383	48.3
Case #18	0.329	0.284	0.24	183.78	0.348	338.4	41.8
Case #19	0.233	0.227	0.373	499.18	0.412	0.444	43.4
Case #20	0.397	467.3	0.378	354.7	0.388	0.895	44.5
Case #21	0.361	0.657	0.401	255.45	0.39	98.02	48.6
Case #22	0.388	0.607	0.189	263.4	17.8	140.25	78.9
Case #23	0.476	0.412	0.263	370	2.12	500	49.3
Case #24	28.3	32.6	0.269	47.4	0.28	332.4	47.1
Case #25	0.329	0.284	0.24	234.2	0.348	440.5	44.6
Case #26	0.367	0.344	0.344	287.1	0.411	108.5	43.9
Case #27	0.455	297.9	0.633	33.75	0.377	0.597	37.6
Case #28	0.305	0.13	0.255	176.1	0.329	50.7	44.1
Case #29	0.411	0.27	0.344	190.2	4.06	55.8	45.3
Case #30	0.329	0.13	0.214	89.01	12.3	500	49.3
Case #31	0.201	0.13	0.255	34.19	0.472	322	44.5
Case #32	0.485	0.405	0.225	425.18	5.19	489.25	46.7
Case #33	0.397	467.3	0.378	354.7	0.388	0.895	53.4
Positive	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 43
Negative	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 43

separated from the blood of pregnant women and analyzed for evaluation of the concentration of lupus antibodies. Interestingly most of the patients (24 out of 33) showed increased concentration of lupus antibody in their blood and required receiving anticoagulant treatment. In addition, the association between the blood level of lupus anticoagulant and serum concentration of IgM and IgG for TORCH was studied (Figures 2 and 3). 24.4 per cent of the pregnant women had increased levels of both lupus and CMV IgM antibody (Figure 2 and Table 3). This relation was significantly reduced in pregnant women infected with rubella and toxoplasma as 3.03 and 6.06 % of patients showed increased levels of both lupus and IgM antibody respectively. Finally, an association between TORCH IgG and lupus antibody was evaluated (Figure 3). A high relationship was found between rubella IgG and lupus (75.75%), whereas CMV (57.57 %) and toxoplasma IgG (18.18%) showed and association with lupus antibody.

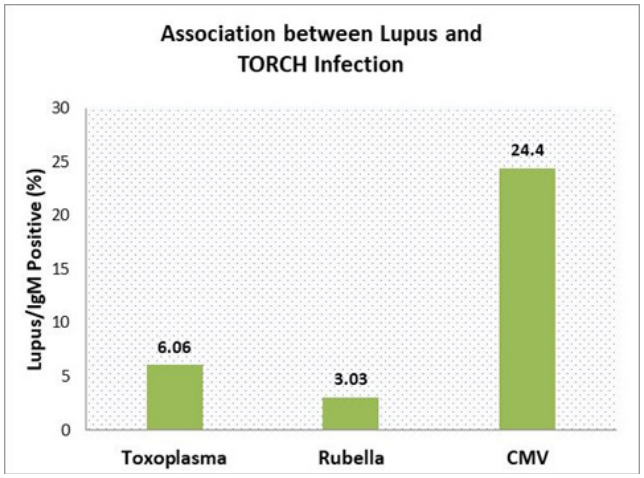

Figure 2. Association of Lupus anticoagulant antibodies with serum concentration of IgM for TORCH microorganisms.

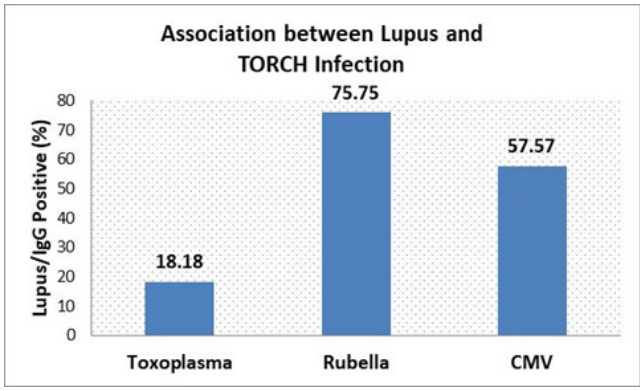

Figure 3. Association of Lupus anticoagulant antibodies with serum concentration of IgG for TORCH microorganisms.

Discussion

These results provide a quantitative analysis of TORCH infections and lupus anticoagulants in pregnant women and demonstrate a strong relationship between the increased levels of IgM and IgG antibodies for CMV alongside IgG for rubella with lupus anticoagulants in these patients. This relation is not found between toxoplasma IgM and IgG and rubella IgM with lupus anticoagulant antibody. The concentration of IgM and IgG antibodies for each of the toxoplasma, rubella and CMV in the blood determines whether the infection is new or become chronic. Arias et al (2008) reported that both IgM and IgG antibodies are produced within 1-2 weeks from the initial exposure to these pathogens and the appearance of the first antibody reveals that the infection is new and lasts for 6 months (11). The second antibody then appears after the concentration of the first antibody declines and indicates that the infection is chronic and remains in the blood for a year (11, 12). Human cytomegalovirus which is also called human herpesvirus 5, has been long identified to cause a number of fetus abnormalities and pregnancy loss worldwide (13, 14-15, 16). In the present study, most of the pregnant women have elevated amounts of IgG antibodies for CMV in their blood. A recent study shows that recurrent abortion is greatly associated with increased levels of CMV IgG antibody (17). Baghel and Inamdar (2020) found that 56% of women diagnosed with increased levels of IgG antibodies for CMV are at higher risk of pregnancy (18). In another study conducted among 116 women with previous miscarriages, the seroprevalence of all types of CMV infection increased (19).

Rubella has been long recognized to produce a teratogenic effect on the fetus during pregnancy which consequently may lead to spontaneous abortion (20). In the current study, most of the pregnant women showed an increase in the serum level of IgG antibodies for rubella. This is reported in the previous studies conducted by Ghazi et al (2002) and EL-MEKKI, and Zaki (1998) (21, 22). Similar to the results of the present study, Rasti and his colleagues (2016) found that 75% of pregnant women with recurrent abortion have increased levels of IgG antibody for rubella antibody (23).

As an intracellular parasite, toxoplasma has been reported by previous investigators to be highly related to abortion during the early stage of pregnancy (24-26). In contrast to these studies, the data here have shown a negative correlation between toxoplasmosis and abortion and the reason for this is not determined.

It is realized by the researchers that one of the causative agents of recurrent abortion during pregnancy could be the increased amount of blood clotting (thrombophilia) in the venous of these patients (27, 28). As an indicator of blood clots, this study determined the amount of lupus anticoagulant antibody in the blood of pregnant women. Similar to the results of the studies conducted by other investigators, the level of lupus anticoagulant antibodies has elevated in the blood of aborted women (29-34).

The most important finding of the current study is the occurrence of increased levels of IgM for CMV antibody and IgG for both CMV and rubella with the increased level of lupus anticoagulant antibody in the blood of pregnant women with recurrent abortion. This indicates that infection with these pathogens may produce thrombophilia and require receiving anticoagulants regularly to avoid an abortion.

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Interest conflict
Nothing to declare

Author's contribution
This study was performed by Hiwa Ramadhan Fatah.

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