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Genetic Study of TORCH Infections in Women with Bad Obstetric History: Multiplex Polymerase Chain Reaction for Detection of Common Pathogens and Agents of Congenital Infections

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OBJECTIVES:
To revealed the incidence of TORCH infections among pregnancy wastage in women which had bad obstetric history (BOH).
METHODS:
The study included 132 women with bad obstetric history. Genetic evaluation for TORCH infections was carried out by specific primers designed for that purpose using PCR method.
RESULT:
Toxoplasma was 36.36%, rubella 20.45%, cytomegalovirus 29.55% and herpes simplex virus 13.64%. Maximum number of cases of abortion 52 (**39.39**%), preterm labor 29 (**21.96**%) was associated with toxoplasma infection, early neonatal deaths 19 (**14.39**%) were maximally associated with toxoplasma and CMV infections. while congenital malformations 14 (**10.6**%) were evident maximally with toxoplasma infection and intrauterine death 8 (**6.06**%).
CONCLUSIONS:
Women with BOH are significantly higher in infection compared with that in control. A previous history of pregnancy wastage, genetic infestation using specific primers for TORCH agent's detection and the serological reaction for TORCH infections during current pregnancy must be considered while managing BOH cases so as to reduce the adverse fetal outcome.

Introduction

Bad obstetric history (BOH) implies previous unfavorable foetal outcome in terms of two or more consecutive spontaneous abortion, history of intrauterine foetal death, intrauterine growth retardation, still births, early neonatal death and/or congenital anomalies. Cause of BOH may be genetic, hormonal, abnormal maternal immune response and maternal infection. Recurrent pregnancy wastage due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms which include the TORCH complex (*Toxoplasma gondii, Rubella virus, Cytomegalovirus, Herpes simplex virus*) and other agents like *Chlamydia trachomatis*, *Treponema pallidum*, *Niesseria gonorrhoeae*, HIV etc. Toxoplasmosis acquired during pregnancy may cause damage to the fetus **[1]** .
Infection with Rubella during pregnancy may lead to congenital malformation in 10-54 percent of cases.**(2)**
The infection caused by CMV in adult is usually asymptomatic but its significance is many times increased when it occurs during pregnancy. However, the rate of primary CMV infection is significantly higher for pregnant women from low socioeconomic group. The mother is the usual source of transmission of HSV to the fetus or newborn. Primary HSV infection during first half of pregnancy is associated with increased frequency of spontaneous abortion, still birth, and congenital malformation.**(3).**
Infections by TORCH agents in women are usually asymptomatic and chronic. The social and reproductive maladjustment because of repeated pregnancy wastages, cost of treatment, and morbidity caused to the infant make the TORCH group of infections a major cause of concern. The prevalence of these infections varies from one geographical area to another **(4)**. Many sensitive and specific tests are available for serological diagnosis of TORCH complex. ELISA for IgM antibodies against these infections is highly sensitive and specific **(5)**.
Nucleic acid testing has allowed more sensitive and specific detection of infectious agents and is being increasingly adopted by diagnostic laboratories **(6)**. The technology is particularly useful in virology as it can replace conventional culture methods that are often expensive and labor intensive, detect fastidious organisms such as hepatitis C virus (HCV), detect low copy number agents such as herpes simplex virus (HSV) in cerebrospinal fluid, and improve turn-around times for detection of treatable agents such as herpes viruses **(7)**.

Toxoplasmosis caused by Toxoplasma gondii, a parasite which can purchase from handling infected domestic animals (cats), milk or eating contaminated meat. The infection can conceded to fetus via the placenta during pregnancy period, it cause infections of the eyes or central nervous system (**8**). **Rubella** is a virus that can cause epidemics most likely in the spring. Between 0.1-2% of newborns will be infected with rubella. The rate of fetal infection varies according to the timing of the mother's infection during pregnancy. Birth defects, however, are most likely (85%) in infants infected during the first eight weeks of pregnancy (**9**). **Cytomegalovirus** (CMV) belongs to the herpes virus group of infections. It can be transmitted through body secretions, as well as by sexual contact; some newborns which acquire CMV through the mother's breast milk Infected infants may have severe problems, such as hearing loss, mental retardation, pneumonia, hepatitis, or blood disorders(**10**). **Herpes simplex virus II** the virus enters the infant through his eyes, skin, mouth, and upper respiratory tract. Infants born with HSV infection, about 20% will have localized infections of the eyes, mouth, or skin, about 50% of infected infants will develop disease spread throughout the body (disseminated) within (9-11) days after birth (**11**).

HSV-2 is sexually transmitted. Symptoms include genital ulcers or sores. In addition to oral and genital sores, the virus can also lead to complications such as infection of the lining of the brain and the brain itself (meningoencephalitis) or infection of the eye especially the conjunctiva and cornea (**12**).

MATERIALS AND METHODS

Study design

Microbial infection during pregnancy can be transmitted to the fetus, resulting in a congenital infection. This study was proposed to analyze/detect the active infection of HCMV by PCR in pregnant women, which are the major contributor in congenital HCMV spread.

Source of samples

A total of 132 blood samples were collected from the gynecology wards in Hospital and out clinic from Babylon city, Karbala city and Baghdad city, the province large hospitals where patients visit from different areas of Babylon province. 5 ml of venous blood was taken in a sterile tube with EDTA; for Nucleic acid extraction process. The samples were divided into two aliquots and were stored at -80°C.

A total of 132 women were investigated. Cases were included in the study group depending on previous history of having 3 or more pregnancy wastages, intrauterine deaths, preterm deliveries, intrauterine growth retardation and unexplained early neonatal deaths. Comprehensive examinations and conventional laboratory investigations were carried out. From each woman 5 ml of venous blood was collected in EDTA container with strict aseptic precautions. DNA was extracted from these samples using (GENOMIC DNA MINI KIT FOR BLOOD/BACTERIAL/CULTUER CELL-GENAID). The DNA was used for genetic evaluation for TORCH infections.

DNA extraction

DNA was extracted in biosafety hood type II from all blood samples with the help of Ultra script DNA extraction kit (GENAID) according to the manufacturer procedure. 100 µl blood was used for DNA extraction and the purified extracted DNA was suspended in 100 µl of elution buffer and stored at -80°C in the refrigerator. **[2]**

Regular PCR

PCR reactions were carried out in a thermal cycler (viriti USA) with Taq DNA polymerase (bioneer and promega USA). The first round of amplification was performed with 5 µl of extracted DNA by using a forward primer and a reverse primer. The reaction mixture for a single reaction consisted of 10X PCR Buffer- 2.0 µl, MgCl2 (25 mM)- 2.4 µl, dNTPs (500 µM)- 1.0 µl, forward primer -1.0 µl, reverse primer- 1.0 µl, dH2O- 11.6 µl, Taq. DNA Polymérase (2 U/µl)- 1.0µ l and extracted DNA- 5.0 µl. The cycling conditions for regular PCR were done for get appropriate descent results.

Electrophoresis

PCR products were electrophoreses in 2.5% agarose gel prepared in 1X TBE buffer (boiled for 2 min in microwave oven and cooled to 55°C), adding ethidium bromide (1 µg/ml) stain and evaluated under ultra violet light. The specific DNA product for TORCH agents of each sample was determined by identifying the specific base pair (bp) amplified DNA bands in comparison with the 100-bp DNA ladder (Promega, USA), used as DNA size marker.

Results and discussion

In this study, women that have no medical history of high blood pressure (hypertension), diabetes and kidney disease were included. A total of 132 women were screened as bad obstetric history (BOH) while 30 as control. Amongst BOH, DNA positive results by PCR; **Toxoplasma** (36.36%), **Rubella** were also detected in women (20.45%), while (29.55%) and (13.64%) women were **CMV** and **HSV** virus positive, respectively, as showed in

table 1.

Table 1: prevalence BOH causative agents

Causative agents	BOH		Control	
	No.	%	No.	%
Toxoplasma	48	36.36	8	26.67
Rubella	27	20.45	4	13.33
Cytomegalovirus	39	29.55	7	23.33
Herpes simplex	18	13.64	11	36.67
Total	132	100.00	30	100.00

The history of the 132 bad obstetric history (BOH) cases consisted of abortion in 52 (39.39%), premature labor in 29 (21.96%), early neonatal death in 19 (14.39%), congenital malformation in 14 (10.6%) and intrauterine death in 8 (6.06%), as **table 2** revealed.

Table 2: Causative agents of TORCH with different presentation of Obstetric history

Patients	Toxoplasma		Rubella		Cytomegalovirus		Herpes simplex		Total
	No.	%	No.	%	No.	%	No.	%	
Abortion (n=52)	23	53.49	6	13.95	11	25.58	3	6.98	43
Preterm labor (n=29)	10	52.63	2	10.53	6	31.58	1	5.26	19
Early neonatal death (n=19)	4	50.00	1	12.50	3	37.50	0	0.00	8
Congenital malformation (n=14)	2	100.00	0	0.00	0	0.00	0	0.00	2
intrauterine death (n=8)	3	50.00	1	16.67	1	16.67	1	16.67	6

High prevalence was recorded in the age group of 20 to 39 years which was 42.05% of toxoplasma infections, followed by the age group of 39 years which was 31.87% with CMV infections, while the age group of 40 to 49 years this formed the lowest ratio.

Table 3: Causative agents of TORCH with age group of women with Obstetric history

Age group	Toxoplasma	Rubella	CMV	HSV
	%			
<19 years	18.60	21.4	32.07	19.4
20 - 29	42.05	34	11.01	23.2
30 - 39	21.90	30.3	31.87	16.9
40-49	11.07	11.6	15.14	36.1
>49 year	6.38	2.7	9.91	4.4

The results showed that 100 percent positive rates were found in all three groups with the TORCH in first, second and third trimesters of gestation, respectively. There was significant difference in TORCH infection among the women in the first and second trimesters of gestation, as previewed in table 4.

Table 4: Prevalence of TORCH agents among pregnant women according trimester ages

Trimester age	Toxoplasma		Rubella		Cytomegalovirus		Herpes simplex	
	No.	%	No.	%	No.	%	No.	%
1st Trimester	25	52.1	9	33.3	13	33.3	7	38.9
2nd Trimester	16	33.3	11	40.7	17	43.6	11	61.1
3'd Trimester	7	14.6	7	25.9	9	23.1	0	0.0
Total	48	100.0	27	100.0	39	100.0	18	100.0

Table 5: Primers used to detect TORCH causative agent

Primers	Toxoplasma	Rubella	Cytomegalovirus	Herpes simplex
Forward	CAG ATG TGC TAA AGG CGT CA	TGC TTT GCC CCA TGG GAC CTC GAG	GAG CCT TTC GAG GAG ATG AA	ATC CGA ACG CAG CCC CGC TG
Reverse	ATT GCC GCA CGA TAC TAG GT	GGC GAA CAC GCT CAT CAC GGT	GGC TGA GTT CTT GGT AAA GA	TCC GGC GGC AGC AGG GTG CT
Size (bp.)	445	321	229	382

Although monoplex PCR and real-time assays have considerable benefits in targeting detection of specific

organisms, they do not necessarily allow detection of the causative agent, due to the specificity of the primer sets used. Increasingly, detection of the causative agent using multiplexes in respiratory specimens (**13, 14**), gastrointestinal specimens (**15**), the eye (**16**), conditions causing lymphadenopathy, cerebrospinal fluid are replacing pathogen specific (versus clinical conditions specific) detection (**17**).

It is obvious that maternal infections show a life-threatening role in pregnancy wastage and their occurrence in patients with BOH is a significant factor. Persistence of encysted forms of toxoplasma in chronically infected uteri, and their subsequent rupture during placentation lead to infection of the baby in the first trimester and often to recurrent miscarriages. In the present study T. gondii, which is a known etiological agent in recurrent pregnancy wastage was found in 14.66% pregnant women with BOH. This is similar to what has been reported earlier is.(**18**)

The high rate of resistance against Toxoplasma may depend upon the environment and life style of the people here. Toxoplasma antibodies are found to be higher in aborters from the rural areas, where contact with soil is common regardless of whether cats are kept pets or not', can be true here also.(**19**)

Rubella is a mild viral infection among children, nevertheless can occasionally infect adults. Primary viral infection during women pregnancy possibly caused fetal harm. In our study rubella infection was 20.45% while other workers report ranging from 4 to 17.77%.(**20**)

The incidence of first trimester miscarriage among the teenagers under this study was higher than the 5.5% miscarriage rate observed among the teenagers in other parts of India (Bhalerao et al., 1990). The teenage pregnancy rates reported from various parts of the world ranged from 8 - 14%.(**21**)

As T. gondii, Herpes Simplex Virus infections also have a statistically significant correlation with the incidence of miscarriages. Both CMV and HSV are well-known to have an intrauterine route of transmission with significant mortality and morbidity. The present study shows rate of 29.55% for CMV in women with BOH. In other studies ranges from 3 to 12.9%. (**22**)

It was suggested that pregnancy may reactivate the latent virus leading to further reproductive wastages. Seropositivity rate for HSV IgM among BOH patients inour study was 8.66%, similar to what has been reported previously. (**23**). On considering the effect of the pathogens on different groups of abortions, Toxoplasma and CMV were found to have high influence on the 'complete abortion' category.

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