Serological analysis for torch infection in women with bad obstetric record

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ABSTRACT

Infant death within utero or at delivery has always been a disgraceful occurrence for perinatal death and remains a dispute in the concern of pregnant women worldwide. Bad Obstetric record implies consecutive spontaneous abortions, history of intrauterine fetal death, intrauterine growth retardation or congenital abnormalities. The purpose of the study is to reveal the evidence of TORCH infections among pregnancy wastage in women which had bad obstetric history and the study also assessed the sero prevalence of TORCH infection in antenatal women of correlated with BOH by using NANO ELISA method. Blood samples for this study were collected from different hospitals in and around Bangalore and Bagalkot city of Karnataka state. From the study it was evident that those maternal infections play a critical role in pregnancy wastage and their occurrence in the patients with BOH is a significant factor. Usage of NANO ELISA technique provides high sensitive, accurate results at economical price.

Key words: Torch, Nano Elisa, Bad Obstetric History

1. INTRODUCTION

Bad obstetric history (BOH) implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early neonatal death and/or congenital anomalies. Infections by TORCH agents in women are usually asymptomatic and chronic. Fetus TORCH infections cause a syndrome characterized by microcephaly, sensorineural deafness, chorioretinitis, hepatospleenomegaly and thrombo-cytopenia. Agglutination tests are easy to

perform for serological observation. Rubella is a well-known as German Measles a viral disease which characterized by patent ductus arteriosus, septal defects, pulmonary artery stenosis, sensory-neural deafness, meningoencephalitis, Intrauterine growth retardation (IUGR), and osseous changes. Less than 5% of pregnant women with primary infection are reported to be symptomatic, and even smaller percentages suffer from a mononucleosis syndrome. Herpes simplex virus (HSV) commonly causes infections of the skin and mucous membranes.

The Present study was aimed to reveal the incidence of ToRCH infections among pregnancy wastage in women which had Bad Obstretic History. This study is also conducted to assess the Seroprevalence of ToRCH infection in antenatal women and correlate with Bad Obstetric History by using nanoelisa method. ToRCH describes a group of clinically similar congenital infections caused by Toxoplasma gondii, Rubella, CMV and HSV1 and 2. Nanowells are much smaller in size (Cubic volume) in comparison to conventional ELISA microwells. As a result, NanoWells require less reagents and are highly cost efficient. Unlike conventional ELISA, Nanowells are not coated directly instead, a special poly amide disc (Solid phase) is inserted and fixed on to each NanoWell. Polyamide covalently binds the capture antigen through impregnation technology. Poly amide solid phase, having very high binding-coefficient, requires limited solid phase surface area to bind capture antigens. This makes the multiplex technology feasible wherein multiple well defined nanospots (Zone of Ag/Ab impregnation) could be accommodated onto a single poly amide disc. The stationary phase of NanoWell can accommodate up to 25 spots per polyamide disc. Capture antigens are impregnated on to a pre-defined zones/spots and that makes Multiplex testing possible. Nanoplex assays are read and interpreted by NanoScan for easy and accurate quantification and interpretation.

2. MATERIALS AND METHODS

A total of 40 blood samples were collected from different hospitals in and around Bangalore and Bagalkot city of Karnataka State. A total of 30 samples were collected from the antenatal woman with Bad Obstetric History (BOH) as the test group and 10 samples were collected from antenatal woman without any pregnancy wastage. Among the test group samples 17 were from antenatal women with history of repeated abortions, 3 from intra uterine death, 2 from intra uterine growth retardation, 4 from congenital anomalies and 4 from preterm deliveries. Cases from other causes of abortions such as Cervical incompetence, Rh incompatibility, Diabetes, Syphilis, HIV, Hepatitis were not included in this study. 2 -3 mL of venous blood is collected aseptically in serum separator vaccutainers and kept in room temperature for 30 minutes to clot. Serum was separated by centrifugation and transferred to plastic aliquots. The aliquots were labeled with information of patient and were stored at-20ºc until tested. Serum samples from both groups of subjects were tested using the commercial kits qualitative immune enzymatic determination of IgM and IgG antibodies for ToRCH infection.

Reagent preparation

Wash buffer

Add 2ml of 7.5 X wash buffer to 13 ml Distilled water.

Reagent 1X Blocker preparation:

Add 0.5 mL 10X blocker to 4.5 ml of 1X Wash buffer.

Sample dilution:

Serum will be diluted with 1X Blocker to give the required dilution of serum.

Preparation of 1X Secondary Antibody:

Add 40 µL 25X Secondary Antibody to 1 ml of 1X Blocker.

Preparation of 1X Detection reagent:

Add 20 µL of 50X detection reagent to 1ml of 1X Blocker.

Preparation of 1X substrate solution:

Add 50 µL of 20X substrate to 1ml 1X substrate buffer.

Test procedure

- Added 40 µL of diluted serum to the wells according to the sample layout.
- Incubated the Nanowells at 37ºC for 30 minutes.

- Aspirated the solution and washed the wells 3 times with 1X wash buffer.
- Added 50 µL freshly prepared 1x Secondary antibody.
- Incubated the Nanowells at 37ºC for 30 minutes.
- Added 50 µL of freshly prepared 1X detection reagent to each NanoWell.
- Incubated the Nanowells at 37ºC for 30 minutes.
- Aspirated the solution and washed the wells 3 times with 1X wash buffer.
- Added 50 µL of 1X substrate solution to each well.
- Incubated the Nanowells at Room temperature for 5 minutes.
- Aspirated the solution and washed the wells once with 1X wash buffer
- Dried the wells at 37ºC for 30 minutes.
- Read the slide on NanoScan instrument and analyzed the results using data analysis software.

3. RESULTS

For the study of ToRCH infection, we collected 40 samples of which 30 patients were antenatal women with Bad Obstetric History (BOH). 10 samples were collected from antenatal women without any pregnancy wastage. All the samples were screened for HIV, HBsAg and HCV through Elisa method at Siddhi Diagnostics, Bagalkot using commercial kits of Meril Diagnostics (P) Ltd. All the samples were non reactive for HIV, HBsAg and HCV.

Normal ranges

NEGATIVE RANGE: 0.0 TO 0.8 IU/ML
BORDERLINE RANGE: 0.9 TO 1.3 IU/mL
POSITIVE RANGE: 1.4 TO 25 IU/mL
STRRONG POSITIVE: More than 25.1 IU/Ml

Table 1: Nano Elisa titre values

		Toxoplasma		Rubella		Cytomegalo virus		HSV	
	Sample No	Ig M	IgG	Ig M	Ig G	Ig M	Ig G	Ig M	Ig G
CONROLS	1	0.1	0	0	1.5	0	0	0	0
	2	0	2.1	0	0	0.1	0	0	0
	3	0.1	0	0	0	0	0	0	0
	4	0	0	0	0.2	0	0	0	0
	5	0.1	2.4	0	0	0	0	0	0
	6	0.1	0	0	0	0	0	0	0
	7	0	0	0.1	0.1	0	0	0	0
	8	1.5	3.5	0	0	0	0	0	0
	9	0.9	4	0	0	0	0	0	0
	10	0	0.5	0	0	0	0	0	0
20-30YRS	11	0.1	0	0	0.1	0.2	7.7	0.2	7.1
	12	0.2	0	0.2	3.7	0	11.4	0	0.9
	13	0.2	10	0.2	11.1	0	0	0.3	0
	14	1	3.2	0	25	0	2.8	0	3.8
	15	0.3	3.5	0	6.2	2	8.2	0.5	2.9
	16	0.2	5.6	0.1	9.2	2	5	0	0.2
	17	2.1	9.3	2	7.7	1.5	8	0.3	6.2
	18	1	4.6	0	0	0.2	3	0	0.9
	19	0	12	0	20	0	0	0	6
	20	2.1	7	0	1.2	0.4	3	0.6	2.8
30-40 YRS	21	0.1	0	0	0.1	0.2	2	0.2	7.1

	22	0	0	0.2	0	0	0	0	0.9
	23	0.2	2	0.2	3	0	0	0.3	0
	24	0	0	0	4	0	0	0	2.2
	25	0.3	3.5	0	6.8	2	8.2	0	0
	26	0.2	0	0.1	0	2	5	0	0
	27	0	7.6	2	4.7	1.5	8	0.3	3.5
	28	1	0	0	0	0.2	3	0	0.9
	29	0	14	0	4	0	0	0	0
	30	2.1	7	0	1.2	0.4	3	0.6	1
>40 YRS	31	0.2	7.2	0	0	0	0	0	0
	32	0	0	2	4.6	0	0	0	6.6
	33	0	0	0	0	0	0	0	0
	34	0.8	2.4	0	0	0	0	0	0
	35	1.7	3.7	0	0	1.2	3.8	0	0
	36	0	0	0	0	0	0	0	0
	37	0.4	5.9	0	0	0	0	0	0
	38	0	0	0	0	0	0	0.2	2.5
	39	0.5	2.6	0	0	0	0	0	0
	40	0	3.6	0	0	0	0	0	0

Table 2: Sero-prevalence of *Toxoplasma gondii*

AGE group	Toxoplasma IgM average	Toxoplasma IgG average
Control	0.23	1.25
20-30	0.72	5.52
30-40	0.39	3.41
>40	0.36	2.54

Table 3: Seroprevalence of Rubella Virus infection

AGE group	Rubella Ig M average	Rubella Ig G average	
Control	0.01	0.18	
20-30	0.47	8.42	
30-40	0.25	2.38	
>40	0.2	0.46	

Table 4: Seroprevalence of Cytomegalovirus infection

AGE group	CMV Ig M average	CMV Ig G Average
Control	0.1	0
20-30	0.63	4.91
30-40	0.35	2.92
>40	0.12	0.38

Table 5: Seroprevalence of Herpes Simplex Virus infection:

AGE group	HSV Ig M Average	HSV Ig G Average
Control	0.1	0.01
20-30	0.19	3.08
30-40	0.14	1.56
>40	0.02	0.91

4. DISCUSSION

The present study shows seropositivity rate of 8.0% for CMV specific IgM in women with BOH. In other studies seropositivity ranges from 3 to 12.9%. Seropositivity rate for HSV IgM among BOH patients in our study was 3%, whereas has been reported previously ranging from 2 to 10%

5. SUMMARY AND CONCLUSION

TORCH infections are associated with recurrent abortion, intrauterine growth retardation, intrauterine death, preterm labor, early neonatal death, and congenital malformation. Delivery wastages of earlier records and positive serological reactions during the present delivery assist management of these cases in order to reduce adverse fetal outcome. Usage of Nano-Elisa technique provides high sensitive, accurate results at economical price.

Peer-review

External peer-review was done through double-blind method.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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