Original Research Article

A community-based prospective cohort study of pregnant women in tribal Gujarat- A methodology

Kranti Vora[1,2,*], Paridhi Gupta[1], Shahin Saiyed[1], Parth Tailor[1], Bhumika Prajapati[1], Senthilkumar Natesan[1]

[1]Dept. of Reproductive and Child Health, Indian Institute of Public Health Gandhinagar, Gandhinagar, Gujarat, India
[2]Visiting Fellow, University of Canberra, Australia

ARTICLE INFO

ABSTRACT

Introduction: Infections acquired during pregnancy increases the risk of maternal child morbidity and mortality. Likewise, maternal stress has undesirable impact on women's health. The aim of this study is to assess the prevalence of TORCH infections and stress among pregnant women in tribal areas of Aravalli district, Gujarat and its impact on birth outcome. This paper outlines the methodology adopted for the research study.

Materials and Methods: A prospective cohort study is ongoing in a tribal block of Aravalli district, Bhiloda. Expecting women will be included to collect data on antenatal care (ANC), household (HH) and post-delivery status of women and children born. Plasma and saliva samples are collected to assess TORCH infection and stress through nested PCR and ELISA assays.

Discussion: As of October 2019, 119 tribal villages & 100,000 populations are covered. This is the first study in India focusing on both TORCH infections and stress during pregnancy, and its birth outcome. This study will add to evidence generation for improving neonatal mortality.

1. Introduction

Infections during pregnancy are major causes of maternal child morbidity and mortality. A well-recognized cause of congenital anomalies, abortion, intrauterine fetal death, intrauterine growth retardation, prematurity, and stillbirth is a TORCH infection.[1] TORCH stands for Toxoplasmosis, Others, including Hepatitis, EBV, Varicella Zoster, Rubella, Cytomegalovirus, and Herpes Simplex virus infection. A high prevalence of these infections has been reported among pregnant women and women of childbearing age.[2] These infections are widely impacting low and middle-income countries.[3]

Generally, chances of transmission of infection are high in the third trimester but during the first trimester, consequences are more serious in a fetus.[4] TORCH infections in the mother are transmissible to the fetus in the womb or during the birth process and cause a cluster of symptomatic birth defects.[5] These infections occur before the woman realizes or seeks medical attention.[1] TORCH is usually asymptomatic and long-lasting infections in pregnant women[6] and hence difficult to diagnose clinically. The detection of specific IgM antibody is the most prominent approach for the identification of TORCH.[7]

The prevalence of TORCH infections differs geographically.[6] In India, pregnant women from low socio-economic backgrounds are possibly exposed to a variety of infections attributable to poor environment and hygiene conditions. Maternal infections have been considered as significant factors in the causation of poor pregnancy outcome.[8]

Maternal stress is one of the most common complications in pregnancy. In developed world maternal stress is identified as an important reason for low birth weight and neonatal mortality. World Health Organization (WHO) defines maternal mental health as a state of well-being

* Corresponding author.
E-mail address: kvora@iiphg.org (K. Vora).

in which a mother realizes her abilities, can cope with the normal stresses of life, can work productively and fruitfully, and can make a contribution to her community".[9] The prevalence of stress during pregnancy has been found to range from 6% to 52.9% in developing countries.[10] It increases the risk of miscarriages and predisposes the mother to perinatal infections, premature labor, hemorrhages, and preeclampsia.[11] Many risk factors contribute to stress during pregnancy. Among them, the previous history of depression, domestic violence, stressful life events, and interpersonal conflicts are important factors.[12]

Indian women also encounter high levels of social and work-related stress, though they have good traditional ways of handling it. No studies have been done in India linking stress in pregancy/its bio-markers and pregnancy outcomes that we know of. It would be useful to document if stress is an important determinant of adverse pregnancy outcomes and developmental issues among children especially in tribal areas in India.

Baseline data of TORCH infections and stress in the local community of Aravalli district in Gujarat is unavailable. Therefore, our objectives are:

1. To assess the prevalence of TORCH infections and its impact on the birth outcome.
2. To assess the prevalence of stress among pregnant women in tribal (marginalized) areas and its impact on maternal and child health.
3. To assess the correlation of stress with bio-markers such as salivary DHEA-S and cortisol.

2. Materials and Methods

2.1. Study design and setting

A prospective cohort study is being conducted from September 2018 to March 2020 (ongoing) in Aravalli district, Gujarat. Aravalli is divided into six blocks, Modasa, Bayad, Dhansura, Bhiloda, Malpur, and Meghraj. The tribal block Bhiloda was purposively selected as 57.57% and 4.82% of its total population (n=239,216) are represented by Schedule Tribe (ST) and Schedule Caste (SC) respectively.[13] Meghraj block was chosen for pilot testing due to its geographic and demographic similarity with block Bhiloda (Figure 1). Ethical approval was obtained by the Institutional Ethics Committee, Indian Institute of Public Health, Gandhinagar, Gujarat. Informed written consent from the participants is taken prior to the interview and sample collection.

2.2. Study population

During the study period, all pregnant women aged 15-49 were considered as eligible participants. To get direct information from the respondents and to test our study
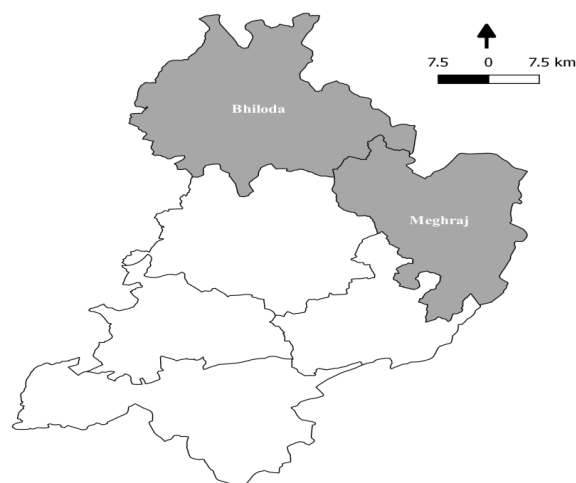

Fig. 1: Study location in Aravalli district

purpose universal sampling method was used.

2.3. Data collection tools and procedure

The questionnaire was developed after reviewing earlier validated and published questionnaires from the National Family Health Survey (NFHS) India. Public health and social sciences experts were consulted to better align the questionnaire with the objectives of our study. Pilot testing was carried out on 81 pregnant women to assess the feasibility of the study protocol and also the appropriateness and comprehensibility of the questionnaires. Errors identified during the pilot study were corrected to ensure consistency in the field.

Fig. 2: Sample collection and follow-up

Pregnant women at any week of gestation are enrolled and recruited with the help of ASHA and ANM. The household list is taken from Sarpanch (head of the village); ASHA and ANM to make sure all the houses are covered. Each household is assigned a unique household ID, generated based on its geographical location (district, block, and village). After enrollment of pregnant women, a team of field investigators collects data using three detailed structured questionnaires on household (HH), antenatal care (ANC), and post-delivery status of women. Demographic,

living and economic status is incorporated in the household questionnaire. The ANC questionnaire enquires about antenatal care, delivery planning, and pregnancy complications. The post-delivery questionnaire includes history of abortion/miscarriage, delivery information, referral details, expenses, maternal morbidity, and government scheme related information. All tools are translated into local language and information is obtained from all the pregnant women through personal interviews.

Enrollment of pregnant women was done on *Mamta* Day at Anganwadi or PHC. Biological samples were collected at the same time. Follow-up was done after delivery. (Figure 2). The PHC MO, ANM, and ASHA provided assistance during sample collection. In addition, a telephone call is made to women whose delivery date is approaching, to determine if they have delivered.

2.4. Database management

Open Data Kit (ODK) is an open source software which allows researchers to generate online questionnaires on tablets and other mobile devices. We used ODK to collect, use, and manage data on tablet devices. Installing of the questionnaire is explained in the online documentation for ODK. It provides drag-drop options for question selection, and features like drop-down lists, question skipping, and data validation. These attributes of ODK help optimize the collection of responses and ensure that investigators do not enter an invalid code or numeric values.

Female field investigators holding a degree of master's in social work were hired and trained in two days of intensive training on the use of ODK application and the e-questionnaire. A mock training and interview session was conducted by a consulted psychiatrist to train our data collectors. All the investigators were experienced in the use of Android devices. Once familiar with the e-questionnaire they were sent for data collection at the study region.

2.5. Data quality assurance

To protect the collected data, each tablet device is passcode enabled, so that the device cannot be used without entering the four-digit code. To ensure the quality of data, supervisor randomly selects households to cross verify the data recorded by field investigators. Inaccurate data and human errors are identified by supervisor every day. After reviewing the completeness of the data and verifying outliers, it is uploaded on the organizational server by data supervisor. Further, data are entered into Microsoft Excel in order to identify any remaining discrepancies.

2.6. Laboratory procedure

For setting up a lab at IIPH-Gandhinagar, a consultant having experience in molecular biology and in the development of molecular diagnostic assays was hired for technical advice and development of standard operating procedures (SOPs) (Table 1). Plasma and saliva samples are collected to assess the prevalence of TORCH infection and stress during pregnancy. Stress is quantitatively assessed by detecting the level of biomarkers such as cortisol and dehydroepiandrosterone sulfate (DHEA-S) through ELISA assays.

The extensive literature review was carried out for the preparation of SOP for the molecular detection of the TORCH organism complex using nested PCR approach. PubMed, Scopus and Google Scholar were searched for selection of the appropriate protocol designing for PCR detection of the TORCH complex. After reviewing the multiple research articles, nested PCR method was used for the qualitative determination of the TORCH infection respectively. In addition, TORCH infections are also assessed by organism specific IgG and IgM ELISA assays.

Table 1: List of standard operating procedure developed for the study

S. No.	SOP
1	Blood Samples Transport & Storage
2	Saliva Sampling, Transportation and Storage
3	Herpes Simplex Virus (HSV1-2) detection
5	Human Cytomegalovirus (CMV) detection
6	Epstein-Barr virus (EBV) detection
7	Hepatitis B virus (HBV) detection
8	Toxoplasma gondii detection
9	Varicella Zoster (VZV) detection
10	Cortisol and DHEA-S detection

2.7. Collection and processing of saliva

The saliva samples of around 3-4 ml from the participating pregnant women are collected by a passive drooling method using a 15 ml sterile centrifuge tube. The saliva is centrifuged to pellet down any food particles or other impurities, supernatant is transferred into sterile 1.5 ml Eppendorf tubes, and stored in -20°C for quantitative estimation of cortisol and DHEA-S by ELISA assays.

2.8. Collection and processing of blood

The whole blood samples of around 6-7 ml is collected in EDTA vacutainer tube from the participating pregnant women. The collected blood samples are transported from the field site to the research lab by maintaining the appropriate temperature and other conditions. The whole blood samples are centrifuged at 2000 RPM for 15 minutes at room temperature to separate the plasma, buffy coat, and RBC layer.

The plasma layer is the top layer after centrifugation, usually semi-transparent and golden in color. Using a sterile Pasteur pipette, the plasma layer is gently collected without disturbing the buffy coat layer and transferred into clear

capped tubes and stored at -80°C.

After centrifugation, the middle white colored layer is known as a buffy coat. A buffy coat suspension is a concentrated leukocyte suspension. It is not mononuclear as the granulocytes are still present. Using a sterile Pasteur pipette, the buffy coat layer is removed gently into a 2 ml tube. The genomic DNA is isolated from buffy coat by using Promega wizard DNA extraction kit, as per the manufacturer's instructions. The DNA is stored at 2–8°C. Isolated genomic DNA is checked for quality by detection of β-globin through PCR.

2.9. Selection of primer pairs and validation of primers

The individual oligonucleotide primer pairs for nested PCR is selected. Designed or selected primer pair is checked for their important parameters such as GC content, melting temperature, annealing temperature, dimer formation or hairpin loop formation using OLIGOANALYZER tool of Integrated DNA technologies.

The molecular detection of the TORCH complex is performed initially through individual reactions using a nested PCR approach.

3. Limitations and Strength

3.1. Participants characteristics

Table 2 shows general characteristics of the participants. As of October 2019, a total of 824 expecting women from 119 tribal villages had been surveyed. The mean maternal age is 24.24 (±3.45) months and parity distribution is 42% primigravida, 53.4% multipara, and 4.6% grandmultipara across the region.

In the block, 72% of the population consists of ST whereas OBC, SC and general category represent 20%, 5.4%, and 2.2% of the total population.

Among the surveyed households 47.8% live in *kuchcha-pucca* house, 29.9% in *kuchcha*/nohouse and only 22.3% in *pucca* houses. In Bhiloda block, only 2.2% households have their own flush toilet whereas 38.4% households either have no access to a toilet or prefer open defecation. Many households use a shared flush or pit toilet.

Limitations of our study include reporting bias in the identification of pregnant women. As data are provided by ASHA and ANM, some pregnant women in our study area may have been missed. Also, less than 5% pregnant women refused to take part in the study. Anemic and all other women in whom difficulty in finding the veins for drawing the blood are excluded from the study.

Another limitation is that saliva and plasma are collected only once for each pregnant woman on the day of interview. Owing to the effect of diurnal variation on cortisol levels, it is necessary to collect saliva and plasma more than once within a day. However, the study objective is to a) categorize the pregnant women based on stress levels

Table 2: General characteristics of the participants

Variable	Percent
Number of Villages covered	119
Number of Pregnant Women covered	824
Maternal Age in Months (Mean ± sd)	24.24 ± 3.45
Parity	
Primi	42
Multipara	53.4
Grand Multipara	4.6
Caste	
SC	5.4
ST	72.4
OBC	20.0
General	2.2
Head of the Household	
Male	91.2
Female	8.8
Type of House	
Pucca	22.3
Kuchcha-pucca	47.8
Kuchcha/No house	29.9
Type of Ration Card	
APL	33.6
BPL	64.8
Antyoday Ann Yojna card	1.6
Toilet Facility	
Own Flush Toilet	2.2
Shared/public Flush toilet; own pit toilet	36.4
Shared/public pit toilet	23.1
No access to toilet/Open Defecation	38.4

associated at the time of sample collection and b) assess the effect of stress on birth outcome. Hence, we have used time specific reference intervals provided by manufacturers (DiaMetra ELISA assays) for cortisol and subsequent stress assessment. For example, the reference range of cortisol in saliva is 3-10ng/ml for morning and 0.6-2.5ng/ml for afternoon/evening sample collection. Similarly, the reference range of cortisol in plasma is 60-230ng/ml for 08:00-10:00 AM and 30-150ng/ml for 4:00 PM sample collection. Likewise, the diurnal variation of DHEA-S during pregnancy is not established and clear. However, limited studies have examined DHEA-S during pregnancy by obtaining samples once a day.[14]

4. Discussion

To our knowledge, this is the first population-based study conducted in Gujarat to describe TORCH infection and stress in pregnant women. Due to the external validity of population based cohort studies, results are generalizable in real life. Another strength of this study is the assessment of two important steroids—cortisol and DHEA-S in pregnant women. Lack of reference points for cortisol in the Indian setting makes it difficult to compare findings. Hence, studies conducted in other countries to assess the cortisol level were

taken as a reference range for our study.

5. Source of Funding

6. Conflict of Interest

None.

References

1. Sadik M, Fatima H, Jamil K, Patil C. Study of TORCH profile in patients with bad obstetric history. *Biol Med*. 2012;4(2):95–101.
2. El-Tantawy N, Taman A, Shalaby H. Toxoplasmosis and Female Infertility : Is there a Co- Relation ? *Am J Epidemiol Infect Dis*. 2014;2(1):29–32.
3. Fauci VL, Squeri R, Genovese C, Alessi V, Facciolà A. The 'Dangerous Cocktail': an epidemiological survey on the attitude of a population of pregnant women towards some pregnancy risk factors population of pregnant women towards some pregnancy risk factors. *J Obstet Gynaecol*. 2019;40(3):330–5.
4. Poudyal A, Poudyal N, Khanal B. Seroprevalance of ToRCH Infection –A Laboratory Profile. *Int J Biomed Res*. 2018;9(4):154–7.
5. Karad D, Kharat A. Seroprevalence of torch infections in bad obstetrics history in HIV and non-HIV women in Solapur district of Maharashtra India. *J Hum Virol Retrovirol*. 2015;2(7).
6. Surpam RB, Kamlakar UP, Khadse RK, Qazi MS, Jalgaonkar SV. Serological study for TORCH infections in women with bad obstetric history. *J Obstet Gynecol India*. 2006;56(1):41–3.
7. Sharma S, Duggal N, Agarwal S, Mahajan RK, Hans C. Seroprevalence of Toxoplasma , Rubella and CMV infections in Antenatal Women in a Tertiary care hospital in North India. *J Commun Dis*. 2015;47(2):23–6.
8. Yasodhara P, Ramalakshmi B, Naidu A, Raman L. Prevalence of specific IGM due to toxoplasma, rubella, CMV and c.trachomatis infections during pregnancy. *Indian J Med Microbiol*. 2001;19(2):52–6.
9. Aeri BT, Arora P. Burden of antenatal depression and its risk factors in Indian settings: A systematic review. *Indian J Med Specialities*. 2019;10(2):55–60.
10. Pantha S, Hayes B, Yadav BK, Sharma P, Shrestha A, Gartoulla P. Journal of Women's Health Care Prevalence of Stress among Pregnant Women Attending Antenatal Care in a Tertiary Maternity Hospital in Kathmandu. *J Women's Heal Care*. 2014;3(5):3–7.
11. González-Ochoa R, Sánchez-Rodríguez EN, Chavarría A, Gutiérrez-Ospina G, Romo-González T. Evaluating stress during pregnancy : Do we have the right conceptions and the correct tools to assess it . *J Pregnancy*. 2018;doi:10.1155/2018/4857065.
12. Engidaw NA, Mekonnen AG, Amogne FK. Perceived stress and its associated factors among pregnant women in Bale zone Hospitals, Southeast Ethiopia: a cross-sectional study. *BMC Res Notes*. 2019;12(1). doi:10.1186/s13104-019-4383-0.
13. Directorate of census operations Gujarat. District census handbook. Available from: http://censusindia.gov.in/2011census/dchb/DCHB_A/24/2407_PART_A_DCHB_AHMADABAD.pdf.
14. Soldin OP, Guo T, Weiderpass E, Tractenberg RE, Hilakivi-Clarke L, Soldin SJ. Steroid hormone levels in pregnancy and 1 year postpartum using isotope dilution tandem mass spectrometry. *Fertil Steril*. 2005;84(3):701–10.

Author biography

Kranti Vora Additional Professor

Paridhi Gupta Biostatistician

Shahin Saiyed Research Associate

Parth Tailor Consultant

Bhumika Prajapati Research Associate

Senthilkumar Natesan Associate Professor