Original Article

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Dentistry Section

Endotoxemia among Postpartum Mothers with Periodontitis Delivering Low Birth Weight Babies: A Case Control Study in Rural Indian Population

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ABSTRACT

Introduction: The presence of circulating maternal endotoxin can arise from pathogenic periodontal bacteria resulting in preterm labour and delivery of Low Birth Weight (LBW) infant.

Aim: The aim of this study was to find if periodontitis induced maternal endotoxemia could be a risk factor for delivery of LBW infant.

Materials and Methods: A total of 60 primiparous mothers between the age group of 18-35 years delivering LBW infants (weight ≤2500 gm) were selected for this case control study. Cases were 30 postpartum mothers with periodontitis whereas controls were 30 postpartum mothers without periodontitis. Periodontitis was defined according to WHO criteria, as pocket probing depth of ≥4 mm in atleast one site. Endotoxin level was assessed by a quantitative end point chromogenic limulus amebeocyte lysate (Lonza QCL 1000®). Odds Ratio (OR) was calculated to see if periodontitis was a risk factor.

Results: Periodontitis increased the risk for occurrence of endotoxemia by more than three times among mothers delivering LBW infants with an OR of 3.21.

Conclusion: Maternal endotoxemia due to periodontal infection can form a possible biological explanation for periodontitis as a risk factor for delivery of LBW babies. Future research is needed to study the effect of endotoxins in periodontal pathogenesis.

Keywords: Endotoxins, Epidemiology, Odds ratio, Risk factor

INTRODUCTION

The LBW delivery continues to be one of the most significant unsolved problems of public health and perinatology [1]. LBW is a major cause of perinatal morbidity and mortality and its rate has been increasing worldwide, reaching 12% in the USA, 5-10% in European countries, and 18.3% in Asian countries, having highest rate in India estimated around 30% [2].

The known risk factors for LBW are young maternal age, low maternal weight gain, low pregravid weight, multiple gestations, gestational diabetes, genitourinary tract infections, drug use, cigarette smoking, excessive alcohol consumption and previous preterm delivery also it is estimated that 50% of the causes are idiopathic [3,4]. Although the pathophysiology of LBW remains unknown, accumulating evidence suggests that subclinical infections and chronic inflammation may account for a majority of LBW deliveries, responsible for 30%-50% of all cases. Periodontal disease may be one such chronic inflammatory condition which could lead to adverse pregnancy outcome [4].

Periodontal disease is an infectious disease resulting in inflammation of gingival and periodontal tissues and progressive loss of alveolar bone, with pocket formation and recession or both and eventually tooth loss if left untreated [5]. The periodontal infection is initiated and sustained by predominantly gram-negative, anaerobic, and microaerophilic bacteria like Actinobacillus actinomycetemcomitans (Aa), Tannerella forsythia (Tf), Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi) that colonise the subgingival area [5]. Periodontal infections may mediate Preterm Low Birth Weight (PTLBW) through one or more of the following mechanisms i.e., contamination of the fetoplacental unit by periodontal pathogens, or through the action of Lipopolysaccharide (LPS) from the periodontal reservoir on the fetoplacental unit, or by the effects of the Inflammatory mediators (Interleukin (IL), Prostaglandins (PGE2), and Tumour Necrosis Factor (TNF) from the periodontal reservoir on the fetoplacental unit [4,6].

Endotoxin is a LPS present on the outer membrane of gram-negative bacteria [7]. The overgrowth of gram-negative bacteria in periodontal pocket causes inflammation and ulceration of pocket epithelium which may result in increased release of LPS into systemic circulation. The LPS further activates the host cells causing an up regulation of transcription of numerous genes and leading to elevated production of an array of cytokine, chemokine and lipid mediators that contribute to acute and chronic inflammatory responses [7-9].

Collectively, case control and cohort studies and systematic reviews [4,6,10,11] indicate that women with periodontitis are about two to four times more likely than healthy women to experience PTLBW infant. Whereas in recent studies [12-14] authors report that after controlling of confounding factors, there is no relationship between LBW infant and maternal periodontitis. The debate still continues and the results are inconclusive. Therefore, there is a need to find out the pathogenesis involved in delivery of LBW from maternal periodontitis. Periodontitis and adverse pregnancy outcomes may be linked through a chronic, systemic inflammatory challenge to the mother and fetus in response to release of endotoxins from mother's oral cavity [8,9]. Alternatively, endotoxemia may affect the uterus more directly through repeated bacteremias with periodontitis-associated microbial species like Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr), Tf, and Pg [15].

To the best of our knowledge, no studies have determined the endotoxin levels in postpartum mothers. It is important to study women delivering LBW to elucidate the role of periodontitis as a risk factor. The proposed hospital-based case control study was done to find if maternal endotoxemia in periodontitis patient is a risk factor for LBW babies and thus elucidates the role of periodontal disease in leading to infant mortality in Jharkhand rural mothers.

MATERIALS AND METHODS

This was a hospital-based case control study (1:1). The design and method of this study was approved by the Vananchal Dental

College Ethical Committee (Protocol No: VDCEC/5487/ETLB). The study was conducted after obtaining written informed consent from every participant. The study participants were recruited among all primiparous women delivering LBW singletons in the Department of Gynaecology and Obstetrics, Government District Hospital, Garhwa, Jharkhand, India. The study was conducted from March 2015 to April 2017.

Study Population

All postpartum mothers from rural population who gave birth to a singleton live born child birth weighing <2500 gm were eligible for this study. All mothers were examined within 48 hours of delivery.

The sample size was calculated according to a previous study with an expected OR of 4 [16], power value of 80% and a type I error rate of 5%. The sample size was calculated to 29 patients but considering drop outs it was rounded to 30 patients in each group. Therefore, a total of 60 postpartum mothers were selected for this case control study.

The cases were those mothers who delivered LBW infant with periodontitis and controls were mothers delivering LBW infant without the presence of periodontitis.

A subject was identified as having periodontitis if Pocket Probing Depth (PPD) of ≥4 mm was present in atleast one site [17]. The mothers should also have atleast 20 teeth and should not have undergone any periodontal therapy in the last six months. Women were excluded if they were younger than 18 years of age or older than 35 years, had systemic conditions like cardiovascular disorders, hepatic deficiency, diabetes, genitourinary tract infection or any other medical condition requiring antibiotic usage. Also, mothers with caesarean deliveries, second or any subsequent deliveries, as well as paired pregnancies were excluded from the study.

The hospital was visited three days/week on a regular basis. The hospital birth register was scrutinised each day to identify potential cases and controls. In order to have a non selected control group, controls were randomly included from postpartum women who delivered LBW child the same day or the day after the case, in the same maternity unit, with the same exclusion criteria, and with a 1:1 case control ratio. Women who refused to participate in the study were substituted with new participants.

Data Collection

A medical and obstetrical data was taken according to the protocol of the study. A Single Examiner (SN) and Data Recorder (RPP) collected data from all the mothers using a structured questionnaire through direct personal interview of the participant. Questionnaire included age, marital status, educational level, information on known risk factors and obstetric factors such as current pregnancy history, maternal age at delivery, prenatal care, tobacco use, alcohol use, genitourinary infections, vaginosis, and gestational age. For nutritional status of the mother, body mass index was calculated. Additionally, each subject reported on her family history considering diabetes (type 1 or 2), gynaecological tumours, and hypertension. The birth weight of infant, type of delivery and date and time delivery were recorded from the hospital database.

Data concerning the periodontal status of the mothers, such PPD, plaque index (PI) [18], Modified Gingival Index (MGI) [19] and Bleeding On Probing (BOP) were measured within 24 hours of delivery. PPD was measured from base of the pocket to gingival margin using a UNC 15 periodontal probe (Hu-Friedy®, Chicago, IL, USA). PI and MGI was measured on six sites of each tooth and given a score from 0-3. BOP was recorded after 15 seconds of probing as present or absent for each site.

Endotoxin Assay

For endotoxin assessment venous blood samples were taken within 24 hours of parturition. Using an aseptic technique, 2 mL of venous blood was collected in a syringe and placed in a heparinised tube. Platelet rich plasma was prepared by centrifugation 2200 gm for 25 minutes at 4°C and the samples were stored at -20°C. All measurements of endotoxin were tested with a quantitative end point chromogenic Limulus Amoebocyte Lysate (LAL) method (Lonza QCL 1000®, Walkersville, MD, USA) according to the manufacturer's instruction. Each assay was performed in duplicate and corrected for its own blank without LAL or substrate. Esherichia coli 0111:B4 reference endotoxin was used for the standard curves. Endotoxemia was considered positive when endotoxin levels were >5 pg/mL and negative otherwise, according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Data entry, data cleaning and validation of data was done by two independent operators (SN, RPP). Cross checking of every tenth interview schedule was done by the principal investigator (SJP). Data entry and analysis was done using the software SPSS (Version 19.0, SPSS® Inc., Chicago, IL, USA). Mean and standard deviation were calculated for each variable. Periodontal variables among test and control sites were calculated using student t-test. All results were considered significant at p <0.05. Odds Ratio (OR) was calculated to identify if periodontitis was a risk factor for endotoxemia.

RESULTS

Clinical characteristics of study population [Table/Fig-1]:

The cases and control were similar in age distribution, education, marital status, and alcohol and tobacco usage. Most of the mothers were below 25 years of age in both the groups and only nine among cases and 11 among control were between age group 25-35 years. Only primary and secondary level of education was completed by the majority of the mothers in both the groups and only few received university level of education. All 30 women in both the groups were married and 22 women among cases and 24 women among controls were employed during their pregnancy. No alcohol usage was reported in any of the groups, while one mother from case and three mothers from control used chewable form of tobacco. More

Maternal characteristics	Cases (n=30) (%)	Control (n=30) (%)
Age		
<25 years	21 (70)	19 (63)
25-35 years	9 (30)	11 (37)
Education level		
Primary level	11 (37)	13 (43)
Secondary level	18 (60)	15 (50)
University level	1 (3)	2 (7)
Marital status		
Married	30 (100)	30 (100)
Unmarried	0	0
Employment during pregnancy		
Yes	22 (73)	24 (80)
No	8 (27)	6 (20)
Tobacco use (Non smoking)		
Yes	1 (3)	3 (10)
No	29 (97)	27 (90)
Alcohol use		
Yes	0	0
No	30 (100)	30 (100)
Number of prenatal care		
>6 visits	24 (80)	21 (70)
>3 visits	6 (20)	9 (30)
BMI (kg/m²)	23.6±2.82	22.3±2.91

[Table/Fig-1]: Characteristics of study population.

than 6 prenatal checkups were completed by 24 mothers in cases and 21 mothers in controls. The body mass index for cases was 23.6±2.8 and controls were 22.3±2.9.

Periodontal status and endotoxemia levels among cases and control

The clinical parameters assessed for cases and controls are described in [Table/Fig-2]. The PPD and PI scores were more in cases when compared to controls (p=0.005). While no significant difference was seen for MGI and BOP. Eight mothers from cases and three mothers from control group tested positive for endotoxemia [Table/Fig-3]. The OR calculated was 3.27. The mothers delivering LBW babies with periodontitis were three times at greater risk for having endotoxemia.

Parameter	Cases (n=30)	Control (n=30)	p-value*
Number of teeth present	24.5±4.86	24.8±4.52	0.40
Probing depth	5.24±0.13	2.1±0.42	0.005**
Modified gingival index	2.6±0.43	2.3±0.40	0.08
Plaque index	2.5±0.62	1.9±0.42	0.005**
Bleeding on probing (%)	82±18.61	75±16.81	0.09

[Table/Fig-2]: Periodontal variables compared between cases and control (Mean±SD).
*Student t-test; **Statistically significant

Endotoxemia	Cases (n=30)	Controls (n=30)	Odds ratio
Endotoxin positive	8	3	3.27
Endotoxin negative	22	27	

[Table/Fig-3]: Odds ratio for endotoxemia.

DISCUSSION

The findings showed that mothers delivering LBW infants with periodontitis had increased endotoxin levels in circulation than mothers delivering LBW infants without periodontitis. For more than two decades the correlation between maternal periodontal disease and adverse pregnancy outcome has been controversial. Several studies and meta-analysis of systematic reviews have shown maternal periodontitis to be a risk factor for LBW [4,6,10,11,20]. Yet the pathophysiological mechanism of periodontitis leading to delivery of LBW babies is still unclear [10].

Several authors [15,21,22] detected high levels of Pg, Tf, Pi, Aa, Fn, and Cr, among premature births compared to term deliveries. Similarly, Blanc V et al., observed periodontal bacteria like Eikenella corrodens and Fn in the placenta [23]. The differences in the distribution and virulence of specific periodontal pathogens may contribute to heterogeneity across studies. Therefore, to overcome this problem the author's assessed bacterial product like endotoxin instead of periodontal pathogen. The literature search showed very few studies assessing the endotoxin level among women with periodontitis [8,9].

The proposed mechanism states that the LPS from periodontal pathogen may gain access to fetoplacental tissue via blood-borne pathways and may elicit an inflammatory response and prostaglandin cascade that causes uterine contraction and precipitate preterm labour or premature rupture of the membranes [8,9]. Two studies on animal model supported the index study finding by demonstrating translocation of periodontal pathogen Pg and Pg LPS to placental tissues, therefore increasing circulating and local proinflammatory markers [24,25]. In-vitro, Pg LPS significantly increased expression of Cyclo-Oxygenase (COX) -2, IL-8 and TNF-α, and HTR-8 trophoblasts in a Nuclear Factor kappa B (NF-κB) dependent fashion [24]. This led to increased maternal blood pressure, induced placental and fetal growth restriction, and increased fetal resorption [25].

In this study, all the postpartum mothers delivering LBW infant were selected from district government hospital, Garhwa, India. Garhwa district is located in the state of Jharkhand and has a high infant mortality rate of 33% and 20.7% of the population delivered LBW infant [26]. Therefore, this population can be considered as naïve and presence of periodontitis and LBW would be a common finding. Literature shows that studies that have been conducted in European countries, where the population has an easily available access to universal healthcare failed to show any positive association between periodontitis and PTLBW [20]. On the contrary, studies from Asian population, especially India showed a significant association between maternal periodontal disease and the risk of PTLBW [6]. For this study, both the groups were homogenous and may be considered free of selection bias. Both the groups were similar in respect to age, had a low socio economic background, mostly below poverty line, and the mothers had never visited the dentist in their lives. None of the mothers were alcohol abusers although one woman from case and three women from control used smokeless form of tobacco during their pregnancy. The women used chewable forms of tobacco like gutkha and khaini, as it is a social taboo for a woman to use tobacco products.

For this study we adopted WHO definition for mild periodontitis [17]. This definition was considered appropriate for this study as it allowed us to identify all mothers with periodontitis. This definition measures PPD, which denotes the progression of periodontitis. Another variation that can cause misclassification is the mouth examination strategy [27]. In the present study, six sites in all the teeth present were examined.

Birth weight is an important indicator to measure the vulnerability of a new born to the risk of childhood illness and chances of survival [28]. If periodontitis is found a significant factor for LBW infants, incorporation of its prevention and treatment in maternal health program could effectively reduce the incidence of infant mortality [28]. These results are of relevance for similar settings and should serve to promote interventions aimed at improving maternal care. Therefore, the government should take necessary measures to increase oral health awareness and implement oral and periodontal examination as a part of antenatal check up. Routine oral prophylaxis should be done for all women who are planning to conceive [9]. These measures can be implemented with minimal costs as they lead to improved systemic and maternal health.

Limitation(s)

The limitation of the study includes several confounding factors responsible for endotoxemia as well as LBW. All the potential known risk factors like women with maternal age under 18 years or over 35 years, women with systemic disease like diabetes and hypertension and genitourinary tract infections were excluded [3], but it was not possible to control all the confounding factors. Another cause of error could be the endotoxin kit used in this study and the results should be interpreted cautiously [29].

CONCLUSION(S)

The study provides evidence that periodontitis may be one of the causes for increased endotoxin production among mothers leading to LBW infant. These finding further highlight the importance of local and systemic bacterial infection among mothers and suggest that prevention or treatment of periodontitis may have beneficial effect on LBW. Future studies with the introduction of these measures, and a follow-up study should be done to assess the impact of these community interventions.

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