JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2020
VOL 11, NO. 1
10.5455/jcmr.2020.11.01.40

RESEARCH ARTICLE

Open Access

Clinical and Microbiological Risk Factors of Periodontal Diseases in the Occurrence of Premature Delivery

Hakobyan M.D[·]., Okoev G.G., Arustamyan K.K. Andriasyan L.H. Sharabchyan R.H.

[1]Teacher at the Department of Prosthodontics of Yerevan State Medical University after M. Heratsi, Microbiologist at Sirmed Medical Center,
[2]Doctor of Medicine, Professor, Head of the Research Center of Maternal and Child Health Protection,
[3]Doctor of Medicine, Professor, Deputy Head of Science at the Research Center of Maternal and Child Health Protection,
[4]Doctor of Medicine, Professor, Head of the Chair of Stomatology of the Armenian Medical Institute,
[5]Microbiologist at Sirmed Medical Center.

ABSTRACT

The aim of the research is a comparative study and contrasting of pathogenic bacterial composition in periodontal pockets and amniotic fluid in new mothers with normal and premature delivery terms.48 women with periodontal diseases underwent clinical and microbiological tests. 28 of the women gave premature births; and the rest 20, term births. The studies showed that the presence of severe periodontium inflammation, especially with a profound destructive pathological component (with the 4-score depth of periodontal pockets and tooth mobility), must be admitted as a risk factor for occurrence of premature delivery. This statement is even truer in cases of detection of prevotella bivia in relatively big amounts (in particular in Armenian population). In its turn, the findings presented above increase the diagnostic significance of microbiological testing of periodontal pocket content in relation to forecasting pregnancy outcome.

ARTICLE HISTORY

Received February 23 2020, Accepted March 17 ,2020
Published June 25, 2020

KEYWORDS

periodontal disease, periodontal pathogens, prevotella bivia, risk factors, premature delivery.

INTRODUCTION

Recent investigations have shown that microbiomes (the collective genetic diversity of bacterial compositions) of ecological niches have certain similarities. Specifically, on the basis of such similarities and other features the conclusion has been drawn that microbiomes of the oral cavity and the intestines can serve as the source for bacterial colonization of placenta [11]. At the same time, experimental studies have established [28] that there are significant differences between the bacterial composition of the microbiomes of amniotic fluid, placenta and vagina, which undoubtedly complicates both diagnostics (when it is done on the basis of studying the vaginal bacterial composition) and interpreting the results of microbiological tests in general. The above-mentioned considerations necessitate the search of alternative diagnostic possibilities, where the focus of particular studying and consideration would be the use of microbiological tests of the content from periodontal pathological pockets.

Studies have shown that at different terms of gestational period, depending on the diet and the peculiarities of hormonal shifts during pregnancy, there can be qualitative shifts in the composition of microbial symbiosis in the body, in particular periodontal pathogenic bacterial forms that directly correlate to quantitative increase in sex hormones [21].

This infection also has etiological significance for bacterial vaginosis and, consequently, for the occurrence of premature labor and random miscarriages. In this case primary importance is given both to the interbacterial imbalances in the mentioned ecological system (the decrease in the amount of lactobacillus and the abrupt increase of anaerobic pathogens) [18;32] and the quantitative and qualitative predominance of anaerobes; specifically Prevotella that can be active both on its own and as a part of a multi-infection – together

[·] **Contact** Hakobyan M.D. Department of Prosthodontics of Yerevan State Medical University after M. Heratsi, Microbiologist at Sirmed Medical Center,

with streptococcus, ureaplasma, colibacillus [24], and also in symbiosis with elatolically active bacteria – Peptostreptococcus, fusobacteria, bacteroides; among this last class prevotella bivia stands out in particular [27]. Studying the proportions of lactobacillus and anaerobic pathogens in new mothers with high and low probability of premature delivery occurrence showed that in the mentioned groups the decreased growth of lactobacillus and the increase in diversity of anaerobes (Prevotella, Peptoniphilus, Streptococcus and Dialister) was observed in 26% and 20% of cases [6]. It is generally considered that 1/3 of premature deliveries are preconditioned by subclinical infections, since in 25-40% of the examined women the signs of chorioamnionitis were revealed; the etiology of this condition is largely caused by prevotella [2]. On the basis of comparative epidemiological and microbiological studies, the increase in the occurrence of premature delivery associated with the imbalance between lactobacillus and anaerobic bacteria (Mobiluncus, Prevotella, Peptostreptococcus, Porphyromonas asaccharolytica, Fusobacterium nucleatum, Mycoplasma hominis, Gardnerella vaginalis) was also described by a number of other researchers [12;14].

Overall, there are quite many researches that assign different species of the prevotella group to the primary periodontal pathogens, point out their probable connection to premature delivery, describe possible mechanisms (including the ones on the basis of studying different pro-inflammatory markers); however, in most cases the role of such species of prevotella as nigrescens and intermedia were considered [20;29].

At the same time, the results of a number of clinical and microbiological tests contradict the presented data in a certain way. Thus, N. Buduneli et. al. [7] established that in view of the premature delivery occurrence, out of all periodontal pathogens P. micris and C. rectus must be admitted the risk factors, whereas certain species of prevotella (specifically nigrescens, which is also an acknowledged periodontal pathogen), as well as A. actinomycetemcomitans, on the contrary, lower the risk of premature delivery occurrence. In its turn, the morphological examination of placentae of women who gave premature birth and had periodontal diseases showed the presence of Fusobacterium nucleatum, but such bacteria forms as Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Aggregatibacter actinomycetemcomitans were not found [3].

On the one hand, the connection between premature delivery and probable translocation of certain pathogenic bacteria must be admitted as an obvious fact, but on the other hand, how much do periodontal diseases in new mothers matter in this regard? In addition, probable hematogenic translocation of bacterial toxins must also be considered as an adverse factor, especially since the results of some studies prove the importance of the cumulative effect of various bacteria on premature delivery occurrence [33].

AIM

The research was a comparative study and contrasting of pathogenic bacterial composition of periodontal pocket and amniotic fluid in new mothers with normal and premature delivery terms.

MATERIALS AND METHODS

The research was a case-control study. Clinical tests were conducted in 48 women aged 20 to 42. The gestational age was defined on the basis of the last period and ultrasound examination. Premature delivery was defined as the one occurring before the 37-th week of the gestational age and with the weight of a newborn lower than 2.5 kg. The subjects were divided into two groups: group A (main) – women with premature delivery (28 women); and group B (control) – 20 women with term delivery. That said, group B included women with approximately the same age and the ones who were clinically diagnosed with inflammatory periodontal pathologies. To eliminate possible interference of additional factors on the studied parameters, the study excluded women with diabetes, hypertension, various pregnancy complications, infectious diseases, and unhealthy habits (smoking, alcohol consumption).

The study was approved by the Ethics Committee of Erevan State Medical University and was conducted in 2018-19 at the Research Center of Maternal and Child Health Protection. In accordance with the Declaration of Helsinki of 2008, all subjects gave their written informational consent to participate in the study. The study was conducted with financial support from the State Science Committee of the Ministry of Education and Science of Armenia No. 18T-1F076 “Systemic influence of the chronic focus of a dental-periodontal complex infection on human body: diagnostic and preventative approaches”.

Clinical testing

Clinical tests were run at the Research Center of Maternal and Child Health Protection. To evaluate the periodontal status of new mothers, a standard set of parameters was used, specifically the parameters of dental mobility, gingival bleeding and the depth of pathological pockets (by Kötschke), and the parameters of gingival inflammation (index PMA by Parma, index GI by Löe-Silness) and oral cavity hygiene (index OHI-S

by Green-Vermillion). In all cases the examinations were conducted on the lower frontal teeth.
To avoid a possible subjective error when defining stomatological parameters, each examination was conducted twice by two qualified specialists separately.

Microbiological testing

Microbiological tests were run at Sirmed Medical Center. The materials for microbiological testing were the contents of periodontal pockets (sampling was conducted with special sterile swabs intended for this procedure and produced by APTACA, Italy) and amniotic fluid (sampling was conducted with regular sterile phials at the time of labor or C-section). The cotton swabs with periodontal pocket biomaterial were soaked in 9 ml of saline solution and culturing was performed. Amniotic fluid was not diluted. Isolation was performed on the following culture media – Table 1.

Table 1: Culture media and detectable microorganisms

Medium	Detectable microorganisms
Blood agar (5% sheep blood agar)	All bacteria
Schaedler K Agar (Sheep Blood 5%)	Gram-positive and gram-negative anaerobes
Schaedler CNA Agar (Sheep Blood 5%)	Gram-positive anaerobes
Schaedler KKV Agar (Sheep Blood 5%)	Gram-negative anaerobes
Chocolate agar	HACEK bacteria and other microorganisms
Chocolate Bacitracin agar	Neisseria spp, Haemophilus spp and other microorganisms
Endo agar	Bacteria of the Enterobacteriaceae family
Mannitol salt agar	Staphylococcus spp
Sabouraud dextrose agar + chloramphenicol	Yeast (Candida) and other fungi

Then cultures were enriched with thioglycol fluid. In cases of no growth after primary isolation, in 24 hours new culturing was made from the enriched medium. Inoculation dishes were put in corresponding conditions for a certain microorganism group: in an anaerobic jar – for anaerobes; in microaerophilic conditions – for corresponding bacteria; other dishes were put in a thermostat at 37^0C.

For the study we used culture media of trademarks Liofilchem (Italy) and BioMerieux (France), as well as an automatic analyzer Vitek 2 compact (France). The results of clinical tests were statistically processed and analyzed by Student's method. We calculated the validity coefficient for difference in values (t), conducted variance analysis (One-Way ANOVA, F-test), bivariant correlation analysis and defined the value of odds ratio. The statistic analysis was conducted with the help of the SPSS Statistics 17 software

RESULTS AND DISCUSSION

The results of the clinical tests of the periodontal tissue state in both groups are presented in Table 2.

Table 2: The parameters of the periodontal tissue clinical state in both groups.

Parameter	Groups					
	A /n=28/	B /n=20/	*t*	*F**	*p*	R**
Age	27,14±1,07	27,80±1,19	0,41	1,563	>0,1	,058
Delivery terms (weeks)	31,78±0,53	39,05±0,19	12,98	-	<0,001	-
Weight of the newborn (r)	1832,14±66,15	3404,5±79,74	15,18	-	<0,001	,826
OHI-S	1,86±0,18	1,4±0,17	1,84	2,308	>0,05	-,153
Gingival bleeding	1,78±0,14	1,7±0,14	0,4	0,427	>0,1	-,123
PMA	2,07±0,17	1,5±0,13	2,71	1,147	>0,01	-,127
GI	2,25±0,14	1,85±0,13	2,1	0,992	>0,02	-,114
Tooth mobility	0,96±0,15	0,65±0,13	1,55	2,173	>0,1	-,135
Depth of periodontal pockets	3,78±0,46	2,7±0,29	2,00	11,057	>0,05	-,288

* - Fisher's ratio test: the parameter "delivery term" was chosen as a variable,
** - Pearson correlation coefficient, correlation was established between the delivery terms and the corresponding parameters in the table.

The studies of the oral cavity hygiene parameters and the clinical state of periodontium showed that intergroup differences (between group A and B) are quite strongly expressed and are statistically

accurate. Thus, in group A the median values of index parameters of oral cavity hygiene (OHI-S) and gum inflammation (GI and PMA) were higher than in group B correspondingly by 25,0 %, 17,28 % and 27,5 %. In this regard the only exception was the parameter of gingival bleeding, which did not differ much between the groups and was virtually the same. As for the parameters that describe the destructive component of a pathological process in periodontal tissue, it must be noted that there were distinct differences between the groups – with the worst values in group A. Specifically, the parameter of tooth mobility in this group was more than 32 % higher than in group B, and the depth of the pathological pocket in subjects of group A was higher by 28,6 %. The significant differences between the groups in relation to the mentioned parameters are also demonstrated by the data of variance analysis that detected the value of Fisher's test (F-test) of 2,173 (in case of tooth mobility) and 11,057 (in case of the depth of periodontal pockets). Regarding the evaluation of periodontal diseases risks and their separate clinical parameters for the premature delivery occurrence, it must be noted that first we should consider the severe forms of periodontitis because the risk factors were the presence of an inflammatory component higher than 2 scores (for GI – OR=1,53 and for PMA – OR=2,2) and, even more so, the expression of the destructive component of periodontal pathology; that is, clinical signs of tooth mobility (OR=1,4) and the depth of pathological pockets higher than 4 scores (OR=5,4).

Table 3: The occurrence rate of microorganisms in periodontal pockets and amniotic fluid

Microorganism species	Tested samples and groups Content of periodontal pockets		Amniotic fluid	
	A (n=28) (quantity/%)	B (n=20) (quantity/%)	A (n=28) (quantity/%)	B (n=20) (quantity/%)
Eikenella corrodens	11 / 39,3	7 / 35,0	-	-
Streptococcus oralis/mitis	28 / 100,0	20 / 100,0	-	-
H.parainfluenzae	25 / 89,3	20 / 100,0	-	-
Veillonella	9 / 32,1	7 / 35,0	-	-
Str.sanguinis	7 / 25,0	6 / 30,0	-	-
Kocuria rosea	3 / 10,7	1 / 5,0	-	-
Neisseria sicca	23 / 82,1	17 / 85,0	-	-
Actinomyces meyeri	7 / 25,0	4 / 20,0	3 / 10,7	-
Prevotella bivia	21 / 75,0	7 / 35,0	20 / 71,4	-
Bacteroides fragilis	3 / 10,7	1 / 5,0	-	-
Candida albicans	8 / 28,6	5 / 25,0	3 / 10,7	2 / 10,0
Propionibacterium propionicus	5 / 17,8	1 / 5,0	-	-
Moraxella group	2 / 7,1	-	-	-
Clostridium sporogenes	3 / 10,7	-	-	-
Fusobacterium nucleatum	6 / 21,4	3 / 15,0	-	-
Staph. Aureus	4 / 14,3	1 / 5,0	-	-
Corinebacterium striatum	3 / 10,7	1 / 5,0	1 / 3,6	-
Prevotella disiens	5 / 17,8	3 / 15,0	-	-
Haemophilus haemolyticus	4 / 14,3	3 / 15,0	-	-
Peptostr. anaerobius	3 / 10,7	-	-	-
Lactobacillus	-	-	8 / 28,6	18 / 90,0
Enterococcus faecalis	-	-	2 / 7,1	-
Pseudomonas aeruginosa	-	-	2 / 7,1	-
Shewanella putrefaciens	-	-	-	4 / 20,0
Staph. epidermidis	-	-	5 / 17,8	11 / 55,0
Gardnerella vaginalis	-	-	2 / 7,1	3 / 15,0
Esherichia coli	-	-	2 / 7,1	-

As for the results of microbiological tests, which are presented in Table 3, first, we must notice that there were 20 species of microorganisms isolated from periodontal pockets, the most common among which were such acknowledged periodontal pathogens as prevotella bivia, eikenella corrodens, veillonella, actinomyces meyeri, fusobacterium nucleatum, and also oral cavity resident flora – streptococcus oralis/mitis, haemophilus parainfluenzae, str.sanguinis, fungi of candida genus. In amniotic fluid there were 11 bacterial species isolated, 4 of which were also found in periodontal pockets (prevotella bivia, actinomyces meyeri, candida, corinebacterium striatum), and the rest of the species were concentrated solely in this biological fluid.

In both groups the most common bacteria found in periodontal pockets were Streptococcus oralis/mitis, Haemophilus parainfluenzae and Neisseria sicca (more than 80 %), but they were never found in amniotic fluid. We have not found any data about potential connection of the above-mentioned opportunistic bacteria to premature delivery occurrence in scientific databases. However, one particular study reports the clinical case of premature delivery with isolation of Haemophilus parainfluenzae in large amounts in vaginal smears [25].

As for such pretty commonly found known periodontal pathogens as Eikenella corrodens, Veillonella and Fusobacterium nucleatum, it must be noted that these bacteria were found at pretty much the same rate in both groups, but only in the contents of periodontal pockets.

Let us focus more on the role of prevotella in the occurrence of premature delivery. Prevotella is a group of rod-like anaerobic and gram-negative bacteria (that comprises approximately 50 microorganism species) that being a part of normal microflora of the oral cavity, upper airways, vagina and other body parts can at the same time cause inflammatory diseases in that body parts.

T. Paramel Jayaprakash et. al. [30] and A. Subramaniam et. al. [34] found bacteria of the prevotella group in amniotic fluid of all women who gave premature births. In experimental conditions (in rabbits) with intrauterine inoculation of the isolated monoculture of Prevotella bivia, the progression of intrauterine infection and premature delivery was observed in 33% of cases [10].

In our study (as can be seen from Table 3) in women who gave premature births prevotella bivia was found in periodontal pockets in 75% of cases. Only in one case this microorganism was not found in amniotic fluid. In group B prevotella bivia was found in almost one third of the subjects, but with no trace of these bacteria in amniotic fluid. A conclusion can be drawn that finding prevotella bivia in periodontal pockets of pregnant women must already be seen as a risk factor for premature delivery occurrence (OR=1,20).

Thus, among the subjects of the group with premature deliveries the following periodontal pathogens were found in amniotic fluid: prevotella bivia in 20 women; Actinomyces meyeri in 3 women; Candida albicans in 3 women; in the rest 2 cases we could not establish any casual relationships between periodontal pathogens and premature deliveries. By the way, the mentioned 2 cases of premature deliveries (not associated with periodontal pathogens) can be the consequence of the presence of such opportunistic forms as Enterococcus faecalis, Pseudomonas aeruginosa and Esherichia coli in amniotic fluid, especially since similar assumptions were set forth by some authors [16;35].

It was established that by activating phospholipase A2 prevotella bivia stimulates the phospholipid metabolism in endometrium, which in itself can be a stimulus for labor [26]. In cases of premature delivery the amount of prevotella bivia and other bacteria in amniotic fluid directly correlates to the amount of certain cytokine in the same fluid – IL-1, IL-6 and IL-8 [23]. Admitting the indisputable contribution of prevotella to causing bacterial vaginosis, A. Briselden et. al. [5] believe that in the issue of the probable mechanism of transferring the infection from lower genital tracts and progression of the disease the increase in activity of bacterial sialidases (neuraminidases) is of particular importance.

Interestingly, prevotella bivia was found in bacteriological testing of placental blood in cases of premature delivery through C-section [4]. This in its turn can attest to the possibility of hematogenic translocation of prevotella bivia into amniotic fluid from other colonization focuses. By the way, this possibility is theoretically not ruled out by some researchers that believe that in cases of inflammatory periodontal diseases pathogenic bacteria (naming fusobacterium nucleatum as an example) can penetrate amniotic space, causing premature delivery [13]. P. Madianos et. al. [22] studied the interconnection of "orange" (Campylobacter rectus, Fusobacterium nucleatum, Peptostreptococcus micros, Prevotella nigrescens, Prevotella intermedia) and "red" (Porphyromonas gingivalis) complexes of pathogenic bacteria and umbilical cord blood antibodies (IgG, IgM) with premature delivery in new mothers. Their findings were pretty curious. For example, the incidence of the mentioned periodontal pathogens in compared groups (term delivery versus premature delivery) statistically were not different, but in premature deliveries the levels of IgM to C. rectus and P. intermedia exceeded the norm by several times. Moreover, the occurrence of premature delivery

was also connected to the absence of IgG to the "red" complex bacteria in the women's blood. On the basis of the obtained results the authors try to confirm the hypothesis that in the absence of maternal immunoglobulin protection a pathogenic periodontal infection can be transferred to the fetus or amniotic fluid, which causes premature delivery. On the basis of certain morphological and biochemical peculiarities of prevotella bivia found in amniotic fluid, some researchers even suggested singling out a new subtype of prevotella – prevotella amnii [19].

In addition, let us note that in previous studies we established that in the content of periodontal pathogenic flora in Armenian population the most pathogenic were bacteria of prevotella and veillonella groups [1].

Since out of all periodontal clinical signs the pocket depth, tooth mobility, the PMA and GI index values turned out to be the risk factors for premature delivery occurrence, we also evaluated the detection rate of the bacterial forms found in amniotic fluid in the group of subjects with high values of these parameters. The results are presented in Table 4.

Table 4: The detection rate of some pathogens in amniotic fluid in group A with high and low values of periodontal parameters.

Periodontal values	Pathogenic bacteria Prevotella bivia (n/%)	Actinomyces meyeri (n/%)	Candida albicans (n/%)
Pocket depth:			
>4 scores (n=18)	15 / 83,33	2 / 11,11	1 / 5,55
<4 scores (n=10)	5 / 50,0	1 / 10,0	2 / 20,0
Tooth mobility:			
>1 score (n=19)	18 / 94,47	2 / 10,52	1 / 5,26
<1 score (n=9)	2 / 22,22	1 / 11,11	2 / 22,22
PMA:			
>2 scores (n=18)	16 / 88,89	1 / 5,55	0 / 0
<2 scores (n=10)	4 / 40,0	2 / 20,0	3 / 30,0
GI:			
> 2 scores (n=23)	20 / 100,0	1 / 4,35	1 / 4,35
<2 scores (n=5)	0 / 0	2 / 40,0	2 / 40,0

As for detection of Actinomyces meyeri in amniotic fluid, it must be noted that we found only one relatively recent study that connects premature delivery due to necrotizing funisitis to the mentioned gram-positive bacterium [36]. Another study described premature delivery associated with acute necrotizing chorioamnionitis caused by Actinomyces spp. [9]. Analyzing scarce scientific data on the contribution of actinomyces bacterial group in the occurrence of premature delivery, S Estrada et. al. [8] conclude that actinomycete infection rarely accompanies pregnancy, but its presence must be admitted as a driving factor for premature delivery.

Scientific literature also describes the cases of premature delivery that are probably associated to candidal chorioamnionitis [15;17;31].

In our studies the detection of the mentioned pathogens in amniotic fluid in women who gave premature birth was quite rare (10,7 %). However, in amniotic fluid of particularly these subjects we found no prevotella bivia, though this pathogen was very common in other cases. This indicates with a certain level of probability that these microorganism forms can be the cause in the occurrence of premature delivery. Though it must be noted that Candida fungi were found in two cases in group B.

CONCLUSION

Thus, the clinical and microbiological tests that we conducted showed that the presence of periodontal inflammation in severe forms, especially with a profound destructive pathological component (with the 4-score depth of periodontal pockets and tooth mobility), must be admitted as a risk factor for occurrence of premature delivery. This statement is even truer in cases of detection of prevotella bivia (in particular in Armenian population). In its turn, the findings presented above increase the diagnostic significance of microbiological testing of periodontal pockets content in relation to forecasting pregnancy outcome.

REFERENCES

1. Andriasyan L.H., Papayan L.R., Zohrabyan A.E., Andriasyan J.L. (2014). Dependence of certain types of periodontal pathogenic microorganisms on ethnicity, *New Armenian medical Journal,* 2014, 8,3, 59-65,
2. Baldwin E., Walther-Antonio M., MacLean A., Gohl D., Beckman K., Chen J., White

B., Creedon D., Chia N. (2015). Persistent microbial dysbiosis in preterm premature rupture of membranes from onset until delivery. *Peer. J.* Nov 26;3:e1398. doi: 10.7717/peerj.1398. eCollection 2015,

3. Blanc V., O'Valle F., Pozo E., Puertas A., León R., Mesa F. (2015). Oral bacteria in placental tissues: increased molecular detection in pregnant periodontitis patients. *Oral Dis.,* 21 (7), 905-12,
4. Boggess K., Watts D., Hillier S., Krohn M., Benedetti T., Eschenbach D. (1996). Bacteremia shortly after placental separation during cesarean delivery. *Obstet. Gynecol.,* 87 (5 Pt 1), 779-84,
5. Briselden A., Moncla B., Stevens C., Hillier S. (1992). Sialidases (neuraminidases) in bacterial vaginosis and bacterial vaginosis-associated microflora. *J. Clin. Microbiol.,* 30 (3), 663-6,
6. Brown R., Al-Memar M., Marchesi J., Lee Y., Smith A., Chan D., Lewis H., Kindinger L., Terzidou V., Bourne T., Bennett P., MacIntyre D. (2018). Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. *Transl Res.* Dec 27. pii: S1931-5244(18)30238-X. doi: 10.1016/j.trsl.2018.12.005. [Epub ahead of print],
7. Buduneli N., Baylas H., Buduneli E., Türkoğlu O., Köse T., Dahlen G. (2005). Periodontal infections and pre-term low birth weight: a case-control study. *J. Clin. Periodontol.,* 32 (2), 174-81,
8. Estrada S.M., Magann E.F., Napolitano P.G. (2017). Actinomyces in Pregnancy: A Review of the Literature. *Obstet Gynecol Surv.* Apr;72(4):242-247. doi: 10.1097/OGX.0000000000000423,
9. Fitzhugh V.A., Pompeo L., Heller D.S. (2008). Placental invasion by actinomyces resulting in preterm delivery: a case report. *J Reprod Med.* Apr;53(4):302-4,
10. Gibbs R., McDuffie R. Jr, Kunze M., Barr J., Wolf D., Sze C., Shikes R., Sherman M. (2004). Experimental intrauterine infection with Prevotella bivia in New Zealand White rabbits. *Am. J. Obstet. Gynecol.,* 190 (4), 1082-86,
11. Gomez-Arango L., Barrett H., McIntyre H., Callaway L., Morrison M., Nitert M. (2017). Contributions of the maternal oral and gut microbiome to placental microbial colonization in overweight and obese pregnant women. *Sci. Rep.,* 6, 7 (1):2860. doi: 10.1038/s41598-017-03066-4,
12. Hecht J., Onderdonk A., Delaney M., Allred E., Kliman H., Zambrano E., Pflueger S., Livasy C., Bhan I., Leviton A. (2008). ELGAN Study Investigators. Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatr. Dev. Pathol.,* 11 (1), 15-22,
13. Hill G. (1998). Preterm birth: associations with genital and possibly oral microflora. *Ann. Periodontol.,* 3 (1), 222-32,
14. Holst E., Goffeng A., Andersch B. (1994). Bacterial vaginosis and vaginal microorganisms in idiopathic premature labor and association with pregnancy outcome. *J. Clin. Microbiol.,* 32 (1), 176-86,
15. Ito F., Okubo T., Yasuo T., Mori T., Iwasa K., Iwasaku K., Kitawaki J. (2013). Premature delivery due to intrauterine Candida infection that caused neonatal congenital cutaneous candidiasis: a case report. *J Obstet Gynaecol Res.* Jan;39(1):341-3. doi: 10.1111/j.1447-0756.2012.01938.x. Epub 2012 Jul 6,
16. Kaambo E., Africa C.W.J. (2017). The Threat of Aerobic Vaginitis to Pregnancy and Neonatal Morbidity. *Afr J Reprod Health.* Jun;21(2):108-118,
17. Khadija B., Abbasi A., Khan S., Nadeem M., Badshah L., Faryal R. (2019). Isolation of pathogenic Candida species from oral cavity of postpartum females, and its association with obstetric and dental problems. *Microb Pathog.* Jun;131:40-46. doi: 10.1016/j.micpath.2019.03.022. Epub 2019 Mar 21,
18. Kimberlin D., Andrews W. (1998). Bacterial vaginosis: association with adverse pregnancy outcome. *Semin. Perinatol.,* 22 (4), 242-50,
19. Lawson P., Moore E., Falsen E. (2008). Prevotella amnii sp. nov., isolated from human amniotic fluid. *Int. J. Syst. Evol. Microbiol.,* 58 (Pt 1), 89-92,
20. Lin D., Moss K., Beck J., Hefti A., Offenbacher S. (2007). Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. *J. Periodontol.,* 78 (5), 833-41,
21. Lin W., Jiang W., Hu X., Gao L., Ai D., Pan H., Niu C., Yuan K., Zhou X., Xu C., Huang Z. (2018). Ecological Shifts of Supragingival Microbiota in Association with Pregnancy. *Front. Cell Infect. Microbiol.,* Feb 15;8:24. doi: 10.3389/fcimb.2018.00024. eCollection 2018,
22. Madianos P., Lieff S., Murtha A., Boggess K., Auten R. Jr, Beck J., Offenbacher S. (2001). Maternal periodontitis and prematurity. Part II: Maternal infection and fetal exposure. *Ann. Periodontol.,* 6 (1), 175-82,
23. Marconi C., de Andrade Ramos B., Peraçoli J., Donders G., da Silva M. (2011). Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. *Am. J. Reprod. Immunol.,* 65 (6), 549-56,

24. McDonald H., Chambers H. (2000). Intrauterine infection and spontaneous midgestation abortion: is the spectrum of microorganisms similar to that in preterm labor? *Infect. Dis. Obstet. Gynecol.,* 8 (5-6), 220-27,
25. Mendz G.L., Petersen R., Quinlivan J.A., Kaakoush N.O. (2014). Potential involvement of Campylobacter curvus and Haemophilus parainfluenzae in preterm birth. *BMJ Case Rep.* Oct 1;2014. pii: bcr2014205282. doi: 10.1136/bcr-2014-205282,
26. Mikamo H., Kawazoe K., Sato Y., Imai A., Tamaya T. (1998). Preterm labor and bacterial intra-amniotic infection: arachidonic acid liberation by phospholipase A2 of Prevotella bivia. *Anaerobe,* 4 (5), 209-12,
27. Mikamo H., Kawazoe K., Sato Y., Tamaya T. (1999). Elastase activity of anaerobes isolated from amniotic fluid with preterm premature rupture of membranes. *Am. J. Obstet. Gynecol.,* 180 (2 Pt 1), 378-80,
28. Moore S., Ericsson A., Poock S., Melendez P., Lucy M. (2017). Hot topic: 16S rRNA gene sequencing reveals the microbiome of the virgin and pregnant bovine uterus. *J. Dairy. Sci.,* 100 (6), 4953-60,
29. Offenbacher S., Lin D., Strauss R., McKaig R., Irving J., Barros S., Moss K., Barrow D., Hefti A., Beck J. (2006). Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: a pilot study. *J. Periodontol.,* 77 (12), 2011-24,
30. Paramel Jayaprakash T., Wagner E., van Schalkwyk J., Albert A., Hill J., Money D.: PPROM Study Group. (2016). High Diversity and Variability in the Vaginal Microbiome in Women following Preterm PrematureRupture of Membranes (PPROM): A Prospective Cohort Study. *PLoS One.* Nov 18;11(11):e0166794. doi: 10.1371/journal.pone.0166794. eCollection 2016,
31. Qureshi F., Jacques S.M., Bendon R.W., Faye-Peterson O.M., Heifetz S.A., Redline R., Sander C.M. (1998). Candida funisitis: A clinicopathologic study of 32 cases. *Pediatr Dev Pathol.* Mar-Apr;1(2):118-24,
32. Romanik M., Martirosian G. (2004). Frequency, diagnostic criteria and consequences of bacterial vaginosis in pregnant women. *Przegl. Epidemiol.,* 58 (3), 547-53,
33. Shira Davenport E. (2010). Preterm low birthweight and the role of oral bacteria. *J. Oral Microbiol.,* Dec 21;2. doi: 10.3402/jom.v2i0.5779,
34. Subramaniam A., Kumar R., Cliver S., Zhi D., Szychowski J., Abramovici A., Biggio J., Lefkowitz E., Morrow C., Edwards R. (2016). Vaginal Microbiota in Pregnancy: Evaluation Based on Vaginal Flora, Birth Outcome, and Race. *Am. J. Perinatol.,* 33 (4), 401-8,
35. Tarana M.N., Shamsuzzaman S.M. (2018). Laboratory Diagnosis of Bacterial Vaginosis and Potential Pathogens Other Than Group B Streptococcus in Vaginal Swab of Pregnant Women in Dhaka Medical College Hospital. *Mymensingh Med J.* Oct;27(4):834-842,
36. Wright JR Jr., Stinson D., Wade A., Haldane D., Heifetz SA. (1994). Necrotizing funisitis associated with Actinomyces meyeri infection: a case report. *Pediatr Pathol.* Nov-Dec;14(6):927-34.