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**INVOLVEMENT OF INFLAMMATORY MEDIATORS IN THE
PERIODONTAL PATHOLOGY DURING PREGNANCY WITH
RISK FOR PREMATURE DELIVERY**

~ ABSTRACT ~

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STATE OF THE ART

Periodontitis is a chronic inflammatory disease caused by an infection of the teeth-supporting tissues. The broad term of “periodontal disease” also includes other conditions, such as the presence of gingivitis, an irreversible state, which is diagnosed by the presence and degree of the gingival inflammation, frequently diagnosed by tooth drill bleeding.

The classical theory of “focal infection” speculates that the oral infection foci might be a factor contributing to the triggering of the systemic inflammatory response. Investigators stated the idea that the translocation of periodontal hematogenic bacteria and their products, or the pro-inflammatory mediators in the periodontal infection sites of fetal membranes, placenta and amniotic cavity, could induce these pathological processes that might lead to complications.

A series of studies in periodontal medicine have focused on finding some connections between periodontal disease and adverse side-effects during pregnancies, such as preeclampsia, low birth weight . [1] or premature delivery.

The bacterial environment in the oral cavity has evolved for thousands of years through a cohabitation in a perfect bacteria-human being symbiosis, beneficial for both sides. There has been proven that the oral bacterial environment in the new-born may nourish the intestinal bacterial environment, similar to the one in the oral cavity, the result being the formation of intestine specific bacteria only 2 weeks after birth. [2]

Lots of studies have reported an association between maternal periodontal disease and the risk over pregnancy evolution [3]. Recent discoveries on bacterial identifying methods have shown that oral niches are replaced by more than 6 billion bacteria, representing approximately 700 species.

The risk for women of developing a periodontal disease during pregnancy, under the influence of maternal hormones, is quite high. Studies indicate that the periodontal infection may lead to fetal and placental exposure when this is coupled with a fetal inflammatory response, possibly leading to a premature delivery [4]. There has been reported that, in pregnant women, periodontitis and/ or gingivitis are simultaneous clinical conditions. As such, the periodontal disease exacerbation and remission during pregnancy determine unpredictable side-effects upon pregnancy.[5].

Ever since 1996, researchers have reported the existence of a relation between maternal periodontal disease and a premature new-born baby. Periodontal disease is an infection with Gram-negative anaerobic bacteria of the oral cavity, affecting up to 90% of the population [6], being observed as higher in pregnant women.

The components of the microbial plaque have the ability to induce the initial inflammatory infiltrate, including lymphocytes, macrophages and PMN. Microbial components, especially lipopolysaccharides (LPS), have the ability to activate macrophages to synthesize and secrete a large number of molecules, including cytokines like IL-1 and TNF-alpha, prostaglandins, especially PGE2, and sub-gingival pathogenic enzymatic agents. Sub-gingival periodontal pathogens affect the local and systemic immune response. The local inflammatory response of these Gram-negative bacteria is characterized by the infiltration of periodontal tissues with inflammatory cells, including polymorphonuclear leukocytes, macrophages, lymphocytes and plasma cells [7].

There have been performed various studies all over the world in order to identify the association between periodontal disease and adverse side-effects during pregnancy in women. A group study performed by Mobeen [8] established that pregnant women have a high level of dental diseases, both moderate and severe ones. Also, fetus morbidity, perinatal and neonatal morbidity increase alongside the severity of the periodontal disease. A study performed by Saddki [9] revealed that the relative risk for preterm deliveries was 4.2 times higher in women with periodontitis, in comparison to those not suffering from periodontitis (95% CI: 2.01-9.04).

There was observed that, in the second pregnancy trimester, the rate of Gram-negative anaerobic bacteria in the dental plaque significantly increases in relation to the aerobic bacteria. In the same way, in the second pregnancy trimester, the level of anti P. Gingivalis serum is associated with premature deliveries and low fetal weight [10].

The part played by the host inflammatory reaction is a critical factor of exaggerated sensitivity and severity. Studies show that the level of reactive C-protein depends on the severity of chronic periodontal infection, being even higher in women with preeclampsia [11].

It is necessary to draw the attention of medical professionals (especially of obstetricians, as, most of the time, they are the only doctors communicating with the patient) in order to advise pregnant women to go to a dentist for evaluation, orientation and treatment, if needed.

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Material and method

The prospective study performed comprised two groups of female patients selected from the cases of the Clinic of Obstetrics-Gynecology within the “Filantropia” Hospital of Craiova, between 2011 and 2014. During this period, the patients were included in the study after filling in the initial evaluation record and signing an informed consent, also being subjected to an investigation protocol through the establishment of a set of quantifiable, specific parameters for case monitoring. There were selected a total number of 109 pregnant women in the 3rd trimester of pregnancy, divided into three groups, such as:

1. a group of 56 pregnant women with gingivitis
2. a group of 26 pregnant women with periodontitis, of which:
 - 16 cases of mild chronic marginal periodontitis = mild CMP
 - 8 cases of medium chronic marginal periodontitis = medium CMP
 - 2 cases of severe chronic marginal periodontitis = severe CMP
3. a control group of 27 healthy pregnant women

For every patient who accepted to be included in the study we performed a personal periodontological control record of the pregnant woman.

At the beginning of the study, we observed that there were no predictable risks for the patients, with obvious advantages. The evaluation of the periodontal status was performed by a clinical general examination, a local examination, and also through paraclinical investigations that may explain any periodontal conditions and may clarify the factors involved in the emergence of clinical signs of the periodontal disease, factors related to the local conditions of the patients included in the study.

For the data processing there was used the Microsoft Excel Program (Microsoft Corp., Redmond, WA, USA), together with XLSTAT for MS Excel (Addinsoft SARL, Paris, France) and the IBM SPSS Statistics 20.0 Program (IBM Corporation, Armonk, NY, USA).

Results

Biostatistical analysis of the studied groups

The demographic aspects and lifestyle have great importance in associating dental pathology with pregnancy complications. The correlation of the mother's age with the periodontal disease shows that age is directly involved and correlated with the dental pathology, thus, with age, there also increases the risk for developing a periodontal disease, as hormonal changes during pregnancy may aggravate a pre-existent gingivitis ($p=0.040$, so $p<0.05$).

Persons with a higher education are usually employed; they have a constant income and tend to use healthy oral services more often. On the contrary, persons with low incomes have financial barriers in accessing oral healthcare, they are less aware of the need of dental care; they have more chances of becoming smokers and having a poor lifestyle. Although there may be observed some variations regarding the education level of the patients in the four sub-groups, overall we cannot state that these differences are statistically significant, as the result of the Chi square test is $p=0.441$ $p> 0.05$.

Pregnant women present a higher risk for cavities, due to different reasons, including high acidity in the oral cavity, together with a tendency for sweet food and prenatal interest decrease regarding the oral healthcare. Also, dental plaque or calculus contains various types of bacteria, some of these being associated with the development of gingival diseases. The statistical analysis showed us that the presence of cavity and calculus in pregnant women is highly statistically significant, $p<0.001$, therefore we may state that the presence of cavities and calculus may be a risk factor for the onset of periodontal disease.

Smoking and alcohol intake are the single major risk factors for periodontal disease that may be prevented. Even though in the specialty literature, smoking is presented as a major risk factor for periodontal disease, in our study we found a statistical representation of this parameter ($p>0.05$). This fact is because it is known that the risk for periodontal diseases increases with the number of the number of cigarettes per day, in our case the pregnant women stating they smoked only 1-3 cigarettes/ day. Instead, we observed that among the four sub-groups there are high significantly differences regarding the alcohol intake $p<0.001$, the patients in the medium and severe CMP groups clearly stating that they were higher alcohol consumers, even though in reduced or moderate quantities, than those in the mild CMP group.

In order to establish whether oral hygiene of the studied patients has influenced the periodontal disease onset, we investigated this parameter and observed that oral hygiene is indeed a parameter significantly varying among the four groups, the result of the Chi square test being highly statistically significant ($p < 0.001$). It was also observed that the greatest difference was found between the control group and all the other groups, and also that the percentage of deficient oral hygiene increased together with the severity of the periodontal condition.

For staging the evaluation of clinical conditions and monitoring the obtained results in various populations, we used the periodontal indices for assessing gingivitis, periodontitis, and also parameters for assessing both of the conditions.

For the gingival index, the plaque index, the gingival bleeding index, Russell's periodontal index, we obtained highly statistical significant differences among the four groups, with values of p ANOVA < 0.001 , with existing differences between any other sub-groups after a subsequent performance of the post-hoc Fisher LSD Test. Also, for all the three compartments, namely gingival, plaque and calculus, from the Ramfjord periodontal affecting index, we obtained highly statistically significant differences among the four groups, with values of p ANOVA $p < 0.001$.

Analysis of various systemic inflammation markers in periodontal disease related to pregnancy

Due to the associations between infection, inflammation and premature delivery, preeclampsia and intra uterine growth restriction, researchers have tried to identify the inflammatory biomarkers that may have a predictive character over these complications.

Justifying documents show that the association between periodontitis and reactive C protein (RCP) is based only on studies in men and non-pregnant women. There are no studies on periodontitis and RCP in pregnant women. This is an important area for the study, as systemic inflammation plays an important part in the pathogenesis of premature delivery, including preeclampsia or intra uterine growth restriction.

By analyzing the distribution of premature delivery according to the type of the periodontal disease, we observed an increase of the incidence of premature delivery in the cases with mild, medium and severe CMP in comparison to the other groups, the result of the Chi square test, $p = 0.00032$, $p < 0.001$, being highly statistically significant.

The statistical analysis of the intrauterine growth restriction (IUGR) distribution has shown that pregnant women in the groups of medium and severe CMP presented a highly significant percentage of IUGR than the other categories, the result of Chi square test being $p=0.00035$, $p<0.001$, highly statistically significant.

The statistical analysis of pregnant women developing preeclampsia showed us that the patients with medium and severe CMP tended to significantly have more cases of preeclampsia than the other groups, the result of the Chi square test, $p=0.00061$, $p<0.001$, being highly statistically significant.

As the cut-off for the reactive C protein, we chose the value of 2.5 mg/dl, a value given by the performed statistical analysis. By performing the ANOVA test for comparing the RCP values, we observed highly statistically significant differences among the groups, $p<0.001$. By continuing the analysis with the post-hoc Fisher LSD Test, we established that the groups with gingivitis and mild CMP had higher significantly values than the control group, and also that they had lower significantly values than the group of medium and severe CMP. The statistical analysis of plasma RPC in pregnant women delivering prematurely showed us that there was no useful connection between the high RCP value and the probability of a premature delivery, even if we found values > 2.5 mg/dl in most of the cases of premature delivery. This test low accuracy, namely 46.79%, as well as its low specificity, 26.03%, make that this test alone show no precision in determining the probability of a premature delivery. Only the predictive negative value may have a practical use, as it shows us that at values under 2.5 mg/dl, only 17.39 % of pregnant women may have a premature delivery. There has also been analyzed the RCP involvement in preeclampsia, but the result of Chi square test exceeded the significant threshold value ($p>0,05$), so there is no useful connection between the high RCP value and the probability of preeclampsia onset. Using a RCP value >2.5 as a test for preeclampsia identifying is not recommended as a single test, as it presents low specificity and accuracy, namely 24.72% and 37.61%, respectively, but the 95% response makes us consider that it may be useful in relation to another test with a specificity over 75%. The predictive negative value of 95.65% shows that, if the RCP value < 2.5 we may almost exclude the preeclampsia onset. The RCP involvement in the intrauterine growth restriction was statistically analyzed and there was observed that the result of the Chi square test was significantly statistical, $p<0.05$, therefore there is the possibility that a high RCP value may be useful in IUGR detection. Using a RCP value >2.5 as IUGR

identification test is not recommended as a single test, as it presents low specificity and accuracy, namely 25.00% and 36.70%, respectively, but the 100% response makes us consider it useful in relation to another test with a specificity over 75%. As practice usefulness, the predictive negative value of 100% shows that, in the case of a RCP value below 2.5, we may exclude the IUGR onset.

There seems that patients with periodontal disease are more susceptible to have a rare form of the gene responsible for fibrinogen expression than the patients with no periodontal diseases.

The fibrinogen values, statistically establishing a cut-off of 5.4 g/l, have been highly statistically different among the 4 groups, p ANOVA <0.001 .

The statistical analysis of the plasma fibrinogen values in pregnant women delivering prematurely has shown a statistically significant result of the Chi square test, namely $p<0.05$, thus there exists the possibility that the identification of a high fibrinogen value may be useful in predicting a premature delivery. Nevertheless, the fibrinogen value > 5.4 does not seem to have any practical usefulness in premature delivery, having a low accuracy, namely 55.05%, a low specificity and response, namely 43.84% and 77.78%, respectively. The predictive positive and negative values do not have a useful statistical value. In pregnant women subsequently developing PE, the result of the Chi square test has exceeded the significance threshold value, $p>0.05$, therefore there is no useful connection between a high fibrinogen value and preeclampsia onset during pregnancy. Neither in PE a fibrinogen value > 5.4 seems to have a practical usefulness in detecting preeclampsia, due to the statistically insignificant values of the used statistical parameters. In IUGR, the result of the Chi square test has been statistically significant $p<0.05$, so there is the possibility that the identification of high fibrinogen values could be useful in detecting IUGR. The statistical analysis of the groups shows us that only the predictive negative value of 95.00% has a practical applicability, 95% of the patients with a fibrinogen value < 5.4 could have pregnancies without IUGR.

CONCLUSIONS

- Maternal age has been directly involved and correlated to dental pathology, the statistical analysis showing that there is a highly statistically significance of this parameter, $p=0.040$, thus $p<0.05$.
- The presence of cavities and calculus may be a risk factor for the periodontal disease onset, p Chi square= 0.000586 , $p<0.001$, which indicates a highly statistically difference.
- In our study, we observed a statistical representativity of the smoking-related parameter, but we also found highly significant differences regarding the alcohol intake, $p<0.001$.
- Oral hygiene is a parameter significantly varying among the four groups, the result of the Chi square test being highly statistically significant ($p<0.001$).
- For the Gingival Index, Gingival Bleeding Index, Rusell's Periodontal Index, Ramfjord Periodontal Index, Gingival Compartment, Plaque and Calculus Compartments, during the statistical analysis we obtained highly statistically differences among the four groups, with values of p ANOVA <0.001 .
- The groups with mild CMP and medium and severe CMP had a celarly higher percentage of premature deliveries in comparison to the other groups, the result of the Chi square test $p=0.00032$, $p<0.001$ being highly statistically significant.
- The statistical analysis showed us that pregnant women in the medium and severe CMP groups presented a significantly higher percentage of IUGR than the other groups, the result of the Chi square test being $p=0.00035$, $p<0.001$, a significantly statistical one.
- The statistical analysis showed us that patients with medium and severe CMP tended to have significantly more cases of preeclampsia than those in other groups, the result of the Chi square test $p=0.00061$, $p<0.001$, being highly statistically significant.
- By performing the ANOVA test for comparing the reactive C protein values, we observed, as expected, highly statistically differences among the groups ($p<0.001$).
- The fibrinogen values were highly statistically different among the 4 groups, p ANOVA, $p<0.001$
- Our study showed a high statistical significance between the periodontal disease and these two markers of systemic inflammation, but they cannot be used as predictive markers for pregnancy complications evolution, except for the case of intrauterine growth restriction.

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