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ORIGINAL ARTICLE

Clinical Parameters and aMMP-8-Concentrations in Gingival Crevicular Fluid in Pregnancy Gingivitis

VICKY EHLERS, ANGELIKA CALLAWAY, WAJIHA HORTIG, ADRIAN KASAJ, BRITA WILLERSHAUSEN

Department of Operative Dentistry, University Medical Center of the Johannes Gutenberg University of Mainz, Mainz, Germany

SUMMARY

Background: **During pregnancy hormonal changes may increase the risk for developing gingivitis. The aim of this study was to evaluate the signs of gingival inflammation and the enzyme activity of matrix metalloproteinase-8 (aMMP-8) in the gingival crevicular fluid of pregnant women.**
Methods: **After approval by the ethics commission, a total of 40 volunteers participated in the study; group 1 (n = 20, age: 32 ± 4 years) with pregnant women, and group 2 (n = 20, age: 30 ± 10 years) with age-matched non-pregnant women as controls. After obtaining anamnestic data, the dental examination included assessment of oral hygiene, gingival inflammation, probing pocket depth, and recession. Gingival crevicular fluid was collected from both groups. A quantitative determination of aMMP-8 concentrations in the gingival crevicular fluid samples was performed.**
Results: **The aMMP-8 values of group 1 were higher (median 6.25 ng/mL aMMP-8 eluate) compared with group 2 (median 3.88 ng/mL aMMP-8 eluate), but the difference was not statistically significant (p = 0.265). Group 1 showed significantly increased probing pocket depths (p = 0.001). Gingival inflammation was present in 80% of the pregnant women, but only in 40% of the control subjects.**
Conclusions: **It was shown that during pregnancy changes related to periodontal health could be observed. Higher aMMP-8 values, elevated probing pocket depths, and an increase of gingival inflammation could be detected in comparison with non-pregnant women.**
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KEY WORDS

gingival crevicular fluid, gingivitis, matrix metalloproteinase-8, pregnancy, probing depth

INTRODUCTION

During pregnancy there is an increased risk for the development of gingivitis because of the change in the hormonal state. Pregnancy gingivitis is defined as gingival inflammation initiated by plaque and exacerbated by endogenous sex steroid hormones [1]. According to the currently accepted periodontal disease classification, pregnancy gingivitis is a gingival disease induced by plaque and modified by systemic factors [2]. It is a common disease that affects 36% - 100% of pregnant women [3,4]. The inflammatory pattern has different clinical appearances; it can range from mild inflammation to severe hyperplasia, pain, and profuse bleeding [5,6]. Although pregnancy gingivitis is not related to the amount of plaque, it needs a minimum of plaque accumulation and does not develop in pregnant women with excellent plaque control [7,8]. Pregnancy gingivitis is typically self-limiting, becomes evident after the second month of pregnancy, peaks during the eighth month, and disappears post-partum with the decline in hormone production [9,10]. Another characteristic is that it carries no risk of developing periodontitis, despite the inflammatory status developed [11,12].

The etiology of pregnancy gingivitis has not yet been clearly understood, although gingival inflammation in pregnant women is clinically and histologically well documented. Furthermore, it is not known why only

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some pregnant women develop signs of gingival inflammation. Four potential mechanisms have been discussed as being responsible for pregnancy gingivitis: an increase in vascular permeability [13], a change to a more susceptible gingival phenotype [14], immunosuppression [15], and changes in the subgingival or supragingival biofilm [11,16].

Commonly used clinical parameters, such as probing pocket depth, bone loss and radiographic evaluation, can effectively describe periodontal disease, but cannot depict the current state of periodontal tissue destruction. Moreover, during pregnancy the use of radiography is strongly limited. It was shown that certain biochemical markers can provide important information regarding periodontal tissue destruction [17-19].

During the pathogenesis of periodontitis, the inflammatory response of the host tissue to the bacterial biofilm leads to a release of matrix metalloproteinase-8 (MMP-8), which acts as a tissue-degrading enzyme [20,21]. MMP-8 is the most important detectable MMP in the gingival crevicular fluid during periodontal degradation processes [22,23]. Tissue-resident TIMPs (tissue inhibitors of matrix metalloproteinases) can inhibit MMP activity through a non-covalent bond to the MMP. Under physiological conditions, TIMPs and MMPs are in equilibrium. As periodontitis develops, this equilibrium is lost and shifts in favor of MMP-8. This results in an increased level of aMMP-8, and hence enhanced collagen breakdown in the affected tissue [24,25]. It was shown in a clinical study that aMMP-8 values of < 8 ng/mL eluate were associated with periodontal health, whereas values > 8 ng/mL eluate reflect sites with signs of inflammation [26]. The activity of human MMP-8 is clearly influenced by the individual's immunological status. Moreover, MMP-8 has become established both clinically and scientifically as a highly specific biomarker for the degree of inflammation of the periodontium [26-28]. In addition to damaging periodontal tissue, it is also associated with bone resorption [29,30]. As a hypothesis, it was assumed that signs of changes in gingival health, caused by hormonal influence during pregnancy, can be detected early, either by classical clinical parameters or by a biochemical marker. Because aMMP-8 is associated with inflammation, the aim of this study was to evaluate the usefulness of this enzyme as a biochemical marker for early detection of pregnancy gingivitis. Furthermore, the onset of classical signs of gingival inflammation and of the active MMP-8 was to be studied in pregnant women from different weeks of pregnancy.

MATERIALS AND METHODS

Subjects

In this study, a total of 40 female adult volunteers (20 pregnant, 20 non-pregnant) with no general or systemic diseases were enrolled. They were chosen from female subjects presenting to the Department of Operative Dentistry, University Medical Center, Johannes Gutenberg University, Mainz, Germany, for their regular control visit every six-months. The inclusion criteria were as follows: no antibiotic therapy within the three months prior to examination, no diabetes mellitus types I and II or other systemic diseases, no allergies, smoking, hypertension or preeclampsia. Originally, 43 pregnant women were considered; however, only 20 subjects met the inclusion criteria. The participants were divided into two groups: group 1 included pregnant participants (n = 20, age: 32 ± 4 years; pregnancy week: 7-10, 17, 19, 20, 22, 24-26, 28, 33-35, 37) and group 2 comprised only female age-matched healthy participants (n = 20, age: 30 ± 10 years). The examination was conducted after explaining the study to participants and obtaining their signed informed consent. The ethics commission approved the study [No. 837.458.08 (6455)], and the evaluation was performed anonymously. The guidelines of the Helsinki Declaration were observed.

Determination of aMMP-8 in gingival crevicular fluid

To prevent contamination with saliva, the crowns of the teeth were cleaned, dried, and isolated with cotton rolls. For the aMMP-8 determination, the tip of a filter-paper sampling strip for absorbing GCF (gingival crevicular fluid) was gently inserted mesially or distally into the gingival sulcus of all four first molars for 30 seconds, avoiding any bleeding from the marginal gingiva (Figure 1). If a first molar was missing, a second molar was chosen.

After collecting the crevicular fluid, the samples were processed individually according to the manufacturer's instructions. The sampling strips were placed into a test tube containing 800 µl of HEPES buffer and eluted for 30 seconds.

Subsequently, the eluate was placed into a cartridge and a quantitative analysis of aMMP-8 was performed using the DentoAnalyzer (Dentognostics, Jena, Germany), a bench-top instrument designed exclusively for determination of this parameter in the dental practice, as previously described in detail elsewhere [31,32].

Clinical examination

The following clinical periodontal parameters were examined on the same teeth where the GCF samples were collected by two calibrated experienced dentists: probing pocket depth, recession, papillary bleeding index (PBI) [33], and plaque index (PI) [34]. Measurements were done with a periodontal probe (Hu-Friedy, Chicago, IL, USA). To record the plaque index, study participants were asked to chew a plaque disclosing tablet (Produits Dentaires, Vevey, Switzerland), distribute it throughout the oral cavity, and finally rinse out twice. After the plaque had been stained, its amount was determined.

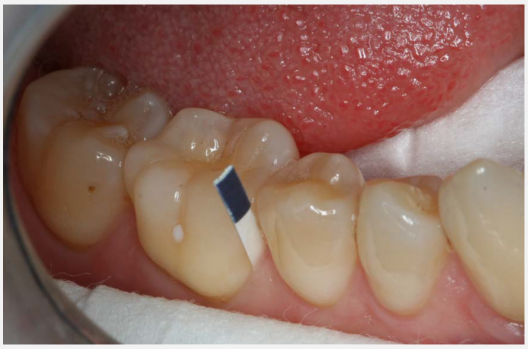

Figure 1. Filter-paper sampling strip for absorbing GCF in situ for evaluating the aMMP-8 concentration.

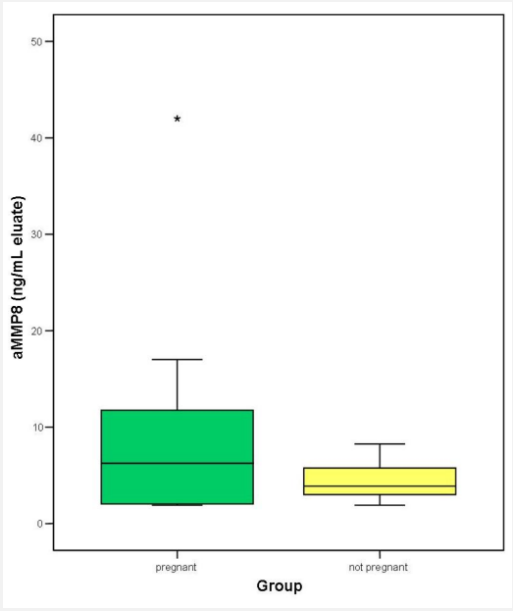

Figure 2. Box-plot diagram of the aMMP-8 concentrations (ng/mL eluate) in pregnant and non-pregnant women. Differences between groups were not statistically significant (p = 0.265).

Statistical analysis

The statistical evaluation was carried out by the Institute for Medical Biometry, Epidemiology and Informatics (IMBEI) at the University Medical Center, Johannes Gutenberg University, Mainz, Germany. All data were statistically analyzed using the program SPSS for Windows version 15.0 (Chicago, IL, USA). For continuous data, which are normally distributed, means (95% confidence interval, CI) and standard deviations (SD) were calculated; for the non-normally distributed continuous data, medians and quartiles were presented. In addition, for discrete parameters, absolute and relative frequencies are given. Comparisons between both groups were performed using the Wilcoxon signed-rank test as the

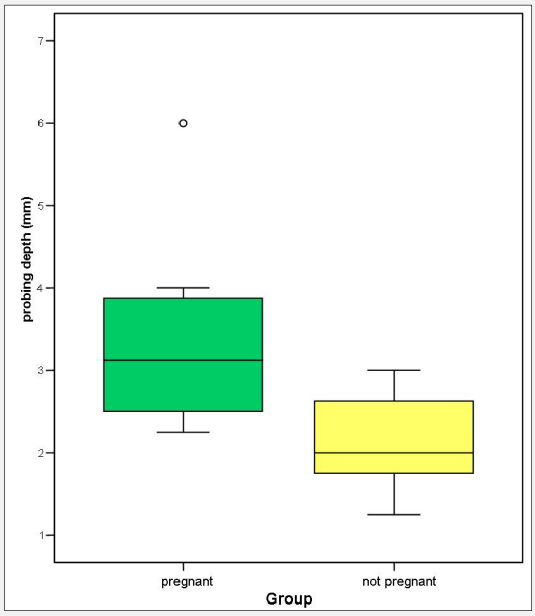

Figure 3. Box-plot diagram of the probing depths (mm) in pregnant and non-pregnant women. Differences between groups were statistically significant (p = 0.001).

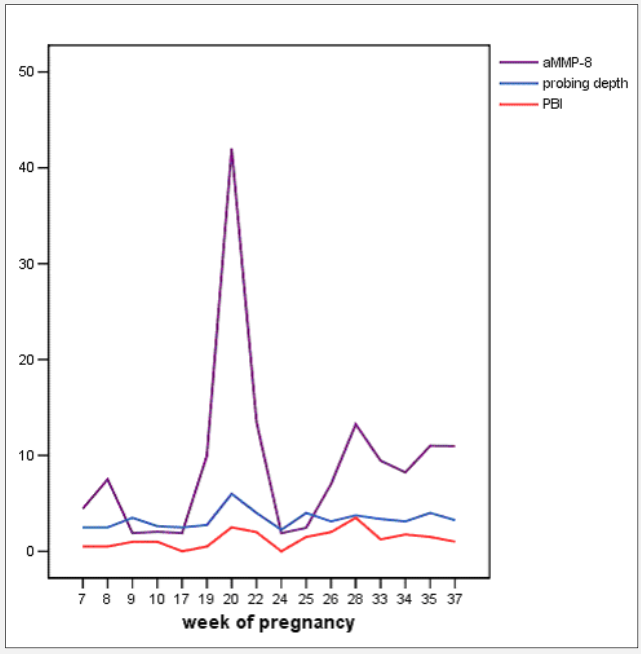

Figure 4. Values from the pregnant women for aMMP-8 (ng/mL eluate), probing depth (mm) and PBI (scores 0 - 4), plotted against the weeks of pregnancy, in which they were examined.

non-parametric test. A significance level of $p < 0.05$ was chosen.

RESULTS

The aMMP-8 concentrations (ng/mL eluate) in the gingival crevicular fluid were determined in all participants. The median aMMP-8 concentration in non-pregnant women (group 2) was 3.88 (Figure 2), mean (95% CI) was 4.41 (3.55 - 5.27), and SD was ± 1.84. In contrast, the median aMMP-8 value in pregnant women (group 1) was 6.25 (Figure 2), mean (95% CI) was 8.71 (4.4 - 13.03), and SD was ± 9.22. Although group 1 had increased aMMP-8 values, the Wilcoxon signed-rank test showed no statistically significant difference between the aMMP-8 levels in both groups ($p = 0.265$). Group 2 showed a median of probing pocket depths of 2.00 mm, whereas in group 1 the median of probing pocket depths was 3.13 mm (Figure 3). The means and SD were 2.19 ± 0.54 mm (group 2) and 3.28 ± 0.90 mm (group 1). There was a statistically significant difference regarding probing pocket depths between both groups ($p = 0.001$).

In group 2, the median of the papillary bleeding index was 0 and in group 1 the median of PBI was 1. Gingival inflammation was present in only 40% of the control subjects, but in 80% of the pregnant women.

When the values from the pregnant women for aMMP-8, probing depth, and PBI are plotted against the weeks of pregnancy, in which these women were examined, it can be seen that each rise in probing depth and PBI is preceded by a rise in aMMP-8 (Figure 4).

In both groups, the subjects had PI scores of ≤ 2, reflecting good oral hygiene. In group 2, the median of the plaque index was 1 and the median of PI in group 1 was 0. Thus, the pregnant women (group 1) showed even better oral hygiene than the control subjects.

The median of recessions in group 2 was 0 mm, group 1 showed a median of recessions of 0.15 mm. These low values reflect periodontal health. The difference between the groups was not statistically significant.

DISCUSSION

The hypothesis that signs of changes in gingival health, caused by hormonal influence during pregnancy, can be detected early, either by classical clinical periodontal parameters or by the concentration of aMMP-8 in GCF as a biochemical marker, is confirmed in part by the results of the present clinical study. The enzyme aMMP-8, which can be considered a highly significant biomarker both clinically and scientifically for the degree of periodontal inflammation [27,28,32], was quantitatively determined in the present study. In pregnant women, aMMP-8 values were higher, probing pocket depths were statistically significantly elevated, and gingival inflammation was observed twice as often in pregnant women compared to the control subjects, although in the former plaque scores were even lower.

To the best of our knowledge, only few studies have been published on pregnancy gingivitis and MMP-8 concentrations in gingival crevicular fluid.

In a clinical study [35], 30 pregnant and 24 non-pregnant women were examined and GCF samples for the determination of MMP-8 and other neutrophilic enzymes were collected, and clinical parameters including bleeding on probing (BOP) and probing depth (PD) were recorded. In the pregnant group, BOP and PD values were significantly increased, but this inflammation was not reflected by enzymes examined in the GCF. The authors concluded that the host response does not seem to activate its own degradative enzymes. In contrast, in the present study the increase in inflammation was accompanied by higher aMMP-8 levels in the GCF of the pregnant women. In another study by the same authors [36], salivary samples from 30 pregnant women were collected and MMP-8 levels were determined using an immunofluorometric assay. During pregnancy, salivary MMP-8 concentrations were significantly lower than postpartum concentrations. Elevated bleeding on probing scores and probing pocket depths were found. Similar observations were also made in the present study.

It was reported [37] that salivary MMP activity (MMP-1, -2, -3, -7, -9, -12, and -13) was low in non-pregnant females and increased in samples taken in the second trimester of pregnancy and at term during active labor. The authors observed that samples collected from women with premature rupture of the membranes before preterm delivery had the highest activity. Salivary MMP activity might be suitable as a predictive marker to identify patients at high risk for premature rupture of the membranes before preterm delivery.

In an in-vitro study [38], the effects of progesterone on MMPs from human gingival fibroblasts were investigated and the production of numerous MMPs (MMP-1, -2, -3, -7, -10, and -13) was found to be significantly reduced by progesterone. The authors concluded that this steroidal modulation of proteolytic enzymes could be an explanation why pregnancy gingivitis typically is not characterized by progression to periodontitis.

To evaluate the severity of periodontal changes during pregnancy [39], 30 pregnant and 24 non-pregnant women were recruited and visible plaque index (VPI), bleeding on probing, probing pocket depth (PPD), and clinical attachment level (CAL) was measured. It was reported that the percentage of BOP was higher in pregnant women than in non-pregnant women. Similarly, in the present study high PBI scores, reflecting gingival inflammation, were seen in twice as many pregnant women than in the control subjects. In the pregnant women, PPD increased without relation to plaque accumulation [39], which was in accordance with the findings from the present study.

In a randomized, multicenter study [40] the periodontal status of 200 pregnant women (mean age = 30 years)

and 200 non-pregnant controls (mean age = 32 years) was evaluated. Pregnant women had significantly higher gingival index (GI) scores and PPD, but no statistically significant differences in plaque index compared with non-pregnant controls. In the present study, statistically significantly higher probing pocket depths as well as more frequent signs of gingival inflammation were also observed in pregnant patients. However, the pregnant women had better oral hygiene, which was reflected in lower plaque index scores. In a study [41] including 2424 pregnant and 1565 non-pregnant women, it was shown that the percentage of pregnant women having pocket depths of 4 or 5 mm was significantly higher than that of non-pregnant women. The increase in pocket depths during pregnancy might be caused by gingival enlargement due to increased vascularization, hormonal influence, gingival hyperplasia, and swelling rather than by periodontal destruction. In the present study, an increase in pocket depths but no recessions were found in pregnant women indicating a lack of tissue destruction.

The influence of hormonal changes during pregnancy on clinical parameters and the presence of exacerbated gingival inflammation were confirmed [42]. It was observed that pregnant women showed an increase in GI despite low plaque index values. The phenomenon of low plaque index scores in pregnant women was also seen in the present study.

A small group of healthy non-pregnant women with generalized chronic periodontitis was examined and a statistically significant correlation between aMMP-8 concentrations in GCF and pocket depths was found [43]. Although the variance was rather weak, the authors conclude that the determination of aMMP-8 concentrations could be useful for diagnostic purposes. Since similar results were found in the present study, aMMP-8 levels might also be useful for early diagnosis of pregnancy gingivitis in addition to conventional clinical parameters.

During pregnancy it is desirable to identify those women at risk of developing severe signs of gingival inflammation as early as possible. Consequently, intensive preventive measures can be applied regularly to make later intervention unnecessary.

CONCLUSION

Within the limitations of this study, the results confirm the susceptibility to gingivitis during pregnancy as reported in earlier studies.

Pregnant women tended to have higher aMMP-8 concentrations compared with non-pregnant subjects. Significantly elevated probing pocket depths and an increase in gingival inflammation were observed in pregnant women. Further studies with larger sample sizes are needed to establish a strong correlation between aMMP-8 concentrations, pocket depths, and signs of gingival inflammation in pregnant women, to be able to make use of this enzyme as a biochemical marker for early detection of pregnancy gingivitis. In addition, longitudinal studies including assessment of aMMP-8 concentrations and other clinical parameters several times during pregnancy and after delivery would provide further information.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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Correspondence:
Dr. Vicky Ehlers
Department of Operative Dentistry
University Medical Center of the
Johannes Gutenberg University Mainz
Augustusplatz 2
D-55131 Mainz, Germany
Tel.: +49-6131/17-3557
Fax: +49-6131/17-3406
Email: ehlersv@uni-mainz.de