Periodontal health status and salivary parameters in pregnancy

Leka'a S., B.D.S. (1)
Leka'a M. Ibrahim, B.D.S., M.Sc. (2)

ABSTRACT

Background: Pregnancy is considered a major risk factor for development and progression of periodontal disease. There are hormonal changes in both estrogen and progesterone hormones in addition to bacterial effect and poor oral hygiene that will enhance development of periodontal disease in pregnant women.

Materials and methods: Seventy subjects were enrolled in the study, the subjects with an age range (20-35) years old without any history of systemic disease. The subjects were divided into 20 non-pregnant women they represent the control group (G I), 30 pregnant women with gingivitis (GII) and 20 pregnant women with periodontitis (GIII).All periodontal parameters (plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level) were recorded and 5ml of unstimulated saliva was collected for each subject. The collected saliva was centrifuged and clear supernatant was collected and kept frozen until biochemical analysis of salivary enzymes which included ALP, LDH and salivary Calcium.

Results: No significant difference in the mean value of salivary ALP between GI and G II, while there is high significant difference between GI and GIII. There was significant difference in the salivary LDH and Ca levels between control group and group II, while there is highly significant difference of salivary LDH and Ca between group I and group III.There was significant difference in the number of bleeding sites, and probing pocket depth (PPD) among all groups. There was increase in the total number of all scores of PPD (score 1,2 and 3) GII and GIII compared to GI.

Conclusions: Thepregnant women revealed more periodontal disease conditions (gingivitis and periodontitis) due to hormonal changes superimposed with microbial infection. Salivary enzymes (ALP, LDH) and salivary calcium are considered as good biochemical markers of periodontal tissue destruction and can be used to evaluate the effect of pregnancy on periodontal health status.

Keywords: pregnancy; saliva; alkaline phosphatase; lactate dehydrogenase; calcium; periodontal disease. **(J Bagh Coll Dentistry 2014; 26(1):128-133).**

الخلاصة

خلفية: يعتبر الحمل عامل خطر رئيسي لتنمية وتقدم الإصابة بأمراض اللثة نتيجة التغيرات الهرمونية في كل من الاستروجين والبروجسترون هرمون بالإضافة إلى تأثير البكتيريا وعدم الاهتمام بصحة الفم و الأسنان عند معظم النساء الحوامل مما يؤدي إلى تعزيز تنمية أمراض اللثة في النساء الحوامل.

المواد والطرق: سبعون امرأة تم إدخالهم في الدراسة تتراوح أعمارهم بين (20-35) سنة من دون أي تاريخللإصابةبالإمراضالجهازية. تم تقسيم النساء إلى مجموعتي الدراسة (30أمرأة حامل مع التهاب اللثة، 20 امرأة حامل مع التهاب دواعم السن) ومجموعة المراقبة (20 امرأة غير حاملوهي تمثل المجموعة الضابطة).وسجلت جميع المعلمات للثة (مؤشر البلاك، مؤشر اللثة، نزيف في اللثة، سبر عمق الجيب وفقدان المرفقات السريرية) وتم جمع عينة اللعاب غير المحفز لكل امرأة. تم تعريض عينة اللعاب للطرد المركزي وجمع اللعاب الطافي الواضح وتم تجميدها إلى وقت التحليل لكل من الإنزيمات اللعابية والكالسيوم.

النتائج: أن متوسطALP انزيم في اللعاب بين المجموعة الضابطة والحوامل مع التهاب اللثةذو فرق غير معنوي، بينما هناك فروق معنوية عالية بين المجموعة الضابطة ومجموعة الحوامل مع التهاب دواعم الس ,هناك اختلاف كبير في مستويات اللعابية في انزيمLDH والكالسيوم بين المجموعة الضابطة ومجموعة الحوامل مع التهاب اللثة، بينما هناك فرق كبير من LDH اللعابية واملاح الكالسيوم بين المجموعة الضابطة ومجموعة الحوامل مع التهاب دواعم السن.وجد فروق ذات دلالة عالية في مؤشر لوحة PLI بين المجموعة الضابطة ومجموعة الحوامل مع التهاب اللثة، بينما هناك فرق كبير عالي بين المجموعة الضابطة ومجموعة الحوامل مع التهاب دواعم السن.هناك فرق كبير للغاية في مؤشر لثوي ,ان هناك اختلاف كبير في عدد من مواقع النزيف، وكان هناك أيضا اختلاف كبير في سبر عمق الجيب تم العثور علىPPD) بين جميع الفئات. كان هناك زيادة في العدد الإجمالي من كل الدرجات من PPD (درجة 2،1 و 3) في الحوامل مع التهاب اللثة مقارنة مع المجموعة الضابطة. الحوامل مع مجموعة اللثة تظهر أيضا زيادة PPD النتيجة (2،1 و 3) مقارنة مع المجموعة الضابطة.

الاستنتاجات: كشفت مجموعة الحوامل تدميرانسجة اللثة وفقدان العظم السنخيأكثر من مجموعة النساء غير الحوامل بسبب التغيرات الهرمونية بالإضافةإلىالتأثيراتالميكروبية التي من شأنها تعزيز التنمية والتقدم في تدمير الأنسجة اللثوية لذلك تعتبر الإنزيمات اللعابية ALP، LDH

INTRODUCTION

Periodontal disease, including gingivitis and periodontitis, is considered to be one of the most common diseases among population and, if left untreated, can lead to tooth loss. The main cause of periodontal disease is bacterial plaque although many other factors such as hormonal changes, diabetes, poor nutrition, smoking, and stress may affect the initiation and progression of gingival and periodontal diseases.

The development of the common periodontal diseases depends mainly on human behavior, and the control of these diseases is greatly supported by the fact that the etiological factors are well documented (1).

(1) Master student, Department of Periodontics, College of Dentistry, University of Baghdad.
(2) Professor, Department of Periodontics, College of Dentistry, University of Baghdad.

Pregnancy is accompanied by an increase in the levels of both progesterone and estrogen which, by the third trimester, reaches levels 10-30 times than seen during the typical menstrual cycle. Changes in the gingiva include an increase inflammation that usually starts during the second to third month of pregnancy and increases in severity through the eighth month, where it decreases along with the abrupt decrease in hormone secretion (2).

Study has been suggested that women who experience periodontal disease during pregnancy may be at risk of having a premature or low birth weight baby. The periodontal disease affects pregnancy outcomes. And he suggested that treating periodontitis during pregnancy may reduce the risks of a preterm birth. Preventing gingival problems from developing during the

stresses of pregnancy also appears to be important in improving the health of mother and baby [3].

Saliva, an important physiologic fluid, containing a highly complex mixture of substances, is rapidly gaining popularity as a diagnostic tool. In the field of periodontology, traditional clinical criteria are often insufficient for determining sites of active disease, for monitoring the response to therapy, or for measuring the degree of susceptibility to future disease progression. Saliva, as a mirror of oral and systemic health, is a valuable source for clinically relevant information because it contains biomarkers specific for the unique physiologic aspects of periodontal diseases [4].

Host responses to periodontal disease include the production of different enzymes that are released by stromal, epithelial or inflammatory cells. There are important enzymes associated with cell injury and cell death like aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatinekinase (CK), alkaline and acidic phosphatase (ALP, ACP), gamaglutamiltransferase (GGT). Changes in enzymatic activity reflect metabolic changes in the periodontium in inflammation [5].

Alkaline Phosphates intracellular enzyme present in most of the tissues and organs, particularly in bones. Their increased activity is probably the consequence of the destructive processes in the alveolar bone, in the advanced stages of the development of periodontal disease remarkable increased activity of ALP in, and after periodontal therapy, the activity of enzyme was restored to the value found in healthy persons. ALP is enriched in the membranes of mineralizing tissue cells (e.g., osteoblasts) and is also present in polymorphonuclear leukocyte (PMN) granules. ALP is produced by some oral bacteria, including gram-negative microorganisms found in the sub gingival plaque,[6].

Lactate dehydrogenase enzyme is indicator of a high level of cellular damage and it is increased activity in saliva is a consequence of their increased release from the damaged cells of soft tissues of periodontium and a reflection of metabolic changes in the inflamed gingival [7].

Calcium (Ca) is the ion that has been most intensely studied as a potential marker for periodontal disease in saliva. A higher concentration of Ca detected in unstimulated saliva from the periodontitis patients. The authors concluded that increase Ca concentration in saliva was characteristic of patients with periodontitis. Nevertheless, the importance of the salivary Ca concentration in relationship to progression of periodontal disease is not defined. Considering the distribution of Ca, this ion appears to hold promise as a marker for periodontal disease [8].

MATERIALS AND METHODS

Subjects included in the study were selected from patients attending to AL-Mustafa Primary Health Center and to AL-Batool Hospital. Seventy subjects were enrolled in the study, the subjects with an age range 20-35 years old. They were carefully informed about the aim of the investigation and they were free to accept or refuse to be examined. Each participant received complete medical and dental history to determine their suitability to the study and all of them had no history of systemic disease all subjects had at least 20 teeth.

The sample include control group (GI) which included twenty non-pregnant women. Study group which included 50 pregnant women in second trimester and subdivided into: Group II which included thirty pregnant women in second trimester with gingivitis.Group III which included twenty pregnant women in second trimester with periodontitis.Un- stimulated (resting) whole saliva was collected before the clinical examination. A sample was collected after an individual was asked to rinse her mouth thoroughly with water.

To insure the removal of any possible debris or contaminating materials we waited for 1-2 min for water clearance. The collected saliva was centrifuged at 3500 rpm for 20 minutes and then the centrifuged clear

Supernatant saliva was collected by micropipette into eppendroftubes and kept frozen and store at- 20`c until biochemical analysis. Samples containing blood were discarded. AII Periodontal variables were recorded on four sites (Mesial, buccal, distal and lingual or palatal for all teeth except the third molar which was excluded. Interproximal areas were probed from buccal part of the tooth with Probe tip parallel to long axis of tooth and positioned inter proximally as close as possible to the contact point; measuring was made to the nearest millimeters. The assessment of dental Plaque was made according to the Plaque Index [9]. The gingival condition was assessed using the criteria of gingival index system. Bleeding on probing done by using a blunt periodontal probe was inserted to the "bottom" of the periodontal pocket and is moved gently along the tooth (root) surface. If bleeding occurs within 30 second after probing the site was given as positive score (1), and a negative score (0) for the non-bleeding site [10]. Probing pocket depth were estimated by using William probe and use a scale for ease of estimation, it involve the following criteria.

Score 0: those include depth from 1-3 mm.
Score 1: those include depth >3-5mm.
Score 2: those include depth >5-7 mm.
Score 3: those include depth >7 mm.

Clinical attachment level (CAL): This was assessed by measuring the distance from comentoenamel junction (CEJ) to the base of the pocket. Attachment level was passed by using Williams's periodontal probe [11] to measure the distance in mm from free gingival margin (FGM) to the CEJ and measure the distance from FGM to the base of the pocket at each site. The attachment level was obtained by subtracting the first measurement from the second one [12].

Biochemical assay for enzyme analysis used kits manufactured by Biolabo which is one of the French leaders of Reagents for Medical Biochemistry of ALP. Atomic absorption spectrophotometer for Medical Biochemistry of Ca was used Human Germany kit for LDH.

Statistical analysis.

The data were processed and analysis using the statistics package social sciences (SPSS.IS.Under window XP and Excel 2003). Both descriptive and inferential statistics were used.

RESULTS

Plaque index (PLI):-

The descriptive statistics for plaque index was shown in Table (1) It was clearly shown that the means of plaque index were elevated in group III compared with group I and II.The mean and SD were 2.34±0.192 in group III , while in group I and II they were 1.275±0.228, 2.123±0.291 respectively.

Inter-group comparison of plaque index using student t-test revealed a high significant difference between group I and group II and between group I and III where the p-value was < 0.05,while there was no significant difference between II and groupIII at p-value < 0.01as shown in Table (2).

Gingival index (GI):-

The mean and SD of gingival index was described in table (3),the mean of gingival index in group III were higher compared with group I and II.It was 2.092± 0.370 in group III, 1.763±0.4 in group II and 0.355±0.176 in group I

Inter –group comparison of gingival index using t-test shown in table (4). Comparison between group I and group II and between group I and III was high significant, while the comparison between group II and group III was significant.

Bleeding on probing (BOP):-

The number and percentage of bleeding on probing for all groups were shown in table (5). The percentage of bleeding sites in group II was (63.592%), while in group III was (76.865%).

Statistical analysis using Chi-square test revealed significant difference of B.O.P scores among all groups as shown in Table (6).

Probing pocket depth (PPD):-

The number and percentage of probing pocket depth for all groups shown in Table (7). The group I shown 100% score 0 percentage of probing pocket depth , while group II shown (50.645%) score o and (46.033%) score 1 and (3.322%) score 2 percentage of pocket depth,whille group III shown (37.810%) score 0 , (60.086%) score 1 , (1.195%) score 2 and (0.909%) score 3 percentage of probing pocket depth

Chi–square test was applied on the PPD scores (0, 1, 2, and 3) for all groups and the result revealed significant difference as shown in Table (8).

Clinical attachment level (CAL):-

The Table (9) illustrates the number and percentage of clinical attachment level for group III, in group III the percentage of score 1 was (24.906%).The percentage of score 2 was (47.466%), the percentage of sites score 3 was (26.720%), while the percentage of score 4 was (0.908%)

Biochemical analysis:

Analysis of alkaline phosphates level (ALP):

The descriptive statistic of ALP for all groups was shown in Table (10). The mean of ALP (IU/L) was higher in group III compared to groups I and II .The highest mean of ALP was found in group III (60.725± 1.929) and the lowest mean was in group I (24.451± 2.472),while the mean of ALP in group II was (25.7±1.835).

Inter-group comparison of the mean ALP shown in Table (11).

Comparison of the mean ALP showed that there was a non significant difference between group I and II, and high significant difference between group I and III, and between group II and III.

Analysis of Lactate dehydrogenase level (LDH):-

The descriptive statistics of LDH for all groups were shown in Table (12). The mean of LDH (IU/L) was higher in group III compared to groups I and II. The highest mean of LDH was found in group III (86.279± 2.970) and the lowest mean was in group I (33.319±1.688),while the mean of LDH in group II was (43.207± 1.388).

Inter-group comparison for the mean of LDH was shown in Table(13).Comparison of the mean LDH showed a significant difference between group I and II, while a highly significant difference found between group I and III , and group II and III.

Analysis of Calcium level (Ca):-

The descriptive statistics of Ca for all groups were shown in Table (14),. The mean of Ca (IU/L) was higher in group I and II compared to group III. The highest mean of Ca was found in group I (4.505± 0.356) and the lowest mean was in group III (1.68± 0.436), while the mean of Ca in group II was (3.334± 0.540).

Inter-group comparison for the mean of Ca was shown in Table (15). Comparison of the mean Ca showed a significant difference between group I and II. On the other hand there was a highly significant difference between group I and III, and between group II and III.

DISCUSSION

This study demonstrates the effect of pregnancy on periodontal health status and on the level of these enzymes in saliva as these enzymes is increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid and saliva. [13]Suggested that the period between weeks 12 and 28 of pregnancy can be characterized by increased susceptibility to plaque bacteria and an inflammatory response in the gingival.Estrogen firstly decreases collagen production and keratinization of gingival epithelium and secondly induces proliferation of fibroblasts and decreases the collagen and no collagen proteins, blocks the turnover of the gingival tissue, thereby reducing the capacity of gingival tissue to repair [14].That leads to increase in the permeability of the epithelial barrier and an increased response to plaque bacteria [15]. Gingivitis due to accumulation of plaque was the most characteristic periodontal condition in this sample and was related to professional level, level of education, and previous periodontal maintenance. These results illustrate the importance of establishing periodontal preventive measures for pregnant women, even though their demographic and clinical characteristics do not differ from those of the general population [16].During pregnancy, progesterone levels increase 10-fold and estrogen levels 30-fold compared to those observed on menstrual cycle due to their continuous production, Physiological changes in metabolism include oral microbial species, immune response and cell metabolism. The increase in progesterone results in greater vascular permeability, gingival edema, crevicular fluid levels and prostaglandin production, which may lead to gingival inflammation [17]. The clinical indicators evaluated in this study were bleeding on probing and pocket depth without loss attachment, which indicated that this may be due to a more pronounced gingival overgrowth in the proximal surfaces of anterior teeth [18].The effect of these changes on the periodontal tissues results in increased gingival swelling. Increased bleeding on probing may be seen in clinical examinations during pregnancy [19].This general increase in CAL in pregnant with periodontitis group was in agreement with [20]. The developing baby draws calcium from the mother's bones. The baby's calcium needs are provided by the mother's diet. When the mother's diet is not sufficient in calcium her body may try to compensate for this lack by drawing some calcium from her bones; however, her teeth will not be affected. While oral health can be affected during pregnancy, it is often because of poor oral hygiene. [21].in present study it was found that salivary alkaline phosphates level increases with increase in periodontal destruction. Total amount of alkaline phosphates levels were significantly higher in periodontitis as compared to healthy and gingivitis sites, similar observations were made by [22]. In this study there was significant difference between the control group and pregnant with gingivitis and highly significant difference present between the control group and pregnant with periodontitis group and this is in agreement with [23]. Intracellular enzyme such as LDH was increasingly released from the damaged cells of periodontal tissue into the GCF and saliva, LDH enzyme can help to monitor the progression of periodontal disease and they appear to be useful to test the activity of periodontal disease [24].

Thepregnant women revealed more periodontal disease conditions (gingivitis and periodontitis) due to hormonal changes superimposed with microbial infection. Salivary enzymes (ALP, LDH) and salivary calcium are considered as good biochemical markers of periodontal tissue destruction and can be used to evaluate the effect of pregnancy on periodontal health status.

REFERENCES

1- Albandar JM. Global risk factors and risk indicators for periodontal diseases. J Periodontol 2000 2002; 29: 177-206.
2- Al-Talib Z. The effects of pregnancy on the periodontal condition of young adult Saudi population. J Egypt Dent.2008; 54: 1-11.
3- American academy of Periodontology (AAP). Epidemiology of periodontal disease (AAP Positonpaper). J Periodontol 2005; 76: 1406-19.
4- Bergstrom J. Pregnancy and periodontal bone loss. J periodontal 1991; 62: 242-6.
5- Battino M. pregnancy and sub gingival dental calculus. J Clin periodontal 2005; 32: 81-8.
6- Calsina G, Roman JM, Echeverria JJ. Effect of pregnancy on periodontal tissues. J Clin Periodontol 2002; 29(8): 771-6.

7- Dawes C. How much saliva is enough for avoidance of xerostomia? J Caries Res 2004; 38: 236-40.
8- Falco MA. The life time impact of sugar excess and nutrient depletion on oral health. J Gen Dent 2001; 49(6): 591-5.
9- Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. J Acta Odontol scand 1963; 21: 533-51.
10- Carranza FA, Newman MG. Clinical Periodontology. 8th ed. W.B. Saunders; 1996.
11- George L, Granath F , Johansson AL, Anneren G, Cnattingius S. pregnancy and risk of spontaneous abortion. J Epidemiol 2006; 17(5): 500-5.
12- Griffiths GS. Formation, collection and significans of gingival crevice fluid. Periodontal 2000 2003; 31: 32-45.
13- Hidalog RV. Pregnancy and periodontal disease. J Periodontol 2000 2003; 3: 50-8.
14- Holtfreter B, Schwahn C, Biffar R, Kocher T. Epidemiology of periodontal disease in the Health in Pomerania. J Clin Periodntol 2009; 36(2): 114-23.
15- Kaufman E, Lamster IB. The diagnostic applications of saliva- a review. J Crit Rev Oral Bio Med 2002; 13(12): 197-212.
16- Kinane DF, Radvar M. The effect of pregnancy on mechanical and antimicrobial periodontal therapy. J Periodontal 1997; 68(5): 467-72.
17- Kingman A, Susin C, Albandar JM. Effect of partial recording protocols on severity estimates of periodontal disease. J Clin Periodontol 2008; 35(8):659-67.
18- Kugahara T, Shosenji Y, Ohashi K. Screening for periodontitis in pregnant women with salivary enzymes. J Obstet Gynaecol Res 2008; 34(1): 40-6
19- Lee JM, Garon E, Wong DT. Salivary diagnostic. J Orthod Craniofac Res 2009; 21(4): 672-6.
20- Mese H, Matsuo R. Salivary secretion, taste and hypo salivation. J Oral Rehabil 2007; 34(10): 711-23. (IVSL).
21- Numabe Y, Hisano A, Kamoi K, Yoshie H,Ito k, Kurihara H. Analysis of saliva for periodontal diagnosis and monitoring. J Periodontology 2004; 40: 115-9.
22- Ozmeric N. Advances in periodontal disease markers. J Clin Chim Acta 2004; 343(1-2): 1-16.
23- Page RC, Beck JD.Risk assessment for periodontal disease. Int Dent J 1997; 47(2): 61-87.
24- Zhou Y, Wang C, Yao W, et al. COPD in pregnant Chinese. Eur Respir J 2009; 33: 509-18.

Table 1: Mean of plaque index in each group

PL I	G1	GII	GIII
Mean	1.275	1.275	2.34
SD±	0.228	0.291	0.192

Table 2: Inter-group comparison by using t-test for mean plaque index.

Groups	t-test	p-value	Significance
Group I and group II	1.923	0.002	HS
Group I and group III	3.013	0.003	HS
Group II and group III	1.306	0.133	NS

Table 3: Descriptive statistics of gingival index in each group.

GI	Group I	Group II	Group III
Mean	0.355	1.763	2.092
SD±	0.176	0.4	0.370

Table 4: Inter group comparison of mean gingival index

Groups	t-test	p-value	Significant
Group I and group II	1.987	0.002	HS
Group I and group III	2.937	0.004	HS
Group II and group III	1.513	0.022	S

Table 5: Number and percentage of bleeding on probing for all groups

Score	Group I		Group II		Group III	
	No	%	No	%	No	%
0	2292	100	1184	36.408	484	23.135
1	0	0	2068	63.592	1608	76.865

Table 6: The chi- square test of bleeding on probing for all groups.

Groups	chi- square	Df	P-Value	Significance
Group I Group II Group III	29.090	2	0.021	S

Table 7: number and percentage of pocket depth for the groups.

Groups	Score 0		Score 1		Score 2		Score 3	
	No	%	No	%	No	%	No	%
Group I	2292	100						
Group II	1647	50.645	1497	46.033	108	3.322		
Group III	791	37.810	1257	60.086	25	1.195	19	0.909

Table 8: The chi- square test of pocket depth for all groups.

Groups	chi- square	Df	P-Value	Significance
Group I Group II Group III	78.081	6	0.013	S

Table 9: Number and percentage of clinical attachment level of chronic periodontitis group.

score	Group III	
	No	%
1	521	24.906
2	993	47.466
3	559	26.720
4	19	0.908

Table 10: Mean and SD of salivary ALP for all groups

Statistics	Group I	Group II	Group III
Mean	24.451	25.7	60.725
SD ±	2.472	1.835	1.929

Table 11: Inter-group comparison by using t-test for mean ALP for all groups.

Groups	t-test	p-value	Significance
Group I and group II	2.192	0.707	NS
Group I and group III	5.193	0.000	HS
Group II and group III	3.136	0.000	HS

Table 12: Mean and SD of salivary LDH for all groups

Statistics	Group I	Group II	Group III
Mean	33.319	43.207	86.279
SD±	1.688	1.388	2.970

Table 13:Inter-group comparison by using t-test for mean LDH

Groups	t-test	p-value	Significance
Group I and group II	2.332	0.028	S
Group I and group III	9.042	0.000	HS
Group II and group III	7.072	0.000	HS

Table 14: Mean and SD of salivary Ca for all groups.

Statistics	Group I	Group II	Group III
Mean	4.505	3.334	1.68
SD±	0.356	0.540	0.436

Table 15: Inter-group comparison by using t-test for mean Calcium

Groups	t-test	p-value	Significant
Group I and group II	1.504	0.032	S
Group I and group III	2.338	0.000	HS
Group II and group III	1.875	0.000	HS