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Assessment of Annexin-1 as A Biomarker For Periodontal Diseases in Pregnant and Non-Pregnant Women

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KEYWORDS

Annexin-1, pregnant, Plaque index, gingivitis, periodontal parameters.

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ABSTRACT

Aim : Assess the level of Annexin-1 and explain the relationship between ANX-1 level and periodontal parameters. **Subjects and methods:** 40 female patients (20 pregnant and 20 non-pregnant) were classified into four groups. Group I: 10 pregnant female patients suffering from gingivitis. Group II: 10 pregnant female patients suffering from periodontitis. Group III: 10 non-pregnant female patients suffering from gingivitis. Group IV: 10 non-pregnant female patients suffering from periodontitis. All patients were received conventional periodontal therapy and evaluated clinically at baseline, 4 and 12 Ws. The laboratory evaluation of ANX-1 level was done at baseline, 4 and 12 Ws. The data were collected, tabulated, and statically analyzed by SPSS (Statistical Package for Social Science). **Results:** The clinical parameters plaque index, gingival index, probing depth, and clinical attachment level were recorded at baseline, 4 and 12 Ws. In all groups, statistically significant differences were shown in four groups at different intervals when compared to baseline. Plaque index between the four groups representing no statistically significant difference at different intervals. Gingival index shows a statistically significant difference in pregnant and non-pregnant groups at different intervals. Probing depth shows a statistically significant difference in group I when compared with other groups at different intervals. Clinical attachment level shows a statistically significant between pregnant groups and non-pregnant groups at different intervals. ANX-1 level shows a statistically significant difference in group I when compared with other groups at baseline**. Conclusions:** ANX-1 in GCF showed marked elevated levels with significant differences during pregnancy with gingivitis

INTRODUCTION

Periodontal diseases are the most common oral diseases that affect up to 90% of the worldwide population, gingivitis the mildest form of periodontal diseases [(1)], It is generally accepted that gingivitis if left untreated, may ultimately progress to periodontitis in a subset of individuals [(2)].

Several host responses play an important role in determining the progression of the inflammatory lesion. At the cellular level, exposure to bacterial products and lipopolysaccharide elicit activation of monocytes/macrophages that promote the secretion of cytokines such as interleukin (IL)-1 resulting in the release of matrix metalloproteinases (MMPs)[3-6].

During pregnancy immunological changes have been considered as a risk factor, responsible for periodontal conditions[7]. Proinflammatory cytokines play a central role in the progression of gingival inflammation [8].

Annexin A1 a member of the annexin superfamily of protein, is a 37 kDa calcium-dependent phospholipid-binding protein [9].

AnxA1 is a glucocorticoid-regulated protein with anti-phospholipase activity, but the protein shows many other anti-inflammatory and pro-resolving properties especially in pregnant women [10].

METHODOLOGY

This study was designed as a randomized controlled clinical trial, carried out on 40 female patients (20 pregnant and 20 non-pregnant) who suffered from gingivitis or mild to moderate periodontitis. All patients were selected from those attending the out-patient clinic, Oral Medicine and Periodontology Department, Faculty of Dental Medicine, Al-Azhar University, Assiut Branch.

All subjects were

1. Free from any systemic diseases, not smokers, and not lactating patients.
2. Acceptable for oral hygiene instructions, cooperative and had ≥20 teeth.
3. The pregnant patient in the second trimester.
4. Pregnant patients not with gestational diabetes mellitus and preeclampsia.
5. Non-pregnant patients do not receive contraceptive pills.

Patients were divided into four equal groups.

Group I: 10 pregnant female patients suffering from gingivitis. **Group II**: 10 pregnant female patients suffering from mild to moderate periodontitis. **Group III**: 10 non-pregnant female patients suffering from gingivitis. **Group IV**: 10 non-pregnant female patients suffering from mild to moderate periodontitis.

Conventional periodontal therapy All patients were received conventional periodontal therapy according to the type of the disease.

Periodontal Evaluation The periodontal health status of each female patient was evaluated at baseline, 4, and 12 weeks after conventional periodontal therapy using the following clinical parameters: Plaque Index (PI), Gingival Index (GI), Propping Depth (PD), and Clinical Attachment Level (CAL) [11-14].

Biochemical Evaluation

Samples collection and preparation

- (GCF) samples were obtained by paper points size 30# in cervical of the teeth with the deepest probing depth.
- Just 5 minutes before collecting the (GCF) samples from the patients at baseline, 4 Ws and 12 Ws, asked to rinse their mouth with tap water 3 times.
- The collected GCF was immediately transferred to an Eppendorf tube containing phosphate buffer solution and transported to the laboratory.
- The samples were frozen at -80 degrees till they were assayed for ANX-A1 level using (ELISA) according to the manufacturer's instructions.

Statistical analysis:

The data were collected, tabulated, and statistically analyzed.

RESULTS

The changes in PI, GI, PD, CAL, and ANX-1 level during the observation periods of the present study were illustrated in table (1) Showing mean± SD and Unpaired t-test for the four groups and table (2) Showing Paired t-test for the four groups.

Plaque index (PI)

There was a statistically significant difference between (Group I) and (Group II) at baseline where (p= 0.026). There was a statistically significant difference between (Group I) and (Group IV) at baseline where (p= 0.045) only. No statistically significant difference at different intervals between the four groups.

Gingival index (GI)

A statistically significant difference between (Group I) and (Group III) after 4 and 12 weeks where (p= 0.008) and (p= 0.002) respectively. A statistically significant difference between (Group I) and (Group IV) after 4 and 12 weeks where (p= 0.032) and (p= 0.006) respectively. A statistically significant difference between (Group II) and (Group III) after 4 and 12 weeks where (p= 0.016) and (p= 0.002) respectively. A statistically significant difference between (Group II) and (Group IV) after 12 weeks where (p= 0.006). No statistically significant difference between other groups at different intervals.

Probing Depth (PD)

A statistically significant difference between (Group I) and (Group II) after 4 and 12 weeks where (p= 0.035) and (p= 0.006) respectively. A statistically significant difference between (Group I) and (Group III) after 12 weeks where (p= 0.007). A statistically significant difference between (Group I) and (Group IV) after 4 and 12 weeks where (p= 0.028) and (p= 0.040) respectively. No statistically significant difference between other groups at different intervals.

Clinical Attachment Level (CAL)

There was a statistically significant difference between (Group I) and (Group II) at baseline, after 4, and 12 weeks where (p= 0.000). There was a statistically significant difference between (Group I) and (Group IV) at baseline, after 4, and 12 weeks where (p= 0.000). There was a statistically significant difference between (Group II) and (Group III) at baseline, after 4, and 12 weeks where (p= 0.000). There was a statistically significant difference between (Group III) and (Group IV) at baseline, after 4, and 12 weeks where (p= 0.000). No statistically significant difference between other groups at other different intervals.

Annexin-A1 (ANX-1) Level

There was a statistically significant difference between (Group I) and (Group II) at baseline and after 4 where (p= 0.000) and (p= 0.036) respectively. There was a statistically significant difference between (Group I) and (Group III) at baseline where (p= 0.000). There was a statistically significant difference between (Group I) and (Group IV) at baseline where (p= 0.000). A statistically significant difference between (Group II) and (Group III) after 4 weeks where (p= 0.001). There was a statistically significant difference between (Group II) and (Group IV) at baseline where (p= 0.006). There was a statistically significant difference between (Group III) and (Group IV) at baseline and after 4 weeks where (p= 0.006) and (p= 0.003) respectively. No statistically significant difference between other groups at other different intervals.

Table (1): *Showing Mean± SD and Unpaired t-Test of Plaque Index, Gingival Index, Probing depth, Clinical Attachment Level, and Annexin-A1 level in the four groups.*

	PI			***Unpaired t-Test***					
FOLLOW UP GROUPS / STUDIED GROUPS	**BASELINE**	**4 WEEKS**	**12 WEEKS**	**BASELINE VS 4 WEEKS**		**BASELINE VS 12 WEEKS**		**4 WEEKS VS 12 WEEKS**	
	Mean± SD	**Mean± SD**	**Mean± SD**	t	p	t	p	t	p
Group I	1.740 ± 0.598	0.378 ± 0.060	0.373 ± 0.057	7.996	**0.000**	7.966	**0.000**	1.763	**0.112 ns**
Group II	2.285 ± 0.384	0.423 ± 0.041	0.413 ± 0.037	17.082	**0.000**	16.911	**0.000**	2.061	**0.069 ns**
Group III	2.140 ± 0.636	0.385 ± 0.070	0.364 ± 0.064	9.796	**0.000**	9.817	**0.000**	7.692	**0.000**
Group IV	2.240 ± 0.423	0.410 ± 0.038	0.388 ± 0.043	15.025	**0.000**	15.369	**0.000**	7.150	**0.000**
	GI ***Unpaired t-Test***								
Group I	2.182 ± 0.529	0.414 ± 0.050	0.401 ± 0.052	11.662	**0.000**	11.770	**0.000**	3.323	**0.009**
Group II	1.974 ± 0.386	0.397 ± 0.034	0.386 ± 0.031	14.122	**0.000**	14.038	**0.000**	14.038	**0.004**
Group III	1.714 ± 0.684	0.339 ± 0.061	0.313 ± 0.055	6.937	**0.000**	6.966	**0.000**	6.697	**0.000**
Group IV	1.930 ± 0.572	0.361 ± 0.051	0.334 ± 0.043	9.510	**0.000**	9.512	**0.000**	7.038	**0.000**
	PD ***Unpaired t-Test***								
Group I	3.043 ± 0.736	2.123 ± 0.738	1.585 ± 0.562	7.405	**0.000**	7.932	**0.000**	4.804	**0.001**
Group II	3.417 ± 0.438	2.737 ± 0.425	2.207 ± 0.280	6.615	**0.000**	11.946	**0.000**	6.693	**0.000**
Group III	3.325 ± 0.514	2.505 ± 0.260	2.206 ± 0.312	5.694	**0.000**	6.538	**0.000**	4.539	**0.001**
Group IV	3.492 ± 0.584	2.768 ± 0.434	2.103 ± 0.484	13.258	**0.000**	14.461	**0.000**	6.525	**0.000**
	CAL ***Unpaired t-Test***								
Group I	0.378 ± 0.258	0.240 ± 0.191	0.132 ± 0.095	4.096	**0.003**	4.339	**0.002**	3.438	**0.007**
Group II	2.685 ± 0.546	2.120 ± 0.497	1.677 ± 0.432	26.052	**0.000**	9.646	**0.000**	4.222	**0.002**
Group III	0.360 ± 0.227	0.288 ± 0.193	0.157 ± 0.117	5.774	**0.000**	5.729	**0.000**	5.280	**0.001**
Group IV	2.632 ± 0.522	1.922 ± 0.452	1.355 ± 0.262	20.252	**0.000**	14.436	**0.000**	9.176	**0.000**
	ANX-1 Level ***Unpaired t-Test***								
Group I	527.472 ± 15.313	206.530 ± 23.8	175.855 ± 10.870	50.769	**0.000**	104.013	**0.000**	5.991	**0.000**
Group II	240.499 ± 12.849	186.550 ± 14.4	170.703 ± 13.886	10.432	**0.000**	13.088	**0.000**	6.139	**0.000**
Group III	252.402 ± 31.097	221.949 ± 23.3	182.890 ± 36.501	7.014	**0.000**	93.884	**0.000**	63.591	**0.000**
Group IV	214.424 ± 22.869	186.180 ± 23.7	164.314 ± 16.580	6.028	**0.000**	7.639	**0.000**	3.890	**0.004**

*ns: Non-significant if **P-value** is greater than 0.05*

Table (2): *Showing Paired t-Test of Plaque Index, Gingival Index, Probing depth, Clinical Attachment Level and, Annexin-A1 level in the four groups.*

Paired t-Test of PI						
	t	**p**	**t**	**p**	**t**	**p**
G I VS G II	-2.423	**0.026**	-1.938	**0.068 ns**	-1.873	**0.077 ns**
G I VS G III	-1.451	**0.164 ns**	-0.244	**0.810 ns**	0.321	**0.752 ns**
G I VS G IV	-2.158	**0.045**	-1.426	**0.171 ns**	-0.681	**0.505 ns**
G II VS G III	0.614	**0.547 ns**	1.472	**0.158 ns**	2.088	**0.051 ns**
G II VS G IV	0.248	**0.807 ns**	0.711	**0.486 ns**	1.396	**0.180 ns**
G III VS G IV	-0.412	**0.685 ns**	-0.998	**0.332 ns**	-0.985	**0.337 ns**
Paired t-Test of GI						
G I VS G II	1.002	**0.330 ns**	0.864	**0.399 ns**	0.770	**0.451 ns**
G I VS G III	1.711	**0.104 ns**	3.006	**0.008**	3.657	**0.002**
G I VS G IV	1.021	**0.321 ns**	2.325	**0.032**	3.111	**0.006**
G II VS G III	1.049	**0.308 ns**	2.646	**0.016**	3.654	**0.002**
G II VS G IV	0.202	**0.842 ns**	1.857	**0.080 ns**	3.086	**0.006**
G III VS G IV	-0.767	**0.453 ns**	-0.885	**0.388 ns**	-0.949	**0.355 ns**
Paired t-Test of PD						
G I VS G II	-1.379	**0.185 ns**	-2.278	**0.035**	-3.131	**0.006**
G I VS G III	-0.992	**0.334 ns**	-1.543	**0.140 ns**	-3.055	**0.007**
G I VS G IV	-1.510	**0.149 ns**	-2.382	**0.028**	-2.210	**0.040**
G II VS G III	0.429	**0.673 ns**	1.472	**0.158 ns**	0.005	**0.996 ns**
G II VS G IV	-0.325	**0.749 ns**	-0.165	**0.871 ns**	0.584	**0.566 ns**
G III VS G IV	-0.678	**0.507 ns**	-1.646	**0.117 ns**	0.564	**0.580 ns**
Paired t-Test of CAL						
G I VS G II	-12.081	**0.000**	-11.171	**0.000**	-11.044	**0.000**
G I VS G III	0.169	**0.867 ns**	-0.564	**0.580 ns**	-0.525	**0.606 ns**
G I VS G IV	-12.228	**0.000**	-10.846	**0.000**	-13.890	**0.000**
G II VS G III	12.433	**0.000**	10.863	**0.000**	10.744	**0.000**
G II VS G IV	.223	**0.826 ns**	.934	**0.363 ns**	2.014	**0.059 ns**
G III VS G IV	-12.606	**0.000**	-10.509	**0.000**	-13.231	**0.000**
Paired t-Test of ANX-1 level						
G I VS G II	45.398	**0.000**	2.269	**0.036**	0.924	**0.368 ns**
G I VS G III	25.095	**0.000**	-1.463	**0.161 ns**	-0.584	**0.566 ns**
G I VS G IV	35.969	**0.000**	1.913	**0.072 ns**	1.841	**0.082 ns**
G II VS G III	-1.119	**0.278 ns**	-4.079	**0.001**	-0.987	**0.337 ns**
G II VS G IV	3.143	**0.006**	0.042	**0.967 ns**	0.934	**0.363 ns**
G III VS G IV	3.111	**0.006**	3.397	**0.003**	1.465	**0.160 ns**

DISCUSSION

The purposes of the present study were to assess the clinical parameters and the level of Annexin-1 in different conditions in pregnant and non-pregnant women. The selected patients to participate in the present study ranged in age between (20 – 35) since teen pregnancies and pregnancies in women aged over 35 years are a risk and associated with systemic alterations [15].

Patients included in the present study suffered from gingivitis or periodontitis with pocket less than 6 mm in depth because many studies have suggested that a probing pocket depth of < 6 mm is a clear indication for surgical intervention [16].

In the present study, all patients had teeth more than 20 due to tooth loss is associated with are severe periodontal diseases and dental caries. [17]

The pregnant women to include in this study were in the second trimester of pregnancy due to a study that had shown that the most obvious changes in periodontal measurements occur in these periods [18].

Also, it is important to avoid elective dental care if possible during the first trimester and the last half of the third trimester.

Hyperventilation begins in the first trimester as well as the main gastrointestinal changes are nausea, vomiting, and heartburn which are due to mechanical changes resulting from an enlarging fetus, in combination with hormonal changes [19].

In the last half of the third trimester danger of premature delivery exists because the uterus is very sensitive to external stimuli. The second trimester is the safest time in dental care and the management of pregnant women [20].

The patients of the control group were not receiving contraceptive pills because many studies reported the negative effects of oral contraceptives on periodontal health [21]. Additional study has shown that (OCP's) create a condition that resembles a pregnant state with a higher prevalence of gingival inflammation, loss of attachment, and periodontal disease progression [22].

In this study instead of the general information that can be obtained from saliva, more precise information about the inflammatory status at a specific site can be obtained from GCF. This oral fluid has a great capacity to serve as a site-specific and point-of-care diagnostic specimen for periodontal disease and healing-related markers [23].

Regarding (PI) and (GI), there were statistically significant differences in all groups for the amount of plaque accumulation and gingival inflammation after treatment when compared to the baseline especially in PI.

Regarding GI, there were statistically significant differences during different intervals when compared to baseline in all groups with significant differences in pregnant groups when compared to non-pregnant groups after 4 and 12 weeks. The recurrence of gingival inflammation in pregnancy can be explained by hormonal changes particularly the marked increases in estrogen and progesterone which act as a risk factor for activation of specific types of virulent periodontal pathogens as well as modification of the host immune response.

The present study showed statistically significant differences in PD in all the studied groups at different intervals after conventional periodontal therapy when compared to the baseline. While the comparisons between the groups revealed a significant difference of probing depth in pregnant with gingivitis group when compared to other groups after 4 and 12 weeks that can be explained by that, the increased probing depth in pregnant with gingivitis is mainly by the inflammatory exudate accumulation with subsequent gingival overgrowth that reduced after treatment in this group.

Regarding CAL, there were statistically significant differences in all groups at different intervals after periodontal therapy when compared to the baseline. In addition, there were statistically significant differences between the gingivitis groups when compared to periodontitis groups at the observational periods due to the differences in the clinical presentation of the two forms of periodontal diseases.

These results are in agreement with previous studies reported, that basic periodontal therapy is effective in removal of plaque that reduces the risk of tooth loss, slow down disease progression, reducing PD, and CAL [24,25].

The levels of ANX-A1 in the gingival crevicular fluid were significantly reduced in all groups after periodontal treatment when compared to the baseline.

As ANX-A1 is a glucocorticoid-regulated protein with anti-phospholipase activity, the protein shows many other anti-inflammatory and pro-resolving properties [14]. It was also observed that during pregnancy, ANX-A1 levels in GCF were significantly higher in gingivitis only when compared to non-pregnant women exhibiting gingivitis as well as both pregnant and non-pregnant women exhibiting periodontitis.

These results were in consistency with other studies conducted on oral health in pregnant and non-pregnant women which assed ANX-A1 levels in saliva and found the levels of ANX-A1 significantly higher in gingivitis compared with health [26].

Also, salivary ANXA1 levels were significantly higher in pregnant women compared with non-pregnant women exhibiting gingivitis in saliva, these findings collectively denote that hormonal changes during pregnancy have a particular impact on ANXA1 regulation on the substrate of gingival inflammation. The association between estrogens and ANXA1 has been documented in vitro [27].

CONCLUSIONS

- ANX-A1 in GCF showed marked elevated levels with significant differences during pregnancy with gingivitis.
- Gingival crevicular ANX-A1 levels are regulated as a part of the gingival inflammatory response in pregnant and non-pregnant women.
- Basic periodontal therapy is sufficient to improve both clinical and biochemical parameters in gingivitis and mild to moderate periodontitis.

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تقييم مستوي انكسين 1 كعلامة حيوية للأمراض حول السنية لدي النساء الحوامل وغير الحوامل

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الملخص :

الهدف: الهدف من الدراسة هو تقييم مستوي انكسين 1 كعلامة حيوية للأمراض حول السنية لدي النساء الحوامل وغير الحوامل.

المواد والطرق: تم اختيار 40 مريضة عشوائيا (20 حامل و 20 غير حامل). تم تقسيم جميع المرضى إلى أربع مجموعات: المجموعة الأولى: ضمت 10 مرضى حوامل يعانين من التهاب اللثة وقد تلقين علاج اللثة الأساسي. المجموعة الثانية: ضمت على 10 مرضى حوامل يعانين من التهاب الأنسجة حول السنية وقد تلقين علاج اللثة الأساسي. المجموعة الثالثة: ضمت 10 مرضى غير حوامل يعانين من التهاب اللثة وقد تلقين علاج اللثة الأساسي. المجموعة الرابعة: ضمت 10 مرضى غير حوامل يعانين من التهاب الأنسجة حول السنية وقد تلقين علاج اللثة الأساسي. بعد ذلك. تم تقييم جميع الحالات إكلينيكيا قبل العلاج وبعد 4 و 12 أسبوعًا من العلاج. تم إجراء التقييم المختبري لمستوى انكسين 1 قبل العلاج وبعد 4 و 12 أسبوعًا من العلاج.

النتائج: تم تسجيل القياسات الإكلينيكية مثل: دليل قياس الطبقة الجرثومية و دليل قياس التهاب اللثة و قياس عمق الجيوب اللثوية و قياس مستوى المرفق السريري قبل العلاج وبعد 4 و 12 أسبوعًا من العلاج في جميع المجموعات. ظهرت فروق ذو دلالة إحصائية في كل المجموعات على فترات مختلفة عند مقارنتها قبل بدأ العلاج. دليل قياس الطبقة الجرثومية تبين عدم وجود فروق إحصائية بين المجموعات. دليل قياس التهاب اللثة وجود فروق أحصائية بين الحوامل وغير الحوامل خلال فترات العلاج المختلفة. قياس عمق الجيوب اللثوية وقياس مستوى المرفق السريري تبين وجود فروق إحصائية في جميع المجموعات بعد العلاج عند مقارنتها قبل بدأ العلاج. قياس مستوي انكسين 1 تبين وجود فروق إحصائية لدى النساء الحوامل الذين يعانون من التهاب اللثة عند مقارنتهم بباقي المجموعات قبل بدأ العلاج . الخلاصه: انكسين 1 مرشح كعلامة حيوية في السائل الثوي للفحص المبكر غير الجراحي لالتهاب اللثة أثناء الحمل.

الكلمات المفتاحية: انكسين 1 ، حامل ، مؤشر البلاك ,التهاب اللثه، مقايس اللثه.