

## Evaluation of the Effect of Diacerein on IL-1 $\beta$ and Osteocalcin levels in GCF of Chronic Periodontitis Patients: A non-randomized controlled clinical trial

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### Abstract

This work has been conducted to evaluate the influence of diacerein (a potent anti-inflammatory drug) both clinically and on IL- $\beta$  and osteocalcin levels in gingival crevicular fluid (GCF) in patients with chronic periodontitis.

The current experiment included forty-five patients allocated to three equal groups: Group I (n=15) included patients with chronic periodontitis who were treated with both scaling and root planing (SRP) only; Group II (n=15) involved patients with chronic periodontitis who were treated with SRP besides systemic diacerein administered orally and Group III (n=15) healthy volunteers with no periodontal disease. PI (plaque index), GI (gingival index), PD (pocket depth), and CAL (clinical attachment level) were recorded. In addition, IL-1 $\beta$  and osteocalcin levels in GCF were measured before treatment and after 1 and 2 months' post-treatment.

The results revealed the significant change and improvement of all the measured clinical parameters with the reduction of IL-1 $\beta$  and osteocalcin levels especially in the group in which diacerein was given.

Diacerein has a playful therapeutic effect in management of chronic periodontitis.

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### Introduction

Periodontal disease is one of the most common diseases affecting human dentition. Although periodontitis is an inflammatory process that is initiated by the plaque biofilm, the majority of periodontal tissue destruction is caused by exaggerated host response to plaque micro-organisms and their products with a resulting localized immune reaction<sup>1</sup>.

This localized immune response results in the recruitment of different inflammatory cells and the release of various inflammatory mediators comprising proinflammatory cytokines (i.e., IL-1, IL-6, TNF $\alpha$ ) and the liberation of a series of lytic enzymes i.e. matrix metalloproteinases (MMPs) and prostaglandins (PG). This finally leads to connective tissue loss and alveolar bone resorption due to activation of osteoclasts<sup>2</sup>.

IL-1 is one of the inflammatory mediators capable of stimulating diverse biological effects, involving inflammatory, metabolic, physiologic, hemopoietic, and immunologic reactions<sup>3,4</sup> potentially involved in the pathogenic mechanisms of periodontal tissue destruction and bone resorption<sup>5</sup>. IL-1 was detected in gingival crevicular fluid (GCF) from diseased periodontal sites and its level correlated with periodontal disease activity<sup>6,7</sup>.

Osteocalcin, a protein found in extracellular matrix of bone and dentin which is

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involved in regulation of mineralization in bones and teeth; is commonly described as a specific marker of osteoblastic function and bone turnover<sup>8</sup>. Several studies have been conducted for assessment of GCF osteocalcin level in different periodontal diseases. They reported that osteocalcin levels in GCF may act as a marker of periodontal disease activity at diseased sites<sup>9, 10, 11, 12, 13</sup>.

Mechanical debridement alongside antimicrobial treatment, both local and systemic, is one of the backbones in periodontal treatment procedures. Plaque elimination by scaling and root planing (SRP) focuses on one arm of the pathogenesis by diminishing the bacterial load, and accordingly the bacterial products that drives the inflammatory reaction in the host tissues. Notwithstanding, the bacterial burden is never totally removed after SRP, and re-colonization by bacterial species happens again<sup>14</sup>.

In addition, these treatment procedures fails to stop or overcome host reaction driving toward tissue destruction because of continuous stimulation with bacteria and their toxic products<sup>15</sup>. This prompted the introduction of host modulatory therapies (HMTs), which could enhance therapeutic goals, arrest disease progression, giving more predictable outcomes, and potentially even work as preventive measures against the development of periodontitis<sup>16, 17</sup>.

Diacerein is a purified derivative of anthraquinone that is extracted from plants with special characteristic anti-inflammatory and analgesic activities<sup>18</sup>. Diacerein reduces association of IL-1 to IL-1 receptors to form heterodimer complexes, represses IL-1 and its related downstream events including inducible nitric oxide synthase (iNOS) synthesis, stromelysin-1, collagenase, MMPs-1, -3, -9 and -13 and their activities<sup>19, 20</sup>.

Moreover, diacerein impairs activation of IL-1 due to the inhibition of the IL-1-converting enzyme (ICE)<sup>21</sup>. Diacerein also interfere with chemotaxis and phagocytosis of immune cells<sup>22</sup>, suppression of osteoclasts differentiation due to blocking of IL1-b, and reduction of synthesis of resorptive factors in terms of modulating RANKL/osteoprotegerin (OPG) expression<sup>23, 24</sup>.

Furthermore, diacerein reduces the production of osteocalcin, urokinase, and IL-6 in human subchondral bone osteoblasts that contribute to modulating bone

formation/resorption in joints<sup>24, 25</sup>. The functions of diacerein have been investigated several years ago and its beneficial effect has been mainly related to tissues/cells for osteoarthritis treatment<sup>26, 27, 28, 29</sup>. It was suggested that both the periodontal disease and arthritis are quite parallel in nature. Therefore, it is highly applicable for diacerein to have a major role in management of periodontal disease<sup>2</sup>.

To our knowledge, limited studies have been carried out to examine the possible role of diacerein in curing of periodontal disease in humans. Hence, the goal of this contemplate is to evaluate the effect of diacerein on the clinical parameters and 1L- $\beta$  and osteocalcin GCF levels in patients with chronic periodontitis.

## Materials and methods

### Subjects Selection:

The current study included forty five subjects, 15 periodontally healthy individuals and 30 patients with chronic periodontitis recruited from the outpatient clinic, Department of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Cairo University and the outpatient clinic at the National Research Centre (NRC), Giza. The Medical Research Ethical Committee (MREC) at the National Research Centre, Giza, Egypt had approved on the protocol of this contemplate. Written informed consent was taken from each patient after explaining the procedure along with the risks and benefits.

All subjects were selected to be systemically healthy according to the modified Cornell Medical Index<sup>30</sup>. To be included in the study, the periodontally healthy subjects had to have at least 24 natural teeth with no probing pocket depth (PD) and clinical attachment level (CAL) > 3 mm and have less than 20% of the sites with bleeding on probing (BOP).

Chronic periodontitis patients (CP) were diagnosed on the foundation of the periodontal classification of the American Academy of Periodontology (2000)<sup>31</sup> with the following inclusion criteria: The patients had at least 20 natural teeth with a minimum of two sites of a probing depth (PD)  $\geq$  5 mm and clinical attachment level (CAL)  $\geq$  4 to 6 mm with radiographic evidence of bone loss  $\geq$  3 mm.

Exclusion criteria included: patients who have received any kind of periodontal treatment

or have been using antibiotics or NSAIDs (non-steroidal anti-inflammatory drugs) in the previous six months, pregnancy and lactation, smoking and patients with hypersensitivity to anthraquinone derivatives.

All subjects were screened by comprehensive periodontal examination and full periodontal charts were obtained along with full mouth radiographic examination.

Chronic periodontitis patients (CP) were allocated into one of two groups:

Group I: consisted of 15 patients who were treated with scaling and root planing (SRP) alone.

Group II: consisted of 15 patients who were treated with SRP in addition to systemic diacerein (Eva Pharma, Egypt) 50 mg tablets administered orally twice daily for 2 months.

The periodontally healthy individuals were assigned as group III which consisted of 15 subjects and didn't receive any treatment.

#### **Clinical Periodontal Assessment:**

The following clinical periodontal parameters were recorded by the first investigator at the most periodontally affected tooth at baseline and again 1 and 2 months after treatment for groups I and II: Plaque index (PI)<sup>32</sup>, gingival index (GI)<sup>33</sup>, pocket depth (PD)<sup>34</sup> and clinical attachment level (CAL).

Gingival index and plaque index measurements were performed at 4 sites per tooth (buccal, mesial, distal and lingual). The scores from the four areas of the tooth were added and divided by four to give the GI and PI for the single tooth.

PD was measured as the distance between the gingival margin and the apical end of the pocket utilizing the Michigan 0 with Williams' markings periodontal probe nearly in a line with the vertical axis of the tooth until the blunt end contacted the bottom of the pocket and recorded in mm. CAL was measured from the cemento-enamel junction (CEJ) till the apical end of the pocket utilizing also the Michigan 0 with Williams' markings periodontal probe and recorded in mm.

PD and CAL measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) and all the

measurements were approximated to the most elevated whole millimeters.

#### **Collection of Gingival Crevicular Fluid (GCF):**

GCF samples were collected from the deepest sites with PD  $\geq$  5mm at baseline and 1 and 2 months after treatment in all the patients in the three groups. A sterile curette was carefully used to evict the supragingival plaque from the surfaces before GCF sampling. These surfaces were washed with water spray, carefully desiccated using an air syringe and were isolated with cotton rolls. GCF was sampled by utilizing filter paper strips (Perio-paper, IDE Interstate, Amityville, NY, USA) carefully inserted into the crevice until mild resistance was felt and left there for 30 seconds.

Extra care was taken to elude any kind of injury especially mechanical one. Strips contaminated with blood were discarded. Immediately, the volume of the sample was measured with the aid of a calibrated Periotron 8000 (Oraflow Inc., Amityville, NY, USA). After volume measurements, the strips were placed into sterile eppendorf tubes containing 300  $\mu$ L PBS (Phosphate buffered saline). Immediately after all the GCF samples were stored at  $-20^{\circ}$ C until subsequent analysis. The third investigator was responsible for the collection of all the GCF samples. The records from the Periotron 8000 were transferred to an actual volume ( $\mu$ L) by reference to the standard curve.

#### **Treatment phase:**

Group I and II received full mouth SRP done by the second investigator using ultrasonic scalers and hand instruments under local anesthesia, completed over 2 visits. Patients were given careful instruction in self-performing of plaque control measures: twice daily tooth brushing with a brush and tooth paste and once daily interdental cleaning using triangular wooden tooth picks and/or interdental brushes in order to keep on having good dental health and plaque control. For each patient the oral hygiene was checked every two weeks for two months.

Group II received 50 mg tablets administered orally twice daily for 2 months. They were advised to take one capsule in the middle of the meal with a glass of water. Blister packs of pills having the 50 mg diacerein were given to the subjects. Then follow up was carried out every 2 weeks for 2 months.

On counting the tablets retained in the blister packs, proper evaluation of the medication's obligation was considered. Plaque detection tablets and flossing procedures assessment aided in evaluation of the bacterial plaque (BP) control in every follow up visit were carried out.

#### **Determination of IL-1 and Osteocalcin levels in GCF samples:**

GCF samples were eluted from the strips by placing them in 300 $\mu$ L of PBS. GCF samples were analyzed for IL-1 $\beta$  and osteocalcin levels were assessed by utilizing commercially available human enzyme-linked immunosorbent assay (ELISA) kits (AvioBion, Ani Biotech Oy, Vantaa, Finland). Procedures were carried out according to the manufacturer's instructions in the kit. The amounts of IL-1 $\beta$  and osteocalcin in each sample were calculated based on the dilutions and the results were expressed as total amount in the 30 seconds of the GCF samples.

All ELISA calibrations were performed in duplicate. It is a sandwich-type ELISA where a monoclonal anti-human IL-1 $\beta$  and osteocalcin, adsorbed onto microwells, to bind IL-1 $\beta$  and osteocalcin in the samples were used. Accurate calculation of the results was carried out utilizing the standard curves involved in every assay kit. The color intensity was measured at 450 nm. IL-1 $\beta$  and osteocalcin were aided in determining their total amount of determined in picograms (pg) and calculations of the concentration in each sample were performed by dividing the total amounts of either IL-1 $\beta$  or osteocalcin by sample volume (pg/ $\mu$ L). The analysis of the GCF samples was the responsibility of the fourth investigator.

#### **Statistical Analysis:**

Numerical results were presented as mean and standard deviation (SD) values. Data's normality was determined by utilizing Kolmogorov-Smirnov test of normality. Mann Whitney U test was essential for approaching the comparison of PI, GI, PD, and CAL before and after treatment in both diseased groups. Student t-test was utilized to compare the IL-1 $\beta$  and osteocalcin levels between the diseased groups at different study time periods. Student t-test was also of use to resemble the IL-1 $\beta$  and osteocalcin limits at different timelines with their healthy controls in both the diseased groups.

The percentage of change between

baseline and different follow up times was explored between the diseased groups using either Mann-Whitney or student t-test according to the normality of the data. Correlating the clinical parameters and the IL-1 $\beta$  and osteocalcin levels was tested using Pearson product moment and Spearman's rank- order correlation. The significant level was set at  $p \leq 0.05$ . Statistical analysis was carried out with SPSS 18.0, Chicago, IL, USA.

#### **Results**

This study included a total of forty five subjects, 15 healthy individuals (6 males and 9 females) with mean age  $40.5 \pm 0.18$  and 30 chronic periodontitis patients divided into two groups. Group I (SRP only) consisted of 15 patients (5 males and 10 females) with mean age  $46.45 \pm 0.57$ . Group II (SRP + diacerein) consisted of 15 patients (8 males and 7 females) with mean age  $44.26 \pm 0.15$ .

Three patients in group II complained from mild abdominal pain which subsided three days from starting the diacerein treatment. Another two patients complained from diarrhea only for two days after the drug administration.

#### **1. Clinical parameters:**

Table (1) shows the mean  $\pm$  standard deviation (SD) and p-values for clinical parameters (PI, GI, PD and CAL) before and after treatment in both groups I and II. Table (2) shows percent of changes in clinical parameters between baseline and 1 month also baseline and 2 months after treatment in both groups.

Table (1) also revealed that there was no significant difference between both groups in baseline values for all the clinical parameters (PI, GI, PD and CAL). Significant improvements in PD, CAL were observed at 1 and 2 months after non-surgical periodontal therapy in both groups ( $p < 0.01$ ) with more significant changes in group II compared to group I. group II showed more significant % of reduction in PD ( $72.57 \pm 7.76$  %) than that found in group I ( $61.81 \pm 9.49$ %) between baseline and 2 months. Group II also exhibited highly significant greater % of gain in CAL ( $64.33 \pm 19.58$ %) than group I ( $38.33 \pm 18.46$ %) between baseline and 2 months as presented in table (2).

	Time	Group I	Group II	Mann-Whitney U test p-value
PI	Baseline	2.8 ± 0.42	2.6 ± 0.52	0.342
	1 month	1.9 ± 0.32	1.5 ± 0.53	0.057
	2 month	1.6 ± 0.52	1.1 ± 0.32	0.022*
GI	Baseline	2.6 ± 0.52	2.5 ± 0.53	0.661
	1 month	1.8 ± 0.42	1.3 ± 0.48	0.028*
	2 month	0.6 ± 0.52	0.1 ± 0.32	0.022*
PD (mm)	Baseline	5.9 ± 0.99	5.8 ± 1.03	0.796
	1 month	3.2 ± 0.63	2.4 ± 0.70	0.019*
	2 month	2.2 ± 0.42	1.6 ± 0.52	0.015*
CAL (mm)	Baseline	4.2 ± 0.79	4.3 ± 0.82	0.744
	1 month	3.5 ± 0.53	2.8 ± 0.63	0.021*
	2 month	2.5 ± 0.53	1.5 ± 0.85	0.009*

\*Statistically significant different, p-value ≤ 0.05.

**Table 1.** Shows the mean ± SD and p-values of PI, GI, PD, CAL measurements of both group I and II at baseline, 1 and 2 months post-treatment. Significance was set at p ≤ 0.05.

	Period	Group I	Group II	p-value
PI	Between baseline and 1 month	30.00 ± 18.92	43.33 ± 11.65	0.048*
	Between baseline and 2 months	40.00 ± 26.29	56.67 ± 11.65	0.148
GI	Between baseline and 1 month	30.00 ± 17.21	48.33 ± 12.29	0.015*
	Between baseline and 2 months	75.00 ± 22.57	96.67 ± 10.54	0.018*
PD	Between baseline and 1 month	44.43 ± 14.88	57.71 ± 14.24	0.056
	Between baseline and 2 months	61.81 ± 9.49	72.57 ± 7.76	0.012*
CAL	Between baseline and 1 month	15.00 ± 14.14	34.67 ± 9.16	0.002*
	Between baseline and 2 months	38.33 ± 18.46	64.33 ± 19.58	0.007*

**Table 2.** shows the significance of mean percentages of change in PI, GI, PD, CAL between before and after treatment in both groups I and II.

**2) Assessment of IL-1β and osteocalcin levels: as shown in table (3) and (4)**

	Time	Group I	Group II	Healthy Controls	Student t-test p-value
IL-1β level (µg/ml)	Baseline	11.48 ± 0.96	12.39 ± 2.07	4.08 ± 0.70	0.230
	1 month	9.01 ± 0.62	7.99 ± 1.40	4.08 ± 0.70	0.049*
	2 month	6.57 ± 1.08	5.18 ± 1.49	4.08 ± 0.70	0.028*
Osteocalcin level (µg/ml)	Baseline	13.44 ± 3.90	12.23 ± 3.37	2.59 ± 0.30	0.467
	1 month	10.10 ± 2.85	7.8 ± 2.07	2.59 ± 0.30	0.054
	2 month	6.89 ± 2.81	3.34 ± 1.50	2.59 ± 0.30	0.003*

**Table 3.** Shows the means ± SD values of IL-1β and osteocalcin levels of groups I, II and the healthy controls.

At baseline, both IL-1β and osteocalcin levels were significantly greater (p < 0.001) in chronic periodontitis groups (I and II) compared to that with healthy periodontium with no significant difference between group I and II. At 1

month as well as 2 months, non-surgical periodontal therapy resulted in significant drop in the level of IL-1β in both groups with group II showing more significant % of reduction in IL-1β level (58.01 ± 9.71%) than group I (42.40 ± 9.11%) between baseline and 2 months.

	Period	Group I	Group II	p-value
IL-1β Level	Between baseline and 1 month	21.34 ± 4.16	34.68 ± 10.43	0.003*
	Between baseline and 2 months	42.40 ± 9.11	58.01 ± 9.71	0.002*
Osteocalcin Level	Between baseline and 1 month	24.07 ± 7.41	35.83 ± 4.37	0.001*
	Between baseline and 2 months	47.43 ± 15.94	72.12 ± 9.23	0.001*

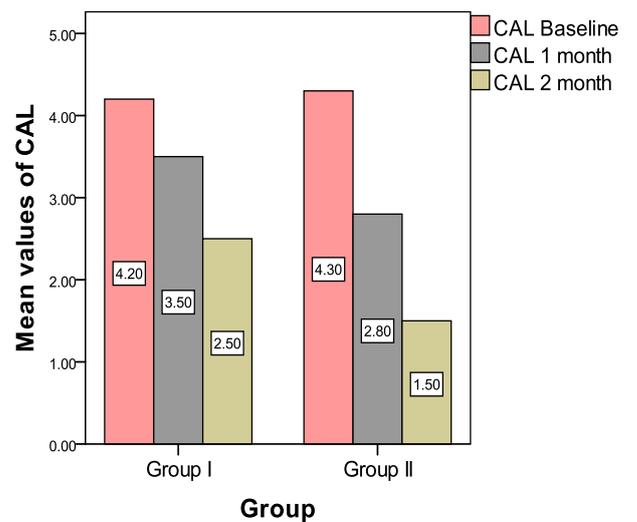
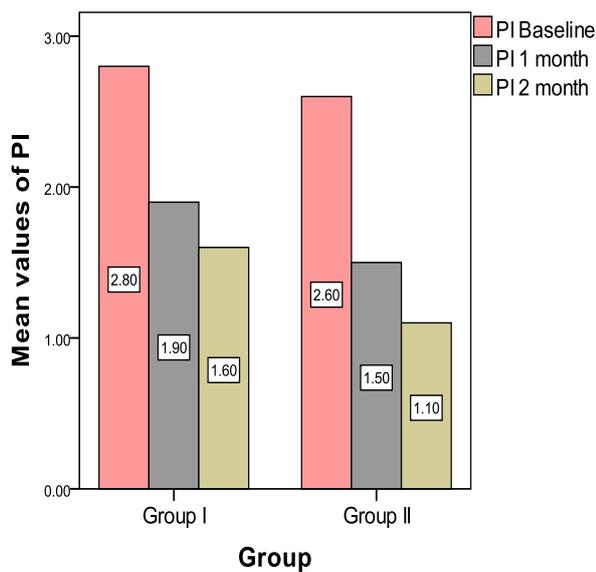
**Table 4.** Shows the significance of mean percentages of change in IL-1β and osteocalcin levels between before and after treatment in both groups I and II.

As for osteocalcin, there was no statistically significant decrease of osteocalcin level in both groups after 1 month with p-value of 0.054. However, both groups showed significant fall in the level 2 months after periodontal therapy (p-value of 0.003) with group II (72.12 ± 9.23%) having more significant % of reduction than group I (47.43 ± 15.94 %) between baseline and 2 months.

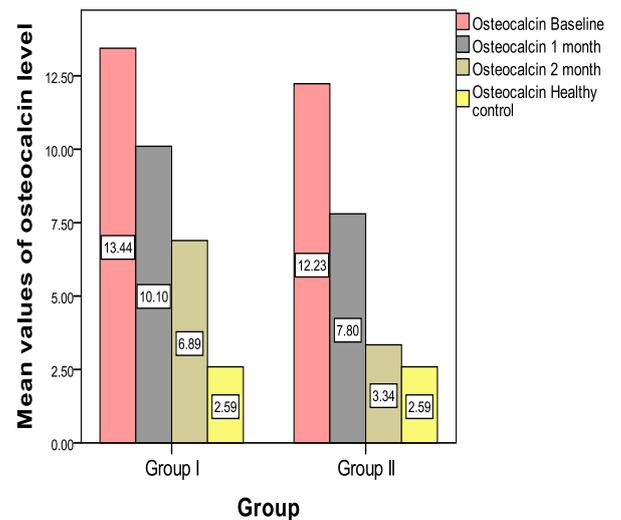
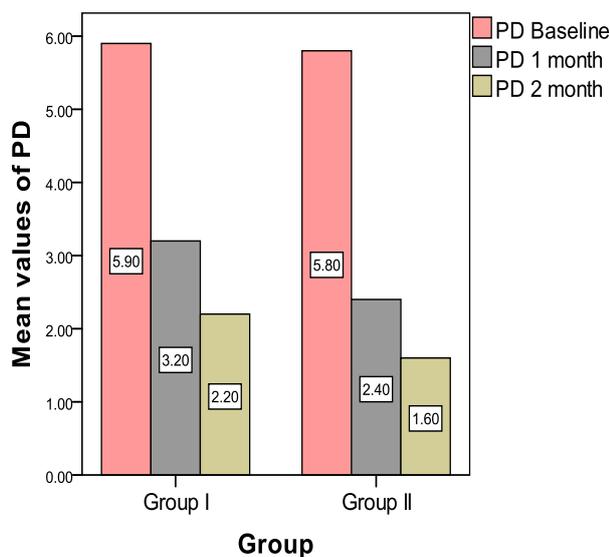
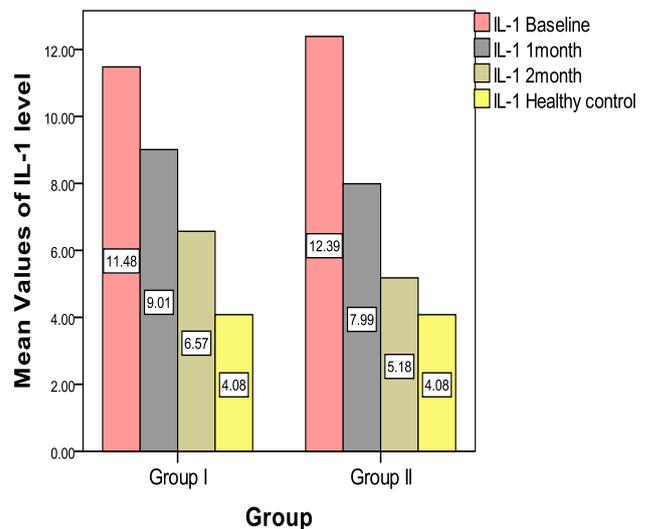
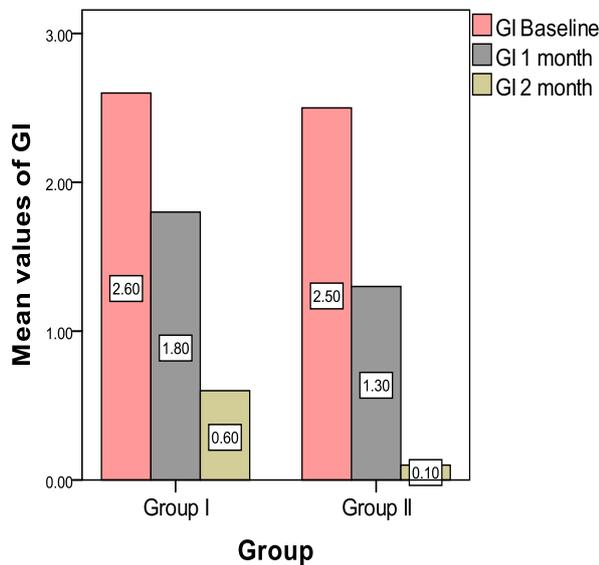
Correlation between the different measured clinical parameters and the levels of IL-1β and osteocalcin revealed the positive correlation between the parameters and the level of IL-1β with a statistically remarkable p-value 0.001. Meanwhile, no statistically significant correlation existed between the clinical parameters and the level of osteocalcin with p-value 0.329 was apparent as reported in table (5).

		PI	GI	PD	CAL
IL-1 level (µg/ml)	Correlation coefficient	0.510	0.137	0.397	0.697
	p-value	0.021*	0.565	0.083	0.001*
Osteocalcin level (µg/ml)	Correlation coefficient	0.273	0.546	0.258	0.230
	p-value	0.244	0.013*	0.271	0.329

**Table 5.** shows the correlation between the measured clinical parameters and the levels of IL-1β and osteocalcin.



**Figure 1.** Shows the mean values of PI, GI, PD and CAL in both groups I and II.



**Figure 2.** shows the mean values of IL-1 $\beta$  and osteocalcin values in both groups I and II as compared to the healthy controls.

## Discussion

Periodontitis is an inflammatory disease initiated by plaque bacteria<sup>1</sup>. The microbial virulence factors produced by periodontal pathogens mount a host immune reaction with resultant release of inflammatory mediators including pro-inflammatory cytokines (IL-1, IL-6, TNF- $\alpha$ ) and prostaglandins (PGE<sub>2</sub>), which can induce extracellular matrix degradation and alveolar bone resorption<sup>2</sup>.

Although inflammatory host response is essential for host defense against bacterial inflammation, exaggerated and prolonged reaction is destructive for the functional periodontal tissues<sup>3</sup>. Therefore, Much attention has been focused on drugs that are not primarily aimed at the palliation of disease symptoms, but modulate the host immune response to the causative factor<sup>35</sup>.

Anti-cytokine therapy for periodontal diseases especially targeting pro-inflammatory cytokines, that is, TNF- $\alpha$ , IL-1, and IL-6, because these are essential for the initiation of the inflammatory immune reaction and are produced for prolonged periods in periodontitis<sup>3</sup>. Diacerein is one of the drugs that have IL-1 $\beta$  inhibitory activity, and it was developed mainly for the treatment of osteoarthritis. Diacerein; through its active metabolite rhein; was found to effectively inhibit the synthesis of cytokines in vitro and reduces the level of the bioactivity of the IL-1 $\beta$  receptor<sup>36</sup>.

To the best of our knowledge, this is the first clinical trial testing the clinical potency of diacerein as an anti-inflammatory drug in management of chronic periodontitis and evaluating its effect on the clinical parameters (PI, GI, PD and CAL) and GCF levels of IL-1 $\beta$  and osteocalcin.

In both of periodontitis groups, all clinical parameters showed statistically significant reduction 2 months after treatment, however diacerein group showed more significant improvement than the non-diacerein group. Similarly, Chapple and Mathews<sup>37</sup> obtained a significant reduction in PD and sites of bleeding on probing (BOP) following non-surgical therapy. This finding was also in agreement with results of Grant et al<sup>38</sup> who confirmed the success of non-surgical therapy by observed reduction in whole mouth (PD) and percentage of bleeding sites on probing.

Cugini et al<sup>39</sup> attributed that to the fact that the main aim of non-surgical periodontal treatment, including instrumentation and effective oral hygiene instruction (OHI), is to prevent tooth loss via the effective and continued prevention of periodontal disease progression, achieved by the reduction of the microbial burden that is present around the periodontal tissues. Effective periodontal therapy disrupts the subgingival plaque biofilm, allowing a shift in the microbial populations to those more commonly associated with health.

Interleukin-1 (IL-1) is a potent bone-resorbing cytokine and was known as osteoclasts activating factor<sup>40</sup>. IL-1 has been detected in both periodontal tissues and GCF in patients with periodontal disease<sup>41</sup>; however results regarding the effect of non-surgical periodontal therapy on GCF IL-1 levels are still conflicting.

In the results of the current study, IL-1 $\beta$  level was significantly higher in GCF of chronic periodontitis patients than in individuals with healthy periodontium. Similarly, Elavarasu et al<sup>42</sup> reported that the IL-1 $\beta$  levels in GCF were significantly induced in samples from chronic periodontitis patients. Faizuddin et al<sup>43</sup> found values of the concentration of IL-1  $\beta$  in the GCF of patients with chronic periodontitis much higher than those values obtained in subjects with a clinically healthy periodontium.

Our results showed that there was a statistically significant reduction in IL-1  $\beta$  level 2 months after scaling and root planing. This was in agreement with Gamonal et al<sup>44</sup> who demonstrated the IL-1 was found in the GCF of chronic periodontitis patients and declined 2 months after scaling and root planning. In line with the current results, Oh et al<sup>45</sup> also found that GCF IL-1 $\beta$  level was significantly lower than baseline at 2 months after initial therapy at shallow and deep sites in chronic periodontitis patients.

The marked reduction of IL-1 $\beta$  in GCF following treatment clearly suggests a relationship between active disease and IL-1 $\beta$  production. Successful therapy resulted in lower IL-1 $\beta$  levels together with an evident recovery of the periodontal tissues.

Our result was not in line with Al-Shammari et al<sup>46</sup>. They reported that IL-1 $\beta$  levels did not decrease significantly following mechanical treatment. Reinhardt et al<sup>47</sup> also found no significant differences in IL-1 levels 6

months after scaling and root planing. Lambert et al<sup>48</sup> suggested that changes in IL-1 levels following treatment may be dependent upon the subjects' composite genotype for the polymorphic IL-1 $\beta$  gene cluster. Significant reductions in IL-1 concentrations after SRP were only detected in subjects who did not possess the periodontitis-associated genotype.

Diacerein group showed more significant reduction in GCF IL-1 $\beta$  than non-diacerein group. In a study conducted by Zaki et al<sup>49</sup> on experimentally induced periodontitis in rats, there was a significant decrease in IL-1 $\beta$  level in both treated groups recording that the decrease was more in the diacerein group which is in accordance with our results.

The current results can be explained by Verbruggen<sup>50</sup> who found that diacerein directly inhibited IL-1 synthesis and release in vitro and down modulated IL-1 induced activities and have been shown to possess disease modifying effect in experimental models of osteoarthritis. Furthermore, Khady et al<sup>51</sup> reported that diacerein also inhibited IL-1 induced expression of bone degrading enzymes thereby accounting for disease modifying property of diacerein.

Osteocalcin level is determinant of bone formation and it has been also proven that it has a major effect on bone resorption in vitro<sup>52</sup>. Our results showed that GCF osteocalcin level was higher in periodontitis group than in subjects with healthy periodontium.

GCF level of osteocalcin in periodontal disease is highly controversial. Kunimatsu et al<sup>53</sup> did not ascertain osteocalcin in GCF of cases having gingivitis, although in periodontitis GCF osteocalcin was remarkably detected the same as our study. Lee et al<sup>53</sup> proved similar GCF osteocalcin levels in both diseased and healthy regions in cases with chronic periodontitis. Wilson et al<sup>54</sup> could not discover osteocalcin in GCF of untreated periodontitis cases. Kinney et al<sup>55</sup> discovered elevated amount of osteocalcin in GCF from periodontitis regions compared to those appeared in both healthy, the same as was shown in our study. Becerik et al<sup>56</sup> concluded that variations in osteocalcin levels among different studies ; may be due to inability to differentiate between sites undergoing attachment loss and others in a "bone loss arrest" state, when clinical signs of periodontal disease ( CAL, increased PPD, bleeding on probing) are present, but no activity was detected.

## Conclusions

Our results showed that osteocalcin level in GCF was significantly reduced 2 months after treatment in both periodontitis groups. This was in accordance with Matouga et al<sup>57</sup> whose study showed a significant reduction in GCF osteocalcin level in chronic periodontitis patients after SRP. The osteocalcin in gingival fluid appeared to be correlated with periodontal disease activity and bone turnover as evidenced by significant elevations in GCF osteocalcin during the more active periods of bone loss. A drop in the levels of GCF osteocalcin after periodontal therapy showed that osteocalcin increased with bone resorption and decreased with periodontal healing.

Our results were in disagreement with those of Golub et al<sup>58</sup> who failed to detect any changes in osteocalcin levels after periodontal therapy in patients with chronic periodontitis. This may be related to the adjunctive use of antibiotic therapy in their study which eliminated the microbial insult and provided a favourable environment for periodontal healing. In the current study, diacerein group showed more percent reduction in the osteocalcin level than do the non-diacerein group. Consistent with our findings, Pelletier et al<sup>59</sup> found that diacerein statistically inhibited the production of osteocalcin in human subchondral bone osteoblasts that contribute to modulating bone formation/resorption in joints.

## Declaration of Interest

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