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A Comparative Evaluation of Clinical Efficacy and Salivary and Gingival Crevicular Fluid Interleukin – 6 Levels with Herbal and Probiotic Host Modulation Therapy in Chronic Periodontal Disease

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Abstract

Background

Host modulation is fast gaining popularity as one of the most safe and effective therapeutic modalities in a number of systemic ailments and also periodontal disease. Of these, herbal agents and probiotics have emerged as popular therapeutic agents. The present study was attempted to comparatively evaluate for the first time the effects of both these agents on chronic periodontitis.

Materials and methods

In this double blinded randomized controlled clinical trial, 96 systemically healthy patients with chronic periodontitis between the ages of 25-55 years were randomly divided into 3 groups. Following scaling and root planing, group 1 was administered the herbal immunomodulator tablets twice daily for 2 weeks, group 2, probiotic tablets twice daily for 2 weeks and group 3 was not administered any agent.

Changes in gingival index, gingival bleeding index, pocket depth and interleukin – 6 (IL-6) levels in saliva and GCF were assessed at day 0, at 1 month, at 3 months and 6 months following which statistical analysis was done.

Results

Statistically significant reduction ($p<0.001$) in clinical parameters and GCF IL-6 levels at 1, 3 and 6 months respectively within all 3 groups was observed. However, there was a significant reduction in salivary IL-6 levels within group 3 only. Group 1 showed better clinical results and salivary IL-6 reduction compared to group 2 and 3.

Conclusion

Both herbal and probiotic immunomodulators were effective when used as adjuncts to scaling and root planing in chronic periodontitis patients.

Keywords: Periodontitis; Herbal immunomodulation; Probiotics; Interleukin – 6; ELISA

Introduction

Immunomodulators are becoming very popular in the natural health industry worldwide as people start to realize the importance of a healthy immune system in the maintenance of health and the prevention and recovery of disease. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases [1]. Complex interactions of the host defense (immune) mechanisms and plaque pathogens play an important role in the onset and progression of periodontal disease [2]. Among different bacterial antigens, lipopolysaccharide (LPS) is a potent activator of macrophage [3]. LPS is known to evoke wide range of signalling pathways in macrophage and other cell types leading to the production of inflammatory mediators [4]. Such inflammatory mediators consist of proinflammatory cytokines like tumour necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6, IL-8 and other mediators like nitric oxide (NO), prostaglandin etc [5], which have been found responsible for the cellular and tissue damage leading to inflammation [6].

Interleukin-6 (IL-6) is an important cytokine involved in the regulation of host response to tissue injury and infection [7]. It is produced by a variety of cells, such as monocytes, fibroblasts [8], osteoblasts [9], and vascular endothelial cells [10] in response to inflammatory challenges [11]. It plays an important role in B-cell differentiation [12] and in T-cell proliferation [13], while IL-6, synergistic with interleukin-1β (IL-1β), induces bone resorption [9].

Conventional periodontal treatment consists of scaling and root planing, which is aimed at elimination of plaque with no effect on the host mediated tissue destruction to continued bacterial challenge [14]. A number of agents such as chemically modified tetracyclines (CMTs) [15] and NSAIDs [16] have been tried and tested to control the tissue destruction caused by inflammatory processes in periodontal disease. Herbal drugs have also shown the capacity to control the production of proinflammatory mediators thereby managing many inflammatory processes [17].

Septilin®§ is an herbal drug preparation which has shown some potency to modulate immune functions in animal models [18]. A recently

conducted pilot study on the host modulating effects of Septilin is suggestive of a promising prospect [19].

In vitro studies have investigated the mechanism of Septilin[§] in regulating the production of proinflammatory mediators such as TNF-α, IL-6, IL-8, NO, COX-2 and PDE4 in LPS stimulated macrophage and monocyte cell lines [20].

Probiotics have been shown to modulate the host immune response by interacting with and strengthening the immune system and helping prevent disease [21,22].

It was therefore decided to comparatively evaluate for the first time, the immunomodulatory effects of an herbal agent (septilin[§]) and probiotics (sporlac[¶]) as an adjunct to scaling and root planing in patients with periodontal disease. Till date, evidence with regard to evaluation of IL-6 in periodontal treatment outcomes is limited. Thus it was also decided to assess the effects of host modulation on salivary and GCF IL-6 levels.

Materials and Methods

Following the approval of the Ethical Committee, Bangalore Institute of Dental Sciences, Bangalore, 96 patients between the age groups of 25-55 years were selected from the outpatient department of Periodontics of the institute for this double blinded randomised controlled clinical trial during the period from January 2011 to September 2011 on the basis of the following criteria:

1. Patients with generalised or localised chronic periodontitis having probing pocket depth of >5 mm.
2. Patients otherwise systemically healthy.
3. Patients with no history of illness or drug intake in the last 6 months.

Patients with compromised immune system, deleterious habits such as smoking, betel nut/paan chewing, pregnant or lactating women and physically or mentally challenged individuals were excluded from the study.

The 96 selected patients were further randomly divided into 3 groups of 32 patients each

Group 1 – Septilin[§] tablets were administered twice a day for 2 weeks following scaling and root planing.

Group 2 – Sporlac[¶] tablets (lactic acid bacillus tablets delivering 60 million spores) were administered twice a day for 2 weeks following scaling and root planing.

Group 3 – only scaling and root planing was done and no agent was administered.

In all the groups, the patients were asked to follow routine oral hygiene measures of brushing twice daily and flossing. No antimicrobial mouth rinse was prescribed.

Periodontal parameters viz gingival index (GI)(Loe & Silness 1963), gingival bleeding index (GBI) (Ainamo & Bay 1975) and pocket depth (PD) were assessed prior to scaling (at day '0'), at 1 month, 3 months and at 6 months post therapy.

Saliva and GCF samples for IL-6 analysis were collected on day 0, 1 month, 3 months and 6 months and analysed using Human IL-6 Elisa kit #. Saliva was collected by passive drool method and stored under refrigeration at -20°C.

GCF was collected from the selected sites with micro- capillary pipettes** and also stored under refrigeration at -20° C after being wrapped in an aluminium foil to prevent desiccation.

Prior to analysis, the GCF samples were diluted 10 times with a diluent buffer as per the instructions from the manufacturer in the ELISA kit. The samples were also further duplicated to ensure accurate results.

Statistical analysis was carried out using Z-tests with SPSS software version 13 as the sample size was greater than 30 in each group.

Results

Changes in clinical parameters

The change in mean GI, GBI and PD from pre-treatment (baseline) was found to be statistically significant within all the three groups when compared at 1, 3 and 6 months (P<0.001) respectively (Tables 1A, 2A and 3A).

However, group I showed highly significant difference in mean GI and GBI compared to both group II and III at 1, 3 months and at 6 months respectively (P<0.001). However, there were no significant changes observed in GI and GBI between group II and III when compared to each other at the various time intervals (Tables 4a and 4b).

With regard to PD, the differences in mean values were highly significant in group I compared to group II and III (P<0.001) and in group II compared to group III at 1, 3 and 6 months respectively (Table 4C).

Changes in biochemical parameters

There was no significant change in the salivary IL-6 levels from pre-treatment (baseline) to 1, 3 and 6 months respectively (p >0.05) both within group I and group II (Tables 1B and 2B). However, the changes were highly significant at 1, 3 and 6 months (p<0.001) within group III (Table 3B). On the contrary, the GCF IL-6 levels showed statistically significant change from pre-treatment (baseline) to 1, 3 and 6 months within group I (p<0.001) (Table 1B) and also from pre-treatment (baseline) to 3 and 6 months within group II (p<0.001) (Table 2B). In addition, as with salivary IL-6, GCF IL-6 levels also showed highly significant changes within group III at 1, 3, 6 months respectively (p<0.001) (Table 3B).

The salivary IL-6 levels in the three groups were compared with each other significant difference was observed between group I and III (P<0.001) at 1 month, 3 months and 6 months and between group I and II (p<0.001) at 3 and 6 months (Tables 4B and 5B). However, no significant difference was observed in the GCF IL-6 levels when the 3 groups were compared with each other at 1, 3 and 6 months respectively (p>0.05) (Tables 4B and 5B).

Discussion

Periodontal disease has a considerable contribution to the immune system and cytokines in its pathogenesis. At present, the strongest evidence for cytokines functioning in networks in periodontal pathogenesis exists for IL-1b, TNF-a, IL-6 and RANK/RANKL/OPG, and ongoing research is elucidating the role of other cytokines and chemokines in periodontal inflammation [23].

[§] ***Himalaya Drug Company, Bangalore, India.***

[¶] ***Uni- Sankyo Ltd, Maharashtra, India.***

[#] ***KrishGen bio systems, Mumbai, India***

** ***Sigma Aldrich, Milwaukee, United States of America.***

Parameter	Time Interval	N	Mean Difference	Z	P-value
GI	Pre testing vs 1 month	32	0.204	4.934	<0.0001*
	Pre testing vs 3 month	32	0.229	4.934	<0.0001*
	Pre testing vs 6 month	32	0.225	4.934	<0.0001*
GBI	Pre testing vs 1 month	32	0.22	4.934	<0.0001*
	Pre testing vs 3 month	32	0.23	4.934	<0.0001*
	Pre testing vs 6 month	32	0.23	4.934	<0.0001*
PD	Pre testing vs 1 month	32	4.337	4.934	<0.0001*
	Pre testing vs 3 month	32	4.647	4.934	<0.0001*
	Pre testing vs 6 month	32	4.756	4.934	< 0.0001*

(*) denotes significant difference
Table 1a: Clinical parameters – group I

Parameter	Time Interval	N	Mean Difference	Z	P-value
SAL- IL6	Pre testing vs 1 month	32	0.036	-0.224	0.8228
	Pre testing vs 3 month	32	0.043	0.429	0.6679
	Pre testing vs 6 month	30#	0.042	1.385	0.1661
GCF IL6	Pre testing vs 1 month	32	0.021	4.037	< 0.0001*
	Pre testing vs 3 month	32	0.027	3.401	0.0007*
	Pre testing vs 6 month	32	0.024	3.906	< 0.0001*

(*) denotes significant difference
Two pairs of values have zero difference
Table 1b: Biochemical parameters–group I

A great deal of evidence exists with respect to the immunomodulating effects of probiotics in periodontal disease [21,22]. Probiotic bacteria have been suggested to provide health benefits to the host by providing nutrients and cofactors to the host, competing directly with pathogens, interacting with pathogen virulence factors, and stimulating host immune responses [22]. Herbal agents are also fast gaining popularity [17] as effective immunomodulating agents. Long-term use of such herbal anti-inflammatory drugs has been found to be safer than chemical anti-inflammatory drugs. Septilin® is an Ayurvedic herbal preparation containing various herbs and minerals. It contains numerous medicinal plants which possess immunomodulatory properties that aid in strengthening the immune system and potentiate the non-specific immune responses of the body [24,25]. Various studies have shown Septilin® to be effective in respiratory tract infections, tonsillitis, and other infections. Septilin has also shown promise as a host modulator in periodontal diseases [24,25]. It was therefore decided to clinically evaluate and compare whether the host modulation effects of these 2 agents on the periodontal disease process were effective when used as an adjunct to scaling and root planing.

The most commonly used methods to detect the presence and quantities of inflammatory markers such as cytokines in the serum and other fluids are the gingival crevicular fluid (GCF) and saliva. Because these fluids are derived from serum, some molecules present in blood also appear in them [26]. Recently, there has been growing interest in diagnosis based on saliva analyses, because saliva has a simple and non-invasive collection method. Oral fluid sampling is safe for the operator and the patient, and has easy and low-cost storage [27]. Moreover, increasing evidence indicates that detection of GCF derived mediators in saliva may serve as a means of rapid screening for periodontal disease [27]. However, evidence also indicates that GCF is comparatively more sensitive than saliva as the markers are detected in higher concentrations [28], therefore, it was decided to assess and compare for the first time, the clinical effects and the immunomodulatory effects of these agents following SRP on the salivary and GCF levels of the inflammatory cytokine IL-6 which plays a prominent role in periodontal tissue destruction.

All three groups, showed significant improvement in the clinical signs of periodontal disease viz. gingival index, gingival bleeding index and probing pocket depth, during the various time intervals. This is in line with evidence justifying anti-inflammatory effects of Septilin® [24,25] and probiotics [22] and also with literature suggesting that scaling and root planing can alter and improve clinical signs of periodontal disease [29].

However, when the 3 groups were compared with each other, the septilin® group showed significant improvement in clinical periodontal parameters over the probiotic group and the SRP group at 1, 3 and 6 months. The probiotic group was significantly better than the SRP group with regard

Parameter	Time Interval	N	Mean Difference	Z	P-value
GI	Pre testing vs 1 month	32	0.142	4.934	< 0.0001*
	Pre testing vs 3 month	32	0.152	4.934	<0.0001*
	Pre testing vs 6 month	32	0.157	4.934	< 0.0001*
GBI	Pre testing vs 1 month	32	0.12	4.934	< 0.0001*
	Pre testing vs 3 month	32	0.14	4.934	< 0.0001*
	Pre testing vs 6 month	32	0.14	4.934	< 0.0001*
PD	Pre testing vs 1 month	32	3.659	4.934	< 0.0001*
	Pre testing vs 3 month	32	3.831	4.934	< 0.0001*
	Pre testing vs 6 month	32	3.862	4.934	< 0.0001*

(*) denotes significant difference
Table 2a: Clinical parameters – group II

Parameter	Time Interval	N	Mean Difference	Z	P-value
SAL- IL6	Pre testing vs 1 month	32	0.008	0.766	0.4437
	Pre testing vs 3 month	32	0.009	0.448	0.6542
	Pre testing vs 6 month	32	0.010	0.168	0.8666
GCF IL6	Pre testing vs 1 month	32	0.011	1.794	0.0728
	Pre testing vs 3 month	28@	0.014	3.681	0.0002*
	Pre testing vs 6 month	30#	0.012	3.468	0.0005*

(*) denotes significant difference
Two pairs of values have zero difference
@four pairs of values have zero difference
Table 2b: Biochemical parameters – group II

Parameter	Time Interval	n	Mean Difference	Z	P-value
GI	Pre testing vs 1 month	32	0.1647	4.747	<0.0001*
	Pre testing vs 3 month	32	0.1647	4.747	<0.0001*
	Pre testing vs 6 month	32	0.1759	4.756	<0.0001*
GBI	Pre testing vs 1 month	32	0.1212	4.934	<0.0001*
	Pre testing vs 3 month	32	0.1395	4.934	<0.0001*
	Pre testing vs 6 month	32	0.1406	4.934	<0.0001*
PD	Pre testing vs 1 month	32	1.3812	4.934	<0.0001*
	Pre testing vs 3 month	32	2.0468	4.934	<0.0001*
	Pre testing vs 6 month	32	2.8344	4.934	<0.0001*

(*) denotes significant difference
Table 3a: Clinical parameters – group III

Parameter	Time Interval	n	Mean Difference	Z	P-value
SAL- IL6	Pre testing vs 1 month	31#	0.0008	3.99	<0.0001*
	Pre testing vs 3 month	32	0.0015	2.85	0.004*
	Pre testing vs 6 month	32	0.0021	2.971	0.003*
GCF IL6	Pre testing vs 1 month	30#	0.0064	3.708	0.0002*
	Pre testing vs 3 month	29#	0.0068	4.391	<0.0001*
	Pre testing vs 6 month	30#	0.0071	4.249	<0.0001*

(*) denotes significant difference
1/2/3 pairs of values have zero difference
@four pairs of values have zero difference
Table 3b: Biochemical parameters – group III

to pocket depth reduction only and not the other two parameters. This justifies the role of Septilin® as an effective immunomodulator as it directly affects the cytokine levels [24,25]. Although studies have shown probiotics to reduce pro-inflammatory cytokines [21,22] their host modulation effect is mainly directed towards microbial shift. Moreover, with SRP, the clinical efficacy is a direct effect of removal of local irritants and resolution of the inflammation is then a result of host factors which is a gradual process.

The salivary IL-6 levels showed no significant changes within both the septilin and the probiotic groups at the various time intervals. However, there were significant changes within the SRP group at 1, 3 and 6 months. On the contrary, GCF IL-6 levels showed more significant change at the various time intervals (i.e.1,3,6 months) within the septilin group, at 3 and 6 months within the probiotic group and also at 1,3,6 months within the SRP group. GCF has been suggested to be a more sensitive marker than saliva [28]. In all likelihood, it could be deemed possible that saliva may have been contaminated or diluted by other factors resulting in variation in the values. However, SRP group showed significant changes with regard to salivary IL-6 levels at the various time intervals. This is in accordance with literature suggesting salivary levels of the biomarkers are significantly reduced following nonsurgical therapy [30]. One plausible suggestion could be that since the selected patients were systemically healthy, removal of local irritants probably triggered adequate host responses to cause significant reduction in IL-6 levels. This also further reiterates the fact that the analysis of saliva may offer a cost-effective approach to assessment of periodontal disease in large populations [31].

Interestingly, when the salivary levels of the cytokine were compared between the 3 groups, statistically significant differences were observed between the septilin group over the SRP group at 1,3 and 6 months and between septilin group and probiotic group at 3 and 6 months. No such differences were observed between the probiotic group and the SRP group at any of the time intervals. Host modulation therapies have been developed and proposed to block pathways responsible for periodontal tissue destruction [32], which Septilin has shown, as it effectively regulates inflammatory cytokines. On the other hand, it has also been suggested that probiotics as an adjunct to mechanical debridement might be an effective approach for the treatment of periodontitis as it controls oral microbiota to induce a beneficial shift away from pathogens [33]. With probiotics, the immunomodulatory effect is directed towards microbial shift rather than cytokine reduction thereby explaining the better cytokine reduction obtained with septilin. This is in contrast to the findings of Twetman et al. who have suggested a decrease in proinflammatory cytokines with the use of probiotic chewing gum [34].

In contrast, when the GCF IL-6 levels were similarly compared, no significant differences in all the three groups at any of the given time intervals were observed. These observations though diverse, to some extent do endorse the suggestions, that saliva although more suitable for qualitative assessments, can be reliably used for quantitative analysis of biomarkers [27]. Increasing evidences also indicate that detection of GCF derived mediators in saliva may serve as a means of rapid screening for periodontal disease [28] as GCF collection methods may at times, is cumbersome. Alternatively, it would be prudent to suggest that these variations could also be the result of sampling or laboratory errors or irregularities in the patient's compliance with regard to the agents prescribed. The above factors could thus be attributed to limitations of the study, which there is a need to overcome, in possible future directions. Thus, whether saliva or GCF is the diagnostic fluid of choice is still debatable as the study has revealed varied possibilities.

Conclusion

There has been a paradigm shift in the treatment protocols for periodontal disease with host modulation therapy (HMT) being among the more promising options. The encouraging results obtained with both herbal (septilin®) and probiotic (sporlac®) immunomodulators, strongly suggest that these agents definitely hold potential. Nevertheless, further extensive research on the beneficial effects in immunocompromised patients, in patients with aggressive periodontitis and on a larger cross sections of population over a longer period of time needs to be carried out and assessed.

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Time Interval	Group	n	Mean	Std dev	SE of Mean	Comparisons	Mean diff	Z	P-value
pre-treatment	I	32	1.919	0.267	0.047	I V/S II	-0.090	-2.040	0.0414*
	II	32	2.009	0.284	0.050	I V/S III	0.140	-2.604	0.0092*
	III	32	2.059	0.241	0.043	II V/S III	0.05	-1.396	0.1627
1 MONTH	I	32	1.716	0.209	0.037	I V/S II	-0.151	-2.497	0.0125*
	II	32	1.867	0.264	0.047	I V/S III	0.179	-3.087	0.0002*
	III	32	1.895	0.226	0.040	II V/S III	0.028	-0.993	0.3206
3 MONTHS	I	32	1.691	0.186	0.033	I V/S II	-0.166	-2.980	0.0029*
	II	32	1.857	0.220	0.039	I V/S III	0.204	-3.651	0.0003*
	III	32	1.895	0.212	0.038	II V/S III	0.038	-1.235	0.2169
6 MONTHS	I	32	1.694	0.177	0.031	I V/S II	-0.158	-2.765	0.0057*
	II	32	1.852	0.239	0.042	I V/S III	0.189	-3.436	0.0006*
	III	32	1.883	0.222	0.039	II V/S III	0.031	-1.195	0.02322

Table 4a: Comparison of clinical parameters between the 3 groups: gingival index

Time Interval	Group	n	Mean	Std dev	SE of Mean	Comparisons	Mean diff.	Z	P-value
pre-treatment	I	32	0.95	0.091	0.016	I V/S II	-0.030	-0.350	0.7263
	II	32	0.98	0.031	0.005	I V/S III	0.032	-0.349	0.7272
	III	32	0.982	0.032	0.006	II V/S III	0.002	-0.497	0.6195
1 MONTH	I	32	0.73	0.095	0.017	I V/S II	-0.17	-5.101	<0.0001*
	II	32	0.86	0.029	0.005	I V/S III	0.131	-5.154	<0.0001*
	III	32	0.861	0.029	0.005	II V/S III	0.001	-0.027	0.9786
3 MONTHS	I	32	0.72	0.094	0.017	I V/S II	-0.16	-5.154	<0.0001*
	II	32	0.84	0.032	0.006	I V/S III	0.124	-5.154	<0.0001*
	III	32	0.844	0.032	0.006	II V/S III	0.004	-0.027	0.9766
6 MONTHS	I	32	0.72	0.090	0.016	I V/S II	-0.16	-5.315	<0.0001*
	II	32	0.84	0.028	0.005	I V/S III	0.121	-5.154	<0.0001*
	III	32	0.841	0.028	0.005	II V/S III	0.001	-0.027	0.9766

Table 4b: Comparison of clinical parameters between the 3 groups: gingival bleeding index

Time Interval	Group	n	Mean	Std dev	SE of Mean	Comparisons	Mean diff	Z	P-value
pre-treatment	I	32	7.584	0.949	0.168	I V/S II	0.300	-1.148	0.2510
	II	32	7.284	0.891	0.157	I V/S III	- 0.244	-0.846	0.3978
	III	32	7.340	0.946	0.167	II V/S III	0.056	-0.201	0.8405
1 MONTH	I	32	3.247	0.619	0.109	I V/S II	-0.378	-2.691	0.0071*
	II	32	3.625	0.524	0.092	I V/S III	2.712	-6.731	<0.0001*
	III	32	5.929	1.081	0.191	II V/S III	2.304	-6.456	<0.0001*
3 MONTHS	I	32	2.937	0.416	0.074	I V/S II	-0.516	-3.973	<0.0001*
	II	32	3.453	0.529	0.093	I V/S III	2.357	-6.771	<0.0001*
	III	32	5.294	0.932	0.165	II V/S III	1.841	-6.194	<0.0001*
6 MONTHS	I	32	2.828	0.468	0.083	I V/S II	-0.594	-4.148	<0.0001*
	II	32	3.422	0.570	0.101	I V/S III	1.678	-6.094	<0.0001*
	III	32	4.506	0.989	0.175	II V/S III	1.084	-4.517	<0.0001*

Table 4c: Comparison of clinical parameters between the 3 groups: pocket depth

Time Interval	Group	n	Mean	Std dev	SE of Mean	Compar-isons	Mean differences	Z	P-value
pre-treatment	I	32	0.222	0.073	0.013	I V/S II	0.014	0.671	0.5029
	II	32	0.208	0.032	0.006	I V/S III	0.000	-0.940	0.9252
	III	32	0.222	0.45	0.008	II V/S III	0.014	-2.2027	0.0427
1 MONTH	I	32	0.186	0.033	0.006	I V/S II	-0.014	-1.369	0.170
	II	32	0.200	0.016	0.003	I V/S III	0.025	-2.262	0.0237*
	III	32	0.211	0.040	0.007	II V/S III	0.011	-1.275	0.2022
3 MONTHS	I	32	0.179	0.027	0.005	I V/S II	-0.020	-3.033	0.0024*
	II	32	0.199	0.018	0.003	I V/S III	0.03	-3.778	0.0002*
	III	32	0.209	0.036	0.006	II V/S III	0.010	-1.758	0.0787
6 MONTHS	I	32	0.180	0.038	0.007	I V/S II	-0.018	-2.819	0.0048*
	II	32	0.198	0.015	0.003	I V/S III	0.028	-3.550	0.0004*
	III	32	0.208	0.033	0.006	II V/S III	0.010	-1.450	0.1471

Table 5a: Comparison of salivary and GCF IL-6 levels between the 3 groups: Salivary IL-6 levels

Time Interval	Group	n	Mean	Std dev	SE of Mean	comparisons	Mean difference	Z	P-value
pre-treatment	I	32	0.229	0.066	0.012	I V/S II	0.015	-0.564	0.5728
	II	32	0.214	0.033	0.006	I V/S III	-0.007	-0.286	0.7884
	III	32	0.222	0.052	0.009	II V/S III	0.008	-0.295	0.7680
1 MONTH	I	32	0.208	0.048	0.008	I V/S II	0.005	-0.591	0.5545
	II	32	0.203	0.021	0.004	I V/S III	0.007	-0.872	0.3830
	III	32	0.215	0.048	0.008	II V/S III	0.012	-0.537	0.5913
3 MONTHS	I	32	0.202	0.004	0.008	I V/S II	0.003	-0.725	0.4685
	II	32	0.199	0.023	0.004	I V/S III	0.013	-1.450	0.1472
	III	32	0.215	0.048	0.008	II V/S III	0.016	-1.691	0.0908
6 MONTHS	I	32	0.204	0.045	0.008	I V/S II	0.002	-0.993	0.3207
	II	32	0.202	0.021	0.004	I V/S III	0.011	-1.329	0.1840
	III	32	0.215	0.048	0.008	II V/S III	0.027	-0.725	0.4686

Table 5b: Comparison of salivary and GCF IL-6 levels between the 3 groups: GCF IL-6 levels

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