

**Comparison of Efficiency of Ozone and Chlorhexidine Subgingival Irrigation in Orthodontic Patients for Controlling Gingival Inflammation**

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**Abstract**

Ozone therapy is gaining wider acceptance in dentistry. Its antimicrobial and anti-inflammatory properties have been recognized in periodontal patients. So, the aim of the study was to compare the clinical effects of subgingival irrigation with Ozonated water to Chlorhexidine solution on gingivitis in orthodontic patients and also to correlate the clinical effects with the inflammatory marker- lactate dehydrogenase enzyme (LDH) activity in GCF. A double-blind clinical study was conducted on 30 subjects for 28 days. A split-mouth design was used in this study for subgingival irrigation. Clinical parameters such as plaque index, gingival index, gingival bleeding index, probing pocket depth and LDH enzyme activity were measured at baseline followed by subgingival irrigation with 0.01 mg/l of ozonated water on right maxillary quadrant and 0.02% of chlorhexidine solution on left maxillary quadrants. These parameters were again assessed on 14th and 28th day. Results showed a significant(  $P < 0.01$ ) reduction of all the clinical parameters and GCF levels of LDH enzyme activity after subgingival irrigation with both. However

when compared between the ozone and chlorhexidine irrigated side, ozonated water showed a highly significant reduction of clinical parameters and LDH activity. Subgingival ozone irrigation can be an effective method to reduce gingival inflammation in orthodontic patients.

**Keywords:** Gingival inflammation, Ozonated water, Chlorhexidine solution, LDH.

**Introduction**

Fixed orthodontic treatment tends to promote dental plaque accumulation and gingival inflammation. Orthodontic appliances pose difficulties in maintaining optimal oral hygiene. In time, plaque accumulation around orthodontic appliances may lead to gingivitis, enamel decalcification and white spot lesion formation [1-4]. In order to overcome these problems, numerous preventive strategies are used and recommended in the literature [5-7]. These strategies are mainly focused on the elimination of the cariogenic microflora or the mechanical removal of the plaque [8].

The most common method for controlling the growth of dental plaque is mechanical. However, the effectiveness of

mechanical method is limited by factors such as individual motivation, inaccessibility to deeper periodontal pockets, concave tooth surfaces and the margins of restorations. Alternatives to these methods are being sought. Chlorhexidine is a broad spectrum antiseptic with marked antimicrobial effects on Gram-positive and Gram-negative bacteria, some viruses, fungi etc. It has become an important oral antibacterial agent and adjunct to periodontal therapy<sup>[9, 10]</sup>. Subgingival chlorhexidine irrigation reduced gingival inflammation in orthodontic patients also<sup>[11-13]</sup>.

Ozone therapy is amongst these strategies and nowadays the ozone treatment is gaining wider acceptance in dentistry. Irrigation of ozonated water has been tried for its antimicrobial as well as anti-inflammatory effects in treatment of periodontitis<sup>[14, 15]</sup>. With regards to application of ozone very few studies have been attempted. In the field of orthodontics, ozone gas has been tested for its anticaries effect and also for its effect on the shear bond strength of orthodontic brackets to enamel<sup>[16, 17]</sup>.

Only one study has been conducted to determine the beneficial clinical effects of ozonated water on gingival inflammation in orthodontic patients<sup>[18]</sup>. No literature exists till date to compare the efficiency of chlorhexidine and ozone on gingival inflammation in orthodontic patients. During orthodontic treatment, increased lactate dehydrogenase (LDH) enzyme levels in gingival crevicular fluid (GCF) was seen.<sup>[19-21]</sup>

Hence, the objectives of the study include evaluation and comparison of the effects of single sub-gingival oral irrigation with ozonated water and 0.2% chlorhexidine on clinical parameters such as Plaque Index (PI), Gingival Index (GI) and Gingival Bleeding Index (GBI). A second objective of the study was to assess and compare the effects of oral irrigation with ozonated water and 0.2%

chlorhexidine on LDH enzyme activity in GCF.

## **Materials & Methods**

### **Study Sample**

A total of 30 subjects aged in between 21-23 yrs were enrolled in the study. The participants had no relevant medical history and had not taken antibiotics nor used antibacterial mouth rinse within the last month. The inclusion criterion was, having been under fixed orthodontic appliance for a minimum of 3-months.

The participants were informed about the study well in advance and informed consent was obtained. Ethical clearance was obtained from the ethical committee of the C.K.S. Theja Institute of Dental Sciences & Research, Tirupati

### **Method**

This study was conducted for a period of 28 days. The study period was divided into three-time intervals i.e baseline (day 0), day 14, day 28. A split-mouth design was used in this study for subgingival irrigation. The right and left maxillary quadrants were irrigated with ozonated water and chlorhexidine solution respectively and were designated as experimental and control groups.

### **Clinical procedure**

At the baseline, clinical parameters such as Plaque Index<sup>[22]</sup>, Gingival Index<sup>[23]</sup>, Gingival Bleeding Index<sup>[24]</sup> were recorded at distofacial, facial, mesiofacial and entire lingual gingival marginal surfaces of all the teeth present. Also Probing Pocket Depth was determined using William's periodontal probe for the same surfaces. The value was registered to the nearest millimetre boundary/division. All the recordings of clinical parameters were made by the same calibrated examiner, who was blinded as to the treatment condition. GCF sample was collected from both sides of maxillary quadrant followed by subgingival irrigation.

### **Ozone irrigation**

The right half of the upper quadrant was irrigated with 0.01 mg/l ozonated water that was released from "Kent Ozone Dental jet TY- 820"(Pure water House, Bangalore, India). A single pulsating stream of ozonated water was released from the device. At a noise output of < 70 dB and water outflow of >450 ml.

### **Chlorhexidine irrigation**

The other half of the quadrant was irrigated with 0.2% chlorhexidine solution released from "water pik". A 22-gauge blunt needle was bent and attached to the tip of the nozzle of watepik. Irrigation was done at low-pressure setting.

### **GCF sampling procedure**

Collection of GCF was done by placing 1-3ml calibrated volumetric microcapillary pipettes obtained from Sigma Aldrich Chemical Company, USA (Catalog No.p0549). A standardized volume of 2ml GCF was collected, by placing the tip of the pipette extracrevicularly (unstimulated) for 5-20 minutes, using the calibration on the micropipette from each test site. After collection, the samples were stored in a refrigerator at -20°C at SVIMS, Dept. of Anthropology, Tirupati for biochemical analysis.

### **Patient's Instructions**

After irrigation, the patients were directed to follow oral hygiene habits regularly, using a standard Ortho toothbrush and paste provided to them. Instructions were given to the patients to report on 14th and 28th day.

### **Lactate dehydrogenase activity determination**

All the vials containing GCF samples were put in the spectrophotometric apparatus (Cyber UV-1, Cyberlab, USA) which implements high precision/resolution with high precision sine driver monochromator, stable voltage and signal process by adopting noise reduction circuit. It can be adopted by stand alone model or PC control mode. The software included in UV can be used in general

quality and quantity analysis. The minimum sample requirement for the system is 2-10µl (microlitre). A reagent of 50µl (Infinite LDH reagent, ACCUREX) was added to the cassettes for determination of various enzyme activity. It is a 2- liquid reagent system, from which working solution is prepared in the ratio of 4R1:1R2. It estimates LDH enzyme activity in 21/2 min at 37°C, at 340nm. By means of this apparatus, the total volume of GCF was expressed in µl. The LDH activity was calculated as total LDH unit activity (milli unit per sample [mU per sample]) by using the formula: GCF volume (µl) + LDH.

### **Statistical analysis**

Statistical data was subjected to Independent T-test to compare between test and control at various durations, Pearson correlation test to correlate between clinical and biochemical parameters, and ANOVA test to analyze the differences among group means. For all the tests, P>0.05 was considered statistically significant.

### **Results**

The results of the study are summarized in Tables 1-13. The interpretation of clinical and microbial data is done on the baseline, 14th and 28th day. A significant reduction was observed for all the clinical parameters, with both ozone and chlorhexidine irrigation. (Table 1-3).

The higher percentage of reduction was observed in PI (71.4%), GI (74.6%) and GBI (93%) using ozone irrigation as compared to chlorhexidine. No such drastic reduction in PPD was observed when a comparison was made between experimental and control side on 14th and 28th day (Table 4).

Besides clinical parameters, ozonated water irrigation also caused significant reduction GCF LDH enzyme activity from baseline to 14th day, from 14th to 28th day and also from baseline to 28th day. The percentile reduction of LDH (63.7%) using ozone was appreciable as compared

to chlorhexidine (45.7%) from baseline to the 28th day (Table 5).

Results also showed a statistically significant positive correlation between the concurrent changes of clinical parameters and LDH values on both sides.

### Discussion

This double-blind prospective clinical study was carried out to evaluate and compare the clinical effects of a single subgingival irrigation with 0.01 mg/l ozonated water on gingivitis and 0.2% chlorhexidine in patients with fixed orthodontic appliances and also to correlate the clinical parameters with the LDH activity in GCF.

Results of the present study showed significant reduction for all the clinical parameters from baseline to 14th day, from 14th to 28th day and also from baseline to 28th day both on ozonated water irrigated side ( $p < 0.01$ ) and chlorhexidine irrigation side ( $p < 0.01$ ). The results followed an expected pattern as seen in the study conducted by Dhingra and Vandana<sup>[11]</sup>

Schlagenhauf et al<sup>[25]</sup> showed that ozonated water was highly effective in killing both Gram +ve and -ve oral microorganisms in vitro.

However, when compared between the ozone and chlorhexidine irrigated side, ozonated water irrigated side showed a highly significant reduction of mean PI, GI, GBI scores, but mean PPD score showed no significant change. A higher % reduction of PI, GI, GBI was also reported by Kshitish and Laxman<sup>[16]</sup>, using ozone as compared to chlorhexidine in patients with chronic and aggressive periodontitis. Furthermore, they have reported that the percentile reduction of Actinobacillus actinomycetemcomitans (Aa) (25%) using ozonated water was observable as compared to no change in Aa occurrence using chlorhexidine solution.

The possible explanation for the fall in the PI and GI related with subgingival ozone irrigation may be because

of the antibacterial effect on the plaque microorganisms or by derangement of plaque in subgingiva rather than an immediate killing of microorganisms. Huth et al<sup>[26, 27]</sup> showed that NF- $\kappa$ B action in oral cells in periodontal ligament tissue from root surfaces of periodontally damaged teeth was stopped following incubation with aqueous ozone (20 $\mu$ g/ml) suggesting that it has an anti-inflammatory capacity.

There was a notable decrease in GBI % scores in this study at both 14th and 28th day. It cannot be ruled out that even though this reduction was maintained throughout the study duration of 28 days, GBI scores might not have been maintained if the duration of the study was longer. A prolonged observation period will allow a better estimation of extinction of the effect.

Reduction in probing depth seen at 14th day (33.4%) and 28th day (44%) on both sides could have resulted from a reduction in gingival inflammation.

In the present study the LDH marker in GCF was selected because its activity may increase in teeth with orthodontic appliances even though they do not experience any orthodontic tooth movement, perhaps as a consequence of gingival inflammation produced due to the presence of plaque retentive appliances<sup>[28]</sup>.

There was a significant reduction in ozone and chlorhexidine irrigation in LDH enzyme activity from baseline to the 14th day, from 14th to 28th day and also from baseline to 28th day. The baseline GCF LDH levels on experimental and control side were 416.643 mU/sample and 419.307 mU/sample respectively. These results were in concordance with Dhingra and Vandana<sup>[11]</sup>. Nonetheless, when compared among ozonated water and chlorhexidine solution, there was highly significant reduction of levels observed on ozonated water compared to chlorhexidine ( $p$  value  $< 0.01$ ). The presence of high total LDH enzyme unit action at baseline in the present

study is in congruence with the results of Serra et al<sup>[29]</sup> who observed increased LDH levels in GCF.

The GCF LDH levels in our study reduced significantly from 416.643 mU/sample to 151.077 mU/sample at 28th day after ozone irrigation ( $p < 0.01$ ) and from 419.307 mU/sample to 227.363 mU/sample on chlorhexidine irrigation side ( $p < 0.01$ ).

In comparison, ozonated water showed a highly significant reduction in LDH concentration ( $p < 0.01$ ). The concurrent changes between the changes in GCF LDH values and the clinical parameters and PPD between baseline and 28th day, were also seen using Spearman's correlation coefficient, which revealed a significant correlation on both sides.

At the end of one month, reduction in inflammation of gingiva in orthodontic patients was appreciable with a single subgingival irrigation of 0.01 mg/l ozonated water as compared with 0.2% chlorhexidine. Thus, subgingival ozone irrigation can be an efficient method that can be performed on orthodontic patients during their monthly visits to reduce the gingival inflammation.

### Conclusion

Considering the constraints of this present study with regard to short duration, ozone can be considered as promising anti-inflammatory agent in the periodontal therapy. Further long term studies are required to assess adequate efficacy of ozone with respect to the frequency and duration of application.

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**Table 1:** Median Plaque Index (PI) on experimental side and control side at three time intervals

Day	Side	No. Of samples	Mean	Q1	Q3	Median	%Reduction	P value
Day 0	Experimental	30	2.243	2.20	2.33	2.30	-	0.116**
	Control	30	2.127	1.90	2.30	2.20	-	
Day 14	Experimental	30	1.190	1.10	1.30	1.20	46.8	0.000*
	Control	30	1.670	1.50	1.80	1.70	21.2	
Day 28	Experimental	30	0.647	0.58	0.80	0.70	71.4	0.000*
	Control	30	0.997	0.88	1.20	0.95	53.3	
*significant **non-significant								

**Table 2:** Median Gingival Index (GI) on experimental side and control side at three time intervals

Day	Side	No. Of samples	Mean	Q1	Median	Q3	%Reduction	P Value
Day 0	Experimental	30	2.130	2.00	2.10	2.30	-	0.277**
	Control	30	2.040	1.80	2.10	2.23	-	
Day 14	Experimental	30	1.200	1.13	1.20	1.23	43.6	0.000*
	Control	30	1.660	1.60	1.70	1.80	18.6	
Day 28	Experimental	30	0.540	0.40	0.50	0.73	74.6	0.000*
	Control	30	1.030	0.80	1.10	1.23	49.5	
*significant ; **non-significant								

**Table 3:** Median Gingival Bleeding Index (GBI) on experimental side and control side at three timeintervals.

Day	Side	No. Of samples	Mean	Q1	Median	Q2	% Reduction	P Value
Day 0	Experimental	30	13.030	8.00	12.00	13.00	-	0.000*
	Control	30	12.870	14.50	20.00	24.00	-	
Day 14	Experimental	30	4.500	3.75	4.00	8.00	65.4	0.000*
	Control	30	10.130	7.75	10.50	12.00	21.2	
Day 28	Experimental	30	0.870	0.00	0.00	0.50	93.0	0.000*
	Control	30	5.470	3.00	8.00	8.00	57.4	
*significant ; **non-significant								

**Table 4:** Mean Probing Pocket Depth (PPD) on experimental side and control side at three time intervals.

Day	Side	No. Of samples	Mean	S.D	% Reduction	t Value	P Value
Day 0	Experimental	30	2.600	0.621	-	0.000	1.000**
	Control	30	2.600	0.621	-		
Day 14	Experimental	30	1.730	0.538	33.4	0.000	1.000**
	Control	30	1.730	0.538	33.4		
Day 28	Experimental	30	1.430	0.626	45	0.000	1.000**
	Control	30	1.430	0.626	45		
** non-significant							



**Table 5:** Mean LDH Values (mU/sample) in GCF on experimental side and control side at three time intervals

Day	Side	No. Of samples	Mean	S.D	% Reduction	t Value	P Value
Day 0	Experimental	30	416.643	28.376	-	-0.553	0.584**
	Control	30	419.307	28.779	-		
Day 14	Experimental	30	291.643	21.197	30	-21.765	0.000*
	Control	30	366.723	7.679	12.6		
Day 28	Experimental	30	151.077	14.105	63.7	-17.641	0.000*
	Control	30	227.363	17.723	45.7		

\*significant \*\*non-significant

**Table 6:** Results of Spearman’s Correlation test between GCF- LDH & Clinical parameters on experimental side – Day 0

	N		GI	PI	GBI	PPD	LDH
GI	30	r	1.000	0.403	0.294	0.428	0.978
		p		0.027	0.114	0.018	0.000
PI	30	r	0.403	1.000	0.538	0.482	0.336
		p	0.027		0.002	0.007	0.069
GBI	30	r	0.294	0.538	1.000	0.622	0.316
		p	0.114	0.002		0.000	0.089
PPD	30	r	0.428	0.482	0.622	1.000	0.420
		p	0.018	0.007	0.000		0.021
LDH	30	r	0.978	0.336	0.316	0.420	1.000
		p	0.000	0.069	0.089	0.021	

r spearman’s value

**Table 7:** Results of Spearman's Correlation test between GCF- LDH & Clinical parameters on experimental side – Day 14

	N		GI	PI	GBI	PPD	LDH
<b>GI</b>	30	r	1.000	0.194	0.168	0.292	0.762
		p		0.304	0.374	0.117	0.000
<b>PI</b>	30	r	0.194	1.000	0.117	0.161	0.116
		p	0.304		0.538	0.397	0.541
<b>GBI</b>	30	r	0.168	0.117	1.000	0.206	0.276
		p	0.374	0.538		0.275	0.140
<b>PPD</b>	30	r	0.292	0.161	0.206	1.000	0.215
		p	0.117	0.397	0.275		0.255
<b>LDH</b>	30	r	0.762	0.116	0.276	0.215	1.000
		p	0.000	0.541	0.140	0.255	

**Table 8:** Results of Spearman's Correlation test between GCF- LDH & Clinical parameters on experimental side – Day

28.

	N		GI	PI	GBI	PPD	LDH
<b>GI</b>	30	r	1.000	0.118	0.268	0.109	0.941
		p		0.536	0.152	0.567	0.000
<b>PI</b>	30	r	0.118	1.000	0.051	-0.02	0.695
		p	0.536		0.788	0.915	0.039
<b>GBI</b>	30	r	0.268	0.051	1.000	0.014	0.782
		p	0.152	0.788		0.940	0.001
<b>PPD</b>	30	r	0.109	-0.02	0.014	1.000	0.818
		p	0.567	0.915	0.940		0.025
<b>LDH</b>	30	r	0.941	0.695	0.782	0.818	1.000
		p	0.000	0.039	0.001	0.025	

**Table 9:** Results of Spearman’s Correlation test between GCF- LDH & Clinical parameters on experiment side – Day 0

	N		GI	PI	GBI	PPD	LDH
<b>GI</b>	30	r	1.000	0.548	-0.088	0.227	0.704
		p		0.002	0.643	0.227	0.000
<b>PI</b>	30	r	0.548	1.000	0.079	0.139	0.633
		p	0.002		0.677	0.464	0.000
<b>GBI</b>	30	r	-0.088	0.079	1.000	0.148	0.429
		p	0.643	0.677		0.434	0.027
<b>PPD</b>	30	r	0.227	0.139	0.148	1.000	0.378
		p	0.227	0.464	0.434		0.039
<b>LDH</b>	30	r	0.704	0.633	0.429	0.378	1.000
		p	0.000	0.000	0.027	0.039	

**Table 10:** Results of Spearman’s Correlation test between GCF- LDH & Clinical parameters on control side – Day 14

	N		GI	PI	GBI	PPD	LDH
<b>GI</b>	30	r	1.000	0.510	0.131	0.093	0.980
		p		0.004	0.492	0.625	0.000
<b>PI</b>	30	r	0.510	1.000	0.304	0.245	0.539
		p	0.004		0.102	0.192	0.002
<b>GBI</b>	30	r	0.131	0.304	1.000	0.523	0.890
		p	0.492	0.102		0.003	0.001
<b>PPD</b>	30	r	0.093	0.245	0.523	1.000	0.499
		p	0.625	0.192	0.003		0.005
<b>LDH</b>	30	r	0.980	0.539	0.890	0.499	1.000
		p	0.000	0.002	0.010	0.005	

**Table 11:** Results of Spearman’s Correlation test between GCF- LDH & Clinical parameters on control side – Day 28

	N		GI	PI	GBI	PPD	LDH
<b>GI</b>	30	r	1.000	0.365	-0.037	-0.211	0.948
		p		0.047	0.847	0.262	0.000
<b>PI</b>	30	r	0.365	1.000	0.073	-0.059	0.863
		p	0.047		0.701	0.755	0.001
<b>GBI</b>	30	r	-0.037	0.073	1.000	0.623	0.634
			0.847	0.701		0.000	0.004
<b>PPD</b>	30	p	-0.211	-0.059	0.623	1.000	0.489
		r	0.262	0.755	0.000		0.006
<b>LDH</b>	30	p	0.948	0.863	0.634	0.489	1.000
		r	0.000	0.001	0.004	0.006	

**Table 12:** KRUSKAL WALLI’S TEST for control side

	Days	N	Median	(Q1-Q3)	Chi square	p value
<b>GI</b>	0 Days	30	2.10	1.80-2.23	68.092	0.000*
	14 Days	30	1.70	1.60-1.80		
	28 Days	30	1.10	0.80-1.23		
<b>PI</b>	0 Days	30	2.20	1.90-2.30	69.537	0.000*
	14 Days	30	1.70	1.50-1.80		
	28 Days	30	0.95	0.88-1.20		
<b>GBI</b>	0 Days	30	20.00	14.50-24.00	44.039	0.000*
	14 Days	30	10.50	7.75-12.00		
	28 Days	30	8.00	3.00-8.00		
<b>PPD</b>	0 Days	30	3.00	2.00-3.00	35.656	0.000*
	14 Days	30	2.00	1.00-2.00		
	28 Days	30	1.00	1.00-2.00		
<b>LDH</b>	0 Days	30	448.2	380.22-520.40	4250.6	0.000*
	14 Days	30	379.25	290.56-422.90		
	28 Days	30	214.55	150.20-299.60		

**Table 13:** Kruskal Walli’s Test For Experimental Side

	Days	N	Median	(Q1-Q3)	Chisquare	p value
<b>GI</b>	0 Days	30	2.13	2.0-2.30	79.726	0.000*
	14 Days	30	1.20	1.13-1.23		
	28 Days	30	0.50	0.40-0.70		
<b>PI</b>	0 Days	30	2.30	2.20-2.33	77.310	0.000*
	14 Days	30	1.20	1.10-1.30		
	28 Days	30	0.70	0.58-0.80		
<b>GBI</b>	0 Days	30	12.00	8.0-13.0	66.736	0.000*
	14 Days	30	4.00	3.75-8.00		
	28 Days	30	0.00	0.00-0.50		
<b>PPD</b>	0 Days	30	3.00	2.00-3.00	35.656	0.000*
	14 Days	30	2.00	1.00-2.00		
	28 Days	30	1.00	1.00-2.00		
<b>LDH</b>	0 Days	30	643.30	480.10-720.30	5120.00	0.000*
	14 Days	30	304.15	220.55-410.45		
	28 Days	30	160.00	90.00-240.00		