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Comparative Evaluation of the Efficacy of Ozonated Water and Chlorhexidine Irrigation along with Scaling and Root Planing and Scaling and Root Planing alone in Chronic Periodontitis Patients
A Clinico-Microbiological Study

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Abstract:- Periodontal disease is a multifactorial inflammatory disease associated with oral anaerobic species like Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in the subgingival environment. Traditionally elimination of periopathogen containing biofilms was done by scaling and root planing. Subsequently adjunctive antimicrobial agents such as topical antibiotics and antiseptics were used along with scaling and root planing. Chlorhexidine was commonly used as an adjunct in periodontal therapy. But prolonged use of chlorhexidine may cause mucosal desquamation, tooth staining, altered taste sensation, impaired wound healing and reduced attachment of fibroblast. In recent decades, ozone is considered to be an alternative oral antiseptic agent because it is a stronger antimicrobial agent and does not induce microbial resistance. hence the aim of the study was to compare and evaluate the effect of scaling and root planing with and without oral irrigation using 0.2% chlorhexidine and o.8% of ozonated water on clinical and microbiological parameters in chronic periodontitis. A randomized controlled trial, 45 patients were examined and were divided into 3 groups depending on the treatment plan. Scaling and root planing alone in group 1, chlorhexidine irrigation along with scaling and root planning in group 2, and ozonated water irrigation along with scaling and root planning in group 3.the deepest pocket present was taken as the site for clinical evaluation and microbiological assessment. The assessment was carried out on the baseline, at 20 days and 40 days. on clinical evaluation, group 3 showed considerable reduction on 20th and 40th days, followed by group 2 and group 1. On Microbiological examination, group 3 showed highest reduction on the 20th day followed by group 2 and group 1, but by the 40th day group 2 showed better results followed by group 3 and group 1. It was concluded that ozonated potential has a potential antimicrobial action on chronic periodontitis and can be used as an adjunct to scaling and root planning for better action. ozonated water irrigation can also be considered as an alternative treatment modality for aggressive periodontitis

Keywords:- *Chronic Periodontitis;Ozonated Water Irrigation; Subgingival Irrigation; Chlorhexidine Irrigation;Porphyromonas Gingivalis; Aggregatibacter Actinomycetemcomitans.*

I. INTRODUCTION

Ozone a naturally found gas in the upper atmosphere filters potentially damaging ultraviolet light from reaching the earth's surface. It is triatomic molecule, consisting of three oxygen atoms and molecular weight 47.98 g/mol. It has many applications in various fields including the field of medicine [1].

Ozone therapy was accepted as an alternative medicine in the USA since 1880 and has been used since then in 20 countries for over 130 years. During World War I, ozone was used for treating post traumatic gangrene and infected wounds [2]. E. A. Fisch was the first dentist to use ozone in his practice in 1930s. he used ozonated water during dental surgeries to aid in disinfection and better wound healing. Ozone therapy can be defined as a versatile bio-oxidative therapy in which oxygen/ozone is administered in gaseous form or dissolved in water or oil bare, to obtain therapeutic benefit [3].

A. Biological actions in the body

Ozone has several known biological actions in the human body, such as immunostimulating, analgesic, antihypoxic, detoxicating, antimicrobial (bactericidal, virucidal and fungicidal), bioenergetics, and biosynthetic (activation of the metabolism of carbohydrates, proteins, lipids) hemostatic, etc[4].

1) Antimicrobial effect:

It is a result of the action on cells by damaging the cytoplasmic membrane. This action is selective to microbial cells and is effective against antibiotic resistant strains. Gram positive bacteria are more sensitive to the action of ozone than Gram negative bacteria. The viricidal activity is due to the inhibition of synthesis of viral proteins[1].

2) Immunostimulating Effect:

Ozone influences cellular and humoral immune system by stimulating proliferation of immunocompetent cells and synthesis of immunoglobulins respectively. It also activates the function of macrophages and increases sensitivity of microorganisms to phagocytosis following which cytokines are released. They in turn activate other immune cells, setting off a cascade of positive change throughout the immune system, which is stimulated to resist diseases. It also produces interleukins, leukotrienes and prostaglandins which is beneficial in reducing inflammation and helps in wound healing[1].ozone in high concentration causes immunosuppressive effect and in low concentration causes immunostimulating effect[5].

3) Antihypoxic Effect:

Ozone brings about a rise in the po_2 in tissues and improves the transportation of oxygen in the blood, resulting in a change in cellular metabolism, activation of aerobic processes. Repeating low doses of ozone can activate enzymes (superoxide dismutase, catalases, dehydrogenase, and glutathione peroxidases)which can protect organisms against the action of oxygen free radicals. It also prevents formation of erythrocyte aggregates and increases their contact surface for oxygen transportation. Its ability to stimulate circulation can be used in circulatory disorders.[5] ozone improves the metabolism of inflamed itssues by increasing their oxygenation and reducing local inflammatory processes.

4) Biosynthetic Effect:

Ozone activates the mechanisms of protein synthesis. On a cellular level there is an increase in mitochondria and ribosomes which elevates regeneration potential and functional activity of the tissues[1].

5) Vasodilatation:

Ozone causes secretion of vasodilators like nitrous oxide, responsible for dilation of arterioles and venules[1].

The average concentration of ozone used in treatments is 25g per ml of oxygen/ozone gas mixture that translates into 0.25 parts of ozone to 99.75 parts of oxygen. This concentration effectively kills bacteria, fungi, viruses and parasites. According to most authors, a 10sec application of ozone causes destruction of 99% bacteria and 20sec application causes destruction of 99.9% bacteria.

B. Modes of Ozone Delivery

1) Gaseous Ozone:

It is frequently used in restorative dentistry and endodontics[6].

2) Ozonated Water:

It is effective against microorganisms, and also less expensive compared to other chemical cleansers. Hence can be used to control oral infections and various pathogens[4].

3) Ozonized Oil:

Sunflower ozonized oil also seems extremely convenient. The wide accessibility of sunflower oil makes this form of ozone, a competitive antimicrobial agent[4].

Many chemical adjuncts like chlorhexidine are widely used to improve the outcome of mechanical therapy. Chlorhexidine is a broad-spectrum antiseptic with pronounced effects on both gram positive and gram-negative bacteria, some viruses and fungi. Chlorhexidine has extensively been proved to show better results than mechanical debridement alone. The various modes of delivery include – rinsing, using oral irrigators and subgingival irrigation at different concentrations (2%, 1.2% and 0.2%). The adverse effects of chlorhexidine include – taste changes, tooth staining, sore mouth and /or sore throat, Tongue irritation and wheezing/shortness of breath [7]. An alternative approach to conventional antimicrobial or antiseptic agents in the suppression of subgingival bacteria is to inhibit their growth by changing the subgingival environment, which has shown to be highly anaerobic with a prevailing low oxygen tension[6]. This has led to the concept of oxygenating the periodontal tissues as a means of therapy, a concept which has been periodically revived since first advocated by Dunlop in 1913[1]. The agents that have been used are molecular oxygen [8], hyperbaric oxygenation [9] and hydrogen peroxide [10]. It has been shown that repeated subgingival oxygen irrigation in previously untreated deep periodontal pockets resulted in changes in the subgingival micro flora and lead to significant healing of the periodontal conditions [1].

Hence this study is designed to compare the efficacy of ozonated water and chlorhexidine irrigation along with the scaling and root planning and scaling and root planning alone on clinical and microbiological parameters.

II. AIMS AND OBJECTIVES

- Compare the effect of scaling and root planing with and without subgingival irrigation on clinical and microbiological parameters.
- Compare the effect of a single episode of subgingival irrigation with 0.8% ozonated water and 0.2% chlorhexidine on clinical parameters such as Plaque Index, Gingival Index, Bleeding on probing and Probing Pocket Depth.
- Assess and compare the effect of single episode subgingival irrigation with 0.8% ozonated water and 0.2% chlorhexidine on specific periopathogenic

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microorganisms such as bacteria, including Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg) and Tannerella forsythia (Tf) and Prevotella intermedia (Pi).

III. METHODOLOGY

A total of 45 patients aged 30-60 years, of both gender visiting the outpatient department of A.J. Institute of Dental Sciences, Mangalore, were enrolled for the study. All the 45 patients were suffering from chronic periodontitis (AAP International Workshop for Classification of Periodontal Diseases,1999) and minimum of 20 teeth were present in each case, 4 sites with 5-6mm of pocket depth and 6 sites that bled on probing. Patients suffering from known systemic conditions predisposing to periodontal disease, patients with history of periodontal surgical and non-surgical therapy 6 months prior to the onset of the study, aggressive periodontitis, antibiotic therapy/ chemotherapeutic mouth rinses/oral irrigation in the last 6 months, smokers, pregnant or lactating patients were excluded. The 45 Patients were randomly divided into 3 equal study groups (15 patients each) depending on the treatment provided. In the first group only scaling and root planing was done, in the second and third groups, subgingival irrigation with 0.2% chlorhexidine and 0.8% ozonated water was done respectively along with scaling and root planing. The study protocol was reviewed and ethical clearance was provided by the 'Ethical Committee' of A.J. Institute of medical sciences. The study was carried out by a single examiner throughout the study period.

A. Periodontal Status Evaluation

All patients were informed about the procedure being performed and an informed consent was obtained. A printed Performa was used to collect demographic data of the patient. The Performa also included the clinical parameters to be recorded along with other intraoral findings.

Full mouth scaling and root planing was done in all the 3 groups using ultrasonic scaler unit (satellite.Inc), prior to which clinical and microbiological parameters were recorded for all the 3 groups. The site with the deepest pocket and highest score was taken as the site of evaluation. The same clinical and microbiological parameters were assessed on 20th and 40th days for all groups. In the second group, along with full mouth scaling and root planing, subgingival irrigation with 0.2% chlorhexidine solution delivered via "WATER PIK" with a medium power setting for 7-10 minutes. In the third group subgingival irrigation was carried out with ozonated water using "KENT OZONE DENTAL JET TY-820" irrigation device. A fine 20-gauge needle was bent and attached to the tip of the jet and the needle was inserted 3mm sub gingivally. Irrigation was carried out for 7-10 minutes.

For homogeneous maintenance of oral hygiene among all the 3 groups, after irrigation the patients were instructed to perform regular oral hygiene habits like brushing twice daily using the roll-on technique for a minimum of 2 minutes. A standard toothbrush and toothpaste were provided to them for the same. The patients were instructed to report subsequently on the 20th and 40th days.

1) Clinical Parameters:

The parameters used were Plaque Index given by Silness And Loe (1964) with the help of an explorer and mouth mirror[11], Gingival Index given by Loe and Silness (1963)[11] and Modified Sulcular Bleeding Index given by Mombeli et al[11].

2) Probing Pocket Depth:

The depth of the pocket was recorded as the distance between the free gingival margin and the base of the sulcus using Williams graduated periodontal probe.

3) Clinical Attachment Level Measurement:

The clinical attachment level was recorded as the distance between the cemento-enamel junction and base of the pocket using Williams graduated periodontal probe.

Probing Pocket Depth and Clinical Attachment Loss were recorded in all the teeth except the third molars. The parameters were assessed at baseline, 20th and 40th days.

B. Microbiological Assessment Procedure

After clinical assessment of all the 45 patients, microbiological analysis was carried out using their plaque samples. Supragingival plaque removal was done for all the teeth with sterile set of instruments, immediately after which the site selected for sampling were dried and isolated. The selected site was sampled for subgingival microflora with the help of sterile absorbent paper point. The samples were dispensed in separate vials containing Reduced Transport Fluid (RTF), which was used as a carrier medium for the plaque samples. The vials were closed and labelled. The labelled vials were sent for microbiological examination within 24 hours. The samples were vortexed and inoculated in culture media according to the requirement in enriched and selective media. The samples were quantified for Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans and Prevotella intermedia by bacterial culture method.

C. Statistical Ananlysis

The results were expressed as mean ± SD and proportions as percentages. Intergroup and intragroup comparisons were made by ANOVA and KRUSKALWALLIS test. A P-value of 0.05 or less was considered for statistical significance.

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IV. RESULTS

A. Periodontal Status

➢ *Comparison of Plaque and Gingival Index between the three Groups on 20th Day.*

All the subjects were regularly monitored for oral hygiene maintenance and instructions for plaque control were emphasized at each visit. All the three groups did not show significant reduction in plaque scores (P>0.376) overall it was found that there is decrease in mean plaque index from baseline to 20th day. Highest reduction in mean Plaque index was recorded in Group III (1.000), followed by Group II (1.000) and Group I (1.067) respectively (Table 1). There was a significant reduction in the mean gingival index from baseline to 20th day between all 3 groups(O<0.014). Highest reduction in mean gingival index was recorded in Group II (0.600), followed by Group III (0.667) and Group I (1.267) respectively (Table 2).

➢ *Comparison of plaque and gingival index between the three groups on 40th day.*

The reduction in the plaque index was significant in all the groups. The highest reduction was noted in Group III (1.067), followed by Group II (1.200), and Group I (1.400) respectively (Table 1). Highest reduction in mean gingival index was observed in Group III (0.600), followed by Group II (1.200) and group I (1.600) respectively. The difference in the mean gingival index between the groups was statistically very highly significant(P<0.001). this indicated that Group III has the greatest reduction and highest significance up to 40th day (Table 2).

Time	Group	N	Mean	Std. deviation	F	P
Pre	**I**	15	2.2667	0.59362		
	II	15	2.2667	0.45774	0.08	0.920
	III	15	2.3333	0.48795		
20th	**I**	15	1.0667	0.25820		
	II	15	1.0000	0.00000	1	0.376
	III	15	1.0000	0.00000		
40th	**I**	15	1.4000	0.50709		
	II	15	1.2000	0.41404	3.56	0.049 sig
	III	15	1.0667	0.25820		

Table 1:- Plaque Index

Time	Group	N	Mean	Std. deviation	F	P
Pre	**I**	15	2.7333	0.45774		
	II	15	2.3333	0.48795	2.61	0.085
	III	15	2.4667	0.51640		
20th	**I**	15	1.2667	0.45774		
	II	15	0.6000	0.63246	4.75	0.014
	III	15	0.6667	0.81650		
40th	**I**	15	1.6000	0.50709		
	II	15	1.2000	0.56061	10.23	<0.001vhs
	III	15	0.6000	0.73679		

Table 2:- Gingival Index

➢ *Comparison of modified sulcular index (MSBI) between the groups on the 20th day. (Table 3)*

A reduction in mean MSBI was recorded in Group III (0.200), followed by Group II (1.733) and Group I (1.933) respectively. The difference in the mean MSBI was statistically highly significant (P<0.001) and overall it was found that there was a decrease in the mean MSBI from baseline to 20th day with greatest reduction in Group III.

➢ *Comparison of modified sulcular index (MSBI) between the groups on the 40th day. (Table 3)*

Higher reduction in MSBI was recorded in Group III (0.200), followed by Group II (1.667) and Group I (1.867) respectively. The difference in the mean MSBI was statistically very significant (P<0.001). This indicated that there was consistent reduction in the mean MSBI from 0 to the 40th day in all the three groups with Group III having the greatest reduction

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Time	Group	N	Mean	Std. deviation	F	P
Pre	**I**	15	3.6000	0.73679		
	II	15	3.6667	0.72375	5.361	0.008hs
	III	15	2.8000	0.94112		
20th	**I**	15	1.9333	0.79881		
	II	15	1.7333	0.70373	31.015	<0.001vhs
	III	15	0.2000	0.41404		
40th	**I**	15	1.8667	0.63994		
	II	15	1.6667	0.61721	38.743	<0.001vhs
	III	15	0.2000	0.41404		

Table 3

- *Comparison of the probing pocket depth between the three groups on the 20th day. (Table 4)*

The reduction in the pocket depth values in all three groups was not significant from baseline to 20th day. Higher reduction was recorded in Group III (3.7333), followed by Group II (3.8667) and Group I (4.2667) respectively. Overall there was a decrease in mean probing pocket depth from baseline to 20th day, with greatest reduction and highest significance in Group III.

- *Comparison of the probing pocket depth between the three groups on the 40th day. (Table 4)*

Highest reduction was seen in Group III (2.5333), followed by Group II (3.6667) and Group I (4.2000) respectively. The difference in the mean pocket probing depth was statistically very significant (P<0.001). This indicated that there was consistent reduction in the mean pocket probing depth from 0 to the 40th day in all the three groups with Group III having the greatest reduction.

Time	Group	N	Mean	Std. deviation	F	P
Pre	**I**	15	5.5333	0.51640		
	II	15	5.6000	0.63246	0.221	0.803
	III	15	5.6667	0.48795		
20th	**I**	15	4.2667	0.88372		
	II	15	3.8667	0.63994	2.056	0.141
	III	15	3.7333	0.70373		
40th	**I**	15	4.2000	0.77460		
	II	15	3.6667	0.61721	19.449	<0.001 vhs
	III	15	2.5333	0.83381		

Table 4

- *Comparison of clinical attachment loss (CAL) between the three groups on the 20th day. (Table 5)*

Highest reduction in mean CAL was recorded in Group III (3.6667), followed by Group II (3.7333) AND Group I (5.0000) respectively. The reduction in mean CAL between the groups from baseline to 20th day was statistically significant (P<0.027) and the greatest reduction and highest significance was found in Group III.

- *Comparison of clinical attachment loss (CAL) between the three groups on the 40th day. (Table 5)*

Higher reduction in mean CAL was recorded in Group III (2.5333), followed by Group II (3.4667) AND Group I (4.6667) respectively. The difference in the mean CAL consistently decreased from 0 to 40th day with Group III having the highest reduction.

Time	Group	N	Mean	Std. deviation	F	P
Pre	**I**	15	6.3333	1.39728		
	II	15	5.5333	1.50555	1.15	0.33
	III	15	5.6667	1.71825		
20th	**I**	15	5.0000	1.30931		
	II	15	3.7333	1.57963	3.94	0.027sig
	III	15	3.6667	1.49603		
40th	**I**	15	4.6667	1.63299		
	II	15	3.4667	1.80739	5.68	0.006hs
	III	15	2.5333	1.76743		

Table 5

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B. Microbiological parameters

➢ *Comparison of Tanerella forsythia between the three groups on the 20th day. (Table 6)*

Highest reduction in the mean Tf was recorded in Group III (0.000), followed by Group I (3.667) and Group II (4.933) respectively. The difference in mean Tf between the groups was statistically significant (P<0.016). There was reduction in mean value of Tf from baseline to 20th day with greatest reduction and highest significance in Group III.

➢ *Comparison of Tanerella forsythia between the three groups on the 40th day. (Table 6)*

Highest reduction in mean Tf was recorded in Group II (4.800), followed by Group III (5.800) and Group I (6.6667) respectively. The difference in mean Tf was statistically not significant(P<0.739). This indicated that there was regrowth of the organism from the 20th to 40th day with Group II having consistent reduction and highest significance.

Time	Group	N	Mean	Std. deviation	H	P
Pre	**I**	15	10.2667	11.31034		
	II	15	17.4000	21.27977	0.56	0.755ns
	III	15	15.5333	27.60659		
20th	**I**	15	3.6667	5.56349		
	II	15	4.9333	7.66687	87.28	0.016 sig
	III	15	0.0000	0.00000		
40th	**I**	15	5.8000	8.01071		p=
	II	15	4.8000	8.30834	0.6	0.739ns
	III	15	6.6667	8.19988		

Table 6

➢ *Comparison of Porphyromonas gingivalis between three groups on 20th day. (Table 7)*

Higher reduction in the mean Pg was recorded in Group III (0.000), followed by Group II (6.627) and Group I (15.933) respectively. The difference in mean Pg between the Groups from baseline to 20th day was statistically very highly significant (P<0.001) with greatest reduction and highest significance in Group III.

➢ *Comparison of Porphyromonas gingivalis between three groups on 40th day. (Table 7)*

Highest reduction in mean Pg was recorded in Group II (7.267), followed by Group I (16.000) and Group I (29.800) respectively. The difference in the mean Pg between the groups was statistically significant(p<0.001). There was a regrowth of organisms from 20th to 40th days in all the three groups with Group II having greatest reduction and highest significance.

Time	Group	N	Mean	Std. deviation	H	P
Pre	**I**	15	37.7333	36.79376		
	II	15	22.2000	20.25269	0.99	0.61ns
	III	15	39.2667	43.3396		
20th	**I**	15	15.9333	18.18346		
	II	15	6.2667	9.98904	26.43	<0.001vhs
	III	15	0.0000	0.00000		
40th	**I**	15	29.8000	30.37198		<0.001vhs
	II	15	7.2667	16.30279	10.32	
	III	15	16.0000	15.83396		

Table 7

➢ *Comparison of Prevotella intermedia between the three groups on the 20th day. (Table 8)*

The difference in the mean Pi between the groups was statistically significant (P<0.008). highest reduction in mean Pi was recorded in Groups III (0.000), followed by Group II (1.200) and Group I (7.000) respectively. There was a significant decrease in mean Pi from baseline to 40th day with greatest and highest significance in Group III.

➢ *Comparison of Prevotella intermedia between the three groups on the 40th day. (Table 8)*

Highest reduction in mean Pi was recorded in Group II (1.200), followed by Group III (4.867) and Group I (10.733) respectively. The difference in mean Pi was statistically not significant(P<0.065). This indicated that there was regrowth of the organism from the 20th to 40th day with Group II having consistent reduction and highest significance.

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Time	Group	N	Mean	Std. deviation	H	P
Pre	**I**	15	18.0000	23.57056		
	II	15	6.5333	7.53910	1.25	0.535ns
	III	15	11.0000	12.82297		
20th	**I**	15	7.0000	13.75811		
	II	15	1.2000	2.21037	9.55	0.008hs
	III	15	0.0000	0.00000		
40th	**I**	15	10.7333	19.16644		
	II	15	1.2000	2.48424	5.46	0.065ns
	III	15	4.8667	5.55321		

Table 8

- *Comparison of Aggregatibacter actinomycetemcomitans between the three groups on the 20th day. (Table 9)*

The difference in mean Aa between the three Groups was statistically not significant (P<0.184). highest reduction in mean Aa from baseline to 20th da was recorded in Group III (6.0000, followed by Group II (6.933) and Group I (14.067) respectively.

- *Comparison of Aggregatibacter actinomycetemcomitans between the three groups on the 40th day. (Table 9)*

Highest reduction in mean Aa was recorded in Group III (2.533), followed by Group II (8.133) and Group I (18.933) respectively. The difference in mean Aa was statistically significant(P<0.04). This indicated that there was regrowth of the organism IN Group II and Group I but IN Group III reduction and highest significance was seen at the 40th day.

Time	Group	N	Mean	Std. deviation	H	P
Pre	**I**	15	31.0000	28.99261		
	II	15	35.8667	29.65002	0.19	0.909 ns
	III	15	39.8667	39.16425		
20th	**I**	15	14.0667	16.01547		
	II	15	6.9333	10.27804	3.39	0.184sig
	III	15	6.0000	11.13553		
40th	**I**	15	18.9333	11.50238		
	II	15	8.1333	11.47585	4.45	0.04 sig
	III	15	2.5333	4.35671		

Table 9

V. DISCUSSION

The results of the current study showed that, there was a higher mean of reduction in clinical parameters such as plaque index, gingival index and modified gingival index in the OZ (group 3) and CHX (group 2) in comparison to SRP (group 1) on the 20th and 40th days. However, the mean reduction of gingival index, plaque index, and pocket robing depth was higher in OZ as compared to CHX and SRP up to the 40th day. These results were in accordance to with the study conducted by KSHITISH et al in 2010 for 18 days in which a higher percentage of plaque index (12 %), gingival index (29 %) reduction using ozone irrigation as compared to CHX was observed [7].

Another study conducted by K. Dhingra and K.L. Vandana IN 2011 evaluated the effect of ozonated water irrigation in orthodontic patients with gingivitis on 15 patients. The assessment concluded that a single episode of ozonated water irrigation was successful in reducing the gingival inflammation [12]. The results of this study were similar to the results obtained by the current study.

Also, Dodwad et al in 2011 compared the effect of oral irrigation with ozonate water, 0.2% chlorhexidine and 10% povidone iodine in patients with chronic periodontitis on 30 patients. At the end of 1-month time period a higher reduction percentage of clinical parameters and higher reduction in spirochetes was seen with ozonated water as compared to chlorhexidine and povidone iodine. The study concluded that local ozone application can serve as potent atraumatic antimicrobial agent to treat periodontal disease [13].

In the microbiological aspects of the present study a study conducted by Nagayaoshi et al (2004) showed that ozonated water (0.5-4mg/l) was highly effective in killing both gram positive and gram-negative oral microorganisms such as Porphyromonas endodontalis and Porphyromonas gingivalis [14]. K. Dhingra (2011) in his study also showed that ozonated water was effective against Candida or Enterococcus fecalis and periodontopathic bacteria such as A. actinomycetamcommitans and P. gingivalis [12].

Similarly, Huth et al (2011) found significant reduction in periodontal pathogens namely P. gingivalis, Parvimonas micra, Tannerella forsythia on irrigation with gaseous/aqueous ozone as compared to 0.2% CHX [15]. It

was also found that by the 40th day, chlorhexidine was sustained better than ozone and prevented the regrowth of organisms better than ozone, therefore, was more effective than ozone for bacteria like Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia. This may be due to better substantivity property of chlorhexidine. Lander PE IN 1986 concluded by his study that the effect of a single episode of chlorhexidine irrigation had its peak action at 2 to 4 weeks [16]. Similarly, our present study showed maximum effect of chlorhexidine on microbial growth between 20th and 40th days, suggesting that chlorhexidine has a sustained effect on microorganism up to 40 days.

Ozone showed greater reduction in the reduction of Aggregatibacter actinomycetemcomitans when compared to chlorhexidine even on the 40th day. This may suggest the use of ozone as a better adjunct than chlorhexidine in the treatment of aggressive periodontitis. In accordance with our results, a study done by Ramzy et al (2005) found a significant improvement in Pocket probing depth, Plaque index, Gingival index, treated by Scaling and Root Planing along with ozone application in patients with aggressive periodontitis [17].

Despite the substantivity of chlorhexidine, ozonated water irrigation has shown better results in terms of clinical parameters and microbial count of A.a up to the 40th day and showed better antimicrobial action on P.g, T.f, P.i up till the 20th day. Considering the limitation of this study in terms of short-term duration, ozone can be considered as a promising antimicrobial agent in periodontal therapy, further long-term studies are required to adequately assess the efficacy of ozone in vivo.

LIMITATIONS OF THE STUDY

- The study was of a short duration i.e. 40 days, hence long-term effect of these irrigants still needs to be assessed.
- Only single episode of subgingival irrigation was performed in 40 days, hence the substantivity of the subgingival irrigation has not been assessed.
- The antimicrobial activity of ozone on the other periodontopathogens needs to be assessed.

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