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Original Research Article.

Comparative Evaluation of Changes in Oral Micro Flora in Patients Undergoing Fixed Orthodontic Treatment Following Different Oral Hygiene Protocols

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ABSTRACT

Background: Patients who receive the orthodontic treatment usually neglect their oral hygiene that consequently led to periodontal problems as they do not follow the oral hygiene instructions properly and especially the use of mouth wash. Moreover, it is difficult to perform conventional cleaning so an adjunct mouthwash must be incorporated.

Aim: To investigate the relative buildup of microorganisms, particularly Streptococcus mutans and Candida albicans on different teeth in the maxillary arch in patients wearing fixed orthodontic appliances and to compare the reduction in counts of Streptococcus mutans and Candida albicans in patients maintaining oral hygiene by brushing and Chlorhexidine (0.2%) mouthwash as compared to those where warm saline rinses were used as an adjunct to brushing.

Materials and Methods: 50 Patients were divided into two groups randomly. One group received oral hygiene instructions about brushing with an orthodontic brush thrice daily and along with it using 20 ml Chlorhexidine mouth wash at night. While the other group received instructions on the use of warm saline rinse at night using a disposable Cup with a half tea spoon of salt. Samples were collected in a similar manner after 3 months of giving oral hygiene instructions. The reduction in the count of microorganisms is noted by calculating CFU. Data obtained was put for statistical analysis.

Results: The results of our study showed a significant reduction in levels of Streptococcus mutans on the surfaces of incisors and highly significant reductions on canines and molars. This was attributed more to its mechanical cleansing action and slightly to its bactericidal action. Our study also showed varying responses of Candida albicans to warm saline rinses and mouthwash, indicating a non-specific action against fungi.

Conclusion: Warm saline rinses are of definite advantage as an adjunct to brushing in patients undergoing fixed orthodontic treatment. However, much of this action can be attributed to its mechanical cleansing action.

Key words: Orthodontic Treatment, Chlorhexidine Mouthwash, Normal Saline, Streptococcus Mutans, Candida Albicans, Oral Hygiene.

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INTRODUCTION

Periodontal complications are one of the most observed side effects associated with orthodontics[1]. Oral health plays an important role in general health and quality of life. The oral cavity is unique in having a communication with the external environment as well as the internal alimentary tract of the individual thus harbouring the microorganisms of various kinds many of which are symbiotic, or as commensals, while some are also pathogenic. Oral cavity represents a complex and highly

organized ecosystem relied on delicate balance between the environment and microorganisms. The existence of specific ecological niches, where the bacteria can grow and metabolize regulates the maintenance of this balance and prevents environmental changes[2]. However, in case, where oral ecosystem is imbalanced, an uncontrolled colonization of bacteria can cause the consequent generation of pathological conditions[3,4]. Furthermore, microbiological studies have revealed substantial changes in the bacterial composition of the sub gingival dental plaque, indicating that orthodontic treatment is possible to change the equilibrium of oral micro flora and increase bacteria accumulation[5]. Streptococci are often present in oral cavity in large numbers due to their association with dental plaque. Amongst various species of streptococci, S mutans is of particular concern because of its association with dental plaque and dental caries[6].

Candida species are present as normal commensals in human beings at various sites including the oral cavity however under certain conditions where the immunity of the host is compromised, they become pathogenic[7]. Amongst the various Candida species, Candida albicans is of particular interest due to its association with dental plaque[8].

MATERIALS AND METHODS

This study was done in the Department of Oral and Maxillofacial Pathology, Kothiwal Dental College and Research Centre, Moradabad, UP, India. The current study was undertaken to evaluate the efficacy of Chlorhexidine mouthwash and warm saline gargles as an adjunct to mechanical cleansing in patients being treated with fixed Orthodontic appliances.

50 patients undergoing fixed orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics were evaluated for Streptococcus mutans and Candida albicans counts. Subsequently 25 of these patients were instructed to do warm saline rinse for 3 months while 25 patients were instructed to use Chlorhexidine mouthwash once daily in addition to thrice daily brushing for the same period. The samples were taken to the microbiological laboratory of the college and incubated onto Mitis Salivaris Agar for Streptococcus mutans and Sabouraud Dextrose Agar for Candida albicans.

Study population included patients undergoing fixed orthodontic treatment. Consecutive patients were screened. Those fulfilling the inclusion and exclusion criteria were included in the study.

The reduction in the count of microorganisms was noted by calculating CFU.

Sampling of the Specific teeth surfaces was done. The teeth chosen were Maxillary Central Incisor, Maxillary Canine and Maxillary first Molar on right side. The sampling was done with the help of sterile explorer. Individual samples were transferred in to test tubes containing 2 ml of normal saline (Stock solution) and immediately transferred into the microbiological laboratory.

METHODOLOGY

Involved 2 steps:

1) **ISOLATION** (preparation of serial dilutions: 10^{-1}, 10^{-2} with dilution factor 10 and 100)[9]and mixing the dilution into the dilution plates by spread plate method[10].

Under laminar air flow, from the dilution tube, with the help of micropipette, 100µl of sample was poured onto the petri plate

CFU COUNT

The incubated plates were inspected for no. of colony forming units and were subsequently counted and CFU was calculated as follows[10]:

$$\text{CFU/ml} = \frac{\text{(No. of colony forming units x dilution factor)}}{\text{Volume inoculated}}$$

2) IDENTIFICATION

After incubation, when growth was seen on the culture plates, the colour, shape and size of growth was recorded. Also, the gram staining was done and morphology and type of bacteria was checked under microscope. Further confirmation of the bacteria was done by the Biochemical tests.

The data obtained was put to statistical analysis using Paired or Dependent t-test.

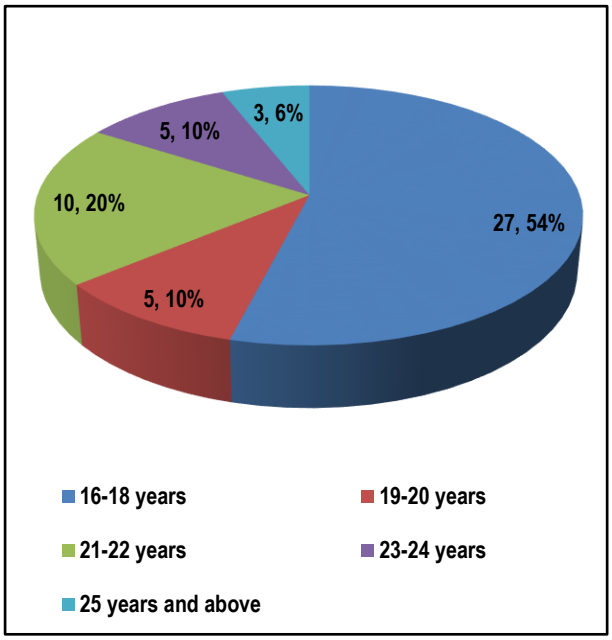

Fig 1: Age wise distribution of patients.

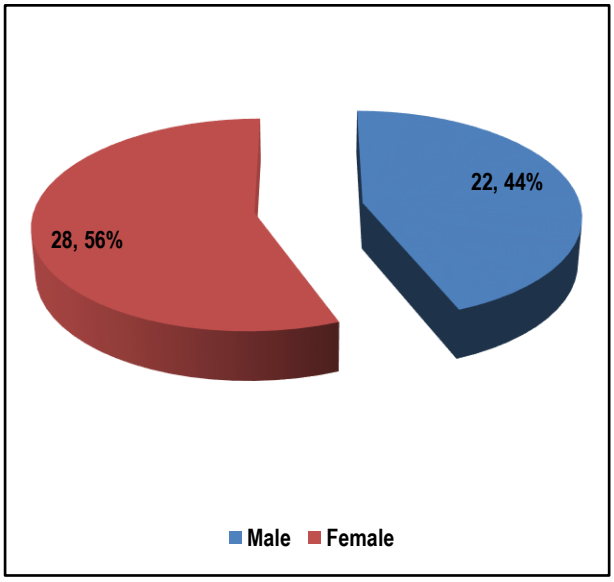

Fig 2: Distribution of total males and females.

Table 1: Incisor Tooth (Candida albicans)

				Effect after warm saline gargles			
Incisor tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	2.58	1.91	0.00	5.30			
After rinse	2.42	1.72	0.00	5.00	0.16	1.342	0.180#
				Effect after mouth wash			
Incisor tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	2.58	1.91	0.00	5.30			
After rinse	1.71	1.12	0.00	5.00	0.87	2.898	0.018*

Table 2: Canine Tooth (Candida albicans)

				Effect after warm saline gargles			
Canine tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	6.06	3.10	0.00	10.30			
After rinse	5.38	2.96	0.00	9.70	0.68	2.232	0.026
				Effect after Chlorhexidine mouth wash			
Canine tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before gargle	6.05	3.10	0.00	10.30			
After gargle	3.23	1.79	0.00	5.70	2.82	2.375	0.018

Table 3: Molar Tooth (Candida albicans)

				Effect after warm saline rinses			
Molar tooth	**Mean CFU count (x10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	12.82	4.85	7.00	21.00			
After rinse	11.48	4.44	6.00	19.00	1.34	2.588	0.010*
				Effect after Chlorhexidine mouthwash			
Molar tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	12.82	4.85	7.00	21.00			
After rinse	7.14	2.92	4.30	11.00	5.68	2.521	0.012*

Table 4: Incisor Tooth (Streptococcus mutans)

				Effect after warm saline			
Incisor tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	4.28	2.45	0.00	9.60			
After rinse	3.70	2.13	0.00	8.00	0.58	2.400	0.016*
				Effect after Chlorhexidine mouthwash			
Incisor tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	4.32	3.25	0.00	9.60			
After rinse	2.34	1.71	0.00	5.00	1.98	2.944	0.001**

Table 5: Canine Tooth (Streptococcus mutans).

				Effect after warm saline rinses			
Canine tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	17.74	8.51	0.00	31.30			
After rinse	15.50	7.20	0.00	27.00	2.24	3.301	0.001*
				Effect after Chlorhexidine mouth wash			
Canine tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	17.93	8.51	0.00	31.30			
After rinse	9.77	5.21	0.00	21.00	8.16	3.413	0.001*

Table 6: Molar Tooth (Streptococcus mutans)

				Effect after warm saline			
Molar tooth	**Mean CFU count (x10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	24.35	10.21	7.00	42.00			
After rinse	20.39	8.99	6.00	36.00	3.96	3.313	0.001*
				Effect after Chlorhexidine mouthwash			
Molar tooth	**Mean CFU count (x 10^3)**	**SD**	**Minimum**	**Maximum**	**Mean difference**	**Wilcoxon sign-rank test**	**p-value**
Before rinse	24.24	10.21	7.00	43.00			
After rinse	13.04	6.51	3.00	28.00	11.20	3.409	0.001*

RESULTS

CANDIDA ALBICANS

Incisor Tooth

The results of our study did not show any statistically significant reduction (p=0.180) in colony counts of Candida albicans (2.42x10^3) on the surface of central incisor teeth after warm saline rinse.

However, after the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Central incisor teeth (1.71x10^3) was significant (p=0.018). (Table 1)

Canine Tooth

The results of our study show a significant reduction (p=0.026) in colony counts of Candida albicans (5.38x10^3) on the surface of Canine teeth after warm saline rinse.

Even after the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Canine teeth (3.23x10^3) was significant (p=0.018). (Table 2)

Molar Tooth

The results of our study show a significant reduction (p=0.010) in colony counts of Candida albicans (11.48x10^3) on the surface of Molar teeth after warm saline rinses.

After the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Molar teeth (7.14x10^3) was highly significant (p=0.012). (Table 3)

STREPTOCOCCUS MUTANS

Incisor Tooth

The results of our study showed a significant reduction (p=0.016) in colony counts of Streptococcus mutans (3.70x10^3) on the surface of central incisor teeth after warm saline rinse.

After the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Central incisor teeth (2.34x10^3) was highly significant (p=0.001). (Table 4)

Canine Tooth

The results of our study show a highly significant reduction (p=0.001) in colony counts of Streptococcus mutans (15.50 x10^3) on the surface of central teeth after warm saline rinse.

After the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Central teeth (9.77 x10^3) was highly significant (p=0.001). (Table 5)

Molar Tooth

The results of our study show a highly significant reduction (p=0.001) in colony counts of Streptococcus mutans (20.39x10^3) on the surface of Molar teeth after warm saline rinse.

After the use of Chlorhexidine mouthwash, the reduction in the colony count on the surface of Molar teeth (13.04x10^3) was highly significant (p=0.001). (Table 6)

DISCUSSION

Brushing and flossing daily are accepted standard oral hygiene instructions which are given to the patients for the control of oral hygiene. However, the maintenance of proper oral hygiene is difficult after the introduction of a fixed orthodontic appliance inside the oral cavity. In these cases, therefore, it is common to prescribe an adjunctive plaque control technique for proper maintenance of oral hygiene. Mouthwashes are commonly used for this purpose. Chlorhexidine is the accepted gold standard for the maintenance of oral hygiene[11]. However, it is generally not prescribed for longer duration as it has got adverse effects related to the staining of teeth.

Mouthwashes containing essential oils have shown a considerable promise in the control of oral hygiene when used as an adjunct to standard oral hygiene protocols in both long and short term studies[12]. In our study, Chlorhexidine mouthwash was used as it can be used for a long term in control of oral hygiene without any considerable side effecs.[11]

In 1929, an independent assessment of this essential oils mouthrinse showed it to have significant bactericidal activity against a variety of microorganisms and concluded it to be safe and effective.[13] Charles et al. reported that Listerine mouth rinse and a Chlorhexidine mouth rinse had similar anti plaque and anti-gingivitis activity.[14]

Essential Oil mouthwashes kill micro-organisms by disrupting their cell walls and by inhibiting their enzyme activity. They prevent bacteria from aggregating with Gram-positive pioneer species, slow bacterial multiplication, and extract endotoxins from Gram-negative pathogens. This can be expected to reduce bacterial load, show plaque maturation, and decrease plaque mass and pathogenicity.[15] Recent studies have shown that bacterial phenotypes are altered when organisms change from a plank tonic to a sessile state. This suggest that an effective mouthwash must also penetrate the plaque bio-film and essential oil containing mouthwash does so.[16]

A study compared the effects of Chlorhexidine mouthwash on S. mutans counts in plaque and saliva. They reported that Chlorhexidine mouthwash produced statistically significant reductions in S. mutans count in plaque and saliva.[17] In our study, we compared the efficacy of Chlorhexidine mouthwash with warm saline gargles in decreasing the counts of specific microorganisms in oral cavity of patients wearing fixed orthodontic appliance. In our study also, Chlorhexidine mouthwash produced significant reductions in S. mutans count.

Warm saline gargles have got Anti-inflammatory and anti-bacterial activity for which they have been commonly used in the treatment

of sore throat. Salt in water works by osmosis which is harmful to the bacteria.

On incisor tooth, rinsing with warm saline gargles resulted in significant reductions in streptococcus mutans count while mouthwash resulted in highly significant reductions. This could be attributed to the mechanical and less bactericidal effects of warm saline as compared to mouthwash which has got active ingredients which kills the bacteria.

On canine as well as Molar teeth, there were highly significant reductions in counts of Streptococcus mutans by using warm saline gargles as well as mouthwash. The canine bracket as well as the bands on molars have hooks, which caused increased difficulty in maintaining oral hygiene by routine brushing. It can be hypothesized that warm saline probably has a good physical cleaning action, and therefore causes highly significant reductions in S. mutans counts.

For Candida albicans, the warm saline rinses did not cause any significant reductions in counts while the Chlorhexidine mouthwash caused significant reductions on incisor tooth. However, significant reduction in counts of C albicans was noted on canine and molar teeth in patients using warm saline rinses, which can again probably be attributed to its mechanical cleansing action.

The results of our study showed a significant reduction in levels of Streptococcus mutans on the surfaces of incisors and highly significant reductions on canines and molars. This was attributed more to its mechanical cleansing action and slightly to its bactericidal action. Our study also showed varying responses of Candida albicans to warm saline rinses and mouthwash, indicating a non-specific action against fungi.

To conclude, we can assume that warm saline rinses are of definite advantage as an adjunct to brushing in patients undergoing fixed orthodontic treatment. However, much of this action can be attributed to its mechanical cleansing action.

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