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Efficacies of Four Different Types of Orthodontic Separators Pertaining Separation, Change in Micro flora and Pain Perception - A Cumulative Comparative Clinical Research

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Abstract:

Objective

Aim of the present clinical research was to compare, analyze and evaluate the amount of separating efficiencies of four different types of commonly used orthodontic separators; evaluate pain perception brought about by the periodontium and count the increase in the growth of common oral microfloral colonies in pre and post placement phases of the separators.

Material and Method

A sample group of 30 periodontally healthy patients, irrespective of gender, between 16 to 25 years, who had never undergone orthodontic treatment, were selected. Elastomeric, Brasswire, Kesling and Kansal Separators were selected for the study. PDL pain perception was evaluated by Visual Analogue Scale (VAS) and a Questionnaire. Microflora count was analysed by Multi-viewing microscope.

Result

Mean separation created by Elastomeric, Brass, Kesling and Kansal Separators were 0.0457 mm, 0.0390 mm, 0.0437mm and 0.0390 mm respectively with maximum separation on day-1 and minimum on day-3. Elastomeric Separators caused maximum and Kesling caused minimum pain. There was almost 50% increase in the microbial count post separator placement.

Conclusion

Elastomeric Separators showed highest mean separation in comparison to other separators and recorded highest pain perception. Maximum pain was observed on the 1st day and minimum on the 3rd day. Significant increase in microflora was recorded pre and post placement of the separators.

Keywords: *Orthodontic Separators, Elastomeric Separators, Brass Separator, Kesling Separator, Kansal Separator, PDL, Leaf Gauge, Oral Microflora, Multiviewing microscope, Visual Analogue Scale (VAS)*

I. INTRODUCTION

Fixed Orthodontic treatment at times may prefer 'banding over bonding' of molars [1, 2] due to larger masticatory forces. Placement of 177.8 μ thick stainless steel orthodontic molar band [3-4] in the PDL space with average thickness [5, 6] of 118μ, without proper tooth separation may exert wedging forces on PDL space beyond physiological limit creating hyalinization [7, 8] areas causing discomfort or pain [3].

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Separation [9] is an orthodontic procedure which aims at slightly loosening the tight interproximal contacts to create space for the placement of Orthodontic Bands [8]. Hence studies are required to evaluate the effective separation of proximal contact points with minimum pain perception [10], inflammation and microbial growth.

Dr Thurow [11] was the first to mention Elastic Separators. Dr Begg [1] introduced separating springs but was popularized by Dr. Anderson for rapid separation within few hours. The earliest attempts for tooth separation were done by Angle [12] and Case [13] who used brass wire and separating tape, respectively. The most recent separator is a self-secured 'two-in-one' Kansal Separator introduced by Dr Sudhanshu Kansal et al [13] in 2012. Contemporary era has multiple separators in orthodontics each one of them having own merits and demerits.

Present study analysed, compared and evaluated functional efficacies of four different types of commonly used orthodontic separators for maximum separation, minimum pain perception and least changes in the oral microfloral commensals [14- 15].

1.1 Objectives:

This clinical research was undertaken:

A. For comparative evaluation of separation efficiency of four different commonly used orthodontic separators for the insertion of orthodontic bands, in an interval of three consecutive days, as measured by 2 leaf gauges of different sensitivities.
B. To evaluate the pain perception brought about individually by the different separators used in the study for three consecutive days.
C. To evaluate and compare the changes in the growth of microbial colonies commonly predominant in the oral cavity, pre and post placement of the separators [18] used for the study.

II. MATERIALS AND METHODS

The present clinical research was conducted in the dental college after gaining consent from the Ethical Committee of the institution.

A sample group of 30 patients irrespective of gender were selected under following criterias:

1. Age between 16 to 25 years.
2. No previous history of Orthodontic Treatment.
3. No caries or restoration on the proximal surfaces of 1st and 2nd permanent molars and 2nd premolars.
4. Healthy periodontium
5. No previous history of extractions and generalized spacing.
6. Good interproximal tooth contacts at the site of separator placement.

Armamentarium used was divided into three groups:

I. To analyze and compare the separated space:
   A. Four different Orthodontic Separators selected were (Fig-1)

Figure 1: Armamentarium

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Top Row - Left to Right: Brass Wire, A. J. Wilcock Wire, Kesling, Kansal and Elastic Separators.
Bottom Row- Left to Right: Periodontal Explorer, Waldent Plyer, Mathew Plyer, Leaf Gauge and Sterile Cotton Swab

1. Elastomeric Separator (Libral Traders, India)
2. Brasswire Separator (26 gauge)
3. Kesling Separator (0.016 inch- A. J. Wilcock)
4. Kansal Separator (0.016 inch- A. J. Wilcock)

B. Two Leaf Gauges (sensitivity: 0.01 to 0.5mm and 0.1mm) for measurement of the separated spaces.
C. Waldent and Mathew's plyers for separator placement.

II. To evaluate and compare pain perception post separator placement:
A. Visual Analogue Scale (VAS) (Fig-2)
B. Questionnaire Chart (Fig-3)

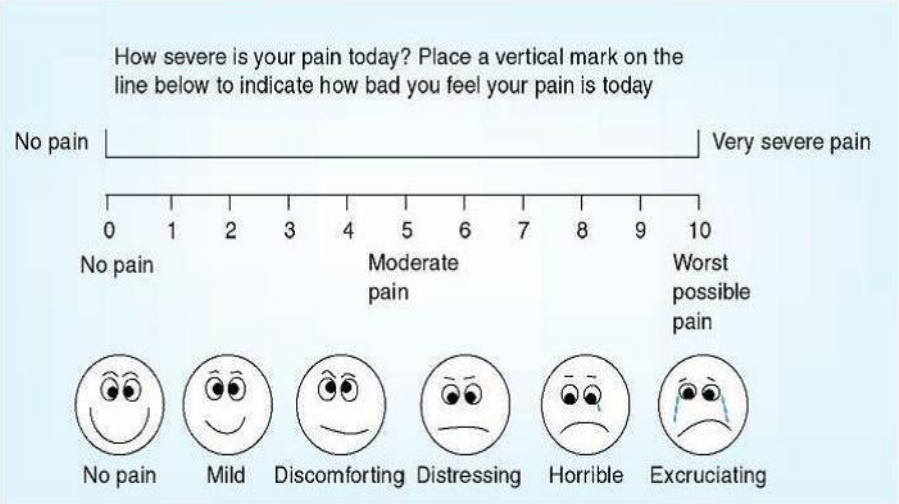

Figure 2: Visual Analogue Scale (VAS)

Questionnaire

S. No.	Question	Duration	Options		
1.	Has separator hurt so much that you have changed your food habits to soft food like curd, banana, poha etc.	Day- 1	Yes	No	
		Day- 2	Yes	No	
		Day- 3	Yes	No	
2.	Has it hurt so much that your leisure activities were influenced, e.g. music, sports, time with family/friends?	Day- 1	Yes	No	
		Day- 2	Yes	No	
		Day- 3	Yes	No	
3.	Has it hurt so much that your work was influenced?	Day- 1	Yes	No	
		Day- 2	Yes	No	
		Day- 3	Yes	No	
4.	Has it hurt so much that you have been awake in the night?	Day- 1	Yes	No	
		Day- 2	Yes	No	
		Day- 3	Yes	No	
5.	Are you absolutely sure that what you are experiencing is pain & not pressure or discomfort?	Day- 1	Pain	Pressure	Discomfort
		Day- 2	Pain	Pressure	Discomfort
		Day- 3	Pain	Pressure	Discomfort
6.	Has it hurt so much that you have had to take painkillers?	Day- 1	Yes	No	
		Day- 2	Yes	No	
		Day- 3	Yes	No	

Figure 3: Questionnaire Chart

III. To culture, count and compare growth of oral microflora pre and post separator placement:

A. Sterile cotton swab stick for sample collection. (Fig-1)
B. Culture Dishes for the preparation of various culture media. (Fig-4)
C. Nutrient Agar Media for the culture of Lactobacillus, Staphylococcus group, Pseudomonas and Leptotrichia Buccalis.

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D. Blood Agar Culture Media for Streptococcus
E. Sabourauds Dextrose Agar Culture Media for Candida Albicans and spores.
F. Laminar Air Flow Equipment (Fig- 4)
G. Microscope (Multiviewing Microscope: CXR5; AC110V- 60Hz, 60 VA,T250V, 500mA) (Fig- 5)
H. Glass Slides for Smear. (Fig-6)

Figure 4: Laminar Flow Chamber with Culture Dishes

Separators [16] were placed in the mouth by proper respective plyers after proper documentation (Fig- 7). The amount of separation at mesial and the distal interproximal area of the 1st permanent molar in each quadrant were recorded separately by two leaf gauges on each day for three consecutive days. The pain perception brought about by the different separators were evaluated by a VAS [17] (Fig-2) and a Questionnaire Chart (Fig- 3). Pain perception was recorded by a set of six questions had to be answered on each day of the separator placement and was to be completed at home at the same time every day. VAS used in the study measured the severity of pain, if any, on a scale 10 cm in length and weighted at both ends by a descriptive terminology, e.g. 'No Pain' which signified zero, to 'Low Pain' which signified 5 to worst possible 'High Pain' coded at 10. The VAS was scored by measuring in millimeter from the left hand end of the line to the vertical mark made by the patient in response to each question [4, 5]. Smears (Fig-6) from the culture of pre and post swab samples of separator placement were analysed under oil immersion multiviewing microscope (CXR5; AC110V- 60Hz, 60 VA, T250V, 500mA) with 1000x magnification after following the protocols of the incubation.

Figure 5 Microscope (multiviewing microscope) CXR 5; AC 110 V, 60 Hz. 60 V A, T250V, 500mA

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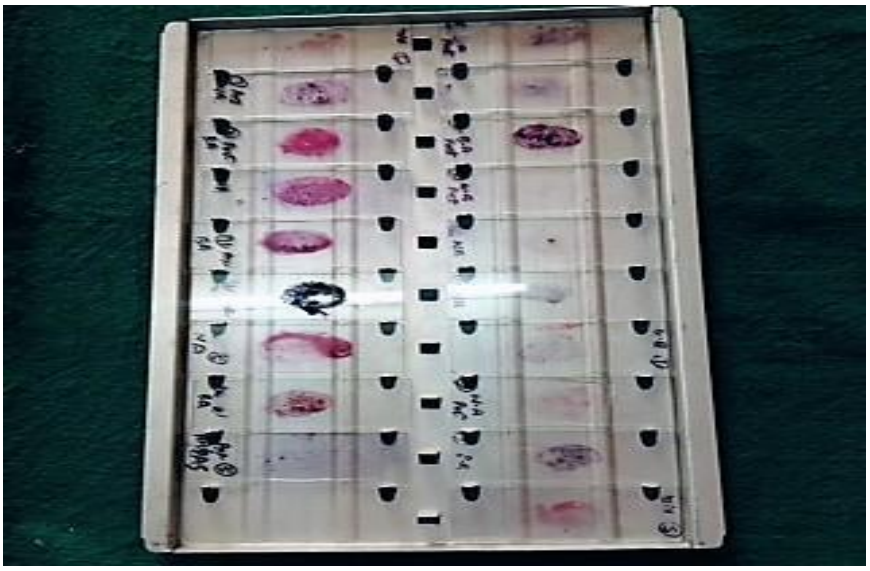

Figure 6: Smears Obtained From Culture Growth n Glass Slides for Microscopic Examination

Figure7: Placement of Different Separators in Patient's Oral Cavity

III. STATISTICAL ANALYSIS

ANOVA with Tukey's Multiple Comparison Test (parametric data), Kruskal-Wallis test with Dunn's Multiple Comparison Test (nonparametric data) and Chi-Square Test (Categorical Data) was applied using SPSS (version 16) and Graph Pad (version 5) statistical software. A p-value ≤ 0.05 was considered statistically significant.

Result

The amount of separation, pain perception and the microbial count related to four different types of separators were analyzed and compared for three consecutive days from the time of insertion in the oral cavity. The datas were then tabulated on an Excel Sheet.

Comparative analysis by ANOVA Test (Table-1) evaluated separation created by Elastomeric, Brass, Kesling and Kansal Separators [16] which were 0.0457 mm, 0.0390 mm, 0.0437mm and 0.0390 mm respectively for day-1; 0.2327 mm, 0.2063 mm, 0.1903 mm and 0.2013 mm respectively for day-2 and 0.3743 mm, 0.2937 mm, 0.2740 mm and 0.3160 mm respectively for day-3. Elastomeric Separator showed highest efficiency in creating separation.

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Table- 1: Comparative Analysis of Separation Created by Different Separators on Different Days

Descriptive				
		n	Mean ± SD (mm)	P value
Day 1	Elastomeric	30	0.0457 ± 0.0141	0.2854 (ns)
	Brass	30	0.0390 ± 0.0109	
	Kesling	30	0.0437 ± 0.0208	
	Kansal	30	0.0390 ± 0.0179	
Day 2	Elastomeric	30	0.2327 ± 0.0746	0.1578 (s)
	Brass	30	0.2063 ± 0.0673	
	Kesling	30	0.1903 ± 0.0690	
	Kansal	30	0.2013 ± 0.0842	
Day 3	Elastomeric	30	0.3743 ± 0.0793	0.001 (s)
	Brass	30	0.2937± 0.0597	
	Kesling	30	0.2740 ± 0.0551	
	Kansal	30	0.3160 ± 0.0519	

ANOVA Test, Significant (s), Non-Significant (ns)

Tukey's Multiple Comparison Test (for parametric data) was used to compare the separation for all three consecutive days between Elastomeric vs Brass, Elastomeric vs Kesling and Elastomeric vs Kansal which was tabulated as 0.0067, 0.0020 and 0.0067 respectively. The comparative difference in the separation was non-significant (Table- 2).

Table- 2: Tukey's Multiple Comparison Test

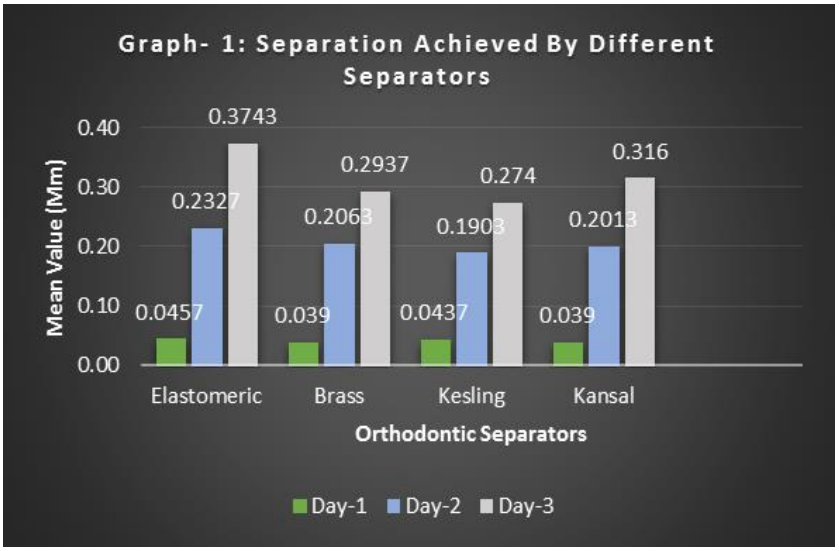
Tukey's Multiple Comparison Test	Day 1		Day 2		Day 3	
	Mean Diff.	Significant	Mean Diff.	Significant	Mean Diff.	Significant
Elastomeric vs Brass	0.0067	ns	0.0263	ns	0.0807	P<0.001
Elastomeric vs Kesling	0.0020	ns	0.0423	ns	0.1003	P<0.001
Elastomeric vs Kansal	0.0067	ns	0.0313	ns	0.0583	P<0.01
Brass vs Kesling	- 0.0047	ns	0.0160	ns	0.0197	ns
Brass vs Kansal	0.0000	ns	0.0050	ns	- 0.0223	ns
Kesling vs Kansal	0.0047	ns	- 0.0110	ns	- 0.0420	ns

Significant (s), Non-Significant (ns)

Graph- 1: Separation Achieved by Different Separators –

A. *Elastomeric Separators: Day 1 of 0.0457mm, day 2 of 0.2327mm and day 3 of 0.374mm.*
B. *Brasswire separators: Day 1 of 0.039mm, day 2 of 0.2063mm, day 3 of 0.2937mm.*
C. *Kesling separators: Day1 of 0.0437mm, day 2 of 0.1903 mm and day 3 of 0.274mm.*

Kansal separators: Day 1 of 0.039mm, day 2 of 0.2013mm and day 3 of 0.316mm.



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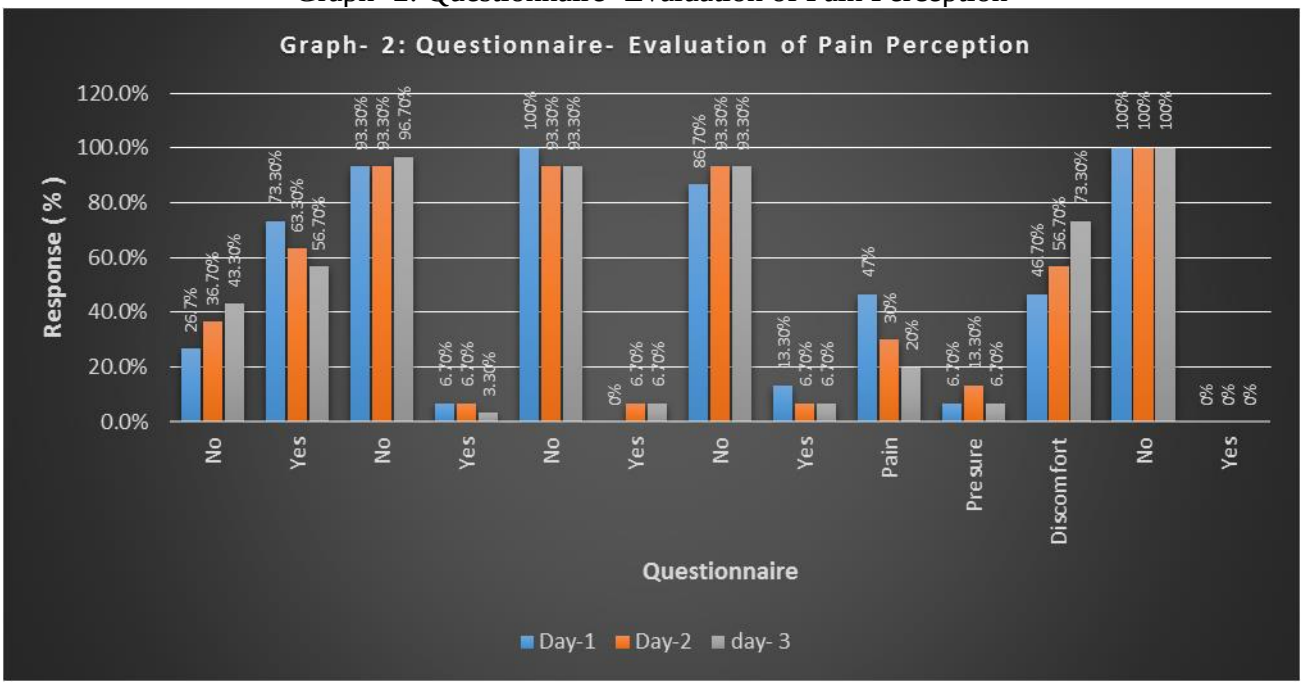
The mean value of separation (Graph-1) by Elastomeric Separators on day-1 was 0.0457 mm; day- 2 was 0.2327 mm and for day-3 was 0.374 mm which when compared to the other types showed the highest separation for the 3rd day. Brasswire Separators achieved a separation of 0.039mm on day-1; 0.2063mm on day- 2 and 0.2937 mm on day- 3, which was less than the Elastomeric and Kansal Separators. Kesling separators showed a mean separation of 0.0437 mm on day-1; 0.1903 mm on day-2 and 0.274 mm on day-3. Kansal Separators showed a separation of 0.039 mm on day-1; 0.2013mm on day- 2 and 0.316 mm on day-3.

Evaluation of the questionnaire indicated that in response for Question No-1, 26.7% of the samples on day-1, 36.7% of the samples on day-2 and 43.3% of the samples on day-3 didn't change their food habits to soft food, while 73.3% on day- 1, 63.3% on day-2 and 56.7% on day- 3 had to change their food preference to soft food. (Table-3, Graph-2)

Table 3: Questionnaire- Evaluation of Pain Perception

Q. No.	Questionnaires	Perception	Day-1		Day-2		Day-3	
1.	Has separator hurt so much that you have changed your food habits to soft food like curd, banana, poha etc.	No	8	26.70%	11	36.70%	13	43.30%
		Yes	22	73.30%	19	63.30%	17	56.70%
2.	Has it hurt so much that your leisure activities were influenced, e.g. music, sports, time with family/friends?	No	28	93.30%	28	93.30%	29	96.70%
		Yes	2	6.70%	2	6.70%	1	3.30%
3.	Has it hurt so much that your work was influenced?	No	30	100.00%	28	93.30%	28	93.30%
		Yes	0	0%	2	6.70%	2	6.70%
4.	Has it hurt so much that you have been awake in the night?	No	26	86.70%	28	93.30%	28	93.30%
		Yes	4	13.30%	2	6.70%	2	6.70%
5.	Are you absolutely sure that what you are experiencing is pain & not pressure or discomfort?	Pain	14	46.70%	9	30.00%	6	20.00%
		Pressure	2	6.70%	4	13.30%	2	6.70%
		Discomfort	14	46.70%	17	56.70%	22	73.30%
6.	Has it hurt so much that you have had to take painkillers?	No	30	100.00%	30	100.00%	30	100.00%
		Yes	0	0%	0	0%	0	0%

Graph- 2: Questionnaire- Evaluation of Pain Perception



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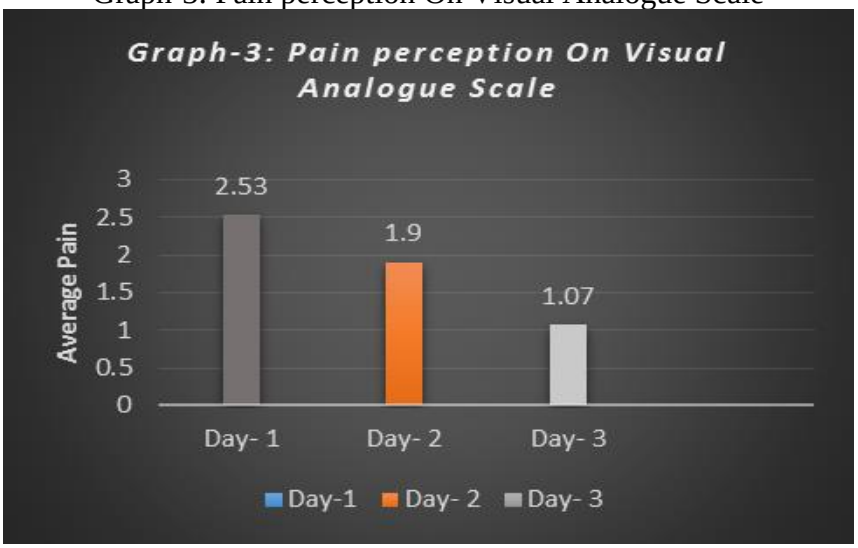
Graph-3: Pain perception On Visual Analogue Scale



In response to Question No -2, 93.3% of the samples on day -1, 93.3% samples on day- 2 and 96.7% on day-3 continued their leisure activities normally whereas 6.70% on day-1, 6.70% on day -2 and 3.30 % on day- 3 complained of impediment of their leisure activities.
In response to Question No-3, 100% of the patients on day-1, 93.3% of the patients on day-2 and 93.3% on day-3 found no difficulty conducting their routine work, while 6.70% on day- 2 and day- 3, found it difficult to go about their daily work.
In response to Question No-4, 13.3% on day-1, 6.7% on day-2 and 6.7% on day- 3 said 'Yes', to being awake at night because of the pain whereas 86.7% patients on day- 1 and 93.3% patients on day- 2 and day-3 said 'No' to the same signifying that they had no difficulty in sleeping at night.

For Question No- 5, 46.7% patients on day- 1, 30.0% on day- 2 and 20.0% patients on day-3 responded by affirmation to pain. 46.7% patients on day-1, 56.7% patients on day- 2 and 73.3% patients on day- 3 said that they felt 'discomfort' and not 'pain'. 6.7% patients on day- 1, 13.3% on day 2 and 6.7% on day- 3 said they felt 'pressure' and neither 'pain' or 'discomfort'.
In response to Question No-6 , 0% patients, on all three days said 'Yes', to having resorted to painkillers and 100% patients on all days said 'No' signifying none of the patients had taken analgesics on any day.
Kruskal-Wallis Test showed significant difference in the pain follow up at day-1, day-2 and day-3 (p=0.0050). The mean pain at day-1, day-2 and day-3 were 2.53, 1.90 and 1.07 respectively, showing significantly high value on day-1 and least on day-3. (Table -4)

Table- 4: Kruskal-Wallis Test Comparison of Pain at Different Days.

Duration	Pain	P value
Day 1	2.53±1.89	0.0050 (s)
Day 2	1.90±1.42	
Day 3	1.07±0.83	

Kruskal-Wallis test, Significant (s), Non-significant (ns)

Dunn's Multiple Comparison Test evaluated statistically significant difference between pain followed up at day-1 and day -3, (p=0.0050). Pain comparison between day-1 and day-2 was non-significant and pain comparison between day-2 and day-3 was non-significant as well. Day- 1 and day-3 showed significant difference. (P<0.01) (Table-5)

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Table-5: Dunn's Multiple Comparison Test for Pain Perception.

Dunn's Multiple Comparison Test	Significance
Day 1 vs Day 2	ns
Day 1 vs Day 3	P<0.01
Day 2 vs Day 3	ns

Significant (s), Non-significant (ns)

VAS, indicated [18] maximum pain of 2.53 on day-1, 1.9 on the day-2 and minimum pain of 1.07 on the day-3.

A comparative evaluation of 'the mean score of pain' by different separators showed 11 patients (36.67%) of Elastomeric; 8 patients (26.67%) of Brasswire ; 8 patients (26.67%) of Kansal and 3 patients (10%) of Kesling separators reporting pain perception (Table-6). Elastomeric Separators showed highest bar graph (Graph-4) of pain perception, followed by the Brasswire and Kansal Separators showing equal scores. Kesling Separators recorded least score of pain perception.

Table- 6: Pain Perception by Different Separators

A. Elastomeric Separators- 11 patients (36.67%)
B. Brasswire Separators- 8 patients (26.67%)
C. Kansal Separators- 8 patients (26.67%)
D. Kesling Separators- 3 patients (10%)

Type of Separators	Pain	
	n	%
Elastomeric	11	36.67%
Brass	8	26.67%
Kesling	3	10.00%
Kansal	8	26.67%

Nutrient Agar culture media was used for the culture of Lactobacillus, Staphylococcus, Psuedomonas, Leptotrichia Buccalis. Chi-square =7.790, df= 3, P value=0.0505, showed 50% of "post- separator placement samples" showed 'High Growth' and 26.7% of samples showed 'Low Growth' of the above microflora. Whereas only 16.7% of "pre- separator placement samples" showed 'High Growth' and 43.3% 'pre- separator placement samples' showed 'Low Growth' of the common microflora for the study. (Table-7)

Table-7: Comparison of Growth of Common Oral Microflora Pre and Post Separator Placement

Nutrient Agar	Pre-treatment		Post-treatment	
	n	(%)	n	(%)
No Growth	3	10.00%	1	3.30%
Low Growth	13	43.30%	8	26.70%
Medium Growth	9	30.00%	6	20.00%
High Growth	5	16.70%	15	50.00%

Chi-square =7.790, df= 3, P value=0.0505, Non-Significant (NS)

Comparative analysis of growth-count from the culture (Graph-5) of Lactobacillus, Staphylococcus, Psuedomonas, Leptotrichia Buccalis [19] was done from pre and post swab samples of separator placement. In "pre-separator placement group", 3 samples (10%) showed "No Growth" of the above microflora.

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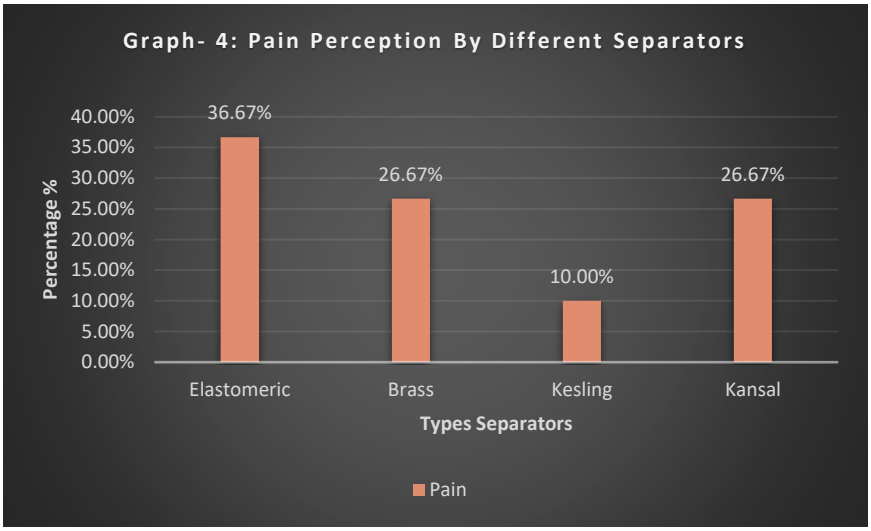
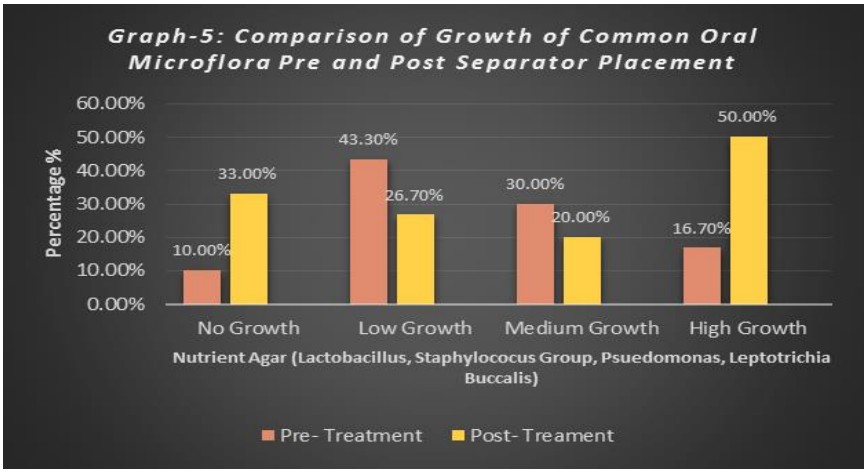
Graph- 4: Pain Perception by Different Separators



Graph-5: Comparison of Growth of Common Oral Microflora Pre and Post Separator Placement



In “post-separator placement group”, only 1 sample (3.30%) showed “No Growth” for the same (Fig-8). In “pre- separator placement group”, 13 samples i.e. 43.30% showed “Low Growth” of the above microflora and in “post-separator placement group” 8 samples i.e. 26.70% showed “Low Growth”. In “pre-separator placement group”, 9 samples i.e. 30% showed “Medium Growth” of the above microflora and in Post-separator placement, 6 samples, i.e. 20% showed “Medium Growth”.

Blood Agar Sabourauds Nutrient Agar Agar

Figure 8: Pre and Post Separator Placement Sample of Culture Media Sample -I

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Blood Agar Sabourauds Nutrient Agar Agar

Figure 8: Pre and Post Separator Placement Sample of Culture Media Sample -III

Table- 8: Result of Culture of Streptococcus Group on Blood Agar Culture Media

Blood Agar	Pre-treatment		Post-treatment	
	n	(%)	n	(%)
No Growth	0	0%	2	6.70%
Low Growth	16	53.30%	4	13.30%
Medium Growth	11	36.70%	20	66.70%
High Growth	3	10.00%	4	13.30%

Chi-square =11.96, df = 3, P value=0.0075, Significant (S)

Table-9: Result of culture of Candida Albicans and its Spores on Sabourauds Dextrose.

A. 30% showed 'high growth' in 'post separator placement samples'.
B. 0% showed 'high growth' in 'pre separator placement samples'.
C. 16.7% showed 'low growth' in 'post separator placement samples'.
D. 60% showed 'low growth' in 'pre separator placement samples'.

Sabourauds Dextrose	Pre-treatment		Post-treatment	
	n	(%)	n	(%)
No Growth	6	20.00%	4	13.30%
Low Growth	18	60.00%	5	16.70%
Medium Growth	6	20.00%	12	40.00%
High Growth	0	0%	9	30.00%

In "pre-separator placement group", 5 samples i.e. 16.7% showed "High Growth" and in "post-separator placement group", 15 samples i.e. 50% showed "High Growth". (Fig-8: sample-I, II, III)
Blood Agar culture media (Table-8) was used for the culture of Streptococcus group. Chi-square =11.96, df= 3, P value=0.0075 (Table- 9) 13.3% samples showed 'High Growth' of Streptococcus group in "post-separator placement group" culture whereas 10% "pre separator placement group" samples showed 'High Growth'. 13.3% samples showed 'Low Growth' of Streptococcus in "post- separator placement group" culture whereas 53.3% samples showed "Low Growth" in "pre- separator placement group" culture.
Evaluating the culture report for Streptococcus (Graph-6) showed 6.7% had 'No Growth' in 'post separator placement samples'. 53.30% showed 'Low Growth' in 'pre separator placement samples'. 13.30% showed 'Low Growth' in 'post separator placement samples'. 36.7% showed 'Medium Growth' in 'pre separator placement samples'. 66.70% showed 'Medium Growth' in 'post separator placement samples'. 10% showed 'High Growth' in 'pre separator placement samples'. 13.30% showed high growth in 'post separator placement samples'. (Fig-9A)

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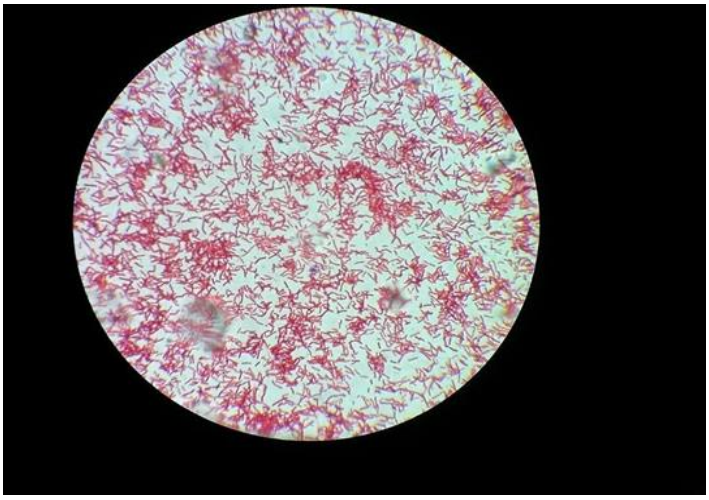
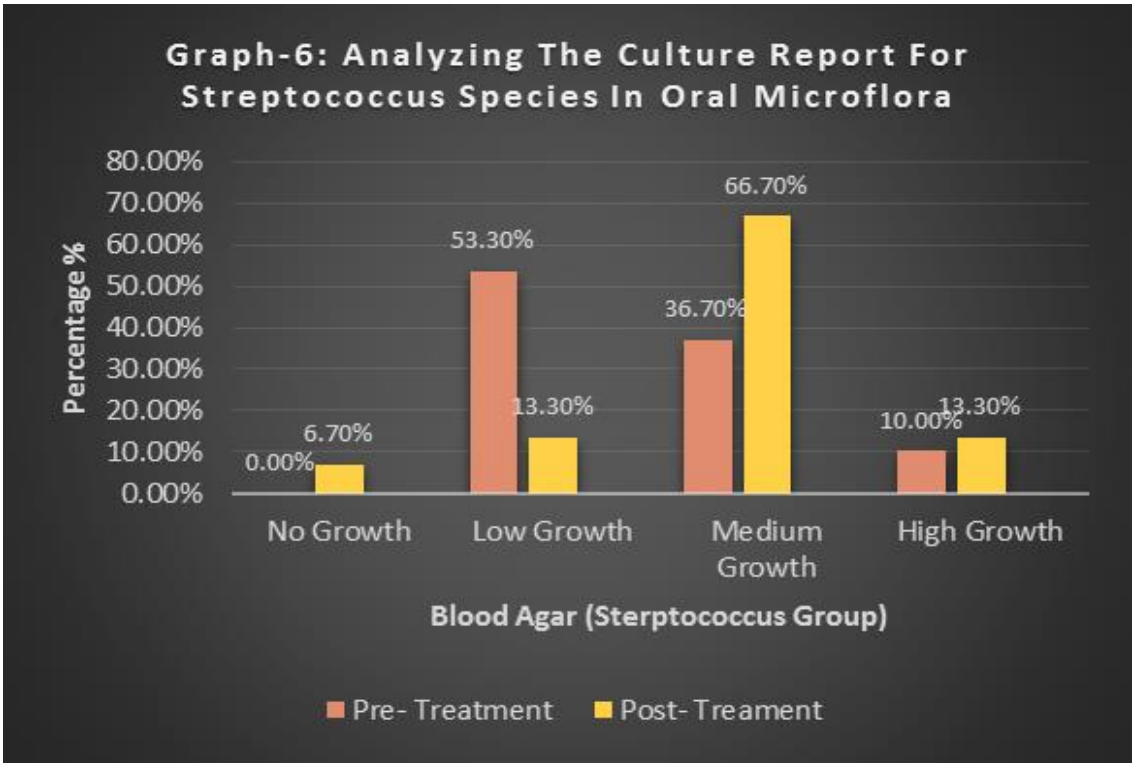


Figure 9: A. Microscopic Slides Showing Growth of Streptococcus Group (At 1000 X Magnification in Oil Immersion Microscope)

Graph-6: Analyzing the culture report for Streptococcus species in oral microflora:

a. 6.7% showed 'no growth' in 'post separator placement samples'.
b. 53.30% showed 'low growth' in 'pre separator placement samples'.
c. 13.30% showed 'low growth' in 'post separator placement samples'.
d. 36.7% showed 'medium growth' in 'pre separator placement samples'.
e. 66.70% showed 'medium growth' in 'post separator placement samples'.
f. 10% showed 'high growth' in 'pre separator placement samples'.
g. 13.30% showed 'high growth' in 'post separator placement samples'.



Sabourauds Dextrose was used for the culture of Candida Albicans and its spores. Chi-square =18.75, df= 3, P value=0.0003, showed (Table-9) 30% had 'High Growth' in 'post separator placement samples'. 0% showed 'high growth' in 'pre separator placement samples'. 16.7% showed 'Low Growth' in 'post separator placement samples'. 60% showed 'Low Growth' in 'pre separator placement samples' (Fig-9B).

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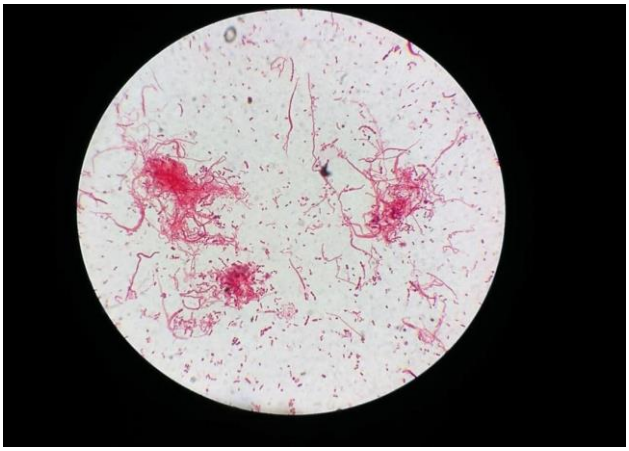
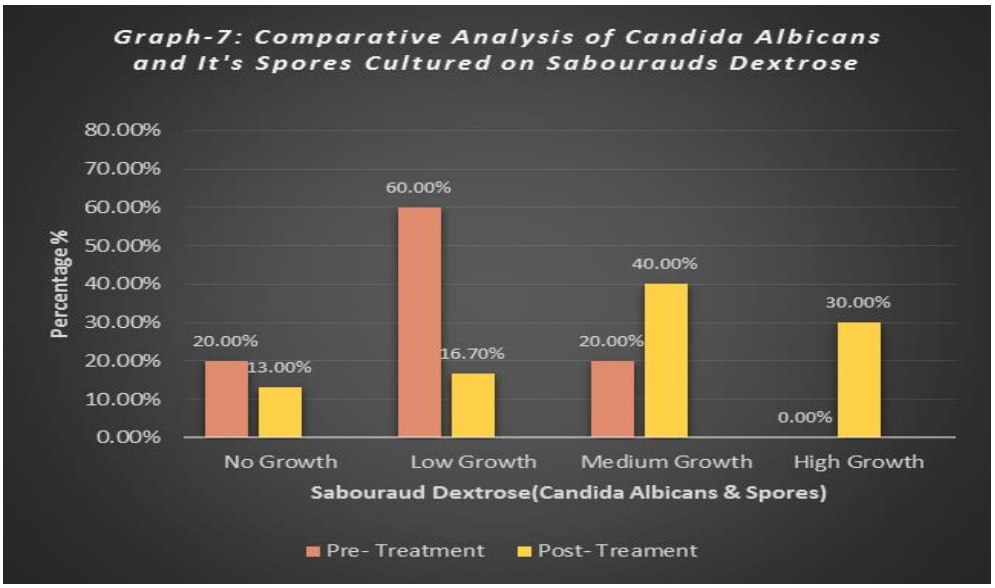


Fig-9:B. Microscopic Slides Showing Growth of Candida Albicans & Spore (1000 X Magnification in Oil Immersion Microscope)

Comparative analysis of culture of Candida Albicans (Graph-7) and its spores on Sabourauds Dextrose showed 20% of 'pre separator placement samples' and 13.3% of 'post separator placement samples' showed 'No Growth'. 60% of 'pre separator placement samples' and 16.70% of 'post separator placement samples' showed 'Low Growth'. 20% of 'pre separator placement samples' and 40% of post separator placement samples showed 'Medium Growth'. 0% 'pre separator placement samples' and 30% of 'post separator placement samples' showed 'High Growth'.

Graph-7: Comparative Analysis of Candida Albicans and It's Spores Cultured on Sabourauds Dextrose



IV. DISCUSSION

Evaluation from the present (Table-1) study suggested increase in separation from 1st to 3rd day after the placement of the separators respectively (Graph-1). This result can be explained from the studies done by Hiroaki Mimura et al [8]. The constant wedging force exerted by separators result in ischemia, inflammation and edema [20] of PDL, creating hyalinization. This resulted in very little initial separation [2] between inter-proximal contacts as on day-1. Gradually osteoclasts and HSP47 heat protein from Endoplasmic Reticulum) were activated. Former resorbed the bone and the protein repaired collagen by forming fibroblast [8]. Simultaneously the force exerted by the separators decayed by 40% -50% from day-1 to day-3 (Akram F Al Huwaizi et al, JBCD- 2008) post separator placement [21, 22]. Depreciation of force within physiological limits of PDL and repair done with time, increased amount of separation on 2nd and 3rd days respectively.

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A comparative analysis (Table-2, Graph-1) in the present study indicated that gross separation was highest at the site of Elastomeric Separators and minimum at the site of Kesling Separators while the separation by Brass and Kansal Separators were in between. Elastomeric Separators are composed of poly-urethane elastomer with excellent shape memory [23]. Comparable diameter amongst separators was greatest too. Akram F Al Huwaizi et al [22] evaluated that Elastomeric Separators exerted highest initial force (790- 660 gram- force in maxilla & 490 gram-force in mandible) with minimum force decay throughout its retention. This enabled maximum gross separation for Elastomeric Separators. Kesling Separator [24] exerted lowest initial force (520- 500 gram-force in maxilla & 280-250 gram-force in mandible) with moderate force decay resulted in minimum separation. One basic reason of less initial force and more force decay in metal spring separators is their mechanics of force exertion due to indeterminate force vectors (Sudhanshu Kansal et al) [26].

Analysis in the present (Table-4, Graph-3) showed maximum pain perception on day-1 and minimum on day-3. The initial pressure exerted on PDL causes compression and inflammation which make PDL sensitive and neuropeptides algogens (Ferreira et al. , 1978 ; Polat et al. , 2005) [27, 28] such as histamine, bradykinin, PGEs, serotonin and substance P are released causing more hyperalgesia (Burston -1962) [29] for first 24 hours and comparatively in 2nd or 3rd days.

Pain perception for Elastic Separators were highest as the amount of force for separation and resultant separation which are directly proportional to the release of the mediators of inflammation [20] and pain. Hence Kesling Separators caused minimum pain perception.

(Force α Neuropeptides Algogens α Pain Perception α Resultant Separation)

Questionnaire revealed that greater discomfort occurred with Elastomeric Separator. This was pertaining to the pathway of placement of the separators. As Elastic Separator traversed through the contact point, while the other springs used gingival and occlusal approach for placement which were more comfortable for the patients [30]. Degree of pain perception is highly personal and variable for the same type of tissue injury. The reason for this variability are still largely unknown [31].

The microbial analysis showed significant increase in the oral microflora from pre to post insertion of separators (Table-7, Graph-5). Systematic review by Alessandra Lucchese et al (2018)32 supports result of the present study. The colonization of bacteria to teeth and other surfaces (Antonelli et al.) [33] occurs with lectin like or hydrophobic ligands of the organism, called “adhesins”. Pellicle on the separators offered microbes extra surface to colonies [34]. Second important reason was compromised oral hygiene [34] even after following dedicated oral prophylactic protocols. Third important reason was the duration (Rossini G et al-2015) [35] of the separators inside the oral cavity promoting bacterial colonization. Fourth reason for increase in oral microflora was peri- periodontal inflammation post placement of the separators [36].

Conclusion

Present study showed that Elastomeric Separators were most efficient followed by Kansal, Brass and Kesling Separators respectively in creating gross separation in three consecutive days after insertion. Minimum separation was created on day-1 and maximum on day-3 after the insertion of the separators. Pain perception after separator placement was maximum on day-1 which gradually decreased through day-3. Kesling Separators caused minimum pain followed by Brass, Kansal and Elastic Separators.

Microbial count from the culture of the swab samples of pre and post separator placement showed significant increase in growth of the oral microflora. There are further scope of studies to show the histological and microbial changes by the use of different orthodontic appliances.

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