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ORIGINAL ARTICLE

Microbiological and Periodontal Status of Patients Enduring Orthodontic Treatment

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ABSTRACT

Objective: To ascertain the probing depths, plaque scores and organism morphotypes in patients enduring orthodontic treatment, as well as the benzoyl-DL-arginine-naphthylamide test scores of periodontopathic bacteria, counting those of red complex bacteria Treponema denticola, Tannerella forsythia and Porphyromonas gingivalis.

Place and Duration: In the Department of Community and Preventive Dentistry, Karachi Medical and Dental College, Karachi for one-year duration from January 2022 to December 2022.

Methods: Plaque samples were taken from 26 patients in this prospective research at baseline and followed up at 30 days and four months following treatment after the appliance was removed. The periodontal pathogens were found using a benzoyl-DL-arginine-naphthylamide assay. The morphotypes were identified using dark field microscopy. At each test interval, the probing depths and O'Leary Plaque Index were evaluated to identify the patients' oral health and periodontal status. The Tukey's Honestly Significant Difference test and analysis of variance were used to analyze the data.

Results: After the insertion of orthodontic appliances, there were significant rise in the probing depths, plaque score, and scores of benzoyl-DL-arginine-naphthylamide at each follow-up visit. But, after the appliances were removed, the levels went back to normal. Increases in large spirochetes (2.5%), small spirochetes (8.1%), fusiforms (6%), filaments (1%) and non-motile rods (10%) were observed during orthodontic therapy, according to dark field microscopy.

Conclusion: Plaque accumulation, probing depth, and microbial activity are all higher in patients receiving orthodontic treatment, which may be a sign of periodontal destruction. The probing depth, benzoyl-DL-arginine-naphthylamide test score and plaque score all came back to baseline levels thirty days after the orthodontic appliance was removed.

Keywords: Porphyromonas gingivalis, orthodontic brackets, bacterial adherence, and Treponema denticola.

INTRODUCTION

Plaque removal, good dental hygiene, and gingival health are all impaired by the mechanical plaque (biofilm) traps introduced by fixed orthodontic appliances[1-2]. This encourages particular changes in the oral environment, such as increased plaque accumulation, lowered pH and higher bacteria counts in the biofilm and saliva[3-4]. Those who don't practice good oral hygiene may develop gingivitis, which can get extremely profound in just 21 days. During orthodontic treatment, patients frequently experience bleeding, gingival hypertrophy, calculus formation and increased plaque accumulation[5-6]. Brackets, bands, elastics and ligature wires stimulate the accumulation of food residues and microbial flora, hence oral hygiene precautions are advised[7-8]. Plaque accumulation around orthodontic appliances over time may result in periodontal disease and dental cavities. Since many orthodontic operations might temporarily cause bacteremia, a higher oral bacteria level raises the risk of periodontal disease and caries in addition to the likelihood of systemic problems[9].

Treponema denticola, Tannerella forsythia and Porphyromonas gingivalis are 3 periodontal bacteria that causes periodontal disease and plaque[10]. These microorganisms are anaerobic periodontal pathogens that can cause periodontal disease. These bacteria are studied in numerous studies which can cause the typical forms of adult periodontitis.[11] The host response to germs is weakened in patients received orthodontic treatment despite the fact that the majority of patients less likely to develop periodontal disease and are young with good immunity[12].

Several researchers have employed the benzoyl-DL-arginine-naphthylamide (BANA) test (developed in Toronto, Canada by Knowell Periodontal Technologies) to quickly screen potential red complex periodontal infections in patients receiving dental treatment[13]. This test is effective and accurate in evaluating the presence of red complex bacteria in the samples of plaques, despite some may disagree with it. The BANA test can be used to identify patients who exhibit vulnerability to these germs and keep an eye out for changes in the bacterial load[14]. Dark field microscopy was utilized to determine the periodontopathogens morphotypes dwelling in the plaque in order to supplement the data from the BANA test[15]. The aim of this study was to assess periodontal health and BANA-positive periodontopathogens in individuals receiving orthodontic treatment.

METHODS

In this prospective research; Plaque samples were taken from 26 patients with mean age of 15 years at baseline and followed up at 30 days and four months following treatment after the appliance was removed. Extensive orthodontic treatment with brackets made of metal placed in the lower and upper arches (arch wire sequence: 0.018-inch NiTi, 0.012-inch nickel titanium [NiTi], 0.018-inch stainless steel [SS], full adult dentition, and SS wires of 0.018 x 0.025-inch), no recent use of antibiotics and history of periodontal disease, and follow-up period of four-months were the selection criteria. All patients gave their signed consent after receiving explanations. At the start of orthodontic treatment, standard oral hygiene guidelines and instructions were provided.

The probing depths were assessed, plaque score was recorded and 7 plaque samples were collected for analysis in the laboratory prior to the use of the pumicing and orthodontic appliances. Plaque samples were taken by a single operator from the interproximal region between the 1st molar and 2nd premolars in each quadrant to ensure consistency. The samples were collected using the scraping action of a Stim-U-Dent tip inserted sub-gingivally. Using linear scribes, the plaque-containing Stim-U-Dent interdental cleaners were scraped onto the inferior reagent matrix. This was done to determine the BANA reaction's and maximum capacity of plaque. Subgingival plaque samples were introduced to the inferior reagent matrix, which had been impregnated with buffered N-BANA. Stabilized Evans black dye was injected into the improved reagent matrix (chromogenic diazo reagent). Any naphthylamide released from the inferior reagent matrix (BANA impregnated strip) allowed to permeate into the superior strip to check whether the reaction has been started. The Evans black dye might then react with naphthylamide to produce a persistent blue-black patch on a light reddish-brown backdrop. The Perioscan

card's sensitivity was increased by incubating the sample for 15 minutes at 55°C. The results were checked for inter-rater reliability 24 hours later. The following criteria were read from the Perioscan reagent test: No background colour indicates a titer of insignificant periodontal pathogens (negative recording or <10,000 colony-forming units). The small and faint blue background indicates weakly positive recording or minimum clinically significant periodontal pathogens (10 000–99 999 CFU). A darker blue colour and distinct background indicates a clinically relevant and significant titer (≥ 100,000 CFU). Data from the aforementioned three categories were categorised as either negative, weakly positive, or positively. Using a stored liquid dental transfer medium and Stim-UDent, the samples of plaque was collected from the premolar/ lower right canine region for dark field microscopy.

The nine periodontal pathogenic morphotypes were quantified and classified during the dark field microscopic investigation; large spirochetes, small spirochetes and intermediate spirochetes, coccoid forms, motile rods, fusiforms, non-motile rods, yeast and filaments were the nine categories that qualified for inclusion. The 400-x magnification factor in microscopy was produced by a 10 x (ocular) and 40 x (nosepiece) magnification. One fourth of the field—which was divided into four equal parts—was used to count 100 microorganisms. Based on their shape, the investigator classified and quantified the organisms. Each organism's percentages in relation to the 100 total counts were calculated. These measurements were taken as the reference point (or "visit 1") for comparing the BANA results.

Probing depth and scoring of plaque: One operator evaluated the selected teeth and their probing depths. The plaque scores were tabulated following the collection of the plaque samples for the BANA test. The lingual and facial surfaces of the lower and upper 1st molars and 2nd premolars were probed on the mesial, middle, and distal sides using a Michigan periodontal probe. In each of the four quadrants, 12 surfaces were therefore probed. The O'Leary Plaque Index 29 was then used to calculate the plaque score after each tooth's mesial, distal, facial, and lingual surfaces were evaluated for plaque on each tooth. By division of the total number of plaque surfaces with total tooth surfaces, the plaque score was computed. Plaque samples were obtained for the dark field microscopic examination and BANA test at each subsequent appointment, and the probing depths and plaque scores were determined precisely.

Plaque scores were calculated as percentages and recorded. For data analysis, the BANA test findings classified as weakly positive, negative, or positive were translated to 0, 1, and 2, correspondingly. Tabulated data included means, SDs, and relative frequencies. Data were analysed with ANOVA with a P value of <0.05 being considered statistically significant. The differences between patient visits or months were analyzed using a Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Figure 1a displays the baseline mean plaque scores and at each follow-up visit. When comparing patient visits, the ANOVA test found significant alterations (P <0.001). The comparison test of Tukey's HSD found that the succeeding four visits (visits 2-5) during orthodontic treatment had significantly higher mean (SD) plaque scores than the initial visit (39.24±15.28%) (48.20±12.57%; 53.98±13.81%; 52.66±14.21%; and 53.41±20.18%) [P<0.05]. The plaque score, which was collected 30 days after the orthodontic appliance was taken out (45.00±14.14%), did not alter significantly from the baseline. Table 1 displays the patients O'Leary Plaque Index scores for all 26 patients at each visit. The ANOVA test found significant patient differences (P<0.001), demonstrating varying differences in plaque scores both within and between patients.

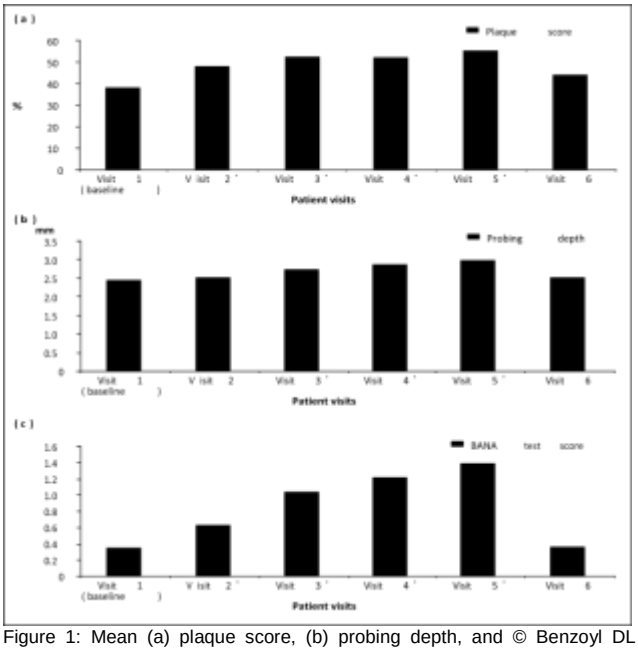

Figure 1: Mean (a) plaque score, (b) probing depth, and © Benzoyl DL arginine naphthylamide

Table 1: Each patient means plaque score at each visit

Patient No.	Plaque score (%)				
	Visit 1 Visit 2 (baseline)		Visit 3	Visit 4	Visit 5
1	39	45	42	53	54
2	25	46	69	49	47
3	36	34	38	30	35
4	52	52	72	81	76
5	60	79	79	50	69
6	21	40	34	40	47
7	25	37	60	42	62
8	60	57	58	71	61
9	24	52	57	41	49
10	56	62	66	63	86
11	68	51	60	61	78
12	50	56	57	63	62
13	20	31	32	38	30
14	32	48	42	50	51
15	28	45	59	52	47
16	37	34	25	39	29
17	58	55	75	82	80
18	52	77	79	49	72
19	20	35	36	42	49
20	24	40	62	39	57
21	61	51	49	62	51
22	29	45	47	50	50
23	45	63	60	54	89
24	51	50	56	71	61
25	52	43	54	63	49
26	26	38	29	37	30

Figure 1b displays the baseline mean probing depths and at each follow-up visit. Using a P<0.001 significance level, the ANOVA test identified differences between the six visits. The Tukey's HSD test found that the mean (SD) probing depths for visits 3 and 5 were substantially higher than the baseline (2.54±0.62 mm) (2.64±0.75 mm and 2.49±0.71 mm, respectively). There were no discernible variations between visits 2 and 30 days after the appliances were removed (2.41±0.67 mm and 2.49±0.61 mm, respectively). Patients' average probing depths varies from 2.1-3.4 mm. Table 2 displays the specific probing depths for 26

patients at each follow-up. The ANOVA test did not find any statistically significant patient differences (P=0.08).

Table 2: Each patient means probing depth at each visit

Patient No.	Probing depth (mm)				
	Visit 1 Visit 2 (baseline)		Visit 3	Visit 4	Visit 5
1	2.54	2.91	2.37	2.46	2.27
2	2.34	2.49	2.81	2.31	3.24
3	2.52	2.58	2.35	2.97	2.41
4	2.31	2.72	2.91	3.11	3.01
5	2.42	2.53	2.73	2.37	2.84
6	2.34	2.21	2.14	2.97	3.11
7	2.69	2.84	2.54	2.22	2.81
8	2.57	2.29	2.68	2.64	2.94
9	2.18	2.39	2.48	2.70	2.49
10	2.28	2.67	2.73	2.48	3.00
11	2.29	2.51	2.49	2.67	3.97
12	2.71	2.66	2.46	2.47	2.75
13	2.81	2.84	2.72	2.77	2.77
14	2.31	2.51	2.48	2.49	2.73
15	2.51	2.39	2.46	2.74	3.89
16	2.62	2.82	2.89	2.19	2.98
17	2.20	2.79	2.43	3.89	3.94
18	2.25	2.56	2.21	2.49	2.74
19	2.31	2.52	2.84	3.18	3.78
20	2.45	2.89	2.46	2.54	2.46
21	2.37	2.19	2.16	2.37	2.89
22	2.34	2.76	2.23	2.91	2.48
23	2.31	2.74	2.61	2.27	3.43
24	2.29	2.84	2.84	2.17	3.52
25	2.71	2.37	2.70	2.91	2.36
26	2.81	2.81	2.61	2.33	2.14

Table 3 displays the unique BANA test results for each visit. The ANOVA test indicated substantial patient differences (P.<001), showing patient variability.

Table 3: Mean benzoyl-DL-arginine-naphthylamide (BANA) test score for each patient at each visit

Patient No.		BANA score			
	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4	Visit 5
1	0.42	0.31	0.40	1.08	1.92
2	0.58	1.11	1.55	1.75	1.07
3	1.40	0.94	1.31	1.85	1.73
4	0.61	0.41	1.80	1.33	1.04
5	1.11	0.68	1.90	1.28	1.44
6	0.20	0.55	1.14	1.92	1.47
7	0.11	1.41	1.94	1.16	1.75
8	0.70	1.27	0.40	1.18	1.11
9	0	0.94	0.90	1.39	1.49
10	0.31	1.41	1.30	1.15	1.67
11	0.27	1.22	0.54	1.11	1.70
12	0.08	0.10	0.99	0.67	1.55
13	0.45	0.45	1.12	1.04	1.44
14	0.87	0	0.08	1.48	1.33
15	0.46	0.70	1.01	1.43	1.22
16	1.71	0.90	0.41	1.67	1.67
17	0.79	0.51	1.09	1.40	1.44
18	1.12	0.44	1.02	1.46	1.20
19	0.21	0.58	0.90	1.84	1.25
20	0	1.41	0.50	1.08	1.11
21	0.87	1.79	1.40	1.88	1.35
22	0.54	0.51	0.90	1.04	1.34
23	0.79	1.01	1.10	1.77	1.31
24	0.45	1.95	1.31	1.38	1.64
25	0.47	0.32	0.94	0.85	1.10
26	0.89	1.74	1.02	1.34	1.20

Plaque samples were examined using dark field microscopy and nine morphotypes. According to Figure 2, the number of tiny spirochetes increased steadily throughout orthodontic treatment, rising from 2.6% at the baseline to 11% at visit 5, or 8% rise in total organisms.

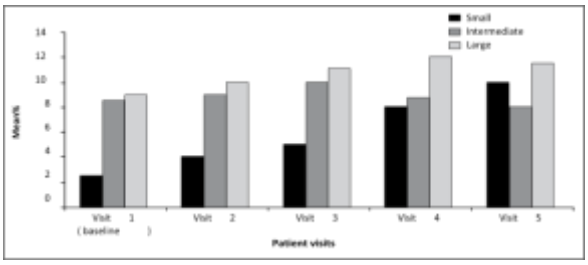

Figure 2: The proportions of large, small and intermediate spirochetes in the plaque organisms over time

The number of intermediate spirochetes increased from 9% at baseline to 12% in visit 3, and then decreased to 7.0% in visits 4 and 5. Large spirochete counts increased by 2.5% from baseline levels of 9.0% to 11.0 at 5TH visit. Motile rod counts decreased steadily from 21% at baseline to 12% at 5TH visit. Coccoid form counts showed a significant decline from 29% at baseline to 13% at 5TH visit. When compared to baseline, the number of non-motile rods increased steadily by 9%, rising to 30% at visit 5. (Fig 3). Fusiform counts increased gradually but steadily from 5% at baseline to 10% in visit 5. Only a slight rise of 1%, from 6% at baseline to 7% on 5th visit was seen in the filament count. Yeasts were counted from 2% at baseline to 4% in 3rd visit before dropping to 3% in 5th visit. (Fig 4).

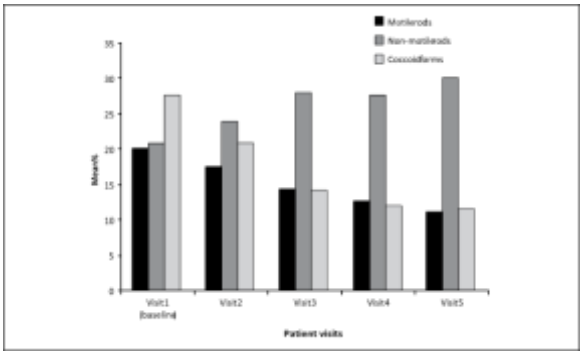

Figure 3: The proportions of non-motile rods, coccoid form and motile rods in the plaque organisms over time

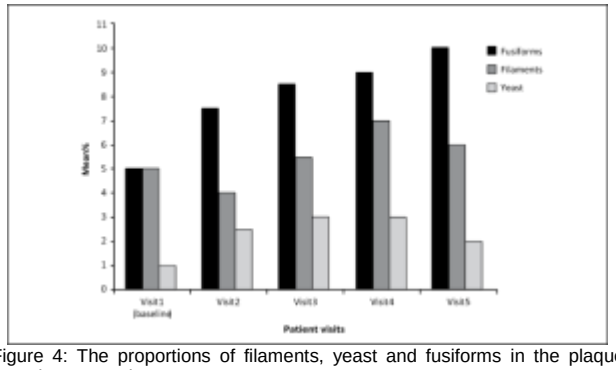

Figure 4: The proportions of filaments, yeast and fusiforms in the plaque organisms over time

DISCUSSION

This study investigated the microbiological and periodontal health of patients receiving orthodontic treatment. The probing depths

and plaque scores were as well analysed to gauge the patients' periodontal health, even though the study's primary objective was to investigate the microbiological state. Several researches have looked into how orthodontic therapy affects periodontal health[16]. These studies mainly pay attention on the effects throughout the course of the treatment, and the observation period was typically brief[17]. The majority of the researchers concluded that periodontal tissues were not permanently harmed by the general gingival changes caused by appliances. The long-term retrospective research came to the same conclusion that orthodontic treatment did not significantly affect anyone. In the current study, it was discovered that individuals receiving orthodontic treatment experienced an increase in plaque scores over time[18]. This study and a number of others have found an increase in plaque scores on teeth. Yet, other study discovered that patients receiving orthodontic treatment either had comparable amounts of plaque or saw a drop from baseline in plaque levels. According to the results of the current study, there was a 17% overall rise in plaque score, with the biggest increase occurring right after orthodontic appliance were installed. The plaque scores varied from person to person[19]. The plaque score for Patient 3 was the same at baseline and at 5th visit. It is possible to achieve ideal cleanliness and plaque control in individuals receiving orthodontic treatment when adequate oral hygiene practices are used[20].

Moreover, the mean probing depth increased with orthodontic treatment, according to this study. While most of the differences were negligible, there were substantial differences between visits 3, 4, and 5 compared to the baseline. Following the use of orthodontic appliances, the probing depths increased more consistently than the plaque scores[21]. This study discovered that orthodontic therapy raised BANA scores. The ratings at visits two through five were noticeably higher than those at the baseline, with the most improvement appearing four months after the orthodontic equipment were initially fitted. It has been established that the 3 potential periodontal pathogens (red complex) T denticola, T forsythia and P gingivalis can be detected using the BANA test (Perioscan).

A thorough image of the microbial communities in the orthodontic samples of plaque is provided by the dark field microscopy findings. In this study, nine morphotypes of common oral microorganisms were used as the sampling. The majority of the first organisms to colonize the biofilm are Gram-positive rods and cocci. The secondary colonizers, which colonize via congregation, are supported by the bacteria that colonise on clean tooth surfaces with adhesins. The secondary bacteria are mostly rods, fusiforms, cocci, filaments, and spirochetes and may be Gram-negative. T forsythia, T denticola and P gingivalis are three of the bacteria that are of concern. The following morphotypes were observed to have increased statistically from baseline in this study: large spirochetes, small spirochetes, fusiforms, filaments and non-motile rods. The fusiforms, tiny spirochetes and non-motile rods had the greatest population growth. Huser et al. discovered a considerable rise in the proportion of motile rods, spirochetes, fusiforms and filaments and a corresponding drop in cocci. These findings are largely comparable with ours, with the exception of the absence of a significant increase in motile rods[22].

The main cause of practically all periodontal and gingival problems is plaque. Plaque management must be stressed as the key to maintaining periodontal health in patients receiving orthodontic treatment. Plaque removal from the tooth and gingival surfaces is made more difficult by orthodontic appliances[23]. Plaque and the patient may coexist in harmony in the oral environment. This equilibrium could, however, lose stability over time and in response to changes in the surrounding environment. In an early plaque, Gram-positive cocci and rods are frequently found[24]. More anaerobic and Gram-negative gradually organisms take their place, which could result in a periodontal response. The environment being altered by orthodontic appliances may cause the biofilm to become imbalanced and pathogenic. Because proper oral hygiene practices are more challenging to maintain while receiving orthodontic treatment, the orthodontic equipment creates mechanical plaque traps where plaques may develop into a pathological state. Hence, encouraging the patient and teaching them about oral hygiene are crucial for a positive orthodontic result[25]. Patients receiving orthodontic treatment frequently run into problems with plaque that could jeopardize the success of their treatment.

CONCLUSIONS

With the insertion of orthodontic appliances, the probing depths and plaque score enlarged with subsequent orthodontic appointments. The BANA positive periodontopathogens counting T denticola, T forsythia and P gingivalis increased concurrently with orthodontic therapy. During orthodontic treatment, populations of small and large spirochetes, non-motile rods, filaments, and fusiforms increased, while all motile rods and coccoid forms decreased, according to dark field microscopy. The BANA score, the probing depths, and the plaque score all nearly reached baseline levels thirty days after the orthodontic appliance was removed.

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