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MICROBIAL CONSTITUENTS AND VOLUME OF GINGIVAL CREVICULAR FLUID IN ADULT FEMALES WITH MALOCCLUSION IN BENIN CITY, NIGERIA.

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Abstract

Microorganisms exist in the mouth, within saliva and periodontal pockets which contain gingival crevicular fluid (GCF). Malocclusion predisposes to a poor oral hygiene and results in stagnation especially in the lower anterior segment. This results in gingivitis which may progress to periodontitis and resultant pocket formation with an increased GCF volume and a pronounced bacterial presence within the GCF. The aim of this prospective study was to determine the bacterial flora and volume of GCF in adult females with malocclusion and compare with a control group of normal women in Benin City, Nigeria. A total of 152 randomly selected women aged 26-65 years, were divided into two groups. Group 1: Malocclusion; n=82 (54%) (Crowding-41, spacing-39 and anterior open bite-2) and Group 2: Normal occlusion; n=70 (46%). GCF volume and content was measured then analysed after being inoculated onto blood, chocolate and MacConkey agar plates which were incubated for 24 hours at 37 degrees C aerobically except chocolate agar, which was incubated in a candle jar. Correlations between age, probing depth, malocclusion and GCF microbial content were determined using the SPSS (version 16) software. Significant values of $P<0.05$ were applied were applicable. The results revealed kliebsiella as the most prevalent microorganism which was isolated in 10(12.2%) and 12 (17.2%) of groups 1 and 2 respectively. There was a higher distribution of both Streptococcus and Staphylococcus in the control group, 10(14.3%) and 4(5.7%) when compared with the malocclusion group in 5(6.1%) and 3(3.7%) respectively. There was however no significant difference between both groups and the different microorganisms in the GCF analysed. There was a highly significant relationship between oral hygiene (OH) and GCF volume ($P<0.01$). Pocket depths of 0.5mm to 7mm were recorded and there was also no correlation between probing pocket depth and GCF microbial composition. kliebsiella was highest in GCF volumes of 0.62μL in women with poor oral hygiene (OH) and in spacing. There was a significant relationship between anterior open bite and microorganisms cultured from the GCF. This study revealed that kliebsiella is the most prevalent anaerobe in gingival crevicular fluid and the highest volume of 1.86μl was observed in adult women with malocclusion in Benin City.

INTRODUCTION

Orthodontic appliances used in the treatment of various types of malocclusion have been shown to alter the GCF volume and possibly microbial composition[1-2]. The evaluation of the pre-treatment microbial constituents and volume of gingival crevicular fluid (GCF) in different types of malocclusion is therefore important in different environments so as to determine the baseline GCF volume and microbial composition. While numerous studies have been carried out to determine the microbial composition and volume of the GCF fluid as it affects and alters periodontal disease[3-5], there appear to be few studies on the microbial composition and volume of GCF fluid in the various types of malocclusion especially crowding, spacing and anterior open bite.

Malocclusion has a high prevalence rate in our environment with values as high as 84.1% reported in Benin City[6]. Crowding which presents predominantly in the lower anterior teeth[4-7] results in plaque accumulation, altered topography of the gingival septum and increased periodontal problems[7]. Many studies have identified and compared plaque accumulation between the different types of malocclusion, mainly mal-aligned lower incisors and its effect on periodontal health [4-5, 7-8]. Plaque accumulation and the resultant increase in GCF flow have been identified in numerous studies as a good indicator of gingival inflammation and periodontal pocket formation [1-3, 8, 9]. This increased flow of GCF washes out the periodontal pocket and provides host-derived substances that determine the subgingival bacterial population[9, 10]. Although studies have been carried out on GCF constituents with few on the bacterial composition[8], there appear to be no studies in our environment on the bacterial composition and volume of GCF in the different types of malocclusion.

Other studies suggested that the bacterial species found in the GCF reflects the bacterial populations detached from subgingival biofilms but there appear to be no available studies on GCF bacterial populations[10]. Asikainen et al determined a wide diversity of bacterial species as part of the GCF in patients to include Streptococcus, Viellonella, Selenomonas, Actinomyces, Treponema, Campylobacter and numerous other microorganisms[10]. Other studies however suggested that GCF did not contain any bacterial content and consists of cell mediators and enzymes with focus on prostaglandins, beta-glucoronidase, neutrophil elastase and aspartase aminotransferase[11]. These studies utilized recent advances to identify species from a complex bacterial community like the oral cavity by using open ended molecular biological methods, DNA extraction and then real-time Polymerase Chain reaction (PCR) for the bacterial groups [10, 11]. Although these methods are more sensitive, they are very technique sensitive and expensive unlike the older methods [18] which are cheap, easy to use, simple and reliable especially in developing countries.

Previous studies on the composition of the oral microflora in our environment were carried out on saliva[12] and identified Streptococcus, Kliebsiella and Staphylococcus as prevalent. Recent studies also suggest that microorganisms like Streptococci and Lactobacilli form

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clones in plaque and salivary microflora and can be monitored as a result of cells released from plaque[13-15]. Over four hundred bacterial species have been identified in the mouth and include Streptococcus viridans, Streptococcus mutans and lactobaccilli [15]. Also, aerobic and anaerobic staphylococci, gram negative diplococci (Neisseriae and Moraxella catarrhalis), and diphtheroids are also resident flora in the mouth [14, 15]. Actinomyces species are normally present in tonsillar tissue and on the gingivae in adults and various protozoa may also be present. Yeast (Candida species) and Veillonella also occur in the mouth [13-15]. There are however mild fluctuations related to total bacterial counts as a result of the oral hygiene and diet but not the composition [5, 9, 12]. However, most of these studies focused on the supra and subgingival microflora within dental plaque in other populations and not on the bacterial composition of GCF.

This study therefore aims to determine the bacterial composition and volume of gingival crevicular fluid in adult females with malocclusion and compared with normal occlusion in Benin City, Nigeria.

MATERIALS AND METHODS

Study population

A total of 152 women were selected after systematic random sampling and were divided into 2 groups as follows:

Group 1 were participants with angles class I malocclusion which included crowding, spacing and anterior open bite.

Crowding: Was defined as overlapping or deflection of erupted permanent teeth limited to the anterior segments of the mandible.

Spacing: Was determined if there was no approximal contact between the lower anterior teeth with a range of 2mm or more within a segment. Spacing as a result of extractions was not considered as spacing.

Anterior open bite: Was determined if there was an actual vertical gap between the upper and lower incisors with the jaw in centric occlusion.

Group 2 which served as the control were participants with normal occlusion.

Inclusion/ exclusion criteria

The selected groups had good general health, were non smokers with no oral disease and had not taken any food, drink or medication at least 12 hours prior to the time of collection of the GCF samples. Exclusion criteria were the absence of restoration or prosthesis on the selected tooth and no prior orthodontic treatment.

Sample collection and processing

Informed and written consent for GCF collection was obtained. Ethical approval was obtained from the Ethics Committee of the University of Benin Teaching Hospital. Intra oral examination was carried out using natural light and disposable mouth mirrors and probes to determine the oral hygiene status and pocket depth of each participant. GCF was taken from each subject between 7am and 10am after isolation with cotton rolls to prevent saliva contamination, utilizing two methods:

GCF volume collection was done with a micro capillary tube (Marienfield Superior ®) 75mm long of known external and internal diameter placed at the entrance of the crevicular sulcus for 20seconds. GCF migrates into the tube via capillary action.

The volume of fluid collected was accurately determined by measuring the distance the GCF had migrated into the known diameter of the tube[16]

GCF for microbial analysis was collected by inserting size 30 sterile paper points into the gingival sulcus of the labial aspect of the middle of the lower central incisor for all participants until a slight resistance was felt and left in situ for 60seconds[16] and sent for microbiological analysis which was done by the same medical laboratory scientist. GCF was inoculated onto blood, chocolate and MacConkey agar plates and then incubated for 24 hours at 37 degrees Celsius aerobically except chocolate agar which was incubated in a candle jar. Emergent colonies were identified according to the criteria by Cowan and Steel (1974)[18].

Data Analysis

Correlations between age, probing depth, malocclusion, GCF volume and microbial content were determined using the statistical package for social sciences SPSS (version 16) software. Significant values of $P<0.05$ were applied where applicable.

Results

A total of 152 female participants aged 26-65-years-of age were included in the study. The 31-40-year-old age group was the highest number in 47 (30.9%) and the 61-65-year-old age group the lowest number in 12 (7.9%). Other age groups included the 26-30-year-old in 20 (13.2%), the 41-50-year-old age group in 40 (26.3%) and the 51-60-year-olds in 33 (21.7%).

Table 1 Shows the distribution of microorganism in both groups with kliebsiella showing the highest distribution in both groups 10 (12.2%) and 12 (17.2%) respectively. Microorganisms were not cultured in the GCF of a high number of participants with malocclusion 63 (76.8%) and control 47 (67.2%) groups respectively. Probing depths of 0.5-7mm were identified with GCF volumes of 0.16-2.17μl. Tables 2 and 3 shows a distribution of GCF volume, probing depth and microorganism in both malocclusion and normal occlusion groups. Oral hygiene was assessed as good in 23 (15.1%), fair in 54 (35.5%) and poor in majority of 75 (49.3%). There was a significant relationship $P<0.01$, between oral hygiene, pocket depth and GCF volume Gingival crevicular fluid volume ranged from 0.16μl to 2.17 μl with pocket depths of 0.5mm to 7mm. Table 4 shows the relationship between oral hygiene and microorganisms.

Discussion

Previous studies on the bacterial analysis of GCF were carried out to identify the types and diversity of detected species[10]. While their study identified a wide diversity to include Campylobacter, Selemonas, Porphyromonas, Catonella, Tanerella, Dialister, Peptostreptococcus, Streptococcus, Leptotrichia, Prevotela, Haemophilius, Fusobacterium, Actinomyces and Eubacterium [10]; this variation in diversity could be as a result of a more technique sensitive method of analysis which concentrated only on samples of GCF from a group of periodontitis patients, and differences in environment and race [10]. Our study was of a larger size and not limited to patients with a disease entity and also included a control group. The difference in diversity of microbial species has been determined by studies[10]which suggested that the bacterial species identified in the GCF is a reflection of the bacterial populations detached from the supra and sub gingival tooth surfaces. This present study however identified Kliebsiella, Streptococcus and Staphylococcus in the GCF.

Table 1: Distribution of micro organism in malocclusion and control groups

	Klieb n%	*Staph* n%	*Strep* n%	*Strep/Stah* n%	No growth n%	Total n%
Malocclusion						
Crowding	3(30)	1(33.3)	4(80)	0(0)	33(52.4)	41(50)
Spacing	7(70)	1(33.3)	1(20)	1(100)	29(46)	39(47.6)
AOB	0(0)	1(33.3)	0(0)	0(0)	1(1.6)	2(2.4)
Total	10(100)	3(100)	5(100)	1(100)	63(100)	82(100)
Control	12(54.6)	1(25)	10(66.7)	0(0)	47(42.7)	70(46.1)
Total	**22**	**4**	**15**	**2**	**110**	**152**

P<0.05

Table 2: Distribution of probing depth, GCF volume and microorganism

GCF vol (µl)	Pocket depth(mm)	Microorganism					Total
		Klieb	Staph	Strep	Strep/Klieb	No growth	
0.16	0.5	2(22.2)	0(0)	0(0)	0(0)	7(77.8)	9(100)
0.31	1	3(13.6)	2(9.1)	3(13.6)	0(0)	14(63.6)	22(100)
0.47	1.5	1(25)	0(0)	0(0)	0(0)	3(75)	4(100)
*0.62	2	12(18.5	2(3.1)	4(6.2)	0(0)	47(72.3)	65(100)
0.78	2.5	0(0)	0(0)	1(25)	0(0)	3(75)	4(100)
0.93	3	2(9.1)	0(0)	2(9.1)	0(0)	18(81.8)	22(100)
1.09	3.5	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)
1.24	4	1(5.6)	0(0)	4(22.4)	1(5.6)	12(66.7)	18(100)
1.55	5	0(0)	0(0)	1(50)	0(0)	1(50)	2(100)
1.86	6	0(0)	0(0)	0(0)	0(0)	3(100)	3(100)
2.17	7	1(50)	0(0)	0(0)	0(0)	1(50)	2(100)
Total		**22(14.5)**	**4(2.6)**	**15(9.9)**	**1(0.7)**	**110(72.4)**	**152(100)**

***P<0.01**

Table 3: Relationship between oral hygiene (using the simplified oral hygiene index) and microorganisms

Microorganism	Oral hygiene			Total
	Good	Fair	Poor	
	n(%)	n(%)	n(%)	n(%)
Kliebsiella	4 (18.2)	7(31.8)	11(50)	22(100)
Staph	0(0)	0(0)	4(100)	4(100)
Strep	1(6.7)	7(46.7)	7(46.7)	15(100)
Staph/strep	0(0)	1(100%)	0(0)	1(100)
No growth	18(16.4)	39(35.5)	53(48.2)	110(100)
Total	**23(15.1)**	**54(35.5)**	**75(49.3)**	**152(100)**

P>0.05

Table 4: Comparative analysis of GCF volume, Malocclusion and control

GCF Vol µl	Crowding n %	Spacing n %	Anterior Open Bite n %	Malocclusion n %	Control n %
0.16	3(7.3)	2(5.1)	0(0)	**5(6.1)**	**4(5.7)**
0.31	4(9.8)	5(12.8)	1(50)	**10(12.2)**	**12(17.2)**
0.47	0(0)	3(7.7)	0(0)	**3(3.7)**	**1(1.4)**
0.62	22(53.7)	15(38.5)	0(0)	**37(45.1)**	**28(40)**
0.78	1(2.4)	2(5.1)	0(0)	**3(3.7)**	**1(1.4)**
0.93	6(14.6)	4(10.3)	0(0)	**10(12.2)**	**12(17.2)**
1.09	1(2.4)	0(0)	0(0)	**1(1.2)**	**0(0)**
1.24	3(7.3)	6(15.4)	1(50)	**10(12.2)**	**8(11.4)**
1.55	1(2.4)	0(0)	0(0)	**1(1.2)**	**1(1.4)**
1.86	0(0)	1(2.6)	0(0)	**1(1.2)**	**2(2.8)**
2.17	0(0)	0(0)	0(0)	**0(0)**	**2(2.8)**
Total	**41(100)**	**39(100)**	**2(100)**	**82(100)**	**70(100)**

P>0.05

Saliva has been shown to contain bacteria shed from supra and sub gingival tooth surfaces and microbial analysis of saliva has identified a wide diversity of bacterial microrganisms[12-18, 19-22]. The bacterial composition of saliva in Nigerians from Edo State was found to include Kliebsiella, Streptococcus and Staphylococcus[12]. This is in agreement with this present study which had a similar bacterial composition. However, while their study had a higher number of Streptococci cultured from both the malocclusion and control participants (79% and 57.9%) respectively[12], this present study had a higher composition of Kliebsiella in 12.2% and 17.2% in both groups. This could be due to a variation in the type of sample cultured. While the former was in saliva, this present study was carried out on GCF. However, studies by Scannapieco[21] identified Kliebsiella in non ambulatory patients with nosocomial pneumonia which is at variance with this study where this microorganism was seen in participants from within the local community.

Various studies have been carried out analyzing the microbial components of the oral cavity with emphasis on saliva and plaque, in normal occlusion and in malocclusion[12-13, 15, 19] with no significant differences observed in both groups. When compared with this present study, there appear to be no significant differences between the microbial content of GCF in normal occlusion and malocclusion. Other studies identified the constituents of the gingival crevicular fluid (GCF) as a result of malocclusion [22] and also the differences in GCF volume[8] during orthodontic treatment. This present study recorded volumes of 0.16-2.17μl with no significant differences in the GCF volume in both malocclusion and normal occlusion which is in agreement with other studies which suggested an increase in GCF volumes as a result of malocclusion[5] with no differences over time between orthodontically treated teeth[8].

There was also no significant difference between crowding and spacing and the various microorganisms cultured in this present study. However, there was a significant difference in the microorganisms cultured from participants with anterior open bite when compared with the control group. Various studies[4-5, 7] demonstrated that crowding in the lower arch results in poor oral hygiene as a result of stagnation areas and thereby predisposes to periodontal disease which has been shown to increase the GCF volume. Other studies have demonstrated that GCF microbial constituents in patients with periodontal disease may be as a result of the bacterial composition of plaque[10]. Although studies have concentrated on the correlation between GCF, crowding and periodontal disease[4-5,7], there appear to be few studies on spacing and anterior open bite.

Microorganisms were not cultured from the GCF in a large number of participants with malocclusion and normal occlusion in this present study. This could be due to the fact that GCF is derived from serum, host cells and oral bacteria but during periodontal inflammation, the mechanism of formation becomes exudative[1-3] and the type of bacteria present in the oral cavity determine the GCF bacterial content[8]. Also, studies by Mullally et al[24] demonstrated that bacteria may or may not be included from the intracrevicular sulcus as a result of the method and site of collection of GCF.

Other studies have identified differences in the constituents of GCF based on the method of collection and analysis [1-4, 11, 16, 17, 25-26]. These

include extracrevicular, intracrevicular superficial[25-27] or intracrevicular deep [28] method of collection. These influence the volume determination and analysis for microbial analysis. Previous studies have utilized a single method for analysis[18] or a combination of methods[4, 10] and found that greater volumes of GCF fluid can be determined using the extracrevicular method with a capillary tube while paper points can be used for microbial analysis with the intracrevicular deep method [6, 11, 12, 14]. This is consistent with findings from this study which utilized two methods for GCF volume; the capillary tube and paper point and the traditional method of microbial determination[18]. The highest GCF volumes of 2.17μl were recorded in pocket depths of 7mm. This is consistent with findings from another study[21] where higher volumes of GCF were associated with deeper pocket depths.

This study determined that Kliebsiella was highest in participants with poor oral hygiene and there was no correlation between oral hygiene and the microbial content of the GCF in both normal occlusion and malocclusion. Other studies identified streptococcus as being more prevalent in saliva[12] and GCF[6] which is in contrast with the findings from this present study where Kliebsiella was the most prevalent microorganism. Microbial analysis of plaque and saliva in patients with poor oral hygiene from other studies identified Actinomyces, Streptococcus, Bacteroides, Actinobacillus[29], Porphyromonas, Canocytophagia, Selemnomonas and Wolinella[18]. The diversity of microorganisms from these studies demonstrates that variations exist probably due to the oral environment and the oral hygiene state of each participant.

Conclusion

Microorganisms can be cultured from the gingival crevicular fluid and there is no difference in the diversity in malocclusion and normal occlusion of participants, although there was a reduction in microorganisms in the malocclusion group when compared with normal; occlusion.
The most prevalent volume of GCF fluid was seen in 0.62μl in both malocclusion and normal occlusion Kliebsiella was highest in participants with poor oral hygiene and there was no correlation between oral hygiene and the microbial content of the GCF in both normal occlusion and malocclusion

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