Isolation and Identification of Candida Species and Bacteria associated with Orthodontic Appliances Experimental in vivo study

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Abstract

This study employed the imprint culture technique to asses the effects of fixed and removal orthodontic appliances on oral carriage .specially the distribution of Candida species in group of adults during fixed and removable orthodontic appliance therapy.75 individuals, mean age=16.5 years, undergoing orthodontic treatment with fixed and removable orthodontic appliances. the experimental group was selected from a larger sample of orthodontic patients who were clinically examined once to obtain baseline data before active treatment.18 males and 19 females were examined with fixed orthodontic appliances. while, 17 males and 21 females were examined with removable appliances .Thereafter, the experimental group was examined three times after 3 and 6 months and over6 months follow-up period after insertion of orthodontic appliance. The whole mouth plaque score was obtained, and the oral cavity was then sampled for Candida species and bacterial species by (imprint culture).

The results of this study showed that streptococcus species were present in higher percentages than other microorganisms, followed by Candida species, then staphylococcus, Pseudomonas and Lactobacillus species respectively. Females showed higher bacterial growth compared with males, and higher mixed growth over single growth in female while male showed the reverse. Number of bacterial growth after 6 months period was higher than 3 months period and both periods were higher than the period of over 6 months, with maximum bacterial growth of streptococcus species followed by Candida, then staphylococcus, Pseudomonas and Lactobacillus species respectively for all time intervals (except that Candida showed the same growth at 3 and 6 months period, and staphylococcus showed the same growth at 6 months and over 6 months period).fixed appliances showed more bacterial growth compared with removable appliance for all isolated microorganism types including Candida species, with maximum growth for streptococcus species .Results also revealed than maximum antibacterial resistance was for streptococcus species for the all four tested antibiotics, followed by Lactobacillus species, while the bacterial resistance for Candida, Staphylococcus and Pseudomonas species was very poor against the all tested antibiotics.

الخلاصة

استخدمت هذه الدراسة طريقةimprint culture technique لتقييم تأثير جهاز التقويم الثابت والمتحرك على الكرايج خصوصا انتشار أنواع الكانديدا لمجموعة من المرضى البالغين خلال العلاج بجهاز التقويم الثابت والمتحرك. تم معالجة 75 شخصا معدل أعمارهم 16.5سنة بجهاز تقويم الأسنان الثابت والمتحرك.المجموعة التي اختيرت للاختبار تم اختيارهم من مجموعة كبيرة لمرضى تقويم الأسنان.تم فحصهم سريريا لأخذ العينات وفحصها وتسجيل النتائج الأولية قبل بداية العلاج التقويمي .18 ذكر و19انثى تم فحصهم باستخدام جهاز تقويم الثابت بينما 17 ذكر و21 انثى تم فحصهم باستخدام جهاز التقويم المتحرك.هذه المجاميع تم فحصها ثلاث مرات بعد ثلاث أشهر وستة أشهر وفوق ستة أشهر باستخدام أجهزة التقويم علما إن البلاك سكور تم

تسجيله للفم قبل اخذ المسحات للكانديدا البيكانس وبقية أنواع البكتريا باستخدام Imprint Culture Tech. أظهرت النتائج المخبرية وجود الستربتوككس بنسبة عالية مقارنة بالأنواع الأخرى ثم الاكانديدا والاستافلوكوكس والسيدو مونص واللاكتوباسلص . عينات الإناث اظهرت نسبة أكثر بنمو البكتريا مقارنتا بالذكور والنمو الخليط أكثر من النمو الانفرادي في الإناث بينما العكس في الذكور. نمو البكتريا بعد ستة أشهر كان أعلى من فترة الثلاثة أشهر وكلا الفترتين كان النمو فيهما أعلى من الفترة مابعد الستة أشهر وأعلى نمو بكتيري سجل للستربتو كوكس ثم الكانديدا وبعدها الستافلوكوكس والسيدو مونص واللاكتو باسلس بالتتابع لكل الفترات الزمنية باستثناء الكانديدا كانت بنفس النمو في فترة 3 و 6 أشهر ثم والاستافلوكوكس أيضا بنفس النمو في فترة 6 أشهر وفوق 6 أشهر . أجهزة التقويم الثابتة أظهرت أكثر نمو بكتيري بالمقارنة بالأجهزة المتحركة لكل أنواع الإحياء المهجرية الدقيقة بضمتها أنواع الكانديدا مع أعلى نسبة نمو سجلت للستربتوكوكس . النتائج المخبرية اطهرت أعلى مقاومة للبكتريا كانت للستربتو كوكس لكل اختبارات المضادات الحيوية الأربعة ثم الالكتوباسلص بينما كانت في أدنى مستوياتها للكانديدا والاستافلوكوكس والسيدومونص في كل اختبارات المضادات الحيوية الاربعة.

Introduction

Intrinsic and extrinsic factors, have an effect on the composition, metabolic activity and pathogen city of the highly diverse micro flora of the mouth of the mouth (Samaranayake, 2002). It has been reported that the presence of a fixed orthodontic appliance greatly inhibits oral hygiene and creates new retentive area for plaque and debris, which in turn predisposes to increased carriage of microbes and subsequent infection (Atack *et al.*, 1996). The high oral colonization by the fungal pathogen Candida albicans in individuals wearing either full or partial removable dentures is well documented (Budtz- Jörgensen, 1990). Many factors contribute to the virulence of Candida albicans, such as phospholipases, protienases, acid phosphatases, etc. However, most investigators agree that hyphal form of Candida albicans adheres better to tissue and acrylic than yeast phase, adherence of Candida albicans to host cell surfaces is considered to be the first and essential step in colonization and infection (Atack *et al.*, 1996;Samaranayake, 2002, Maza *et al.*, 2002; Herro *et al.*, 2002).

Aim of study

The aim of this investigation was to evaluate the prevalence of Candida species and associated microorganisms by using imprint culture technique in group of adults during fixed and removable appliance therapy. Then indicated sensitivity antibiotic test .

Materials and methods

Study design:

In this study only healthy people were included and the medical history of each individual was checked for factors known to affect carriage of Candida i.e. diabetes, anaemia and immunosuppression. Similarly, individuals who smoking or who had currently received treatment with antibiotic ,antifungal or steroid in the past 3 months were excluded. all patients were examined by a single examiner. The age, gender, dietary habits, dental and medical history and oral lesions were recorded. A complete oral examination of both soft and hard tissues was performed. Plaque indices were recorded for each patient, as indicators of oral status, using the criteria of Silness and Löe(1964)

75 swabs were collected from patients (males and females)with mean age 16.5 ± 2 years were analyzed to evaluate gender differences(fig.2).after that patients ready to receive orthodontic therapy, after insertion fixed and removable appliances. All

samples were subjected to bacteriological diagnosis, and the microorganisms isolation and identification were depending on (Collee *et al.*, 1996; MacFaddin, 2000).

Imprint culture technique, designed by Arendorf and Walker(1979),was used to determine the Candidal colonization. sterile plastic pads(1.5 x 1.5 cm²) were dipped in Sabouraud's broth and placed on the cervical third of teeth crown between gum and parts of fixed and removable appliances for 60 seconds. The pad then pressed firmly on to a Sabouraud's agar plate and incubated at 37°C for 48 hours. Candida numbers were determined by visual counting and expressed as colony forming units per mm²(CFU\mm²)yeasts were identified by the Gram stain, germ tube test. As follow ***Diagram:***

Swabbing of upper and lower fixed and removable appliances ⟶ transfer the samples to the lab. by Brain heart infusion broth ⟶ Cultivation samples on basic and differential selective media(blood agar, nutritional agar ,MacConkey agar, Mannitolsalt agar and Sabouroud agar) ⟶ Purification of grew colonies and isolation ⟶ Identification A) colonial morphology B)cellular morphology C) biochemical activity ⟶ The isolates subjecting to anti biotic disc sensitivity test on Muller Hinton agar depending on(Kirby-Baure Methods).

- The following bacteriological culture median and reagents with solution were used in this study(Blood agar-Mast, brain heart infusion broth-Mast,MaConkey agar-Mast, nutritional agar-Mast,sabouraud agar-agar-Mast,muller hinton agar-Mast,mannitol salt agar-Mast,catalase test reagent, oxides test reagent-BDH,grams stain solution, normal saline(0.85%)and antibiotic disc(oxide).
- The following orthodontic materials were used in this study:

a-Orthodontic brackets sets with accessories arch wires, bands and elastics(Roth System-Orthomatrix\ USA).

b-Direct bonding system(Kerr\USA).

c-Stainless steel orthodontic wires and acrylic base plates(one of components of removable appliances)-QD\England.

Results and Discussion:

Among many of microorganisms present in oral cavity as a normal micro flora of the mouth, only five types of these microorganisms were isolated by this study.

The results of this study showed that streptococcus species was present in a higher percentage than other microorganisms (55.22%), followed by Lactobacillus species, while the bacterial resistance for Candida, Staphylococcus Among many of microorganisms present in oral cavity as a normal micro flora of the mouth, only five types of these microorganisms was isolated by this study.

The results of this study showed that streptococcus species were present in higher percentages than other microorganisms (55.22%), followed by Candida species (24.22%), then staphylococcus, Pseudomonas and Lactobacillus species respectively as shown in Fig. (1).

Female showed higher bacterial growth compared with male, and higher mixed growth over single growth in female while male showed the reverse as in Fig. (2).

Number of bacterial growth after 6 months period was higher than 3 months period and both periods were higher than the period of over 6 months, with maximum bacterial growth of streptococcus species followed by Candida, then staphylococcus, Pseudomonas and Lactobacillus species respectively for all time intervals (except that Candida showed the same growth at 3 and 6 months period, and staphylococcus

showed the same growth at 6 months and over 6 moths period) as shown in Fig. (3) and Table (1).

Generally fixed appliances showed more bacterial growth compared with removable appliance for all isolated microorganism types including Candida species, with maximum growth for streptococcus species as in Fig. (4) and Table (2).

Results of the current study also revealed than maximum antibacterial resistance was for streptococcus species for the all four tested antibiotics and Pseudomonas species was very poor against the all tested antibiotics as shown in Fig. (5) and Table(3).

This study, which investigated micro flora during fixed and removal orthodontic therapy ,indicates that the wearing such appliances leads to increased carriage and considerable changes in the oral bacterial population, possibly due to the appliance-induced ecological alterations within the oral cavity. Low plague index and total bacterial count for the baseline patient was not elevated and this associated with good oral hygiene prior to the experiment. After insertion fixed and removable appliances a significant increase in plaque index and bacterial counts according to type of appliance and time of insertion also, the difference very clear according to gender group.

The reasons of this observation: (Addy *et al.,* 1983; Boyd, 1983; Scheie *et al.,* 1984; Alexander, 1991)

A- The presence of orthodontic attachments or parts on the labial and lingual surfaces of these teeth.
B- Parts of orthodontic appliances may interfere with brushing of gingival area.
C- Similar changes occurred with removable and in upper and lower appliance but higher in upper fixed if compare with lower and removable appliances due to stagnation of debris and difficulty to cleaned through brushing ,while in lower arch more easy and role of tongue movements which minimizing chance of colonization with ability of cleaned out side of mouth.
D- The presence of rough-surfaced bonding material act as a plaque trap and a gingival irritant.
E- Colonization increased after insertion orthodontic appliances could partly be due to the patient's attitude and behavior, which made the cleaning difficult .

The results of investigation in this study showing the significant differences in sex group after insertion of orthodontic appliances which leads to alter the ecological environment in the oral cavity by introducing new stagnant areas available for bacterial colonization and retention of substances in tow figures as mixed and single growth. the single growth more than mixed in males and visa versa in females, this observation may be due to hormonal changes and physiological activity differences between them.(Rosenbloom and Tinanoff, 1991; Sukontapatipark *et al.,* 2001).on the other hand, Arendorf and Walker(1979) have shown that the presence of prosthesis or an appliance increase Candidial numbers, not only at the occluded site, but at all mucosal sites sampled. Hence, it is possible that orthodontic appliances may affect oral coli form colonization and carriage in adults. In recent study where the bacterial composition of dental plaque deposits on(metallic and ceramic)compared using 'Checkerboard' DNA-DNA hybridization analysis, 37 bacterial species were isolated, implying that the appliance surface may modify the oral environment and help colonize non-indigenous bacteria(Anhoury *et al.,* 2002).

The use of antibiotics to suppress the growth of bacteria during orthodontic treatment is controversy because this study revealed that different types of microorganisms showed high resistance values to multiple types of antibiotics, while athors were very sensitive to same antibiotics that used in this study.

Conclusion and Suggestions

1- Treatment with affixed and removable appliances may alter the ecology in the oral cavity by introducing new stagnant areas available for colonization and retention of Candida species .
2- The result confirm this by indicating that fixed and removable appliances have a direct effect upon bacterial count and adverse effect upon the prevalence of Candida and coli form carriage in this group of adult.
3- Orthodontic appliances may also interfere with oral hygiene practice.
4- The results of this study indicates that special efforts must be doing and attention has to be paid to control infection may associated with orthodontic appliances.
5- Streptococcus followed by Lactobacillus sp are highly resistant to different commonly used antibiotics while Candida, Staphylococcus and Pseudomonas sp are highly sensitive to these antibiotics. So that further studies are needed to explain the possible reasons for these antibacterial resistance variations.

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