Changes in oral flora of newly edentulous patients, before and after complete dentures insertion

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ABSTRACT

Background: Investigation of the oral flora of edentulous subjects is becoming increasingly important because of recent wide spread use of implants in the treatment of edentulism.

Materials and methods: Twenty eight newly edentulous patients with age ranged between (40-80) years were included in this study. Saliva samples were collected at two intervals; before taking the primary impression, and after one month of functional use of the complete dentures, and assessed microbiologically.

Results: indicated that the total numbers of microorganisms were decreased within the post insertion period with non significant variation in their types, newly edentulous mouth harbored Neisseria species which disappeared after denture insertion, while E. coli, Klebsiella, and Moraxella (Branhamella) were observed after one month of denture wearing. Other microorganisms include Streptococci and Candida was reduced. On the other hand, Staph. aurous, Diphtheroids, Veillonella and Acinetobactor were considered as a part of the normal flora of edentulous patient that unchanged by denture wearing.

Conclusion: Although denture can serve as a colonization site for the various microorganisms with short period of denture use and good oral hygiene the microorganisms did not increase in the oral cavity.


INTRODUCTION

Normal oral flora is taking an important role in keeping the oral mucosa in a health status (1). It is important to fully define the human microflora of the healthy oral cavity before one can understand the role of bacteria in oral diseases. Recent studies reveal that more than 700 bacterial species of phylotypes of which over 50% have not been cultivated, have been detected in the oral cavity. There is a distinctive predominant bacterial flora of the healthy oral cavity that is highly diverse and site and subject specific (2).

As the completely edentulous patients are usually in elderly status, so the age related change in salivary flow will affect the health of oral tissue (3,4), as well as functional use of the complete dentures will affect the salivary flow by changing the occlusal forces. Previous studies suggested that proper replacement of full complete dentures will increase the salivary flow rate along with improved occlusal force (4-6), at the same time it enhances the accumulation of dental plaque and bacteria on the surface of the dentures. Studies revealed bacteria which depend on hard surfaces for attachment and growth will in part re-colonize the mouth if a denture is worn (7,8). Investigations and researches revealed that a number of pathogenic microorganisms which were present in the mouth when the patient was dentate, they were harbored in the oral cavity even when they are in the edentulous state (8-10). Microbial samples from the tongue and saliva suggested that black-pigmented Gram-ve anaerobic microorganisms were present as indigenous oral flora in mouth with or without dentures (11).

More than 300 microbial species coexist in the oral cavity, with Streptococcus spp. and Actinomyces spp. being the predominant cultivable flora in early plaque (4,12). Yeasts such as Candida albicans are usually a minor component of the oral flora, except in situations where the host is compromised, and then oral candidosis may be evident (4,12). Increased numbers of Candida albicans also occur in denture stomatitis (13).

Adhesions present on the surfaces of oral microbial cells are considered necessary for there growth and survival in the mouth (12-15). Probably best characterized are the adherence of oral Streptococci and Actinomyces that enable these organisms to attach to salivary pellicle (12-16). Candida albicans adhere to mucosal surfaces, to plastic, and to acrylic, but modes of attachment are less clearly defined than those in oral Streptococci (14,5). In view of the importance of interbacterial coaggregation in the establishment and maintenance of bacteria such as Strep. viridians with Candida albicans may influence colonization of the oral cavity by the yeast (12). A number of oral and non-oral bacteria can agglutinate with Candida albicans cells (13).

The complete loss of teeth may eliminate some pathogenic bacteria for lack of a suitable habitat for colonization, such as tooth surfaces and subgingival sites (4, 10). For instance, Strep. mutans, the primary etiological agent in caries development and found in oral cavity of dentate people, Strep. mutans cannot be found in edentulous subjects (15). The prevalence of selected putative periodontal pathogens colonizing oral mucous membranes was investigated in 26 denture-wearing subjects with a history of periodontitis, while other previous
studies suggested that none of subjects examined harbored Prophyromonas gingivalis and up to now P. gingivalis has not been found in the healthy oral cavity. Also a high numbers of black-pigmented Gram-ve anaerobes may belong to the indigenous oral flora in edentulous subjects with or without dentures.\(^{11, 16, 17}\)

Different studies suggested that with loss of teeth, bacteria associated with hard subjects for attachment and growth (e.g. Strep. mutans), strict anaerobes generally found in periodontal pockets (black-pigmented Gram-ve anaerobes of the genera P. gingivalis and Prevotella, and Spirochetes) will in part recognize the mouth if the denture is worn.\(^{4, 11, 16, 18}\). The prevalence of salivary Strep. mutans and Lactobacilli, salivary flow rate and the type of dentition were studied in connection with a medical survey of 76-86 year-old inhabitants of Helsinki living at home. High counts of Strep. mutans were found in 68% of wearers of full dentures, as compared with 53% of subjects having natural teeth. High counts of lactobacilli were found in 44% in subjects having removable partial dentures and 39% in subjects having natural teeth. The bacterial counts did not correlate with medicines taken daily nor with diseases among the studied population.\(^{19}\).

**PATIENTS AND METHOD**

Twenty eight newly edentulous patients (16 males and 12 females), their age ranged between (40-80 years) who were non-smokers, non-alcoholic, devoid of systemic diseases and did not use antibiotics before six months included in this study. They visited prosthodontic clinic in the College of Dentistry-University of Sulaimani for construction of complete dentures. Each patient was asked to intake 5 ml distal water and vortex mix it inside the mouth for 1 minute then recollect it in sterilized plastic cups, by spitting method, at two intervals; before construction of the dentures (group1, newly edentulous) and after a month of functional use of their complete dentures (group 2). All patients received similar instruction during complete dentures insertion and along the regular checking and follow up visits. The samples were directly sent for microbiological examination at Sulaimani Public Health laboratory Teaching Hospital, and the following culture media were performed; 1-Blood agar aerobically for Streptococcus , Staphylococcus, and Pneumonococcus. 2-Blood agar anaerobically for Pepto-streptococcus, Peptococcus, and Veillonella 3-Chocolate agar and CO2 for Hymophilus and Neisseria .4-Maconkey agar for Gram –ve bacillae. 5 Sabauroid dextrose agar for Candida. The data were analyzed by using Chi-Square test, P-value <0.05 was regarded as statistically significant.

**RESULTS**

The result clarify that when microorganisms were grouped according to Gram stain bacteria and fungi, the total frequency of positive cultures in these groups among all the studied cases were slightly decreased (from 68 patients to 54) within the post insertion period in comparison to newly edentulous mouth (Figure: 1), with no significant variation in their types (P=0.11, Table: 1).

Thus before denture insertion, Hemolytic and Non-hemolytic Streptococcus as well as Staph. aureus were the more predominant Gram+ve bacteria. After one month period of functional use of complete dentures the positive cases with Non-hemolytic Strep. increased from 8 to 13 patients, while the a-hemolytic type decreased from 24 to 14 patient. On the other hand Staphylococci and Diphtheroids were unchanged. Yet there were no statistical difference in frequencies of Gram +ve microorganisms existence before and after denture insertion (Table 1, P=0.09).

Regarding Gram-ve bacteria, newly edentulous mouth was characterized by Neisseria Spp.(15 53.6%). However after denture insertion these microorganisms were absent, and instead of them E.Coli, Klebsiella pneumonia and Moraxella (Branhamella) catarrhalis were detected (Table 1). Nevertheless, the differences not reach the statistical level (P=0.24).

Concerning fungi, the existence of Candida albicans after one month of complete denture insertion was slightly reduced from 10.7% to %7.2 (Table 1).

**DISCUSSION**

Investigation of the oral flora of edentulous subjects is becoming increasingly important because of recent wide spread use of implants in the treatment of edentulism. The aim of this study was to compare the recovery rate of certain microorganisms that are able to survive in the changed oral ecology of edentulous subjects wearing dentures. Previous studies\(^{4, 11, 16, 18, 19}\) suggested that with loss of teeth, bacteria associated with hard subjects (e.g. Strep. mutans), strict anaerobes generally found in periodontal pockets (black-pigmented Gram -ve anaerobes of the genera P.gnigivalis and Prevotella and Spirochetes), and very fastidious organisms tend to disappear from the oral cavity. Bacteria that depend on hard surfaces for attachment and growth will in part recognize the mouth if the denture is worn.\(^{4, 18}\).
Concerning Gram +ve microorganisms, literature indicated that Strept. mutans is a predominant microorganism isolated from mucosa and saliva of denture wearing edentulous subjects. In our study, Hemolytic and non-hemolytic Streptococcus were also predominant in saliva and denture wearing, its level was higher in saliva compared to its previous reported frequency in mucosa. Furthermore, Strept. pneumoniae are considered as normal commensal in the human upper respiratory tract, up to 4% of the population carry these bacteria in small numbers, they induce inflammatory response and associated with sinusitis. Newly denture wearers according to our study had no chance to harbor these microorganisms in comparison to its existence in previously reported healthy mucosa of complete denture wearers (39%).

On the other hand, Staph. aureus are found in saliva of healthy subjects older than 70 years as well as in the oral mucosa of denture wearer. In this study it exists in saliva of both edentulous patients with or without dentures. Several studies indicate that the level of Lactobacillus in the saliva of edentulous mouth is very low. It constitutes less than 1% of the total flora. This is just in line with our result. But they also remark that these microorganisms return to the same or rise even to higher level than in dentate mouth when dentures are worn. However, we did not record the later finding.

Diphtheroids are normal inhabitant of skin and dental plaque. In this study they were isolated from edentulous mouth, and constitute 10% of the total flora. Their level was unchanged after denture insertion. This is in contrast to its higher level (21%) reported by Al-Aswad study.

Regarding Gram-ve bacteria, commensals Neisseria (Sicca, Flava, and Mucosa) are common habitants in the oral cavity both in the saliva and mucosa. While Moraxella are commensal of human respiratory tract that associated with maxillary sinusitis. In general, Neisseriaceae family, Moraxella “Branhamella”, and Acinetobacter were disappear after denture insertion, i.e.; edentulous patient without denture had great percentage of these microorganisms, and all of them disappeared after one month of denture use. This is in contrast to Al-Aswad findings that indicate greater percentage of Neisseria (39%) in mucosa of denture wearer, and they did not specify other members of this family.

Veillonella species are obligate anaerobic frequently isolated from oral samples (tongue, saliva, dental plaque) that have no pathologic potential. Using culture independent molecular technique, Sreebny indicated that it was among the species common to all sites of healthy human mucosa. In our study Veillonella was detected in edentulous patient with or without wearing dentures, similar to previous mentioned studies. Thus it belongs to the endogenous oral flora in edentulous subjects.

Klebsiella together with E. coli are indigenous to the human respiratory and intestinal tract respectively, and occasionally isolated from the oral cavity, however, they are considered to be transient oral commensals. E. coli, however, are a major agent of sepsis (urinary tract infection and diarrheal disease, as well as neonatal meningitis and septicaemia). Their level in saliva of complete denture wearers was just like that reported previously in mucosa of similar patients. Although we did not isolate them from edentulous mouth, this greatly remark to its association with denture wearing even for the short period of one month. On the other hand Klebsiella occasionally isolated from the oral cavity and hence are considered transient oral commensals. However their percentage like E. coli also increased after denture wearing similar to Al-Aswad findings.

There is hardly any observation on the presence of Actinobacillus actinomycetemcomitans (Aa.) in the edentulous mouth. The oral cavity of edentulous subjects do not contain A. a and P. gingivalis as normal inhabitant, even they were absent around implant in complete edentulous patients. Also Prevotella spp. black pigmented bacteroids seems to be a preferable habitant at the oral mucosa and it considered as normal oral flora of edentulous subjects wearing dentures. Prevotella intermedia were recovered in full denture wearer for longer period time (mean 20 years) and did not detected in subjects of 6.6 years period. This suggested that absence of Prevotella intermedia shortly after extraction may reflect only a temporary event. However; Kulekci et al. identify black pigmented Gram-ve anaerobes in the saliva of both edentulous patients with or without dentures.

Candida albicans are usually minor component of the oral flora. It increased in denture stomatitis and medical compromised patient. Candida albicans adhere to mucosal surfaces, to plastic, and to acrylic. In this study Candida albicans were detected in the saliva in 10.7% of the pre insertion samples and unexpectedly slightly decreased within the post insertion samples to be far less than the level.
registered from oral mucosa in denture wearer in Al-Aswad study (20). The colonization of the oral cavity by the yeast may be influenced by the importance of interbacterial coaggregation in the establishment and maintenance of bacteria such as Streptococcus viridians with Candida albicans (12) also; the number of oral and non oral bacteria can agglutinate with Candida albicans cells (13-15).

It seems that the prevalence of oral flora in edentulous patients revealed a great variation regarding site and method of sample collection, selective medium and enrichment cultivation and interpretations. As well it is affected by day time and period of denture use and period of hours wearing per day. Our patients were new cases who use denture for one month only and wear it at day only. They also keep good oral hygiene as a result of frequent motivation through the period of follow up. Thus denture can serve as a colonization site for the various mentioned microorganisms when there is neglecting oral hygiene with prolong denture use.

CONCLUSION

Newly complete edentulous patients after one month period of functional use of dentures showed no significant difference in type of microorganisms than before insertion. Staph. aureus, Diphtheroids, Veillonella and Acinetobacter were part of the normal flora of the edentulous patient that unchanged by denture wearing. While E. coli, Klebsiella, Moraxella “Branhamella” started to be observed after denture wearing. Other microorganisms include Streptococci and Candida were reduced and finally Neisseria disappeared.

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REFERENCES

Table 1: The number and percentage of positive cultures for different Gram positive and Gram negative microorganisms in 28 completely edentulous patients before insertion and after a month period of functional use of full complete dentures arranged in descending manner.

<table>
<thead>
<tr>
<th>Types</th>
<th>Microorganisms</th>
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<th>Post insertion</th>
<th>P value</th>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Gram +ve</td>
<td>α-hemolytic streptoccci</td>
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<td>85.7</td>
<td>14</td>
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<tr>
<td></td>
<td>Non-hemolytic streptoccci</td>
<td>8</td>
<td>28.6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Staph.aureus (coagulase-ve)</td>
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<td>21.4</td>
<td>6</td>
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<tr>
<td></td>
<td>Diphtheroids</td>
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<td>10.8</td>
<td>3</td>
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<tr>
<td></td>
<td>Streptococcus pneumonia</td>
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<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>1</td>
<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td>Gram -ve</td>
<td>Neisseria Spp.</td>
<td>15</td>
<td>53.6</td>
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<tr>
<td></td>
<td>Veillonella</td>
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<td>14.3</td>
<td>3</td>
</tr>
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<td>Escherichia coli</td>
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<td>Klebsiella pneumonia</td>
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<td>Moraxella (Branhamella) catarhalis</td>
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<td>0</td>
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<td>Fungi</td>
<td>Candida albicans</td>
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<tr>
<td></td>
<td>Total</td>
<td>68</td>
<td></td>
<td>54</td>
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Figure 1. The frequency of positive cultures for different groups of microorganisms in 28 completely edentulous patients before and after one month of functional use of full complete dentures.