Comparison between severe haemophilic A and healthy children in Streptococcus mutans, oral Lactobacilli and Candida albicans counts

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ABSTRACT

Background: Haemophilia is a common hereditary hemorrhagic disorder, however little is known about the oral microflora of hemophilic patients. The purpose of this research is to compare between the viable count of Streptococcus mutans, oral Lactobacilli and Candida albicans from saliva of children suffering from haemophilia A aged (6-12) years and the viable count of the same microorganisms isolated from saliva of healthy subject (healthy subject group) aged (6-12) years.

Materials and methods: - Saliva samples were collected from 30 children with severe Haemophilia A (patients group and 30 healthy children (healthy subject group). Microbial counts of Streptococcus mutans, oral Lactobacilli and Candida albicans were recorded for each group by using colony counter and expressed as colony forming unit multiplied by the dilution factor per millimeter saliva (CFU/ml).

Results: - The present study observed that the viable count of Streptococcus mutans and oral Lactobacilli in patient group was higher than the count of the healthy subject group while no significant differences were observed between the viable count of Candida albicans in patients group and healthy subject group.

Conclusion: - Education, prevention and effort among parents and dental professionals can aid in improving the oral health of Haemophilia children.

Key words: Haemophilia, Streptococcus mutans, Lactobacilli, Candida albicans. (J Bagh Coll Dentistry 2012;24(3):149-153).

INTRODUCTION

Haemophilia is a group of inherited bleeding disorders termed congenital disorder characterized by a lifelong defect in the clotting mechanism. This disease is caused by hereditary deficiency of factor VIII or IX. And it shows an X-linked recessive pattern affecting predominantly males (1). It can further be classified according to the clotting factors affected.

Haemophilia (A) or classic haemophilia is a deficiency of factor (F) VIII that occurs in 85% of patients and has an estimated frequency of 1 in 10000 males in Caucasian population most of haemophiliacs cases were first diagnosed following an episode of severe oral bleeding (2). Thus the dentist may be the first to diagnose patient with haemophilia (3). Dental caries (holes in teeth) occur when the hard tissues of the tooth and dentine are softened by demineralization caused by bacteria on foods especially sugars, producing an acid that demineralizes the hard tooth (4). Streptococcus mutans is a gram positive cocci facultative anaerobic bacterium commonly found in the human oral cavity and it is a significant contributor to tooth decay (5). These streptococci can attach to the proteins covering the tooth enamel, where they then convert sucrose into extra cellular polysaccharides (mutan, dextran, levan) (6). These sticky substances, in which the original bacterial layers along with secondary bacterial colonizers are embedded, form dental plaque. The final metabolites of the numerous plaque bacteria are organic acids that breach the enamel, allowing the different caries bacteria to begin destroying the dentin (7). Oral Lactobacilli (LB) are the most aciduric of the plaque bacteria, but these organisms only predominate by the time the carious lesion has extended into the dentin, this aciduricity best explains the involvement of Lactobacilli in human decay (8). A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of Candida, especially Candida albicans). The first exposure to fungi that most humans experience occurs during birth, when they encounter the yeast Candida albicans (C albicans) while passing through the vaginal canal. C albicans accidentally penetrate barriers such as intact mucus membrane linings, or when immunologic defects or other debilitating conditions exist in the host, these conditions favorable for fungal infections (9).
MATERIALS AND METHODS

- Sample collection: 30 children with severe Haemophilia A, aged (6-12) years that attended Al-Mansur hospital for children in Baghdad medical city and matched with 30 healthy healthy subject children aged (6-12) years were included in this study. Stimulated saliva samples were collected under standard conditions from patient and healthy subject group. Each individual was instructed to chew a piece of Arabic chewing gum (0.4-0.5g) for five minutes to stimulate salivary flow as much as possible then saliva was collected in sterilized screw capped bottles.

- Inoculation: The collected saliva was homogenized by vortex mixer for two minutes. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected for each microbial type and inoculated on the following culture media:
  1. *Mitis-Salivarius Bacitracin Agar (MSB Agar)*, the selective media for *Streptococcus mutans*: 0.1ml was withdrawn from dilutions $10^{-2}$ and $10^{-3}$ using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader on the plates of MSB agar,
  2. The plates were then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hrs at 37°C followed by aerobic incubation for 24hrs at 37°C.
  3. *Rogosa Selective Lactobacilli Agar (RSL Agar)*, this is selective for cultivation of oral LB: The inoculum was withdrawn from $10^{-2}$ and $10^{-3}$ dilutions; 0.1ml from each dilution was inoculated by using pour plate method. The plates were incubated aerobically for 48 hrs at 37°C.
  4. *Sabouraud Dextrose Agar (SD Agar)*, the medium is selective for the cultivation and isolation of *C. albicans*: 0.1ml was withdrawn from dilutions $10^{-2}$ and $10^{-3}$ using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader on the plates of SD agar then the plates were incubated aerobically for 48 hrs at 37°C.

- Identification: -
  a) Colony morphology: - the colony on MSB agar, RSL agar and SD agar were examined directly and under dissecting microscope (magnification ×15).
  b) Morphology of the Microbial Cells: - a colony was picked up from MSB agar, RSL agar and SD agar plates separately under sterilized conditions and subjected to gram’s stain.
  c) Biochemical Tests: - Bacterial colonies of different morphology were picked up from MSB agar, RSL agar and SD agar separately under sterilized conditions using inoculating loop and then inoculated in 10 ml of sterilized (BHI-B) and incubated aerobically at 37°C for 18 hrs. The following tests were conducted:-
    1. Catalase Production test: - this test was conducted on both types of cells (*S. mutans* and LB) separately. Hydrogen peroxide 3% ($H_2O_2$) had been used to detect the activity of catalase enzyme production.
    2. Carbohydrate fermentation test for: *S. mutans* - CTA- mannitol media had been used to test the ability of *S. mutans* to ferment the mannitol which was added in a concentration of 1% to the CTA- mannitol media.
    d) Identification system of API (analytical profile index) strep: - API 20 strep was a standardized system used in the identification of *S. mutans*. It is combining of 20 biochemical tests that offer wide spread capabilities. The strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. The enzymatic tests were inoculated with a dense suspension of organisms made from a pure culture

Microbial counts of *S. mutans*, *LB* and *C. albicans* were recorded by colony counter taking in consideration the dilution factor and expressed as colony forming unit multiplied by the dilution factor per milliliter saliva (CFU/ml)

RESULTS

1. Identification of *Streptococcus mutans*, oral *Lactobacilli* and *Candida albicans* was carried out by:-
   a) Colony morphology.
   1. On the selective MSB agar plates, *S. mutans* colonies appeared light blue in color about 1-2mm in diameter as spherical or ovoid in shape with raised or convex surface.
   2. On the selective RSL agar plates, LB colonies appeared as spindle, star like shaped, circular, ovoid or heart like in appearance, white in color.
3. Colonies of *C. albicans* appeared smooth, creamy in color with a yeasts odor and typically medium sized 1.5-2mm diameter which later develop into high convex, off-white larger colonies after 2 days.

b) Morphological test of bacterial cells.
1. *S. mutans* cells were gram positive, spherical or ovoid in shape, arranged in short or medium length non spore forming chains.
2. Microscopical appearance of LB includes the presence of gram’s positive, non-spore forming rods
3. *Candida albicans* under light microscope are rounded or oval yeast cells which stained Gram positive.

c) Biochemical test: - All colonies of *S. mutans* were catalase negative and had the ability to ferment mannitol. A positive reaction indicated by the change in color of indicator from red to yellow by the formation of acid after incubation.

All colonies of LB were catalase negative.

d) Identification system of API strep: - The reaction read according to the reading table and the identification was obtained by referring to the analytical profile index. The fermentation of carbohydrates was detected by a shift on the PH indicator.

2) The viable count of microbial isolates.
As shown in table 1 there are significant differences between healthy subject group and patients group for the viable count of *S. mutans* isolates

| Table 1: Comparison in viable count of *Streptococcus mutans* $\times 10^3$ CFU/ml in saliva of haemophilia group and healthy subject group |
|-----------------|------------------|------------------|
|       | Viable count | Patient group | Healthy subject group |
| mean  | 20.67         | 22.7            |
| SD    | ±9.54         | ±2.25           |
| P(t-test) | 3.12488 E-14 | P<0.0000        |

Table 2 demonstrate that statistically there are significant differences between patients group and healthy subject group for the viable count of *Lactobacilli* isolates

| Table 2: Comparison in viable count of *Lactobacilli* $\times 10^3$ CFU/ml in saliva of haemophilia group and healthy subject group |
|-----------------|------------------|------------------|
|       | Viable count | Patient group | Healthy subject group |
| mean  | 24.2         | 1.83           |
| SD    | ±11.76       | ±2.98          |
| P(t-test) | 2.14771 E-14 | P<0.00000       |

Result in table 3 revealed that there are no significant differences between patients group and healthy subject group for the viable count of *Candida albicans*.

| Table 3: Comparison in viable count of *Candida albicans* $\times 10^3$ CFU/ml in saliva of haemophilia group and healthy subject group |
|-----------------|------------------|------------------|
|       | Viable count | Patient group | Healthy subject group |
| mean  | 1.9          | 1.0            |
| SD    | ±3.04        | ±0             |
| P(t-test) | P = 0.11 | Not significant |

Results analyzed by using SPSS 15 statistical package (SPSS LTD working UK) T test showed differences between the viable count of *Streptococcus mutans*, oral *Lactobacilli* and *Candida albicans* from saliva of patients group
and the viable count of the same microorganisms from saliva of healthy subject group.

DISCUSSION

Haemophilia is the most common sex linked bleeding disorder worldwide. In developing countries most the patients with haemophilia are in the pediatric age group as they seldom reach adulthood because of inadequate treatment. Haemophilia in these areas are not given the priority it deserves as there are high numbers of other serious health problems.

The results of this recent study showed that the count of Streptococcus mutans and oral Lactobacilli was higher in patients group in comparison to healthy subject group while there is no significant differences between patient group and healthy subject group in Candida albicans and these results disagree the with results of other studies in developed countries like the study of Boyd D. & Kinirons M. who found that the prevalence of caries was low in haemophilia patients.

Moreover, Sonbol et al. also disagree with our results because they found that a significantly greater group of children with severe haemophilia were caries free compared with the healthy subject and the mean number of colony forming units of Streptococcus mutans in healthy subject group was significantly greater than patients group.

The possible explanation for the lower dental caries experience in these developed countries is to the existence of comprehensive haemophilic centers which provide children diagnosed as haemophiliacs, regular dental periodic checkups, a comprehensive preventive dental programme including topical application of fluoride by the delivery of fluoride to teeth topically or systemically in order to prevent dental caries and fissure sealant application which is a method of preventing caries from developing in the pits and fissure of the teeth by sealing them off with a special varnish called fissure sealant and strict dietary and oral hygiene instructions from an early age.

These differences reflect the higher tendency for conservative treatment and the greater concern about health education and preventive measures among both healthcare workers and haemophilic patients and their caregivers in these developed countries.

Oral care of haemophiliacs is not of primary importance in developing countries, as they have tended to receive less oral health care, or of lower quality, than the general population, yet they may have oral problems that can affect their systemic health also. The fear of the dentist to deal with these patients who might be bearing dangerous transmissible viruses as hepatitis and human immunodeficiency virus and the fear from complications like uncontrollable haemorrhage or life-threatening haematomas from simple procedures as anaesthesia and extraction have participated in the exaggeration of their dental problems. These also may be explained by the fact that haemophiliacs either refrain from the use of the tooth brush all together or use it inefficiently to avoid gingival bleeding and that they are more concerned with their medical health than their dental health.

It is clear that education, intervention and prevention make positive changes in oral health of haemophiliacs. A team effort among parents/guardians, children, and dental professionals can aid in improving the oral health of those who may not have the necessary means to dental care.

REFERENCES