Oral Health Status and Caries Related Microflora Among Children with Congenital Coagulation Disorders
(Comparative study)

Abeer M.H.Zwain   Maha M.Misbah Al- Ameen..
Department of Pedodontic and Preventive Dentistry, College of Dentistry, University of Baghdad
Wael S. Al-Alousi

Abstract

Background: Congenital coagulation disorders are hereditary bleeding disorders characterized by a lifelong defect in the clotting mechanism. Maintaining a good oral health might be a problem for those patients because of the potential bleeding tendency. The aims of the present study are to assess the condition of the dental health and the changes in the caries related micro flora in children with these disorders.

Materials and methods: 45 children with congenital coagulation disorder and 45 healthy controlled children matching the study group in age and gender were examined and indices recorded for PI, GI and dental caries (DMFS, dmfs) and caries related micro flora was sampled and cultured for Streptococcus mutans (on mitis salivarius agar supplemented with Bacitracin), Candida species (on sabouraud dextrose agar) and Lactobacilli (inoculated on Glucose yeast extract acetic acid agar) counts, the results then compared between the two groups.

Results: The mean values of the plaque index and gingival index of the study group were more than that of the control group (highly significant difference P<0.01 for PI and a significant difference P<0.05 for GI).

Regarding dental caries, no significant difference was found between the two groups for primary dentition, while for the permanent dentition the study group had significantly higher caries experience than the control group (P<0.01).

The mean values of colony forming units of Streptococcus mutans, Lactobacilli and Candida species in the oral micro flora of congenital coagulation disorder children were higher than that of the healthy children (P<0.01).

Conclusions: children with congenital coagulation disorders have more plaque accumulation, sever gingival inflammation, higher prevalence of dental caries and there is a change in the oral micro flora when dealing with them a great deal of emphasis should be placed on pediatric dentistry. They need regular dental programs and continued proper oral hygiene care can be instrumental in preventing dental treatment of those children.

Key words: Congenital coagulation disorders, hemophilia, dental caries, gingival, plaque index, oral micro flora.

Introduction

Inherited tendency to bleed is an uncommon disorder which termed congenital coagulation disorder (C.C.D). The most common is hemophilia A, hemophilia B affecting approximately 1 in 7500 males and Von Will brand’s disease (White, 1998)

The bodies of these children are unable to produce one or more of the essential clotting proteins. Hemophilia A is a deficiency of factor VIII, hemophilia B or Christmas disease is a deficiency of factor IX, Von Willebrand’s disease caused by a deficiency of von willebrand factor a protein that mediates platelets adhesion (Werner, 1996)

Other factor deficiencies, such as those of factors II, V, and XIII (one case per 1 million) and factor VII (one case per 500,000) are rare and extremely uncommon (McDonald et al., 2004)
In developing countries, most of the patients with hemophilia are in the pediatric age group as they seldom reach adulthood because of inadequate treatment. As there are high numbers of other serious health problems priority, C.C.D in these areas are not given the priority it deserve (Chuansumrit, 2003). Limited data are available about the oral and dental health of C.C.D children and young adults worldwide as most reports deal more with the surgical management of the disease(Cancro & Fischmal, 2000), in addition oral care of hemophiliacs is not of primary importance in developing countries(Billings & Mckee, 1998) and in Iraq there is no previous study concerning the changes in oral micro flora that might occur in children with C.C.D, so as it’s the duty of the dentist to full understand the conditions that effect the ability to provide safe oral hygiene care to those patients, the present study is designed to compare the dental health and streptococcus mutans, Lactobacilli and Candida counts in oral micro flora of children with C.C.D and compare them to healthy controlled children.

**Material and Methods**

Collection of the sample started in September and finished on the end of October 2010, during these two months, 45 children with congenital bleeding disorders aged 7-12 years old that attended the Center of Congenital Coagulation Disorders at Al-Mansur Hospital participated in the present study, any patient had a positive serological test indicating any viral infection was excluded from the study. The control group was healthy children that attended Pedodontic Department at Baghdad College of Dentistry and was matching the study group in age and gender.

A consent form obtained from the parents and an information sheet filled by interviewing them, then the children oral health was examined using artificial light for illumination, a plane mouth mirror and sickle explorer and according to the following criteria: accumulation of plaque deposits assessed using plaque index(Silness & Löe, 1964), for the patients with C.C.D, it is necessary to find an index with least harm to the gingiva in assessing gingival condition; an index that doesn’t depend on the bleeding potential as an assessing point(Sonbol et al., 2001), so the condition of the gingiva was assessed using the gingivitis component of the periodontal disease index(Ramfjord, 1959).

For the assessment of dental caries, all teeth examined using WHO criteria and the indices recorded as DMFS/dmfs(World Health Organization, 1987).

Microbiological samples were obtained from the children by swapping their gingival margins of the buccal surfaces of the last two posterior maxillary teeth, both left and right; the gingival margins of the lingual surfaces of the last two posterior mandibular teeth, both left and right and the dorsum of the tongue with sterile cotton swap (Tankkunnasombut et al., 2009). Each sample was transferred into 1ml of reduced transport fluid (RTF) and transported on ice at 4°C to the oral microbiology laboratory at the college of dentistry, university of Baghdad and plated within 2 hours (Tankkunnasombut et al., 2009).

**Microbiological procedure**

Samples were dispersed by vortex mixer to disrupt bacterial aggregation for 60 seconds then ten fold serial dilutions were prepared using sterile normal saline,(0.1 ml) was withdrawn from dilution 10^{-2}, 10^{-4} and then spread in duplicate by using sterile microbiological spreader on plates of mitis salivarius agar supplemented with Bacitracin
which is a selective media for selection and enumeration of mutans streptococci (Dasanayake et al., 1995) and on sabouraud dextrose agar, a selective media for Candida species growth (Mitchell, 2001) and for Lactobacilli colonies, 1ml from dilution($10^{-2}, 10^{-4}$) was inoculated on Glucose yeast extract acetic acid agar by using pour plate method (Kandler & Weiss).

Then the plates of mitis salivarius agar were incubated anaerobically (by putting them in anaerobic jar with candle which generate CO2) for 48 hours at 37°C followed by aerobic incubation for 24 hours and 37°C (Dasanayake et al., 1995) while the plates of sabouraud dextrose agar were incubated aerobically for 48 hours at 37°C (Mitchell, 2001) and the Glucose yeast extract acetic acid agar plates were incubated aerobically for 48 hours at 37°C (Kandler & Weiss, 1986).

**Identification of the microorganisms**

Preliminary identification of S.mutans colonies was confirmed by colony morphology under dissecting microscope, Gram staining where bacteria appeared as Gram-positive cocci and fermentation of mannitol. Cystine trypticase agar (CTA) had been used to taste the ability of S.mutans to ferment mannitol which was added in a concentration of 1%CTA (Dasanayake et al., 1995; Tankkunnasombut et al., 2009).

The identification of Candida species based on colony morphology that result from cultivation directly on Sabouraud dextrose agar, gram stain and the presence of pseudohyphae yeast colonies that identified by the production of germ tube chlamydomspore (Mitchell, 2001; Pereira et al., 2004).

Lactobacilli were identified by colony morphology under dissecting microscope and gram stain. The colonies of Lactobacilli on the selective media (Glucose yeast extract acetic acid agar) appeared as spindle, star like structure, circular, ovoid or heart like appearance, white or white to yellow in color. All colonies of Lactobacilli were catalase negative. Microscopical appearance includes the presence of gram positive non motile and non spore forming rods (Sonbol et al., 2001; Kandler & Weiss, 1986).

After incubation, microbial colony counting occurred and was expressed as colony forming units per ml (CFU/ml).

Statistical analyses were performed with SPSS package version 10. student’s t-test was applied for comparisons between congenital coagulation disorder and healthy control children. (p<0.05) was considered statistically significance.

**Results**

The sample of children suffering from congenital coagulation disorder was 45 children, aged 7-12 years (mean age 8.4) were included in the present study, most of them were with Hemophilia A (80% of the cases, n=36), 17.8% of the children (n=8) were with Hemophilia B and only 1 child suffered from Von Willbrand’s disease (Table 1). No statistical differences found between different types, severity of the disease and age groups, so they considered as one group. The control group was healthy children matching the studied sample in age (mean age 8.1 years) and gender.

Dental plaque and gingivitis scores in congenital coagulation disorder patients and matched control patients were shown in Table (2), a highly significant difference in plaque index (P<0.01) and a significant difference in gingival index (P<0.05) was found between the two groups.

Table (3) illustrate a comparison between congenital coagulation disorder patients and healthy control patients regarding dental caries, for the permanent dentition the mean
DMFS values for the total study group was significantly higher than that of the control group (P<0.01), in the other hand, no statistical significant difference could be found between the two groups regarding the caries experience represented by the (dmfs) for the primary teeth.

Highly significant differences found between congenital coagulation disorder and matched control patients in the mean number of colony forming units of streptococci mutans, candida species and lactobacilli (P<0.01) as shown in figure (1).

Discussion

Oral disease usually are not life threatening, in spite of that it should be remembered that oral health is one of the most important part of the general body health. In developing and industrialized countries, last decade studies have indicated that dental caries is one of the major health problem in the adult population(Reisine et al., 1984), in addition, recent researches highlighted that oral disorders have emotional and psychosocial consequences as a serious as other disorders(Namal et al., 2008).

In the present study, plaque accumulation around the teeth of children with congenital coagulation disorders were more than that of healthy children (high significant difference in PI scores P<0.01). Moreover, a significant difference (P<0.05) found between the gingival index scores in the two groups (Table 2). This finding is in agreement with that found in Poland and Turkey in which they disclosed that worse dental status and oral hygiene level were seen in children with hemophilia(Mielnick, 1999; Albayrak et al., 2006). In Egypt the value OHI-S (The oral hygiene index simplified) of hemophilic were in the higher end of the ‘fair’ level(Kabil et al., 2007) which is higher than those of the Polish hemophiliacs which were in ‘good’ level (Mielnick, 1999) and higher than that of healthy children. In the other hand Sonbol and his colleague’s results showed that plaque index were significantly greater in the control compared with the hemophilic children in England (Sonbol et al., 2001).

No significant difference found between mean value of dmfs scores in study and control groups, while when comparing DMFS scores, highly significant difference found between them. This finding is consistent with that found in previous Iraqi study(Al-Kubaisi & Al-Aousi, 2006) and in an Egyptian study, in which they concluded that the DMFS and dmfs of hemophilic were significantly higher than those of non-hemophilic children(Kabil et al., 2007) which is also found in Poland and Turkey(Mielnick, 1999; Albayrak et al., 2006). On the contrary, the researches from UK and Northern Ireland revealed that the children with hemophilia have a significant lower prevalence of dental caries compared with matched healthy controls (Boyd & Kinirom, 1997; Sonbol et al., 2001).

Streptococcus mutans and Lactobacilli bacteria is the predominant microorganism responsible for dental caries(Van Houte, 1994) and Candida species is an opportunistic pathogens that invade the mucosa only when there is changing in the oral environment such changes can be brought about by several reasons one of which is systemic diseases(Allen & Beck, 1987), a significant inter correlation was found between Candida species counts and Streptococcus mutans and Lactobacilli counts which is likely due to their acidogencity and acidurity as Candida affected by some strains of Streptococci by competing for attachment sites(Russel et al., 1999), a highly significant differences found between the mean number of colony forming units of these three microorganisms in the oral micro flora of C.C.D children than those of healthy children, this finding is not
agreed with the England study (Sonbol et al., 2001) in which there was no significant difference in the total bacterial counts between the two groups except for the mean streptococcal count, which was significantly greater in the controls.

These results can be explained by the fact that children with C.C.D in Iraq and other developing countries either refrain from the use of the tooth brush all together or use it inefficiently to avoid gingival bleeding and they are more concerned with their medical health than their dental health, and as dental caries is a multi factorial disease initiated by acidogenic microorganism present in the dental plaque, so neglecting the oral health, lack of motivation to exercise daily oral hygiene, in addition to the misconception of parents and physician that bleeding in the mouth will occur due to oral hygiene measures like brushing or flossing and difficulties in getting factor concentrate all these factors will cause the child get used to neglect his teeth providing a suitable environment for the bacteria responsible for dental caries. Time of exposure to the factors promoting caries is also important, so the effect of neglecting oral hygiene on the primary teeth may be minimum but on the permanent it is obvious (Table 3).

On the other hand, in developed countries, the possible explanation of lower caries experience, good oral health and less bacterial counts in the oral microflora is to the existence of comprehensive hemophilic centers which provide children diagnosed as hemophiliacs, regular dental periodic check ups, a comprehensive preventive dental programs including topical fluoride and fissure sealant application and strict dietary and oral hygiene instructions from an early age (Boyd & Kinirom, 1997; Sonbol et al., 2001).

So as a conclusion children with congenital coagulation disorder in Iraq should be advised about the importance of oral care because oral health affects the people physically and psychologically and for them, good oral health is important in prevention of any unnecessary bleeding, patients should be recalled for regular dental visits and preventive dental health programs should be designed for them.

References


Table 1: Distribution of the studied sample of congenital coagulation disorder regarding the disease type, severity and age of the children.

<table>
<thead>
<tr>
<th>Disorder Groups</th>
<th>Severity</th>
<th>Cases No.</th>
<th>%</th>
<th>Mean age (years)</th>
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<tbody>
<tr>
<td>Hemophilia A</td>
<td>Sever</td>
<td>18</td>
<td>40</td>
<td></td>
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<tr>
<td></td>
<td>moderate</td>
<td>10</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>8</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36</td>
<td>80</td>
<td>8.41</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Sever</td>
<td>3</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>3</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8</td>
<td>17.8</td>
<td>7.92</td>
</tr>
<tr>
<td>Von Willbrand’s disease</td>
<td></td>
<td>1</td>
<td>2.2</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>45</td>
<td></td>
<td>8.4</td>
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Table 2: Dental plaque and gingival scores in congenital coagulation disorder patients and matched control patients

<table>
<thead>
<tr>
<th></th>
<th>C.C.D</th>
<th>Control</th>
<th>statistic</th>
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<tr>
<td></td>
<td>Mean +SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>PLI</td>
<td>1.44±0.094</td>
<td>1.32</td>
<td>1.93</td>
</tr>
<tr>
<td>GI</td>
<td>1.013±0.32</td>
<td>0.1</td>
<td>1.38</td>
</tr>
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<td></td>
<td></td>
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</table>

Table 3: Comparison between congenital coagulation disorder patients and matched control patients regarding dental caries

<table>
<thead>
<tr>
<th></th>
<th>C.C.D</th>
<th>Control</th>
<th>statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>DMFS</td>
<td>3.58±0.207</td>
<td>2.99</td>
<td>3.99</td>
</tr>
<tr>
<td>dmfS</td>
<td>5.573±0.395</td>
<td>4.1</td>
<td>5.3</td>
</tr>
</tbody>
</table>
Figure (1): Total bacterial counts (log 10) differences between congenital coagulation disorder and matched control groups.