Dental Caries and gingivitis associated with Mutans Streptococci among children

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Abstract
Background Dental Caries is the most prevalent oral disease among children caused by Streptococci mutans, which produces acids that cause tooth surface breakdown and subsequent formation of cavity which encourage growth of bacterial biofilms and cause the gingivitis. Objective The aim was to assess the prevalence of dental Caries and severity of gingivitis among school children and to correlate it with the Streptococcus mutans counts.

Materials and Methods Five hundred and eighty (580) school children participated in this study. Dental caries was evaluated by the decayed-missing filled Index (dmft) while gingival index was used for assessed gingival health status. Snyder test was used to determine dental caries activity, Microbial counts of Streptococcus mutans were recorded for each group. Results the study indicates that the prevalence of dental caries had decreased with age from (93.2%) in group A students (7-9 years) to (88.3%) and (85.7%) in group B (10-12 years) and group C (13-15 years) respectively. The mean dmft values have increased with age from 4.5 in group A students to 4.8 in group B and 5.1 in group C. Prevalence of gingivitis increased with age, the mean gingival score were between 1.7 and 2.2. The count of Streptococcus mutans had been increased with age ranged from 4.2×10⁴ cfu/ml to 2×10⁵ cfu/ml and children with severe inflammation gingivitis are more likely to have high counts of Streptococcus mutans. Conclusion Bad oral hygiene and moderate gingivitis are highly prevalent among school children. No correlation was found between the mean dmft and the number of Streptococcus mutans (cfu), High Streptococcus mutans levels showed directly co-associated with increased severity of gingivitis at older ages, indicated a positive correlation was found between the presence of gingivitis and the number of S. mutans.

Keywords: Dental caries, Gingivitis, Streptococcus mutans, School children.
Introduction
Dental caries (tooth decay) remain the most prevalent chronic disease in both children and adults, which affect a large population of the world (Petersen, 2003a). Although many studies have focused on tooth decay, the disease still persists among children and adult from poor and disadvantaged backgrounds (Petersen, 2003b). The cost of dental treatment itself places caries among the most expensive of bacterial infections, in addition to the patient loss of time and productivity (Petersen PE et al., 2005). Dental caries develop in the presence of four interacting factors; cariogenic bacteria, sugar, susceptible tooth surfaces and time (Ed, 2014).

Sucrose in food and drink is considered the most fermentable cariogenic carbohydrate and act as a substrate for the synthesis of extracellular (EPS) and intracellular (IPS) polysaccharides in dental plaque (Marsh and Martin, 1992). In biofilm (dental plaque), Endogenous bacteria produce weak acids as metabolic products of fermentable carbohydrates (sucrose). The acids cause fall of critical value of pH and diffuse into the tooth through the water amongst the hydroxyapatite crystals, which are the major composition of tooth enamel and dentin (Bowen, 2002). Once the acid reaches crystal surface, carbonate ion incorporated in the crystal lattice leading to dissolve of Calcium and phosphate and transferee into the surrounding aqueous phase between the crystals resulting in defects and calcium deficient regions and subsequently the destruction of tooth structures (Marsh and Martin, 1992; JD., 2008). Although, the acids produced by bacteria lead to loss of calcium and inorganic phosphorus and fluoride from the enamel, Saliva acts to protect teeth through several mechanisms; such as clearance of food debris and sugar, dilute and neutralize the acid and it is an important natural defense against caries as it aggregation and elimination of microorganisms (Ribeiro et al., 2005; Tenuta et al., 2006; Fejerskov O., 2008).

Gingivitis is a non-destructive disease that occurs around the teeth. Bacterial biofilms that attached to tooth surfaces cause the gingivitis which is the most common form of periodontal disease (Caton et al., 1989). Gingivitis is an inflammation of the gingival tissue which is all soft tissue surrounding the tooth coronal to the crest of alveolar bone. Many research studies indicate that the prevalence of gingivitis forms of periodontal disease is lower in children than in adults (Chauhan et al., 2012). Plaque-induced gingivitis began once the bacterial plaque accumulates in the small gaps between teeth and in the gingival grooves where bacteria produce degradative enzymes, and endotoxin, such as lipopolysaccharide (LPS) or lipoteichoic acid (LTA), that initiate an inflammatory response in the gum tissue resulting an enlargement of gingival and destruction of the gingival tissues, which may progress to destruction of the periodontal attachment apparatus (Page and Schroeder, 1976). In health, the bacterial community in early dental plaque is dominated by Gram-positive bacteria. As plaque matures and gingivitis develops, the communities become increasingly complex with higher proportions of Gram-negative rods (Kistler et al., 2013). Although many bacteria associated with dental caries, Endogenous bacterium such as streptococcus mutans is the most important bacterium in the initiation of this disease while lactobacillus is associated with the active caries episode (Aguilera Galaviz et al., 2005; Cameron AC, 2008).

Many research studies about oral health showed a correlation between streptococcus mutans counts and the prevalence and incidence of dental caries Beighton et al., 1989; Crielaard W, Zaura E, Annemarie AS, Huse SM, Montijn RC and BJF., 2011). Usually prevalence of lactobacilli in saliva is low, but it becomes high once oral cavity
colonized by Streptococci mutans because of production of favorable acid environment for lactobacilli, so the pH-value decreases (Newbrun, 1992). Maintaining good dental health is important especially in children not only because it prevents pain and suffering but also because tooth loss in children can adversely affect how the jaws and teeth function as the child matures.

Materials and Methods
A community based cross-sectional study was conducted from September to December 2016 in Duhok province. Six primary schools and four secondary schools were selected at random. A total of 580 students aged (7-15) years were enrolled. General information data was collected which include gender, sex, age, socioeconomic state, and tooth brushing frequency during our visits to their school. The dmft indices were recorded by the same dentist throughout the research by using mouth mirror and probe.

Microbial analysis
Identification of Streptococci mutans
Unstimulated saliva was collected by placing a sterile 5 ml syringe (without a needle) in the buccal area and applying gentle suction. Samples were collected in the midmorning period (10:00-11:00 a.m.) and were processed within an hour. Approximately 5 ml of the whole saliva was collected and serial dilution was also prepared using sterile saline (1:10, 1:100, 1:1000, and 1:10000). Then 0.1 ml of the dilution 1:10000 was spread over the selective medium Mitis Salivarius Agar (MSA). The inoculated plates were incubated in an atmosphere of 5% CO2 using a gas pack supplied in an anaerobic jar for 48 hours at 37°C followed by aerobic incubation for 24 hours at 37°C. Streptococci mutans was confirmed by typical morphologies under a dissecting microscope, with gram staining and biochemical testing (Hardie and Bowden, 1974). The isolated strains were confirmed using Identification system of API (analytical profile index) API 20 strep (bio Meraux, France). Counts were expressed as colony forming units per ml of saliva.

Determination of dental caries by Snyder Test
Snyder test was used for detection of caries activity and determines a person susceptibility to dental caries (cavities); the susceptibility is correlated with acid production result from fermentation of glucose by cariogenic bacteria such as lactobacillus species on the teeth or in other areas of the mouth. The test used Snyder tubes agar which contains 2% glucose and pH indicator Bromocresol Green. The acidity of agar is about 4.8, which inhibit the growth of most microorganisms, but it is ideal for acidophilus such as Lactobacillus species.
All tubes inoculated with saliva and allowed to incubate in a candle jar at 37°C for 48 hours. If lactobacillus is present in the saliva, it will produce lactic acid from fermentation of glucose, causing drop the pH to about 4.4, which lead to change the color of Bromocresol Green indicator from green to yellow(Snyder, 1941).
Snyder tubes were examined daily for 3 successive days for color change. If the culture shows slight color change, indicate mild susceptibility to form dental caries (+). While significant color change indicate moderate susceptibility (++) and complete color change indicate high susceptibility (+++).
Oral Hygiene Index (dmft index)
The damage and the severity of tooth decay are measured by the universally adopted measurement, known as the Decayed-Missing-Filled Index (dmft) index (Klein, Palmer and Knutson, 1938). The damage to the tooth decay is irreversible, therefore, the visible state of a decayed tooth can be: decayed and left untreated (dt), missing extracted due to decay (mt), or filled (ft). The total number of teeth affected by tooth decay in an individual is the sum of dt + mt + ft which is known as the dmft value. Each adult normal has 32 permanent teeth, and its DMFT value may range from 0-32. Each child normally has 20 (dt, mt, ft are used to denote decay experience in the primary teeth). The severity of tooth decay is measured by the mean dmft values for age 12 and above and the mean dmft value for children below age 6. The mean dmft is useful value in making comparison among population where almost everyone is affected by tooth decay.

Gingival Index scoring
Changes happen in the gingival soft tissues are evaluated by dentist using gingival Index scoring (Löe, 1967). The index based on visual examination include; color, texture, changes in form, spontaneous bleeding. The calculation of the index as follows; for absence of inflammation score (0), for Mild inflammation - slight change in color and little change in texture score (0.1-1) while for Moderate inflammation – moderate glazing, redness, edema and hypertrophy (1.1- 2) and for Severe inflammation - marked redness and hypertrophy, Tendency to spontaneous bleeding score (2.1- 3).

Statistical analysis
Significance of differences in clinical parameters by age group and gender was sought using ANOVA for continuous variables (Caries index, Gingival index and mean dmft), (p<0.05) considered significant(Huntsberger, 1989). All analyses were performed using SPSS version 18.

Results
To best of our knowledge, this is the probably first comprehensive research study for assessment of oral hygiene and gingival health status among school children in Duhok providence. Five hundred and Eighty (580) school going children, 300 (51.72%) boys and 280 (48.27%) girls aged 7-15 years participated in this study. Out of the study population 190 belong to group A (7-9 years), 180 belong to group B (10-12 years) and 210 belong to group C (13-15 years). In total, dental caries is observed in 516 students (89%) and 64 students were caries free (11%) (Table1). In this study, the prevalence of dental caries has decreased with age from group A students (7-9 years, 93.2%) to group B (10-12 years, 88.3%) and group C (13-15 years, 85.7%) (Table1).
The caries prevalence of female 47% (273) was higher than caries prevalence of male 42% (243) (Figure 1). Same results have been reported by previous studies who observed high prevalence of dental caries in girls (Mosha et al., 2006; Mwakatobe and Mumghamba, 2007; Shingare et al., 2012). Our results found no significant differences of caries prevalence among students attend public and private school, the dental caries are more common in students attended public school (47%) than who's attended private school (42%) (Figure1). The caries prevalence in girls attend public school was 25% and in boys was 22%, while the prevalence of caries in girls attend private school was 22% and in boys was 20%.

The mean dmft values have increased with age from 4.5 in group A students (7-9 years) to 4.8 in group B (10-12 years) and 5.1 in group C (13-15 years) as shown in table 2. The similar results have been seen in previous studies in many countries around the world (Shingare et al., 2012; Al-Darwish, El Ansari and Bener, 2014). The Table2 provides whole-mouth clinical scores within each age group. Average gingival scores showed minor differences and were between 1.7 and 2.2. Similar to previous studies (Lang, Schätzle and Löe, 2009) and different from others which reporting
an increase in scores with age (Ababneh et al., 2012). The table also shows that the percentage of children harboring mutans Streptococci was 100% and in all ages. The number of cfu/ml of saliva varied from 104 – 105. The highest count of streptococcus mutans was (2×105 cfu/ml), which observed in the older ages (13-15 years).

Gingival statuses are presented in Tables 3, about 47% of the group A (7-9 year), 78% of the group B (10-12 year) and 86% of group C had gingivitis. The latter had significantly higher mean gingival index, with 53% demonstrating moderate gingival inflammation compared to 48% of group B. The highest mean of mild gingival inflammation had been seen in group A (40%), followed by group C (30%) and group B (29%). No sever inflammation gingivitis observed in group A students in comparison with group B (1%) and group C (3%). Different levels of gingival inflammation could be seen in Figure 2.
Snyder test was used to confirm caries activity among children which was found positive in 88.96% (n=516) children. No Caries activity conducted in 11% (n=64) children. Around 17.93% (n=104) children were found to be highly caries active while 13.79% (n=80) were moderate active and children with low caries active were 57.24% (n=332) as shown in table 4. The results were in accordance with prevalence of gingivitis in different groups. Similar result were found in previous study (Sonika et al., 2012).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N (%) No caries activity</th>
<th>N (%) Low caries activity</th>
<th>N (%) Moderate Caries activity</th>
<th>N (%) High caries activity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 - 9</td>
<td>13 (6.84%)</td>
<td>169 (88.94%)</td>
<td>6 (3.15%)</td>
<td>2 (1%)</td>
<td>190</td>
</tr>
<tr>
<td>10 - 12</td>
<td>21 (11.66%)</td>
<td>77 (42.77%)</td>
<td>32 (17.77%)</td>
<td>50 (27.77%)</td>
<td>180</td>
</tr>
<tr>
<td>13 - 15</td>
<td>30 (14.28%)</td>
<td>86 (40.95%)</td>
<td>42 (20%)</td>
<td>52 (24.76%)</td>
<td>210</td>
</tr>
<tr>
<td>Total</td>
<td>64 (11%)</td>
<td>332 (57.24%)</td>
<td>80 (13.79%)</td>
<td>104 (17.93%)</td>
<td>580</td>
</tr>
</tbody>
</table>

**Discussion**

The reasons suggested behind the decreased of caries prevalence are due to improvement in the oral hygiene because of increase the level of manual dexterity of the children and high oral health awareness (Grewal et al., 2009). Also when tooth erupts into the mouth undergo post-eruptive maturation making teeth more resistant to caries as compared immature tooth. In addition, Saliva play critical role in post-eruptive maturation process (Tandon, 2009). Although no significant difference was found regarding the prevalence of caries in relation to gender. The caries prevalence of female was
higher than male is due to female students skipping breakfast usually leads to more snacking during the day time, beside females have easy access to food supplies. As shown in table 2, the mean dmft values have increased with age; it is probably because of that children with various ages may have different portions of newly erupted teeth which are more prone to caries than longer erupted teeth. In this study it was found that children with severe inflammation gingivitis are more likely to have high counts of Streptococcus mutans than children with light and moderate gingivitis. A higher presence of Streptococcus mutans in children with older age was showed moderate and severe gingivitis, it may derive from a lower oxygen pressure in deep periodontal pockets, Which favors growth of such microaerophilic bacteria (De Soete et al., 2005). Once Streptococcus mutans predominates, it creates new conditions for the remaining microorganisms and develops biofilm. Bacteria within biofilm aggregate and colonize saliva-coated dentin and provide nutrients to other microorganisms. (Davey ME, 2000; Kolenbrander PE et al., 2002; Blehert DS et al., 2003). Our results revealed that the prevalence of caries is in concern with current study and Snyder test can be used as a part of routine assessment of oral health of school children. A large number of students reported no recent dental visits and utilized dental services only in the case of pain or other emergencies, corroborating previous observations (Grewal et al., 2009).

**Conclusion**

Majority of students enrolled in this study have experienced dental caries. The prevalence of dental caries was estimated in 516, 7 to 15 years school children. Following findings can be summarized from this research study:

1. The prevalence of dental caries among school children in Duhok province was 88.9%.
2. Dental caries prevalence has increased with age. The mean dmft values have increased with age from 4.5 in group A students (7-9 years) to 4.8 in group B (10-12 years) and 5.1 in group C (13-15 years).
3. Prevalence of gingivitis increase with age, the mean gingival score was between 1.7 and 2.2.
4. The count of Streptococcus mutans increase with age ranged from 4.2×104 cfu/ml to 2×105 cfu/ml and severe inflammation gingivitis are more likely to have high counts of Streptococcus mutans.

**References**


