Journal of Dental Hygiene Science Vol. 6, No. 3, pp. 159~162 (2006)

한국치위생과학회
THE KOREAN SOCIETY OF DENTAL HYGIENE SCIENCE

Dental Caries Risk Can Be Predicted by Simply Measuring the pH and Buffering Capacity of Saliva

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치아우식과 연관된 타액의 pH와 완충력

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ABSTRACT This study examined the relationship between the quantity of *Streptococcus mutans* and *Lactobacillus* spp. related to dental caries and the degree of acidity in saliva. A total of 240 saliva samples were taken from 80 subjects at the faculty of dentistry in Skopje, Macedonia. The saliva samples were taken by stimulating saliva production stimulation with paraffin chewing. However, no stimulation was applied when obtaining the samples used for measuring the pH. The data showed that in the caries group, *S. mutans* in 1 ml of saliva formed colonies with confluent growth (CFU > 10^6 and 10^4–10^5) in 100% of samples, whereas the *Lactobacillus* spp. formed colonies with confluent growth in 78.3%. In contrast, no colonies with confluent growth (CFU > 10^6 and 10^5) were found in the control group (with healthy intact teeth). In the caries group, the pH of the saliva was slightly acidic (pH = 5.90 – 6.50) and the buffering capacity was very low (below 0.7 ml of saliva per min). On the other hand, the pH of the saliva in the control group was neutral (pH 7.01 – 7.7) and the buffering capacity was high (over 1 ml of saliva per min). The increased number of *S. mutans* and *Lactobacillus* spp. in 1 ml of saliva (above 10^5 CFU or more) from the CRT (Caries Risk Test, Vivadent, Liechtenstein) bacteria test can indicate an increased caries risk as well as slightly higher acidity of the saliva. Overall, these results reveal that the caries risk can be predicted by simply measuring the pH and buffering capacity of saliva, and can be used to monitor the effect of dental hygiene practices with the aim of preventing dental caries.

Key words Buffering capacity, Dental caries susceptibility, *Lactobacillus* spp., pH of saliva, *Streptococcus mutans*,

INTRODUCTION

Bacteria are major factors in dental caries but their presence in the oral cavity does not necessarily lead to caries. The initiation of dental caries depends on the immune response, as well as the number and type of cariogenic bacteria such as *Streptococcus mutans*[1)] and *Lactobacillus* spp.[2)]. In addition, the initiation of dental caries is often associated with dental plaque, oral hygiene, nutrition, quantity and quality of the saliva, as well as other external and internal factors[3, 4)]. The caries process can be described as demineralization that occurs

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when the pH of plaque drops below the critical value of 5.5[5]. Whether a lesion develops will depend on the balance between demineralization and remineralization, in which the latter process is significantly slower than the former in caries cases[4].

The pH in the saliva plays an important role in the life, growth and multiplication of oral bacteria. The number of acidophilic bacteria is increased when the pH in the saliva is very low, whereas the number of the acid-sensitive bacteria is decreased[6]. The increased number of acidophilic bacteria in the dental plaque and saliva above 10^5 CFU colonies, as well as a low pH and CRT (Caries Risk Test)-buffer capacity of the saliva, can indicate a high risk of caries[1, 7-11]. In dental caries experiments in rats, the regrowth of *S. mutans* after chlorhexidine suppression was enhanced by a sucrose diet[12]. Therefore, the aim of this clinical study was to evaluate the quantity of *S. mutans* and *Lactobacillus* spp. in the saliva at neutral and acidic pH, as well as the buffering capacity of the saliva.

MATERIAL & METHODS

Sixty subjects (18-30 years of age) with caries (above 3 of DMFT index) in the oral cavity were selected from the endodontics clinic at "St Kiril and Metodi" University, Faculty of Dentistry, Skopje, Macedonia. The subjects were medically healthy with no systemic diseases and had not used any antibiotics or antiseptic mouthwashes during the last four weeks. The control group of 20 subjects also fulfilled the general criteria but did not have any indication of caries.

The subjects were suggested to stop oral hygiene 24 hours prior to the visit. Stimulated saliva was collected from all patients in the morning. The subjects were given a small piece of paraffin wax (1 cm × 1 cm × 1 cm) and asked to chew for a period of 30 seconds, then to swallow any saliva but not the paraffin. Thereafter, the subjects continued to chew the wax and saliva was collected at one-minute intervals for a total of five minutes. The total levels of stimulated saliva/minute were determined immediately. The accumulated saliva was used for the subsequent tests.

The quantitative presence of *S. mutans* and *Lactobacillus* spp. in the saliva was determined using the CRT-bacteria test (Caries Risk Test, Vivadent, Liechtenstein). The saliva samples for microbiological testing were taken in small sterile bottles, graded at 5 ml and then processed using the Vitel system (Bio Merilux, France). The growth density of the bacteria was evaluated according to the manufacturer's instructions. Bacterial growth was then scored by comparing the results obtained with the standards expressed in colony forming units (CFU) as follows[13]: *S. mutans* scoring: 0 = Very few colonies; 1 = Low, $< 10^5$ CFU; 2 = Medium, $> 10^5$ but $< 10^6$ CFU; 3 = High, $> 10^6$ CFU. *Lactobacillus* spp. scoring: 0 = Very few colonies; 1 = Low, ~10^3 CFU; 2 = Medium, ~10^4 CFU; 3 = High, ~10^5 CFU.

The CRT-buffer capacity of the saliva was measured using specific test-strips (Vivadent, Schaan, Liechtenstein). The CRT-buffer capacity was scored as follows: high (more than 1 ml saliva per minute), medium (1 ml saliva per minute), low (0.7-0.9 ml saliva per minute), and very low (less than 0.7 ml saliva per minute).

The saliva samples, used for determining the pH, were taken during the morning hours as soon as the examinees had arrived using a pH meter (Sutjeska, Yugoslavia). The pH values were then scored as follows: acid (pH < 6.50), slightly acid (pH 6.55-6.99), neutral (pH 7.00), slightly base (pH 7.01-7.51) and base (pH > 7.51). In order to establish a connection between the salivary CRT-buffer capacity and the pH of the saliva, both score values (CRT-buffer capacity and pH) were combined into four different groups of scores: very low (pH < 5.9 and CRT buffer < 0.7), low (pH = 6.55-6.99 and CRT-buffer 0.7-0.9), medium (pH = 7 and CRT-buffer 1) and high (pH > 7.01 and CRT buffer >1)

RESULTS

Fig. 1 shows the results for the quantitative presence of *S. mutans* (Fig. 1A) and *Lactobacillus* spp. in saliva in the caries group (Fig. 1B) and the controls. The control group of subjects showed very low scores for the presence of *S. mutans* colonies, where most of the subjects (90%) had a score of 1. The *Lactobacillus* spp. colonies from the control group were virtually undetectable (55% had score of 0) or were detected at a low level (35%). On the other hand, the *S. mutans* scores for the caries group were mainly high (46.6% scored 2 and 31.6% scored 3) and the *Lactobacillus* spp. scores (58.3% scored 3) indicating an obvious increase in both *S. mutans* and *Lactobacillus* spp. in the caries group compared with the controls.

The salivary pH in the caries group indicated acid (pH 5.90-6.50) and slightly acid (pH 6.55-6.99) pH values in 51.6% and 33.3% of the examinees, respectively (Fig. 2). However, the pH in the control group ranged from slightly basic to neutral, with 55% slightly basic (pH 7.01-7.51) and 30% neutral (pH 7.00). The obtained pH values for the both groups of examinees showed a statistically significant difference ($p < 0.01$).

Fig. 3 shows the CRT-buffer capacity of the secreted saliva within the period of 5 minutes expressed in 1 ml of saliva per minute for the both groups. Low (41.6%) and very low (33.3%) buffer capacities were detected in the caries patient group. In the controls, there was a high (65%) and medium (25%) CRT-buffer capacity, while there were no cases with a very low CRT-buffer capacity. The buffering capacity of the secreted (stimulated) saliva from the both groups of examinees was also significantly different, and there was a correlation

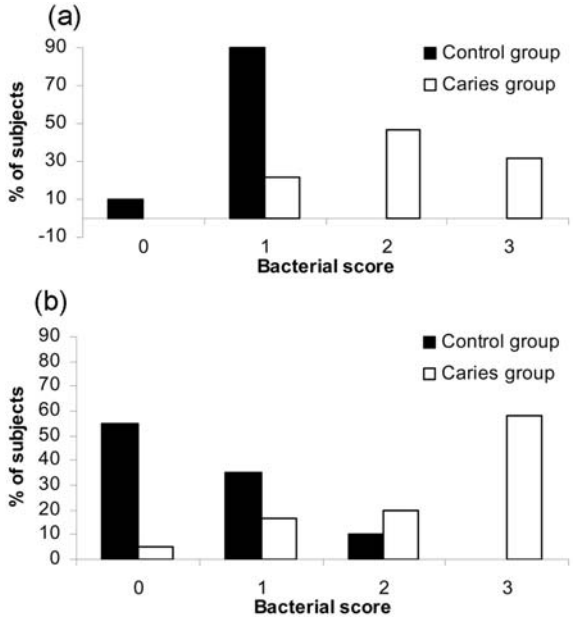

Fig. 1. A: Scores indicate the bacterial growth of *S. mutans* in caries group, not treated dental patients (n = 60) and control group (n = 20) of patients with no caries presence. B: *Lactobacillus* spp. scores in caries group, not treated dental patients (n = 60) and control group (n = 20) of patients with no caries presence. Scores evaluation described in the material and method section

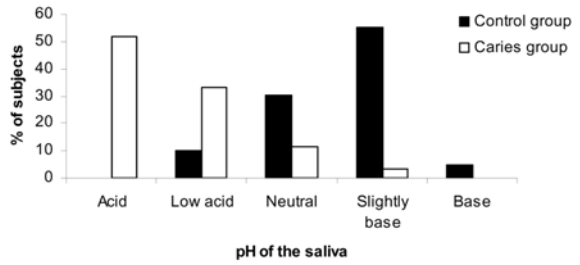

Fig. 2. Obtained pH values of mixed (nonstimulated) saliva from control group (n = 20) of patients with no caries presence and caries group, not treated dental patients (n = 60). The pH values were then scored as follows: acid (pH < 6.50), slightly acid (pH 6.55-6.99), neutral (pH 7.00), slightly base (pH 7.01-7.51) and base (pH > 7.51).

between the buffering capacity and pH.

When the results of pH and CRT-buffer capacity in the saliva for the both groups were compared, there was a correlation between the pH of the saliva and the CRT-buffer capacity (Fig. 4). In the caries group, there was a very low salivary pH (5.90-6.50) and CRT-buffer capacity (less than 0.7 ml saliva per minute), while it was the reverse in the controls (Fig. 4). For the control group, a high pH in the saliva pH 7.01-7.51 (slightly base environment, pH = 7.01-7.51) was associated with a higher CRT-buffer capacity (more than 1 ml saliva per minute).

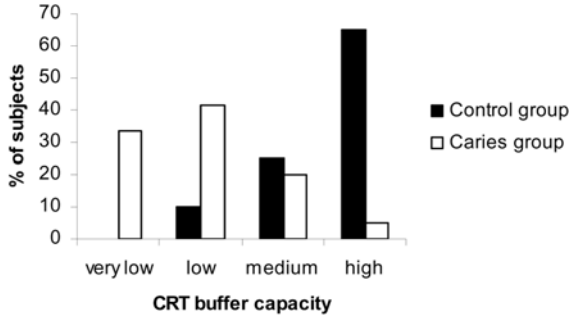

Fig. 3. CRT-buffer capacity of secreted (stimulated) saliva for caries patients (n = 60) within the period of 5 minutes, expressed as ml per minute, compared to controls (n = 20). The CRT buffer capacity was scored as follows: high (more than 1 ml saliva per minute), medium (1 ml saliva per minute), Low (0.7-0.9 ml saliva per minute), and as very low (less than 0.7 ml saliva per minute).

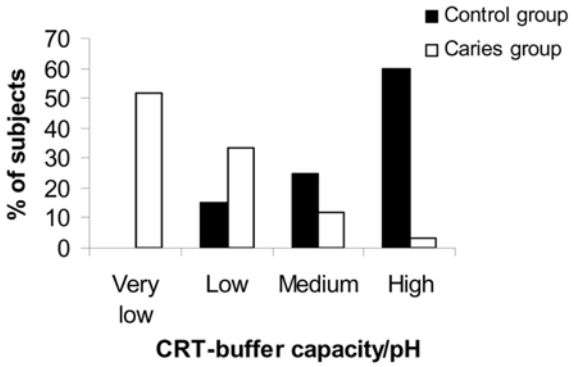

Fig. 4. The comparison of the combined results of pH and CRT-buffer capacity in the saliva for the both groups of examinees, caries patients (n = 60) and controls (n = 20). CRT-buffer capacity and pH score values are combined as follows: very low (pH < 5.9 and CRT buffer < 0.7), low (pH = 6.55-6.99 and CRT buffer 0.7-0.9), medium (pH = 7 and CRT buffer 1) and high (pH > 7.01 and CRT buffer > 1).

DISCUSSION

The saliva samples included *S. mutans* and *Lactobacillus* spp. because they are released from the colonized tooth surfaces into the saliva[7, 9, 11]. According to previous reports, this can also be demonstrated by taking a sample of nonstimulated and stimulated saliva[8, 14]. Considering that the stimulated saliva sample was obtained by chewing paraffin, a large number of *S. mutans* and *Lactobacillus* spp were released from the oral surfaces of the teeth. Hence, there is always a larger number of *S. mutans* and *Lactobacillus* spp. than in nonstimulated saliva samples.

In addition, there are differences between the reported qualitative and quantitative studies as well as differences between the analysis of the samples from the dental plaque and saliva. *S. mutans* and *Lactobacillus* spp. were present in a much greater number in the examinees with caries teeth than in

those with healthy intact teeth (control group).

The optimal pH in saliva has great importance for the growth and multiplication of oral bacteria. The published reports show that the number of acidophilic bacteria increased in the saliva very low pH (acid environment) and the number of the acidosensitive bacteria decreased[8, 15], which was also found in this study. The levels of *S. mutans* and *Lactobacillus* spp. found in 1 ml of saliva correlated with the pH and the buffering capacity of the secreted saliva within the time of 5 min in both groups of examinees. These results suggest that an increased number of *S. mutans* and *Lactobacillus* spp. in the dental plaque and saliva ($>10^5$ CFU) as well as a very low or low pH and CRT-buffering capacity indicate a very high risk of caries.

The CRT-buffer capacity and pH in saliva are of great importance in the pathogenesis of dental caries. The risk of caries can be determined by taking a saliva sample, which can be used to highlight the monitor dental hygiene practices and help prevent the onset of dental caries, particularly with the young population. In addition, this method is very simple, and can be applied to everyday dental practice.

요 약

이 연구의 목적은 타액이 산성일때 치아우식과 연관이 있는 *Streptococcus mutans* 와 *Lactobacillus* spp.의 양을 조사하여 치아우식의 지표로 사용될 수 있는지를 관찰한 외국인을 대상으로 한 임상결과이다. 총 240개의 타액 표본들은 마케도니아(Macedonia), 스코프에(Skopje)에 위치한 St Kiril- Metodi 치과대학병원에서 환자 80명으로부터 얻었다. 표본들은 사전의 파라핀 저작(씹기)법에 의해 타액을 자극하여 얻어졌으나, 타액의 pH의 결정을 위해서 다른 자극은 사용되지 않았다. 치아우식 집단의 타액 1 ml에서 *S. mutans*는 100%의 confluent growth (CFU>10^6와 10^4-10^5) 였으나, *Lactobacillus* spp.는 78.3%였다. 대조표본 집단(건강하고 손상되지 않은 치아를 가짐)에서, confluent growth (CFU > 10^6와 10^5)를 가지는 colony는 발견되지 않았다. 이와 달리, 두 집단 모두(60 + 20)에서 타액의 pH와 타액의 완충력으로부터 얻어진 결과는 상호연관이 있었다. 치아우식집단에서, 타액의 pH 결과치는 약산성(5.90 - 6.50)이었고, 타액의 완충력 역시 매우 낮았다(0.7 ml 이하). 한편, 대조표본 집단에서, 타액의 pH는 중성(pH 7.01 - 7.7)이었고 타액 완충력은 높았다(1 ml 이상). CRT-bacteria 시험법으로부터 얻은 타액 1 ml내(10^5 CFU 이상)의 *S. mutans*와 *Lactobacillus* spp.의 증가된 수는 타액의 산성과 약산성 pH의 결과처럼 치아우식위험을 증가시켰다. 따라서, 타액 pH 법과 완충력 측정법은 치아우식 위험을 예측 할 수 있으며, 치아우식 예방을 위해 유용한 측정법으로 사용될 수 있을 것으로 사료된다.

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(Received August 17, 2006; Accepted September 18, 2006)