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Association between decrease in salivary iron levels and caries experience in children

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ABSTRACT

OBJECTIVE: Human saliva has numerous components that are important for maintaining oral health. We investigated the association between iron levels in saliva in children and their caries experience.

METHODS: We conducted a case-control study that included 186 healthy children, aged 11-14 years. The decayed-filled teeth/decayed-missing-filled teeth (DMFT) score was estimated according to the Brazilian Oral Health Project. The children were divided into two groups: caries-free group (DMFT=0, n=55) and caries experience group (DMFT≥1, n=131). Saliva was collected from each child using the spitting method and analyzed for stimulated saliva flow (SSF), pH, and iron levels. The SSF, pH, and salivary iron levels were analyzed for statistical assumptions of normality and homoscedasticity and compared between groups. All of the statistical tests were performed using R and SPSS software, with a level of significance of 0.05.

RESULTS: No statistically significant differences in mean pH were found between groups (t=-0.0488, p=0.51). Children with caries experience had significantly lower salivary iron levels than caries-free children (w=5088, p<0.0001). The SSF values were significantly different between children with caries experience and children without caries (w=4198, p=0.03).

CONCLUSION: Salivary iron levels were significantly lower in children with dental caries experience, suggesting that salivary iron plays a role in maintaining oral health.

Key words: Saliva; Salivary iron; Caries

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Associação entre a diminuição do nível de ferro salivar e a experiência de cárie em crianças

RESUMO

OBJETIVO: Investigar a associação entre os níveis de ferro na saliva das crianças e sua experiência de cárie.

MÉTODOS: Realizamos um estudo caso-controle envolvendo 186 crianças saudáveis de 11 a 14 anos. O número de dentes cariados, perdidos e obturados (CPO-D) foi estimado de acordo com o Projeto Saúde Bucal Brasil (SBBrasil) e as crianças foram divididas em dois grupos: grupo sem cárie (CPO-D=0; n=55) e experiência Grupo de cárie (CPO-D≥1; n=131). A saliva foi coletada de cada indivíduo usando o método de cuspir e analisada quanto ao fluxo estimado de saliva (SSF), pH e concentração de ferro. As variáveis SSF, pH e ferro salivar foram analisadas pelos pressupostos estatísticos de normalidade e homocedasticidade e comparados entre os grupos. Todos os testes foram realizados utilizando os programas estatísticos R (R Core Team, 2016) e SPSS (IBM, EUA), assumindo um nível de significância de 0,05.

RESULTADOS: Não houve diferenças estatisticamente significativas entre os meios de pH entre os grupos (t=-0,0488; p=0,51). Em relação ao ferro salivar, as crianças com experiência em cárie apresentaram valores significativamente mais baixos de ferro salivar do que aqueles que não apresentavam caries (w=5088; p<0,0001). Além disso, os valores de SSF foram diferentes entre as crianças com experiência de cárie e aqueles sem cárie (w=4198; p=0,03).

CONCLUSÃO: os níveis de ferro salivar foram estatisticamente inferiores em crianças com cárie dentária e presumimos que a jogada de ferro salivar desempenha um papel importante na manutenção da saúde bucal.

Palavras-chave: Saliva; Ferro salivar; Cáries

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INTRODUCTION

Human saliva moistens all oral surfaces and is critical for maintaining the integrity of mineralized tissues and the oral mucosa. It is a biological fluid that plays an important role in maintaining oral health. Saliva has both organic and inorganic components that perform various functions, such as maintaining oral pH balance and exerting antifungal, antiviral, and antibacterial actions [1]. One advantage of using saliva as a diagnostic tool is the diversity of its components and functions, in addition to the non-invasive manner of obtaining samples [2]. Additionally, saliva is well-known to be associated with blood metabolite levels [3] and thus can be useful for diagnosing and monitoring systemic diseases [4].

Organic salivary proteins are known to play an important protective role against periodontal disease and dental caries [5]. Inorganic components, such as calcium, chloride, bicarbonate, and phosphate, are also important for enamel and dentin remineralization [6], but the protective mechanism of other ions, such as iron, has not yet been elucidated.

Among the metals that are present in saliva, iron plays an important role. It is one of the most abundant transition metal ions in saliva [7] and a cofactor of lactoferrin, an important protein with antibacterial activity [8] that is able to strongly bind two iron atoms and salivary bicarbonate, thus conferring strong bacteriostatic activity to the protein [9].

The present study investigated the association of salivary iron levels between children with and without caries experience.

METHODS

Study design

This was a case-control study that was conducted with healthy children, aged 11-14 years, who were randomly selected from 18 different schools from Curitiba, Brazil, between August 2015 and November 2016. Considering a confidence level of 95%, power of 80%, and sample error of 5%, the required number of controls/cases was estimated to be 140 children. A total of 186 children were examined and divided into two groups: caries-free group (decayed-filled teeth/decayed-missing-filled teeth [DMFT]=0, n=55) and caries experience group (DMFT≥1, n=131). The DMFT score was estimated according to the Brazilian Oral Health Project [10]. The exclusion criteria were individuals with a diagnosis of systemic disorders (e.g., anemia or other blood diseases), malnutrition, any other conditions that can influence plasma iron concentrations, and the use of medications (e.g., antidepressants, muscle relaxants, and nonsteroidal or antiinflammatory drugs).

Saliva collection

Saliva was collected from each child using the spitting method. They were instructed not to eat, drink, or perform oral hygiene procedures for at least 1 h prior to saliva collection. Stimulated saliva samples were collected by asking the children to chew on a paraffin blade for 5 min. The children were instructed to gently tilt the head forward and not talk or swallow the saliva that was present in the mouth. Every minute, the children spat saliva into an 80 ml graduated collection flask. Stimulated saliva flow (SSF) was determined by the gravimetric method [11] and is expressed as ml/min. Salivary pH was measured with a portable meter (DM 23, Digimed Analytical Instrumentation, Pembroke Pines, FL, USA). To determine salivary iron levels, we used the Labtest Diagnostic® colorimetric test. A total of 250 µl of the saliva sample was added to 1000 µl of the colorimetric reagent kit. In acidic medium, iron that binds to salivary proteins dissociates and is reduced to a ferrous ion form through the action of hydroxylamine. After adding ferrozine, a bright complex was formed. The absorbance of this complex was measured at 560 nm by a spectrophotometer. All of the tests were performed in triplicate.

Statistical analysis

To compare caries experience according to the salivary parameters SSF, pH, and iron levels, the sample was dichotomized into DMFT=0 and DMFT≥1. The variables SSF, pH, and salivary iron levels were initially analyzed with regard to assumptions of normality and homoscedasticity using the Shapiro-Wilk test and F-test. SSF values were statistically compared between groups using the Welch t-test for independent samples. The pH values and salivary iron levels were compared between groups using the Mann-Whitney nonparametric test. All of the statistical tests were performed using R software (R Core Team, 2016) and SPSS software (Statistical Package for the Social Sciences, Chicago, IL, USA). Values of $p<0.05$ were considered statistically significant.

The study protocol was approved by the local Ethics Committee and adhered to the guidelines of the Declaration of Helsinki. Individuals with adverse oral or systemic manifestations and intraoral prostheses were excluded from the study. Informed consent for participation was obtained from the parents or legal guardians of the participants.

RESULTS

A total of 186 children were included in the study, of which 112 (60.2%) were girls and 74 (39.8%) were boys. Fifty-five children were caries-free, and 131 had caries experience. The characteristics of the sample are shown in **Table 1**. The comparison of salivary pH between children with DMFT≥1 and children with DMFT=0 indicated no statistically significant differences between groups (t=-0.0488, p=0.51; **Table 2**, **Figure 1a**).

Statistically significant differences in salivary iron levels were found between children with DMFT≥1 and children with DMFT=0 (w=5088, p<0.0001; **Table 2**). Children with caries experience had significantly lower salivary iron levels than those without caries (**Figure 1b**). In addition to differences in salivary iron levels relative to caries experience, salivary iron levels also presented a significant negative correlation with DMFT values (r_s=-0.251, p=0.01), and 25% of the variation in the number of teeth that were affected by caries was explained by the variation in salivary iron levels. A statistically significant difference in SSF values was found between children with caries experience and children without caries experience (w=4198, p=0.03). These results indicate that SSF in children with DMFT≥1 was lower than in children with DMFT=0 (**Figure 1c**), although no correlation with the decrease in salivary iron levels was found.

Table 1. Characteristics of the study sample (n=186) recruited from Curitiba, PR, Brazil, 2016.

Characteristic	Mean (SD)	Minimum	Maximum
Age (years)	12 (1.89)	11	14
DMFT	3.16 (0.23)	0	13
Salivary pH	7.73 (0.35)	6.15	8.90
Salivary Iron (µg/dl)	89.76 (3.39)	6.68	241.37
SSF (ml/min)	1.09 (0.38)	0.20	2.90

Table 2. Mean salivary pH, salivary iron level, and stimulated saliva flow.

Salivary parameter	Group		p
	DMFT=0 Mean (SD)	DMFT≥1 Mean (SD)	
pH	7.63 (0.08)	7.73 (0.03)	0.51
Iron (µg/dl)	113.22 (6.82)	79.91 (3.55)	0.0001
Stimulated saliva flow	1.16 (0.06)	1.06 (0.04)	0.03

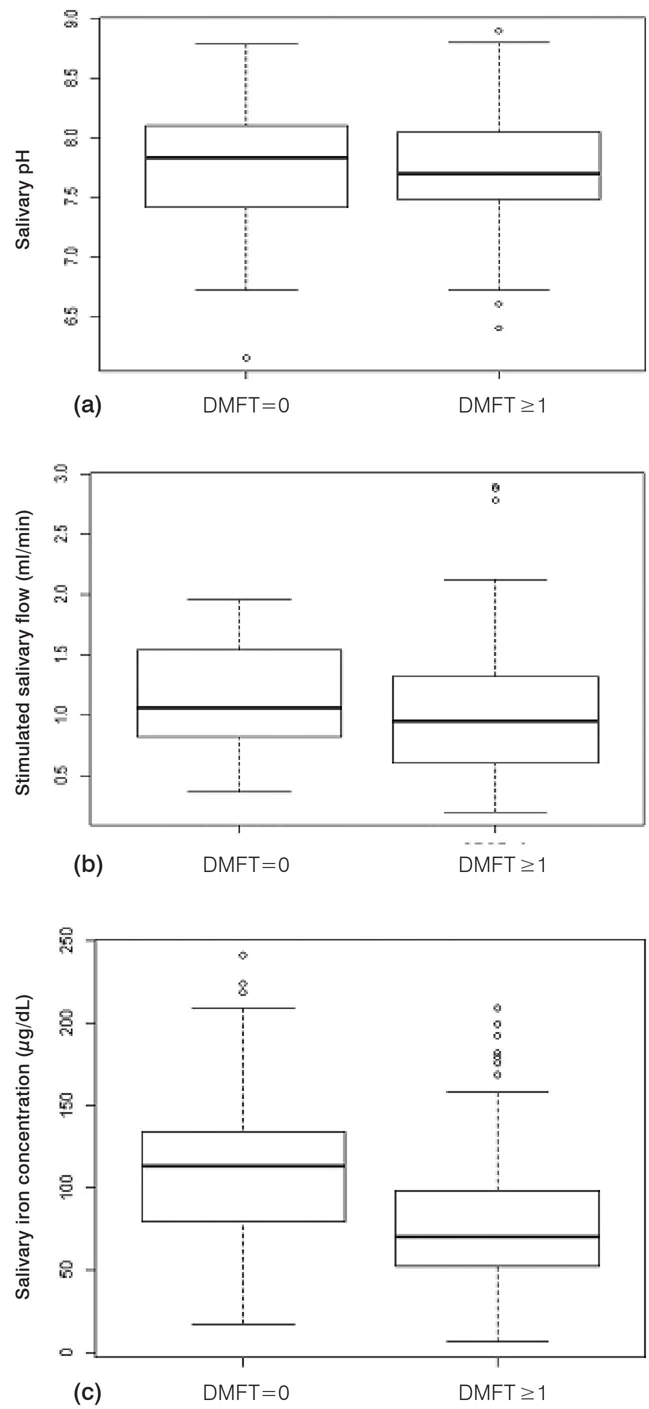

Figure 1. **(a)** Box-plot of salivary pH, **(b)** mean stimulated salivary flow), and **(c)** salivary iron level (µg/dl). Black lines = median. Boxes = 1st and 3rd quartiles. Whiskers = 10th and 90th percentiles. Circles = outliers.

DISCUSSION

The present study found that the decrease in salivary iron levels was associated with caries experience in children. Our previous pilot study was published [12], the results of which were confirmed in the present study. The number of participants increased from 92 in the pilot study to 186 participants in the present study (112 girls [60.2%] and 74 boys [39.8%]) which increased the statistical power of the analyses. Additionally, 55 children were caries-free (DMFT=0), and 131 had caries experience (DMFT≥1). The present findings suggest that salivary iron levels are lower in children with caries experience (w=5088, p<0.0001).

Salivary biomarkers are a promising topic for research. Salivary biomarkers are used for screening purposes in epidemiological studies [13] and are being used to monitor and detect various diseases, such as breast cancer [14], lung cancer [15], celiac disease [16], and chronic renal failure [11]. With regard to oral health, three important salivary variables should be investigated: pH, flow velocity, and buffering capacity. Of these, two were evaluated in the present study: pH and SSF. Surprisingly, salivary pH in both groups was higher than 7.0, with no significant difference between groups (t=-0.0488, p=0.51). To our knowledge, saliva has a lower protective effect when its pH is less than 5.5. However, pH values were higher in all of the children who were examined in the present study. Moreover, a protective effect on the structure of tooth minerals was observed in both groups.

When salivary flow is stimulated, there is a consequent increase in several salivary components, such as proteins, calcium, phosphate, and iron. In the present study, a significant difference in SSF was found between children with and without caries experience (w=4198, p=0.03). These results indicate that SSF in the group of children with DMFT≥1 was lower than in children with DMFT=0, thus confirming previous reports that adequate salivary flow is responsible for protecting mineralized tissues in the mouth [17]. However, no correlation was found between the reduction of SSF and the decrease in salivary iron levels.

The variation in salivary iron levels may be associated with gingival bleeding that is caused by gingivitis and dietary iron deficiency. The former possibility can be ruled out because none of the children had gingivitis. Thus, we assumed that the origin of iron was only glandular. Alterations in dietary iron intake and general iron turnover may also be reflected in salivary iron levels. Systemically, iron deficiency causes anemia. Iron deficiency typically results from the inadequate intake of iron, which is the most prevalent nutritional deficiency worldwide [18]. In the present study, however, we did not assess the influence of diet on salivary iron.

The importance of iron for maintaining bacterial metabolism has been discussed in several previous studies [19,20]. Iron is a trace element that is required for numerous cellular metabolic functions. In saliva, iron is a cofactor of lactoferrin, an important salivary protein with antibacterial activity that strongly binds to two iron atoms and salivary bicarbonate, thus limiting the presence of iron in bacterial cells and making saliva strongly bacteriostatic [8,9,21]. When associated with histatin, it also has antifungal activity [22].

A previous study showed that salivary iron directly influences the oral microbial population. When there is a low availability of iron, certain bacterial species can achieve an ecological advantage [23]. Interestingly, *Streptococci* are bacteria that can grow under limited iron conditions [24], which may explain the caries experience in children with reduced salivary iron levels. Another hypothesis is that the lower availability of salivary iron does not inhibit the activity of the glycosyltransferase enzyme, which is responsible for producing insoluble extracellular polysaccharides that in turn confer an ecological advantage to cariogenic bacteria [25]. Finally, one issue is whether it is possible to predict anemia using saliva samples. Serum iron is used to diagnose and monitor anemia. Thus, future studies should investigate whether saliva can be used to monitor anemia that is caused by iron deficiency.

CONCLUSION

We found that salivary iron levels were significantly lower in children with dental caries experience, suggesting that salivary iron plays an important role in maintaining oral health. More studies are needed to determine the exact functions of salivary iron.

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