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IgA antibodies against *Streptococcus mutans* in breast milk and saliva: Passive Immunity against caries

Anticorpos IgA contra Streptococcus mutans no leite materno e na saliva: Imunidade passiva contra cáries

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ABSTRACT

Objective: The present work aimed to correlate the levels of IgA antibodies reactive with Streptococcus mutans antigens in the saliva and/or in the breast milk and the oral health of lactating. Material and Methods: Breast milk and whole saliva samples were collected from 29 lactating. The oral health was verified using Decayed, Missing, Filled (DMF) scores and the volunteers were separated in three groups: 1) low DMF score; 2) high DMF score with active caries and 3) high DMF score without active caries. The IgA antibodies anti-Streptococcus mutans were analyzed in the samples using ELISA technique. Results: The results showed similar levels of IgA antibodies in all groups, both in milk and saliva. No correlation could be confirmed between the levels of IgA in the saliva and in the breast milk with the oral health of lactating studied. Conclusion: The results suggest that, independently of mother's oral health, the newborn receive the same amounts of anti-Streptococcus mutans IgA by breastfeeding.

KEYWORDS

Breast milk; Immunoglobulin A; Saliva; *Streptococcus mutans*.

RESUMO

Objetivo: O presente trabalho objetivou correlacionar os níveis de anticorpos IgA reativos com antígenos de Streptococcus mutans na saliva e / ou no leite materno com a saúde bucal de mulheres em lactação. Material e Métodos: Amostras de leite materno e saliva total foram coletadas de 29 lactantes. A saúde bucal foi analisada utilizando os índices de CPO e os voluntários foram separados em três grupos: 1) baixo escore de CPO; 2) alto escore de CPO com cárie ativa e 3) alto escore de CPO sem cárie ativa. Os anticorpos IgA anti-Streptococcus mutans foram analisados pela técnica de ELISA. Resultados: Os resultados mostraram níveis semelhantes de anticorpos IgA em todos os grupos, tanto no leite como na saliva. Nenhuma correlação pôde ser confirmada entre os níveis de IgA na saliva e no leite materno com a saúde bucal das mulheres estudadas. Conclusão: Os resultados sugerem que, independentemente da saúde bucal da mãe, o recém-nascido recebe as mesmas quantidades de IgA anti-Streptococcus mutans pela amamentação.

PALAVRAS-CHAVE

IgA; Streptococcus mutans; Saliva; Leite materno.

INTRODUCTION

S treptococcus mutans is considered the main cariogenic microorganism of oral cavity. Its abilities to adhere to teeth surface and to produce high concentration of acids in the presence of sucrose are consistent attributes

associated with cariogenicity [1].

There are different patterns of response against S. *mutans* antigens in children and adults, which can influence the susceptibility to infection and caries development [2-4].

Immunization with different S. *mutans* antigens can induce high levels of IgG in the serum and IgA antibodies in the saliva, tears, colostrums and breast milk, promoting the reduction in counts and adherence of S. *mutans*, and the protection against dental caries [5-8]. The World Oral Health reported that the breast milk could prevent the occurrence of rampant caries in the early childhood [9].

Al Amoudi et al. [10] observed that children with early childhood caries and their mothers had higher levels of sIgA when compared with caries free children and their mothers, showing a positive correlation between secretory IgA of mothers and children.

Since newborn immune system is immature and maternal IgG and IgA, acquired during gestation and breastfeeding, respectively, can help in defense against pathogens, it is important to know the specificity of these antibodies and if the oral health could influence their levels. The present study compared the IgA anti-S. *mutans* levels on the breast milk and on the saliva of lactating women with different oral health.

MATERIAL AND METHODS

Studied population and Clinical analyzes

The study included lactating females who had delivered the day before sample collection. They were informed about the objectives of the present study and signed a free and clarified consent term. This study followed the Resolution MS $n^{o}196/1996$ and was approved by Local Research Ethical Committee (n^{o} 70/99-PH/CEP).

An oral examination was performed by a unique dentist. The clinician analyzed the DMF scores. The patients were divided in three groups: with good oral health – low DMF scores (group 1), with high DMF scores with active caries (group 2) and with high DMF scores without active caries (group 3). The DMF scores between 0 and 3 were considered low and those over 10 were considered high DMF scores. The patients presenting scores between 4 and 10 were excluded of the study.

Sample collection

Breast milk and whole saliva samples were collected from each woman in sterile disposable collectors. The saliva samples were collected without stimulation. The samples were added by proteases inhibitor and conservator and were kept in -20 °C until analyzes.

IgA analyzes

The levels of anti-*Streptococcus mutans* IgA were analyzed using the ELISA technique. The sensitization of ELISA plates was performed using surface antigens of *Streptococcus mutans* ATCC 1910, prepared as described by Leão and Unterkircher [11]. The plates were then incubated with diluted saliva or breast milk (1: 5, 10, 20 and 40), followed by an anti-human IgA labeled with horseradish peroxidase (Sigma, St. Louis, MO). The reaction was developed with orthophenylenediamine (Sigma, St. Louis, MO) and H_2O_2 as substrate. Absorbance was measured at 450 nm.

Statistical analyzes

The optical densities media of each dilutions of each group were compared using ANOVA (Biostat 2.0 program), considering p value < 0.05.

RESULTS

At the end of collections, 29 patients were considered in the study, 10 patients of the group 1, 10 patients of the group 2 and 9 patients of the group 3. The DMF means were 2.4, in the group with good oral health (group 1), and 12.3 and 16.2, in groups with high DMF scores with or without active caries (group 2 and 3), respectively. The results showed similar IgA levels in saliva samples of all analyzed groups (p < 0.05), in all the tested dilutions 1:5, 1:10,1:20 and 1:40 (Figure 1). No correlation could be confirmed between IgA levels in saliva and that in breast milk (p < 0.05). There were also no statistical differences between IgA levels on the breast milk of studied groups (p < 0.05), in all tested dilutions 1:5, 1:10,1:20 and 1:40 (Figure 2).

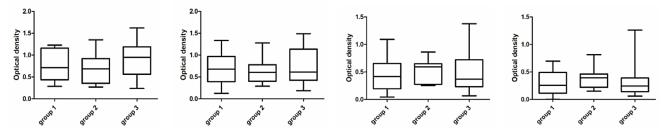


Figure 1 - Levels of anti-S. *mutans* IgA in the saliva of group 1 (low DMF), group 2 (high DMF with active caries) and group 3 (high DMF without active caries). Dilution 1:5, 1:10, 1:20 and 1:40, in the sequence; p values = 0.466, 0.782, 0.879, 0.737, respectively.

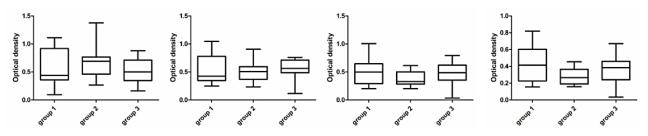


Figure 2 - Levels of anti-S. *mutans* IgA in the breast milk of group 1 (low DMF), group 2 (high DMF with active caries) and group 3 (high DMF without active caries). Dilution 1:5, 1:10, 1:20 and 1:40, in the sequence; p values © 0.405, 0.887, 0.360, 0.180, respectively.

DISCUSSION

IgA against S. *mutans* seems to interfere on the adherence of these bacteria and then protect against dental caries [2]. However, controversial results regarding the correlation of caries, *Streptococcus mutans* and anti-S. *mutans* IgA levels have been reported [5,14-16].

Cogulu et al. [14] observed that patients with Down syndrome had a significantly lower prevalence of caries and significantly higher levels of total salivary sIgA. The authors suggested the hypothesis that higher levels of salivary sIgA could protect against dental caries. Hashizume et al. [15] also observed higher salivary sIgA concentrations in Down syndrome, but this finding did not correlate with caries experience in the study population. However, Yang et al. [16] found significantly higher levels of salivary total sIgA in children with severe early childhood caries and in the elderly subjects with root caries. Ranadheer et al. [5] also observed that s-IgA levels were significantly higher in caries-active group compared with free caries group, suggesting an increase in the

protection mechanism against *Streptococcus mutans* after dental caries.

No significant correlation between caries and anti-*S. mutans* IgA was found in the present work, since individuals with low and high DMF scores, with or without active caries presented similar levels of these specific IgA. These conflicting reports in the literature [5,14-16] regarding caries and anti-*S. mutans* IgA may be attributed to different measurement methods, studied population, time and quality of sample collection, and others.

Newborn infants are known to have a higher frequency of microbial infections when compared with older children and adults, due to immaturity of their immune system [17]. So, passive transfer of IgG and IgA antibodies by placental transfer and breastfeeding is important in protecting newborns against infections [18].

Concentration of IgA in colostrum drops rapidly after birth and varies significantly according to different kinds of exposures [19]. In the present work, although the time of collection was the same for each one (on the second day after birth), the heterogeneity of the patients, with their different profiles of exposure, may have interfered in the results. This can be considered a limitation of the present work. This way, new studies, with more patients and with fewer bias, are necessary to confirm our results.

Streptococci represent the majority of bacteria that initially colonize the oral cavity and the early responses to virulence-associated antigens of *S. mutans* may influence this ability [17]. Although, the present work did not find differences in the levels of IgA anti-*S. mutans* in the breast milk of studied females, these antibodies were present, and would be certainly transferred to the newborn during breastfeeding.

The role of IgA response in protection against dental caries has been still discussed, however, according to our findings, the newborn receives anti-*S. mutans* IgA by breastfeeding, independently of mother's oral health.

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