**IgA antibodies against *Streptococcus mutans* in breast milk and saliva**

**Short title: Passive Immunity against caries**

**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 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.

Key words: Breast milk; Immunoglobulin A ; Saliva; *Streptococcus mutans*.

**Introduction**

*Streptococcus 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**

The study included 29 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 nº196/1996 and was approved by Research Ethical Committee of Univ. Estadual Paulista (nº 70/99-PH/CEP).

**Clinical analyzes**

An oral examination was performed by a unique dentist. The clinician analyzed the carious, restored and lost teeth (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.

**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 -20ºC until analyzes.

**IgA analyzes**

The levels of anti-*Streptococcus mutans* IgA were analyzed using the ELISA technique. 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 H2O2 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**

 The DMF means were 2, 4, in a 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 (Fig 1). No correlation could be confirmed between IgA levels in saliva and that in breast milk. There were no statistical differences between IgA levels on the breast milk of studied groups (Fig 2).

**Discussion**

The mucosal immune system represents the first line of defense to mucosal infection. Immunoglobulins sIgA prevents the binding of a pathogen to these surfaces and high levels of sIgA are associated with lower symptoms of illness [12-13].

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.

Cogulu et al.[14] observed that patients with Down's syndrome had a significantly lower prevalence of caries and significantly higher levels of total salivary sIgA [15]. The authors suggested the hypothesis that higher levels of salivary sIgA could protect against dental caries. However, Yang et al. found significantly higher levels of salivary total sIgA in children with severe early childhood caries and in the elderly subjects with root caries [16]. 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. The same was already found by other authors. These conflicting reports in the literature 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 term 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 different conditions presented in each patient could not be excluded, the time of collection was the same for each one (on the second day after birth), standardizing the possible variations regarding the time of collection.

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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**Conflict of interest:** none.

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