The objective of this study was initially to evaluate the concentration of IgA in salivary samples (taken from 135 children 5-13 years old) by single radial immunodiffusion, and to detect possible correlation between salivary levels of IgA and several parameters (age, gingival and plaque index). No correlation, however, could be found between salivary IgA concentration and any of the parameters analyzed. The same occurred when IgA concentration and number of bleeding gingival areas were analyzed to test the influence (on IgA salivary levels) of the points of major severity in gingival inflammation. It was observed, however, that the extension of the inflamed gingiva was more important, when referring to IgA secretion, than its greatest severity in scattered points of the gingiva. This showed that a new index was necessary to establish a correlation between the concentration of salivary IgA and gingival inflammation.

Key Words: IgA, saliva, gingival inflammation, children.

Introduction

Periodontal disease is currently considered to be an infectious process primarily mediated by dental plaque. This infectious process may take multiple forms according to its specific etiology, which in turn involves a limited number of bacterial species such as Haemophilus (Actinobacillus) actinomycetemcomitans (Zambon, 1985), Bacteroides (Porphyromonas) gingivalis (SLOTS et al., 1985; LOESCHE et al., 1985), Bacteroides (Prevotella) forsythus, Wolinella recta (DZINK et al., 1985), and spirochetes (LOESCHE et al., 1985), as proposed by Bretz and Loesche (1987). Periodontal disease is also assumed to be associated with immunological reactions against the action of bacterial plaque (LEHNER et al., 1967).

Several investigators have attempted to correlate the periodontal index with immunoglobulin levels. No correlation was detected by some, at least with respect to IgA levels in saliva (Shillitoe and Lehner, 1972; CHANDLER et al., 1974; SINGI et al., 1982), whereas others detected a positive correlation between periodontal indices and salivary IgA levels (LINDSTROM and FOLKE, 1973; ØRSTAVIK and BRANDTZÆG, 1975, among others).
Most of the studies carried out thus far have investigated the behavior of immunoglobulin A in relation to periodontal disease, using adults as research subjects. However, one may ask what might be occurring among children, how their immunological system may be acting in the presence of gingival inflammation since periodontal disease is assumed to start in childhood in the form of gingivitis, reaching a peak close to puberty and then progressing to the typical and overt form of periodontitis in adults. Thus, it would be of interest to obtain a better understanding of the immunologic defense mechanism of gingiva during childhood, so as to have the possibility of arresting periodontal disease in its milder form, i.e., gingivitis, perhaps simply by monitoring the immunologic defenses of the organism itself. The great advantage of this approach would be a much longer survival of teeth, with a consequent significant improvement of the general living conditions of the population both in terms of physical and psychological health.

Considering the advantages that might be obtained by this monitoring of organic defenses in terms of the prevention of periodontal disease, and the many doubts still existing about the mechanism of immunologic defense against periodontal disease, the objective of the present investigation was to study the behavior of IgA during the phase of apparent onset of the problem (among 5- to 13-year old children) in order to determine IgA levels in saliva and the possible existence of a correlation between IgA levels and gingival index.

Material and Methods

Collection of material

The study was conducted on 135 healthy children aged 5 to 13 years. Total, non-stimulated saliva samples were obtained from each subject and gingival and plaque indices were calculated.

Saliva samples were collected into 20 x 150 mm tubes, immediately transferred aseptically to sterilized 12 x 75 mm tubes and frozen on dry ice and alcohol. The samples were stored in styrofoam boxes containing dry ice and carried to a freezer where they were left until the time for immunoglobulin measurement.

Gingival and plaque indices

Gingival and plaque indices were obtained before saliva collection according to the method of Løe (1967).

Single radial immunodiffusion

The samples stored in the freezer at -20°C were thawed in a refrigerator for 18 hours, a procedure that, according to Sigman et al. (1989), does not cause losses of IgA. The samples were then centrifuged at 10,400 g (8,000 rpm) for 15 minutes.
IgA levels in saliva were measured by single radial immunodiffusion as described by Mancini et al. (1965) using LC-Partigen radial immunodiffusion plates (Behring). Protein-Standard-Serum Human LC-V, OFTO 3218, lot no. 021818F (Behringwerke AG, Marburg, Germany) was used as standard at 1:2, 1:4 and 1:8 dilutions. Saliva samples and diluted standard serum were applied with an automatic 20-μl micropipette (Lio Serum, Ribeirão Preto, Brazil) and the plates were maintained at room temperature for 15 to 30 minutes for pre-diffusion. After this time, the plates were kept at 23°C in a Fanem BOD incubator model 347 for 48 hours.

The diameters of the precipitation haloes were read by the same observer with the unaided eye and with the aid of a millimeter ruler (Behring) with 0.5-mm precision.

**Results and Discussion**

IgA concentration was measured in 135 saliva samples from children aged 5 to 13 years and the distribution of IgA concentrations by age range is presented in Table 1.

The possible existence of a correlation between the IgA concentrations detected in the saliva samples and the plaque and gingival indices was determined. The possible correlation between IgA levels and number of bleeding gingival sites and age was also determined. Statistical analysis, however, showed no correlation between these parameters.

**Plaque index (PI) and gingival index (GI)**

The lack of correlation between IgA levels and PI and GI [p(r = 0.1091 and p(r = 0.0271), respectively] did not seem to be logical to us since bacterial plaque is known to be able to cause gingival inflammation and the inflammatory conditions of gingiva are intimately linked to the production and secretion of antibodies in saliva.

However, considering how the gingival index proposed by Loe (1967) is calculated, it did not seem rational to calculate something comparable to it in order to determine a "mean inflammation level", a procedure that would distort reality since healthy
sites do not improve or reduce the severity of affected sites. However, in the calculation of gingival index as recommended by Löe (1967), the gingival surfaces with a zero score, i.e., uninflamed surfaces, are also taken into consideration, causing an artificial reduction of the index of gingival inflammation of an individual and preventing a rational correlation between gingival inflammation and the concentrations of secreted immunoglobulins, since healthy gingiva does not contribute to a stimulation of the production of these antibodies.

This ratio is only the prerogative of inflamed regions of the gingiva in which the source of triggering antigens is directly or indirectly located.

To illustrate the above statement, let us imagine a hypothetical individual with severely inflamed left hemiarches (a score of 3), but with healthy right hemiarches (zero score). By the method of Löe, the gingival index of this individual would be 1.5, an index that would therefore be interpreted as mild inflammation reflecting in no way the real clinical picture of this individual, since pathological pictures are usually interpreted more on the basis of the aspect of their points of highest severity than on the basis of a mean of the general picture. These considerations lead to the natural conclusion that, in an attempt to correlate the gingival inflammation present with the level of immunoglobulin in saliva, this level should be solely correlated with the gingival areas that are actually inflamed, while healthy regions should not be considered.

A new concept thus started to take shape along a series of logically linked reasonings that finally led to the suggestion of new indices that would be more appropriate for the interpretation of the possible, and even probable, relationship between IgA levels and gingival inflammation and would permit a more convenient study and understanding of this relationship.

**Number of bleeding gingival sites**

Since the production and/or secretion of immunoglobulins is intimately related to the inflammatory defense process, it was natural to continue to think that, despite the results of the correlation tests, the solution of the problem was somehow linked to gingival inflammation. Thus the first question naturally coming to mind was whether this concentration of immunoglobulin A depended on the severity or local intensity of the gingival inflammatory reaction.

However, once again the same lack of correlation was detected when an attempt was made to correlate IgA levels with number of bleeding gingival sites. This attempt was made because bleeding gingival sites reflect an intensification of inflammation, although it should be kept in mind that in the present study only sites with scores of 2 (bleeding upon stimulation) were counted as bleeding gingival sites, since no case of a gingival index of 3 (spontaneous bleeding) was detected.

Thus, in view of the lack of correlation detected \( r = 0.0836 \), we realized that neither the determination of the conventional gingival index nor the count of bleeding gingival sites represented adequate parameters for the type of experiment carried out even
though we were studying immunoglobulins, i.e., something that is directly related to the levels of oral defense against gingival inflammation.

It was at this point that new concepts were developed, resulting in a new index - immunologic gingival defense index (IGDI) - that permits monitoring the immunologic defenses of the gingiva with respect to IgA as early as during childhood. This index will be presented and demonstrated in the second part of this study.

Conclusions

The present results permitted us to reach the following conclusions:
1. IgA levels in saliva are not correlated with any of the standard indices used (gingival index or plaque index).
2. The severity of gingival inflammation does not seem to affect much the level of IgA in saliva, since the IgA level measured was not significantly correlated with number of bleeding gingival sites.
3. The concentration of IgA in saliva does not change significantly along the age range studied (5 to 13 years).

References


Löe H: The gingival index, the plaque index and the retention index systems. J Periodont 38: 610-612, 1967


Singi LM, Silva OP, Gasparini OT, Moraes N, Marques ALV: Quantificação de IgA na saliva total e de paróitida de indivíduos normais e de portadores de periodontite: estudo comparativo. Estomat Cult 12: 95-102, 1982


Correspondence: Maria Cristina Monteiro de Souza-Gugelmin, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, 14049-903 Ribeirão Preto, SP, Brasil.

Accepted October 28, 1993