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Secretion of Salivary Immunoglobulin A in Relation to Age, Saliva Flow, Mood States, Secretion of Albumin, Cortisol, and Catecholamines in Saliva

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Salivary immunoglobulin A (IgA) is one characteristic humoral factor of the local immune system in the upper respiratory tract. Epidemiological studies emphasize the importance of secretory IgA in the protection from infections of the upper respiratory tract. However, due to high interindividual variability of secretion of salivary IgA, it remains difficult to define normal ranges. This series of studies focused on identification of factors influencing basal secretion of salivary IgA. The results indicate a significant relationship between age and salivary IgA concentration. Children below 7 years have lower salivary IgA concentration than children above 7 years or adults. Furthermore, a significant inverse relationship between saliva flow and salivary IgA concentration was found. Gender, mood states, salivary albumin, salivary catecholamines, and salivary cortisol were not associated with salivary IgA. It can be concluded that for defining normal ranges of salivary IgA, age and saliva flow have to be considered.

KEY WORDS: Salivary immunoglobulin A; saliva flow; age; salivary albumin; salivary cortisol; salivary catecholamines; mood states.

INTRODUCTION

Infectious diseases of the upper respiratory tract are one of the most frequent diseases, lead to significant costs in health system, and are relevant causes of death (1, 2). Salivary immunoglobulin A (IgA) secreted by plasma cells in the submucosa is one characteristic humoral factor of the local immune system in the upper respiratory tract. Epidemiological studies emphasize the importance of secretory IgA in the protection from infections of the upper respiratory tract. Yodfat and Silvian (3) showed in a prospective study that children with marked increases in concentration of IgA under viral infections had a lower incidence of infections in the upper respiratory tract. Rossen *et al.* (4) reported that individuals with a high concentration of IgA in nasal secretions developed fewer symptoms after viral inoculation. McClelland *et al.* (5) found that individuals with high concentrations of salivary IgA less often report symptoms of infections in the upper respiratory tract. Hess *et al.* (6) reported that a high incidence of infections in the upper respiratory tract in children with marked adenoid hyperplasia was related to lower salivary IgA levels.

Salivary IgA has often been seen as a functional parameter of the local immune system in the upper respiratory tract (7, 8). However, it has been difficult to define normal ranges for secretion of salivary IgA. Usually, one obtains large interindividual variability even in healthy populations.

In order to find important factors accounting for this variability, we designed a series of studies, focusing on constitutional factors, such as age and gender; mood states, such as depression and feeling stressed; saliva composition, such as saliva flow and saliva albumin; and hormones in saliva, such as cortisol or catecholamines.

MATERIALS AND METHODS

A series of three studies was designed.

Study I: Influence of Age and Salivary Albumin. In order to study age dependency of secretion of

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salivary IgA, 23 children (age 2 to 15 years) were compared with 10 adults (age 29 to 38 years). All participants were on no medication for at least the last 72 hr before entering the protocol and they were not suffering from systemic disease. Beside salivary IgA, salivary albumin was determined by radial immunodiffusion technique (9) using commercially available test plates RID (low level; Behring Co., FRG).

Study II: Influence of Saliva Flow, Mood States, Gender, and Salivary Cortisol. Eighty-four medical students (age 19 to 28 years; 39 male and 45 female) were asked to work on a mood checklist [a short version of the Adjective List (10)] comprised of 15 dimensions: concentration, activation, dreaminess, anxiety, depression, good mood, sensitivity, irritation, excitement, self-reliance, numbness, sociability, tiredness, introversion, and passiveness.

In addition to salivary IgA, saliva flow was determined by weighing the tubes before and after saliva sampling. Salivary cortisol was assessed by radioimmunoassay using an adopted commercially available test kit (Byk Co., FRG) (11, 12).

Study III: Influence of Salivary Catecholamines. Twenty-eight participants (age 18 to 21 years) were asked for a saliva sample. All participants were on no medication for at least the last 72 hr before entering the protocol and they were not suffering from systemic disease. Beside sIgA, salivary catecholamines, including epinephrine and norepinephrine, were determined using high-pressure-liquid chromatography coupled with electrochemical detection (see Refs. 13 and 14).

Whole unstimulated saliva was used in all studies for the determination of salivary IgA. A major source of salivary IgA is minor salivary glands (15), so using parotid secretion might lead to an underestimation of the actual amount of salivary IgA present in the mouth. Saliva was collected in study I by asking participants to put a cotton wool swab into the mouth for 2 min. Pilot studies showed that some commercially available cotton wool swabs bind salivary IgA and albumin, which can bias their determination. In this study, we used a special preparation of purified cotton wool (provided by Emte Co., Hamburg, FRG) which, in pretests, had the best reproducibility compared with standards. The swab was put into a prepared tube (Salivette, Sarstedt Co., FRG), cooled, centrifuged at 1700 rpm for 10 min, and stored at −18°C.

In studies II and III, participants were asked to collect saliva in their mouth for 2 min and to put it into prepared tubes, which were then stored at −18°C.

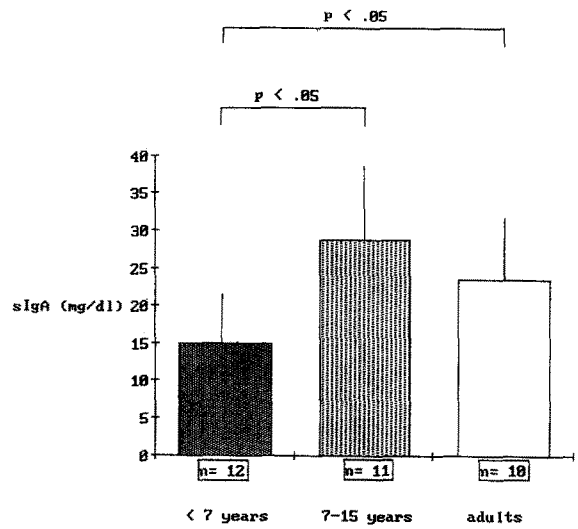

Fig. 1a. Concentration of salivary immunoglobulin A in children below 7 years, in children between 7 and 15 years old, and in adults. Mean and standard deviation are displayed. Analysis of variance showed significant differences across age groups (ANOVA; df = 2,30, $F = 4.695$, $P < 0.05$). P values refer to group comparisons by unpaired t test.

Participants (or, in the case of children, their parents) were advised not to drink or eat for at least 30 min before the appointment.

The concentration of salivary IgA was determined by radial immunodiffusion technique (9), using commercially available test plates (low-level IgA; study I, Behring Co., Mannheim, FRG; studies II and III, Kallestad Co., Freiburg, FRG). Because salivary IgA differs in its physicochemical properties from IgA in serum, a correction factor, introduced by Brandtzaeg *et al.* (16), was used. The concentrations of salivary IgA obtained were multiplied by 3.25.

Saliva samples were inspected for blood contamination prior to further analysis, but no color changes were detected. Saliva samples were obtained in the morning (study I, 9 to 12 AM; studies II and III, 10 AM).

Statistical analysis was done using SYSTAT (17).

RESULTS

The results of study I show significant differences with regard to age. Children below 7 years tend to have lower concentrations of salivary IgA than children older than 7 years or adults (ANOVA, df = 2,30, $F = 4,695$, $P < 0.05$; Fig. 1a). Further analysis

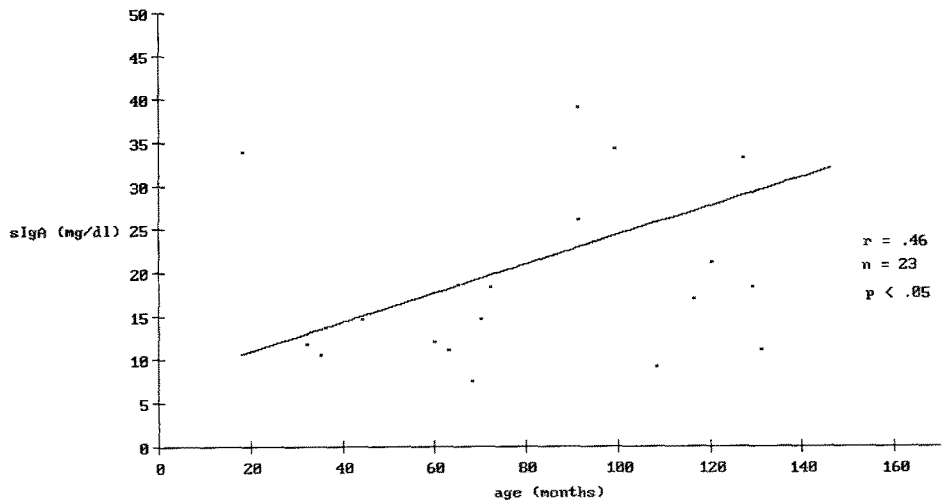

Fig. 1b. Pearson correlation between children's age and concentration of salivary immunoglobulin A.

shows that there is a significant positive linear correlation ($r = 0.49$, df = 21, $P < 0.05$) between children's age and concentration of salivary IgA (Fig. 1b). No significant age differences could be found for salivary albumin and no significant correlation ($r = -0.11$, df = 21, P = ns) between salivary IgA and salivary albumin (Table I).

Study II shows that collecting whole saliva results in a significant negative correlation ($r = -0.27$, df = 82, $P < 0.05$) between saliva flow and concentration of salivary IgA (Fig. 2). Neither significant gender differences nor a consistent correlation pattern between salivary IgA and mood states was found (Table I). Only "excitement" was positively correlated with salivary IgA ($r = 0.26$, df = 82, $P < 0.05$; Table I).

Studies II and III showed no close relationship between salivary cortisol or salivary catecholamines and secretion of salivary IgA (Table I).

Table I. Pearson Correlations between Salivary IgA and Salivary Albumin, Mood States, and Salivary Hormones

	Concentration of salivary IgA (mg/dl)
Study I (n = 33)	
Salivary albumin	n.s.
Study II (n = 84)	
Concentration	n.s.
Excitement	0.26*
Activation	n.s.
Self-reliance	n.s.
Dreaminess	n.s.
Numbness	n.s.
Anxiety	n.s.
Sociability	n.s.
Depression	n.s.
Tiredness	n.s.
Good mood	n.s.
Introversion	n.s.
Sensitivity	n.s.
Passiveness	n.s.
Irritation	n.s.
Salivary cortisol	n.s.
Study III (n = 28)	
Salivary norepinephrine	n.s.
Salivary epinephrine	n.s.

*$P < 0.05$; $n = 84$.

DISCUSSION

It has often been documented that salivary IgA is of importance in protection from infections in the upper respiratory tract. However, because of high interindividual variability, it remains difficult to define norm values, to specify populations at risk, or to prognosticate the course of diseases with recurrent infections, such as recurrent acute otitis media (18, 19).

In this series of studies, we focused on the identification of relevant influences which have to be taken into consideration for the definition of normal ranges. The results indicate a significant relationship between age and salivary IgA concentration. Children below 7 years have lower salivary IgA concentrations than adults, while there is no difference between children above 7 years and adults. Because salivary albumin showed no age dependency, it can be stated that increases in salivary IgA are more likely an effect of maturation of the local immune system rather than due to unspecific changes in the secretion conditions. The results support the view that age-dependent normal

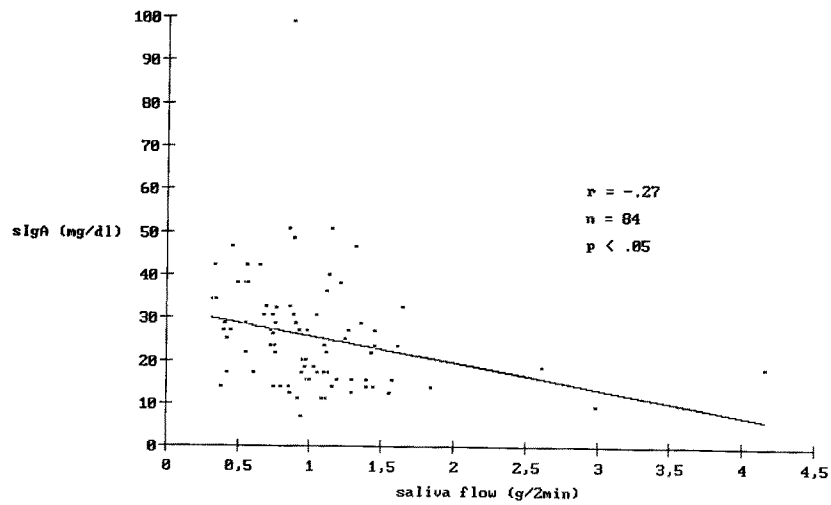

Fig. 2. Pearson correlation between saliva flow and concentration of salivary immunoglobulin A.

values have to be developed (20). Given the epidemiological evidence that children below 7 years have a higher incidence of infections in the upper respiratory tract (1), determination of salivary IgA may be useful to monitor the development of the local immune system.

Furthermore, an inverse relationship between saliva flow and salivary IgA concentration was shown. However, the variance explained by this factor appears to be small (<8%) in unstimulated saliva, when the variation of saliva flow is small. This factor may be of more importance if saliva flow is stimulated, i.e., by gustatory stimuli (16) or paraffin chewing (21).

The relationship between mood and secretion of salivary IgA has received increasing attention (22–25). It has been suggested that secretion of salivary IgA is substantially reduced by negative mood states, such as depression and anxiety. One may conclude that this can invalidate clinical interpretation of measured levels of salivary IgA. On the other hand, most of the psychoneuroimmunological results on salivary IgA were obtained in individuals in long-lasting stress situations, such as prisoners, hospital staff, or students in exam periods. We wondered if mood states in everyday life are associated with basal levels of salivary IgA. In our student sample (n = 84), 14% rated themselves as being somewhat or fairly depressed, and 6% as being somewhat or fairly anxious. Such negative mood states, however, were not associated with secretion of salivary IgA. Moreover, in contrast to our expectations, a significant positive correlation between "excitement" and salivary IgA concentration was found, indicating that excited individuals tend to have even higher salivary IgA concentrations than others. In general, our findings suggest that mood states in everyday life are not related to basal salivary IgA concentration. The picture might change if individuals with severe emotional disorders were studied.

No relationship could be found between salivary IgA and salivary albumin, salivary cortisol, salivary epinephrine, or salivary norepinephrine. This shows that the secretion mode of salivary IgA is different from that of substances which have to be transported from the serum to the saliva (26, 27). Salivary IgA is produced by the local immune system in the upper respiratory tract. Salivary IgA concentrations cannot be extrapolated from blood-derived salivary parameters.

No gender differences could be found. This is in accordance with previous reports (28) indicating that only women shortly before and after giving birth showed increased secretions of salivary IgA.

In conclusion, it can be stated that high intersubject variability of salivary IgA secretion can be explained, to some extent, by age and saliva flow. However, more research must be done to identify additional influential factors in order to define normal ranges.

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