

## Age-Dependent Changes of Salivary IgA and IgE Levels in Healthy Subjects

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### ABSTRACT

**Background:** The secretory immunoglobulin A (IgA) is the first line of defense against pathogens that invade mucosal surfaces. It has been reported that the immune system exhibits profound age-related changes. The aim of this study was to investigate the age-dependent changes of salivary IgA and IgE levels among healthy subjects.

**Methods:** Saliva samples were collected from 203 healthy individuals (aged 1-70 years). The salivary IgA and IgE concentrations were measured by use of ELISA technique and analyzed using the Mann-Whitney U, Kruskal-Wallis and Chi-Square tests.

**Results:** The mean salivary IgA levels were 42.67 µg/ml at age 1-10 years, 82.44 µg/ml at age 11-20 years, 93.5 µg/ml at age 21-30 years, 97.58 µg/ml at age 31-40 years, 106.45 µg/ml at age 41-50 years, 113.47 µg/ml at age 51-60 years and 92.95 µg/ml at age 61-70 years. There was significant difference among mean salivary IgA levels of different age groups ( $P < 0.001$ ). The frequency of subjects with detectable concentrations of salivary IgE increased with increasing age up to 40 years and thereafter decreased. There was also significant difference among the mean salivary IgE levels of different age groups ( $P < 0.001$ ). In adults, the mean salivary levels of IgA and IgE were significantly higher than those observed in children ( $P < 0.0001$  and  $P < 0.002$ , respectively).

**Conclusion:** These results showed that the salivary IgA and IgE levels exhibit age-related changes. Oral immunization may be considered to improve oral immunity when the salivary concentrations of IgA begin to decrease during lifetime.

**Keywords:** Adult, immunoglobulin A, immunoglobulin E, saliva.

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### Introduction

Secretory IgA constitutes the predominant immunoglobulin isotype in secretions, including saliva. It is considered to be the first line of defense of the host against pathogens that colonize or invade mucosal surfaces.<sup>1</sup> Salivary IgA antibodies could help maintain the integrity of the oral surfaces by preventing microbial adherence to epithelial and tooth surfaces, by neutralizing enzymes, toxins and viruses, or by acting in synergy with other antibacterial factors such as lysozyme, lactoferrin, salivary

peroxidase, and mucins.<sup>1,2</sup> Salivary IgA may also prevent the penetration of food antigens in the oral mucosa.<sup>1</sup> Some studies have also demonstrated that a lower incidence of caries resulted from a high salivary IgA concentration.<sup>3,4</sup> In addition, a lower concentration of IgA in saliva has been presented as a risk factor for upper respiratory infection in children and the elderly.<sup>5</sup> Furthermore, lower levels of salivary IgA are associated with increased risk for periodontal disease and caries.<sup>6,7</sup> IgE con-

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centrations have an important role in immunopathogenesis of some allergy and inflammatory reactions. It has been reported that the elevated total serum IgE levels are characteristic of atopic diseases.<sup>8</sup> Several studies indicated that nearly every component of the immune system undergoes dramatic age-associated remodeling, leading to changes that include enhanced as well as diminished functions.<sup>9</sup> However, it has been shown that mucosal immunization is an effective method in increasing the IgA levels in mucosal secretions.<sup>10</sup> Accordingly, it may be possible to induce salivary IgA response via oral immunization, when the salivary IgA concentrations begin to decrease during a lifetime. This strategy could help the maintenance of the salivary IgA levels and improve the oral immunity. To our knowledge, although there are some fragmented studies regarding the association of salivary immunoglobulins and age, however, no papers have been published on the relationship between the long term variation of age and the changes in salivary IgA and IgE levels. This study was conducted to evaluate age-dependent changes of salivary IgA and IgE levels in healthy subjects aged 1-70 years.

## Materials and Methods

We conducted this descriptive study from July 2006 to January 2007 in the Department of Immunology, Rafsanjan University of Medical Sciences and Health Services, Rafsanjan, Iran.

### Subjects

In total, 203 healthy subjects (aged 1-70 years) were enrolled in the study. Subjects with a medical history of recurrent infections, other acute diseases, history of asthma, allergy, atopic diseases, any suspected immunological disorder and chronic illnesses or syndromes were all excluded from the study. Children were recruited from randomly selected kindergartens, schools and health centers of Rafsanjan city. The adults were recruited among students and staff of Rafsanjan University of Medical Sciences. Elderly subjects (> 60 years) were selected from the general population of Rafsanjan city and invited to health centers for medical examinations and collection of saliva. Saliva sampling was performed randomly according to the registration number of participants. The informed consent was obtained from the participant before enrollment in the study. Moreover, this study was

approved by the Ethical Committee of Rafsanjan University of Medical Sciences. The subjects were divided into 7 groups according to their ages (Table 1).

### Collection of the saliva

All saliva samples were collected between 10-11 am to reduce possible circadian interference. Before collecting the saliva, the subjects did not eat or drink for at least 1 h. Unstimulated whole saliva samples were collected at one occasion from the mouth, during a period of 5 min. For saliva collection, subjects were asked to generate saliva in their mouths and to spit into a wide test tube. The saliva samples were placed on ice and all samples were centrifuged for 15 min at 10000 g to remove cells and debris. The supernatants were kept at -70°C until used. Samples were thawed and analyzed by enzyme-linked immunosorbent assays (ELISA).

### Immunoglobulin A quantification in saliva

Measurements of IgA in saliva were performed by sandwich ELISA. In these assays, polystyrene microtitre plates (F96, NUNC, Roskilde, Denmark) were coated overnight at 4°C with 0.2 µg/well of affinity purified rabbit anti-IgA antibodies (Beta, Iran) in 0.05 m NaHCO<sub>3</sub>, PH 9.5. Blocking was performed by use of phosphate buffer containing 0.5% bovine serum albumin (BSA) at RT for 90 min. 100 µl of saliva samples (in duplicate) and standard samples (in duplicate) were pipetted into the microtitre wells. The plates were incubated for 90 min at 37°C. The wells were washed 5 times with washing solution. Then, 100 µl of goat anti-human IgA conjugated with horse radish peroxidase was pipetted into each well, and the plates were incubated for 30 min at 37°C. The wells were washed 5 times with washing solution and tapped dry. A fresh substrate solution, tetramethylbenzidine (100 µl), was added and the plates were incubated for 15 min at room temperature. The enzyme reaction was stopped with 100 µl of 1 N HCl. Results were quantified through a standard curve whose values are expressed as mg/l. The %CV for these ELISA were 3.8%.

### Immunoglobulin E quantification in saliva

Salivary IgE levels were quantified in duplicate by ELISA, using commercial kits (Radim, Italy). Salivary IgE concentration was expressed as IU/ml.

**Table 1.** Age-dependent variations of salivary IgA and IgE levels.

Age group (years)	No.	IgA ( $\mu\text{g/ml}$ ) Mean(SD)	Detectable rate of IgE	IgE (IU/dl) Mean(SD)	P-value (IgA differences)	P-value (IgE differences)
1 (1-10)	28	42.67(38.53)	7(25%)	20.90(18.45)	-----	-----
2 (11-20)	31	82.44(65.48)	12(38.7%)	97.35(169.00)	0.01(vs. group 1)*	0.3(vs. group 1)
3 (21-30)	31	93.5(58.01)	15(48.4%)	101.55(169.21)	0.002(vs. group 1)*	0.2(vs. group 1)
4 (31-40)	32	97.58(54.82)	20(62.5%)	131.27(141.38)	0.001(vs. group 1)*	0.05(vs. group 1)*
5 (41-50)	32	106.45(68.17)	19(59.37%)	116.41(217.37)	0.0001(vs. group 1)*	0.2(vs. group 2)
6 (51-60)	28	113.47(78.78)	12(42.85%)	132.56(237.21)	0.0001(vs. group 1)* 0.05(vs. group 2)*	0.1(vs. group 1)
7 (61-70)	21	92.95(64.26)	8(38.1%)	81.45(94.34)	0.006(vs. group 1)*	0.2(vs. group 1)

\* It is significant at  $\alpha=0.05$ .

### Statistical analyses

Differences in variables were analyzed using the Mann-Whitney U-test, Kruskal-Wallis and Chi-square tests as appropriate and p values less than 0.05 were considered significant.

## Results

### Salivary IgA levels

Table 1 shows the mean salivary IgA levels of different age groups. The mean salivary IgA levels were 42.67  $\mu\text{g/ml}$  at age 1-10 years, 82.44  $\mu\text{g/ml}$  at age 11-20 years, 93.5  $\mu\text{g/ml}$  at age 21-30 years, 97.58  $\mu\text{g/ml}$  at age 31-40 years, 106.45  $\mu\text{g/ml}$  at age 41-50 years, 113.47  $\mu\text{g/ml}$  at age 51-60 years and 92.95  $\mu\text{g/ml}$  at age 61-70 years. Statistical analysis showed significant difference among mean salivary IgA levels of different age groups ( $P < 0.001$ ). These results showed that the mean salivary IgA level increased with increasing age up to 60 years and then decreased. Subjects who were younger than 18 years of age were considered to be children ( $n = 50$ ) and those over the age of 18 years were considered adults ( $n = 153$ ). The mean salivary IgA levels in adults ( $99.84 \mu\text{g/ml} \pm 64.17$ ) was significantly ( $P < 0.00001$ ) higher than that observed in children ( $61.28 \mu\text{g/ml} \pm 58.11$ ).

### Salivary IgE levels

Table 1 also shows the mean salivary IgE levels of different age groups. The positive rates of salivary IgE were 25% at age 1-10 years, 38.7% at age 11-20 years, 48.4% at age 21-30 years, 62.5% at age 31-40 years, 59.3% at age 41-50 years, 42.85% at age 51-60 years and 38.1% at age 61-70 years with a mean titer of 5.22 IU/dl, 37.68 IU/dl, 50.77 IU/dl, 82.04 IU/dl, 69.2 IU/dl, 56.8 IU/dl and 31.02 IU/dl, respectively. There were significant differences among mean salivary IgE levels of different age groups ( $P < 0.001$ ). The positive rates of

salivary IgE were significantly higher in adults compared to children (51.3% vs. 30%,  $P < 0.001$ ). Furthermore, the mean salivary IgE levels in adults ( $58.2 \text{ IU/dl} \pm 146.9$ ) was significantly ( $P < 0.002$ ) higher than that observed in children ( $22.17 \text{ IU/dl} \pm 88.3$ ).

## Discussion

Secretory IgA is considered to be the principal mediator of host defense at mucosal surfaces.<sup>1</sup> We hereby, described the changes of salivary IgA and IgE levels across different ages, from 1 to 70 years. As we observed, the salivary IgA levels increased with increasing age up to 60 years and then decreased. Moreover, the detectable rates and levels of salivary IgE increased from age 1 to 40 years and then decreased. The salivary concentrations of both immunoglobulins were higher in adults compared to children. In some studies, the age-related changes of salivary immunoglobulin concentrations (especially IgA) have been reported. Eliasson et al.<sup>11</sup> investigated the IgA concentrations in secretions of palatal, buccal and labial salivary glands in individuals aged 18-72 years. They have observed that the salivary IgA concentrations in these saliva samples were higher in elderly subjects ( $\geq 65$  year) as compared to subjects aged 18-64 years. Increased whole-saliva IgA concentrations in older ages have been attributed partly to positive age-related effects on IgA concentration in the buccal gland secretion. Weemaes et al.<sup>12</sup> reported that the salivary IgA secretion rate increased during infancy and childhood period at age 1-12 years. Childers et al.<sup>13</sup> determined the concentration of IgA in parotid saliva of healthy children (age 6-12 years,  $n = 14$ ) and healthy adults (age 22-51 years,  $n = 20$ ) and reported that the levels of IgA increased with age. Challacombe et al.<sup>14</sup> showed in healthy adults that the salivary IgA con-

centrations increased with age and reached the maximum levels in the oldest studied group (>80 years). These investigations are nearly consistent with our results. However, genetic and environmental differences and differential oral health may account for some different results obtained in this study and those reported by other investigators. For example, in our study the mean salivary IgA levels decreased after 60 years. Our results regarding decline in salivary IgA concentration after 60 years may be attributed to the increased susceptibility of elderly individuals to oral infectious diseases especially infection with IgA-degrading bacteria.<sup>1</sup> Regarding salivary IgA changes, it has been reported that the stimulus for IgA synthesis at mucosal surfaces appears to be colonization of these surfaces by commensal bacteria-bearing polyclonal mitogens such as lipopolysaccharide and perhaps lipoteichoic acid, since germfree mammals have underdeveloped mucosa-associated lymphoid tissues and lack IgA in their secretions.<sup>15,16</sup> Although IgA is thought to act to exclude extrinsic pathogenic microorganisms, it appears to be without effect on commensal bacteria, since these microbes colonize and persist on mucosal and tooth surfaces despite its presence.<sup>15</sup> The reasons for this persistence are unknown, although immune tolerance and antigenic variation have been proposed. Regarding salivary IgE, our observations were approximately consistent with the pattern of age-dependent changes of serum IgE levels. It has been reported that total serum IgE levels increase from birth to the age of 25–30 years and decline thereafter.<sup>17,18</sup> We observed a marked intraindividual variability of salivary IgE in subjects of all age groups. Since increased IgE is characteristic of atopic disorders, many studies have been conducted to identify genes involved in IgE synthesis. Twin studies suggest that serum IgE levels in children and adults are under strong genetic control.<sup>17</sup> Various variants in many candidate genes have been related to increased or decreased IgE levels; however, the strongest association was observed with IL-13 gene polymorphism.<sup>19</sup> The immunological basis of the age-dependent changes in salivary IgA and IgE concentrations could be partly explained according to the responses of T-helper (Th) and regulatory T (Treg) cells. Th1 cells are characterized by secretion of the cytokines such as IFN- $\gamma$  whereas Th2 cells produce cytokines such as IL-4, IL-5 and IL-13.<sup>20</sup> Th2 cells secretions especially IL-5 are re-

sponsible for IgA production whereas IL-4 and IL-13 are essential for IgE production by B cells.<sup>21</sup> Moreover, Treg cells are defined by their ability to produce transforming growth factor- $\beta$  (TGF- $\beta$ ) which also induces IgA production and inhibits Th2 cell development.<sup>22,23</sup> Regarding the results of this study, it seems that up to age 40 years, the Th2 cell responses are responsible for salivary IgA and IgE production. After that, the greater activation of Treg cells may diminish Th2 cells response and IgE production. On the other hand, hyperactivation of Treg cells that may occur after age 40 years, probably results in higher salivary IgA concentrations through production of TGF- $\beta$ . Consistent with our observations, an enhancement in Th2 cell responses and also an elevation in the percentages of Treg cells have been described during aging.<sup>24</sup> In conclusion, the results of the present study demonstrated the profound age-related alterations in salivary IgA and IgE levels in healthy subjects. These results encourage further studies to elucidate the precise cellular and molecular mechanisms responsible for these changes to improve oral immunity.

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