ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 197 SECTIO D 2004

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The level of secretory IgA in saliva of patients with dental infections

Human saliva contains many components protecting particular tissular structures of the oral cavity against damaging factors, especially those produced by microorganisms (14). In the multifactor defensive system, a special role in the regulation of microflora and natural protection is played by secretory immunoglobulin A (s-IgA). Class A immunoglobulins are produced by plasmatic cells of the mucosa. More than 90% IgA in saliva is of secretory character. The opinion prevails that secretory IgA constitutes the main element of the mucosae protection (8, 14). Immunoglobins A occur in two subclasses: IgA1 and IgA2, differing in the number of amino acids in the hinge region. This structural difference is of crucial biological importance, as it prevents destroying IgA2 by numerous bacteria secreting s-IgA1 inactivating proteases. The effectiveness of s-IgA2 is connected with greater ability to neutralize viruses, greater effectiveness of bacteria agglutination and weakening the adhesion of bacteria to the epithelial cells by blocking receptors on the fimbriae. In seromucous secretions both the subclasses occur in equal amounts.

The level of s-IgA in the saliva changes during human life, and these differences are mainly connected with changes in the amount of secreted resting saliva (14).

In newborns and infants the level of s-IgA is lower than in adults. In this period of life immunoglobulin D fulfills the protective role of oral mucosa. Studies on newborns revealed that IgA occurs in saliva as early as in the first 24 hours after birth, and its level is low and unstable until the 8th month of life. According to S e i d e l et al. the immune system is fully formed in the IgA class at the beginning of the 9th month of life (12). The amount of secreted resting saliva increases with age, until adulthood. With the organism getting older, the amount of secreted resting saliva decreases as a result of the secretory function of salivary glands (12).

The act of chewing increases the flow of saliva and stimulates s-IgA secretion, as well as the transcytose of the oral mucosa epithelial cells. Also in infants' saliva the amount of s-IgA increases under the influence of alimentary stimuli (12). Secretory immunoglobin A dominates in the saliva among the immunoglobins of the remaining classes, but its level is characterized by individual variability. Big individual differences in the saliva IgA level of the examined persons were also noticed. They were influenced by such factors as: time of the day, diet, and mood. In the experiments, the level of s-IgA in saliva was evaluated during and after physical effort, under stress and under the influence of various alimentary stimulators (1, 13).

The presence of pathogenic bacteria in the stoma, despite the constant presence of IgA in the saliva is explained by lesser sensitivity of these microorganisms to the action of immunoglobulin A. Immunoglobins A are responsible for homeostasis, whereas in pathological situations, at increased virulence of microorganisms, additional defensive mechanisms must be mobilized (14). The bacteria causing odontogenic infections are part of the microflora, which is constantly present on the oral

mucosae, dental plaque and in the gingival pockets. Microorganisms taking part in these infections belong to mixed bacterial flora, consisting mainly of aerobic and anaerobic Gram-positive coccuses and anaerobic Gram-negative rods (11). The course of odontogenic infections mainly depends on virulence of the pathogens and the organism's defensive forces. A specific immune response is mobilized when the bacteria invade into the tissues.

The aim of the paper was examining the level of secretory IgA in the patients' saliva, in the course of odontogenic inflammatory states local treatment.

MATERIAL AND METHODS

The study material were 55 patients (37 women and 18 men) aged 12–65 years (33 patients < 30 years of age, 22 patients > 30 years of age) treated in the Outpatient Clinic of Medical University Teaching Hospital of Dental Surgery in Lublin, because of ailments related to the inflammatory process caused by an odontogenic bacterial infection. In these patients, besides the necessary surgical intervention, only local treatment with a natural drug (herbal mixture) was applied (15). The control group consisted of 30 students (20 women and 10 men) of the Department of Dentistry, Medical University in Lublin, aged 22–28 years. Selection of the control group for the conducted studies was aimed at the determination of s-IgA level in persons consciously taking care of the health and proper hygiene of their mouths (teeth, parodontium). The studies and patient treatment were conducted after obtaining the consent of the University Ethical Committee at the Medical University in Lublin and of the particular persons.

For the test we collected mixed, non-stimulated saliva, at least 2 hours after the morning meal, in the amount of 2.5–3 ml. From the patients saliva was collected three times: first – before the beginning of treatment, second – after 3–4 days of therapy, when the patients reported improvement of the pathological state, confirmed in the clinical examination, and for the third time, after 14 days, when all the morbid ailments (symptoms) subsided and the treatment was ended. The saliva collected for tests was centrifuged at 3000 rotations a minute for 15 minutes, then the supernatant was frozen in the temperature of –20°C. The level of A immunoglobulins (s-IgA) in the saliva was determined with the use of an immunoenzymatic test with the application of ELISA method – a set manufactured by Immunodiagnostik-Biomedica (Germany). The level of the examined antibody was expressed in concentration-volume units and reported in mg/dl of the examined material. The t-Student test with a correction for variance non-homogeneity was applied for analyzing statistical significance of the differences between subsequent measurements and the control group. The value of $p < 0.05$ was assumed as the significance limit. Due to variable concentration of saliva, connected with the velocity of its secretion, the obtained results of s-IgA level testing were standardized according to sodium and potassium ions contained in the supernatants. The concentration of sodium and potassium ions was examined by means of flame photometry method, and the values were determined in concentration-volume units mmol/l. The values of measured IgA absolute values to those after standardization, separately, according to sodium and potassium, according to the formula:

$$\text{Standardized value IgA according to } Na+(K+) = \frac{\text{Absolute IgA value in the examined person}}{\text{Absolute } Na^{+}(K^{+}) \text{ value in the examined person}} \times \text{mean } Na+ K+ \text{ value in a given measurement}$$

RESULTS

As the IgA values, standardized according to sodium and potassium ions do not significantly differ from absolute values (Fig. 1), for the sake of simplification they were disregarded in further

considerations. The level of IgA in the patients' saliva was characterized by a significant dispersion of values and sometimes distinctly departed from the mean value, especially in measurements 1 and 3. Distinctly smaller deviations from the average level values occurred in the control group (Table 1).

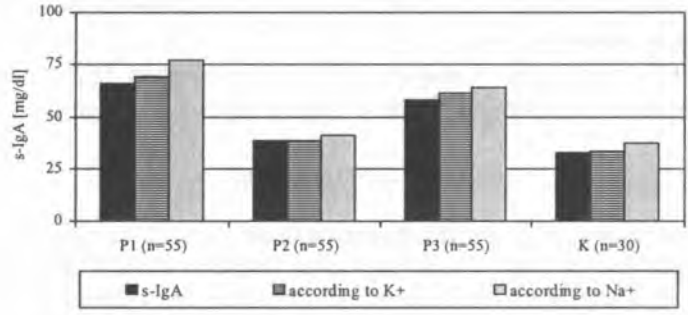

Fig. 1

Table 1. The level of s-IgA in saliva

Examined group	Measurement	Min. (mg/dl)	Max. (mg/dl)	Mean (mg/dl)	SD
Group of patients	1	0	576.0	66.1	103.4
	2	0	120.0	38.8	24.1
	3	4.31	380.23	58.1	58.7
Control group		12.8	72.0	32.7	13.0

The mean s-IgA level in each of the three measurements in the patients was higher than the results obtained in the control group. The highest level occurred in measurement 1 (66.1 mg/dl) and was significantly higher *($p < 0.05$). In measurement 2 the mean level was significantly lowered (38.8 mg/dl), whereas in measurement 3 it increased again (58.2 mg/dl) and was statistically significantly higher **($p < 0.01$). Despite the fact that the mean s-IgA level in saliva in measurement 3 was lower than in measurement 1, yet the level of significance was lower because of smaller standard deviations (smaller dispersion of results) in measurement 3 (Fig. 2).

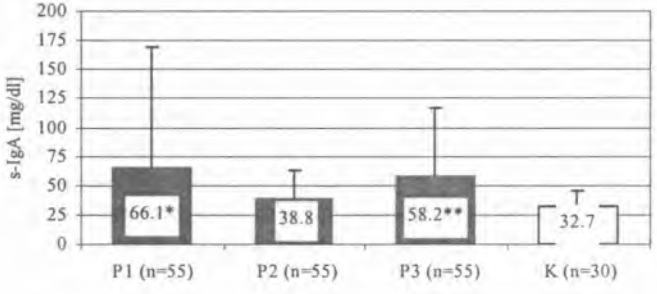

Fig. 2

The analysis of s-IgA dependence on sex in the control group (20 men and 10 women) revealed a small and slightly higher level of it in men, as compared to the level observed in women. However, in the patients (37 women and 18 men), the s-IgA level in saliva was characterized by high variability. The highest mean level (81.1 mg/dl) occurred in female patients in measurement 1, and differed significantly *($p < 0.05$) as compared to the control group of women (31.1 mg/dl). Also statistically significant **($p < 0.01$) was also the difference between measurement 3 in patients (52.6 mg/dl) and

the control group of women. Despite the fact that the average s-IgA level was lower than in measurement 1, the significance level was lower because of smaller standard deviations in measurement 3. In measurement 1 the mean s-IgA level in women was statistically significantly higher *($p < 0.05$) as compared to the level observed in the group of the examined men (35.1 mg/dl) (Fig. 3).

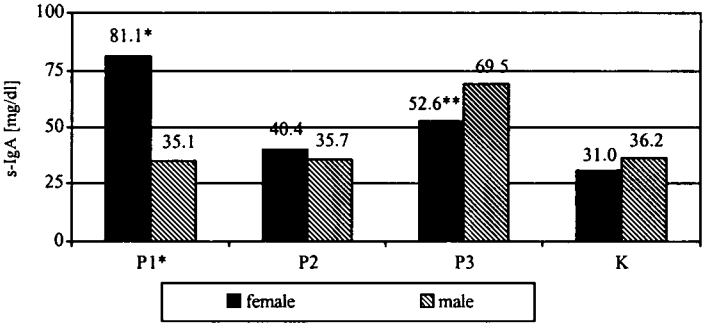

Fig. 3

Similar results were noticed when comparing two age groups: group I – up to 30 years (33 patients), group II – more than 30 years (22 patients). The control group consisted of persons younger than 30 years. The highest mean s-IgA level occurred in the younger group (81.1 mg/dl) in measurement 1 and was statistically significantly higher *($p < 0.05$) as compared to the control group. Also in measurement 3 in patients younger than 30 years of age the mean s-IgA level (55.7 mg/dl) was statistically significantly higher **($p < 0.01$) as compared to the control group. The lower significance level in measurement 3 was connected with a smaller standard deviation than in measurement 1 and it probably also resulted from the fact that most of the examined persons were women from the younger age group (Fig. 4).

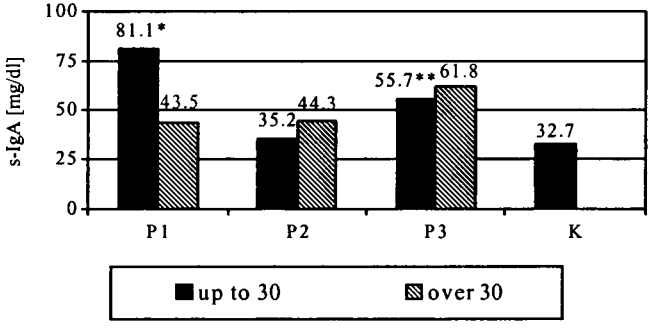

Fig. 4

DISCUSSION

Saliva may be a diagnostically important systemic fluid, as it is easily available for testing with the use of non-invasive methods (14). In various pathological states the level of s-IgA was examined as the effect of immune response. Examinations of patients with upper respiratory tract infections revealed the increase of the IgA level in saliva and serum (10). Also in patients with recurrent aphtae the increase of IgA level in saliva and serum was revealed (2). It was also noticed that general anesthesia and surgical procedures influence the defensive system of the stoma. It was found that after bone marrow transplant the level of IgA in saliva rapidly decreases (5), whereas after cardiologic operations on open heart the flow of saliva decreases, the amount of bacterial flora in the stoma increases and the level of s- IgA in saliva significantly grows (7).

In our own work we examined the level of s-IgA in saliva as the response of this immunological link in the patients' defensive systems in the course of odontogenic inflammatory complications induced by bacterial infections. All the examined patients were unburdened with any other diseases, neither were they pharmacologically treated, so they might constitute an appropriate study population for the evaluation of immune response in IgA class during the course of odontogenic inflammations treatment.

Examinations of s-IgA in the patients' saliva revealed the increase of its levels in all three measurements during the course of treatment, as compared to the control group. In the test conducted after 3–4 days of treatment the level of s-IgA in saliva distinctly decreased in relation to the s-IgA level determined in the measurement performed before the commencement of treatment. Similar observations were reported by Glesson et al. conducting IgA-EBV assays in saliva of a group of swimmers revealing predispositions to inflammatory states of the upper respiratory tract. In their studies they observed the increase of IgA-EBV concentrations at the onset of the infection, whereas in the period of its development they found that the level of IgA-class antibodies decreased (6). Various bacterial strains living in the stoma produce enzymes, which intensely degrade s-IgA1 (4).

The decrease of the s-IgA level in the patients' saliva in the second measurement, observed in our own studies, might have been caused by damaging class A immunoglobulins by bacteria taking part in odontogenic inflammatory processes. The results of studies also revealed substantial individual; differences of the s-IgA level in the patients' saliva. The individuality of s-IgA level in the saliva, stressed by many authors, was observed in the treated patients as much more conspicuous than in the control group. This may be the evidence for individually different responses of the defensive system in the course of these infections. The level of s-IgA remained high also after the treatment had been finished, but the individual differences were smaller as compared to the tests performed before commencement of the therapy. In long-term studies many authors observed the state of slow return of the normal level of immunoglobulins in saliva (3, 5, 9).

In the first test (before the commencement of treatment) the s-IgA level in women's saliva was higher as compared to the results obtained in men, which would suggest the existence of sex-related differences in the immune response of IgA class to odontogenic infections. Also comparing two age-groups (group I – up to 30 years, group II – more than 30 years) to the control group, consisting only of young persons, may indicate higher efficiency of the defensive system in class IgA in young people. The performed tests of the s-IgA level in the patients' saliva indicate reactions taking place in a specific immune system during the course of odontogenic infections.

CONCLUSIONS

1. During the course of odontogenic infections the s-IgA level in the patients' saliva increased and was characterized by significant individual variability.

2. Before the commencement of treatment, the average s-IgA level in saliva was statistically significantly higher $*p < 0.05$, and after the end of treatment it was $**p < 0.01$, as compared to the control group.

3. It was found that before the commencement of treatment women responded with greater increase of s-IgA level in saliva as compared to men, and this difference was statistically significant $*p < 0.05$, whereas in the control group the s-IgA in men's saliva was slightly higher than in the control group of women.

4. In women the s-IgA level before the commencement of treatment was statistically significantly higher $*p < 0.05$, as well as after the end of treatment, $**p < 0.01$, as compared to the control group of men.

5. It was found that s-IgA level in saliva of patients up to 30 years of age before the commencement of treatment was statistically significantly higher *$p < 0.05$, as well as after the end of treatment **$p < 0.01$, as compared to the control group, comprising exclusively young persons.

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SUMMARY

In this study the level of s-IgA in saliva of the patients with dental infections was measured. In the treatment only herbal drug was applied locally after the surgical procedures. The samples of saliva were taken three times: I – before treatment, II – after 3–4 days of the treatment and III – after the treatment. The level of s-IgA in saliva was measured according to ELISA method. Results were compared with 30-people control. Statistical analysis was done according to t-Student test. The level of s-IgA in saliva differed a lot between patients. Mean level of s-IgA in saliva of the patients was statistically higher before treatment and just after the treatment as compared to control (*$p < 0.05$, **$p < 0.01$). It

was also shown that the level of s-IgA in women before treatment was higher as compared to man. The level of s-IgA was statistically higher in younger age group in comparison with older age group. The statistical differences between women treated and the control in measurements I and II were found (*$p < 0.05$; **$p < 0.01$).

Badanie poziomu wydzielniczej IgA w ślinie pacjentów leczonych z powodu zębopochodnych stanów zapalnych

W pracy badano poziom s-IgA w ślinie u pacjentów leczonych z powodu zakażeń zębopochodnych. U chorych poza zabiegiem chirurgicznym stosowano wyłącznie leczenie miejscowe lekiem ziołowym. Ślinę pobierano do badań trzykrotnie – przed rozpoczęciem leczenia, po 3–4 dniach stosowania terapii i w 14 dni po zakończeniu leczenia. Grupę kontrolną stanowiło 30 ochotników. Poziom s-IgA w ślinie oznaczano metodą testu immunoenzymatycznego ELISA. Testem t-Studenta badano statystyczną istotność różnic między kolejnymi pomiarami a grupą kontrolną. Poziom s-IgA w ślinie u pacjentów charakteryzowała duża zmienność osobnicza i średni poziom s-IgA był statystycznie istotnie wyższy *$p < 0{,}05$ przed rozpoczęciem leczenia oraz po jego zakończeniu **$p < 0{,}01$ w porównaniu z grupą kontrolną. Stwierdzono, że u kobiet poziom s-IgA przed rozpoczęciem leczenia był istotnie wyższy *$p < 0{,}05$ w porównaniu z mężczyznami. Statystycznie istotnie wyższy był też poziom s-IgA u kobiet w dwóch pomiarach (*$p < 0{,}05$, **$p < 0{,}01$) oraz u pacjentów w młodszej grupie wiekowej (*$p < 0{,}05$, **$p < 0{,}01$) w porównaniu z odpowiednimi grupami kontrolnymi.