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Human Salivary Acidic Proline-Rich Proteins (APRP-1/2) In Adult Patients With Dental Caries

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Abstract

Background: Acidic proline-rich proteins (APRPs) are manifested in human saliva in various phenotypes and represent its important component. The unique structure of their two isoforms. APRP-1/2 as well as their coupling to hydroxyapatite and formation of the acquired enamel pellicle are well known. Nevertheless, role of APRP-1/2 in adult patients with dental caries still remains unclear. The aim of this study was to analyze the levels of APRP-1/2 in saliva of adult patients with dental caries.

Patients and Methods: The studies were conducted on 106 adult patients which were qualified to individual groups on grounds of dental examination and calculation of DMFT index. Group 1 (control) included 18 caries-free patients (DMFT=0). Group 2 included 20 persons (DMFT=2.3 ± 1.0) with very low intensity of caries. Group 3 included 20 patients (DMFT=6.2 ± 1.3) with low intensity of caries. Group 4 comprised 24 patients (DMFT=10.9 ± 1.8) with moderate intensity of caries. Group 5 included 24 patients (DMFT=19.5 ± 3.5) with high intensity of dental caries. Concentrations of APRP-1/2 in saliva were estimated using PRH2 ELISA kit (MyBioSource).

Results: In persons of group 1 (control) concentration of APRP-1/2 averaged at 15.2 ± 2.6 ng/ml. This concentration did not statistically differ from results obtained in patients of groups 2. On the other hand in patients of groups 3, 4 and 5 mean values of APRP-1/2 concentrations amounted respectively to: 18.6 ± 3.2 ng/ml, 35.4 ± 4.6 ng/ml and 39.8 ± 5.1 ng/ml. The obtained values of APRP-1/2 were significantly higher than results obtained in group 1 ($p<0.05$; Mann-Whitney test). In parallel the numerical force of women and men examined in the distinguished groups manifested no significant differences ($p>0.05$; test for two independent proportions).

Conclusions: High levels of APRP-1/2 in saliva of adult patients may be involved in intensification of the caries process.

Keywords: Oral health; Dental health; Saliva; Salivary proline-rich proteins; Dental caries

Introduction

Acidic proline-rich proteins (APRPs) accounting for 20-30% of all human salivary proteins are secreted by both parotid and submandibular/sublingual glands [1]. APRPs belong to the highly unique family of proline-rich proteins – PRP, characterized by high proline content (25 to 42% of total amino acids), which affects protein conformation with a prominent preference of β-sheet structure [2,3]. Currently, a group of 10 distinct APRPs can be distinguished (APRP-1/2, APRP-3/4, parotid isoelectric-focusing slow/fast variant– PIF-s/f, parotid acidic protein – Pa, the double-band slow/fast isoform– Db-s/f and PC peptide), encoded by two genetic loci, PRH1 and PRH2 on chromosome 12 at p13.2 [4]. The PRH2 locus has two alleles and is responsible for the synthesis of principal isoforms of APRP-1/2 representing precursors of APRP-3/4 [3,5]. The PRH1 locus has three different alleles encoding the PIF-s, Pa and Db-s isoforms [5]. Despite studies, recently also those with application of proteomics, the role of different APRP phenotypes, and particularly of APRP-3/4. PIF-f and Db-f in oral cavity continues to be unclear. In turn, it is well known that APRP-1/2 manifest high affinity for hydroxyapatite and are involved in the formation of dental-acquired pellicle [6-8]. It is also suggested that salivary APRPs may affect susceptibility to dental caries [9].

Therefore, the currently undertaken study aimed at obtaining data on the range of APRP-1/2 quantitative alterations in full unstimulated saliva of adult patients as compared to intensity of dental caries.

Patients and Methods

Patients grouping

The studies were conducted on 106 patients, 20-35 years of age, which were qualified to individual groups on grounds of dental examination and calculation of DMFT (Decayed Missing and Filled Teeth) index. Evaluation of caries intensity took into account the obtained values of DMFT index, in line with the WHO criteria [10], presented in Table 1.

DMFT Index	Caries Experience
1-4.9	Very Low
5-8.9	Low
9-13.9	Moderate
>13.9	High

Table 1: Criteria of dental caries intensity.

Group 1 (control) included 18 caries-free patients (20-26 years of age; 10 women and 8 men; DMFT=0). In none patient of the group detectable caries or fillings were detected. The remaining groups included patients with dental caries. Group 2 included 20 persons (20-32 years of age; 12 men and 8 women; DMFT=2.3 ± 1.0) with very low intensity of caries. Group 3 included 20 patients (21-35 years of age; 11 men and 9 women; DMFT=6.2 ± 1.3) with low intensity of caries. Group 4 comprised 24 patients (20-35 years of age; 15 men and 9 women; DMFT=10.9 ± 1.8) with moderate intensity of caries. Group 5 included 24 patients (20-35 years of age; 14 men and 10 women; DMFT=19.5 ± 3.5) with high intensity of dental caries.

Groups	DMFT index Mean values ± SD	P-value	Salivary APRP-1/2 Mean values in ng/ml ± SD	P-value
1&2	0 2.3 ± 1.0	<0.001	15.2 ± 2.6 15.9 ± 2.1	Ns (p=0.355)
1&3	0 6.2 ± 1.3	<0.001	15.2 ± 2.6 18.6 ± 3.2	<0.001
1&4	0 10.9 ± 1.8	<0.001	15.2 ± 2.6 35.4 ± 4.6	<0.001
1&5	0 19.5 ± 3.5	<0.001	15.2 ± 2.6 39.8 ± 5.1	<0.001
2&3	2.3 ± 1.0 6.2 ± 1.3	<0.001	15.9 ± 2.1 18.6 ± 3.2	<0.004
2&4	2.3 ± 1.0 10.9 ± 1.8	<0.001	15.9 ± 2.1 35.4 ± 4.6	<0.001
2&5	2.3 ± 1.0 19.5 ± 3.5	<0.001	15.9 ± 2.1 39.8 ± 5.1	<0.001
3&4	6.2 ± 1.3 10.9 ± 1.8	<0.001	18.6 ± 3.2 35.4 ± 4.6	<0.001
3&5	6.2 ± 1.3 19.5 ± 3.5	<0.001	18.6 ± 3.2 39.8 ± 5.1	<0.001
4&5	10.9 ± 1.8 19.5 ± 3.5	<0.001	35.4 ± 4.6 39.8 ± 5.1	<0.01

Table 2: Comparison between groups in the means ± standard deviations of the DMFT and salivary APRP-1/2.

The patients qualified to the studies were generally healthy, with no general or chronic diseases in anamnesis. Moreover, the exclusion criteria included fungal infection in oral cavity, destructive periodontal diseases, bruxism, alcoholism and smoking of cigarettes. Within three weeks preceding the study, the patients were not subjected to hygienization procedures or to use of anti-bacterial mouth washes.

Ethical approval and consent to participate

This study was approved by the Medical Ethics Committee at the University of Medical Sciences in Poznań, Poland. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Salivary sample collection

Saliva was collected by the standard method. Samples from the subjects were collected between 8:00 and 11:00 a.m. All subjects abstained from eating and drinking for 2 h. Unstimulated whole saliva was collected for 10 min by a spitting method. Saliva samples were homogenized and clarified by centrifugation at 3.000 × g for 15 min at 4°C. The aliquots of clarified supernatants were stored at -70°C for the APRP-1/2 measurements.

Determination of APRP-1/2

Salivary APRP-1/2 were quantitated using an immunoenzymatic technique (ELISA). The PRH2 ELISA kit (MyBioSource, San Diego) was applied, manifesting high specificity for detection of salivary APRP-1/2 and high sensitivity: 0.55 ng/ml. The tests were performed as recommended by the manufacturer. Values of absorbance depending on estimated APRP-1/2, were read at the wavelength of A=450 nm using Reader 250 (bioMerieux). The results were obtained from standard curves. Every estimation of salivary APRP-1/2 was repeated three times and the obtained mean represented individual result for the patient.

Data analysis

The results obtained in the studies were analyzed using the Statistica v.12 software. In the comparative analysis of salivary APRP-1/2 levels in the studied groups the nonparametric Mann-Whitney test was used. In analysis of shares of examined women and men the test for two independent proportions was used.

Differences with P-values higher than 0.05 were considered non-significant.

Results

APRP-1/2 was detected in full unstimulated saliva in all the examined groups; the results are listed in Table 2.

In group 1 of caries-free individuals concentration of APRP-1/2 averaged at 15.2 ± 2.6 ng/ml. In turn, in patients with dental caries salivary concentration of APRP-1/2 was analyzed in the context of caries intensity, expressed by values of DMFT-index. The obtained mean values of DMFT-index in the distinguished groups of patients proved to be statistically distinct and amounted to, respectively: group 1-0; group 2-2.3 ± 1.0; group 3-6.2 ± 1.3; group 4-10.9 ± 1.8 and group 5-19.5 ± 3.5. In turn, mean levels of salivary APRP 1/2 in individual groups of patients amounted to, respectively. 15.2 ± 2.6 ng/ml in group 1; 15.9 ± 2.1 ng/ml in group 2; 18.6 ± 3.2 ng/ml in group 3; 35.4 ± 4.6 ng/ml in group 4 and 39.8 ± 5.1 ng/ml in group 5. Comparative analysis of the mean APRP-1/2 values between individual studied groups disclosed significant differences except of the absence of

statistical significance between groups 1 and 2. The results are listed in Table 2. Moreover, the obtained results allowed to draw the exponential curve of dependence between DMFT index and APRP1/2 (Figure 1).

In turn, analysis of numerical forces manifested by studied women and men in the distinguished groups demonstrated no statistically significant differences ($p>0.05$).

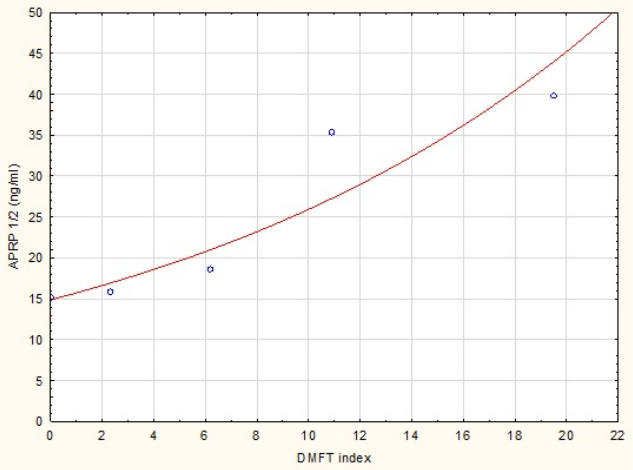

Figure 1: Exponential curve of dependence between DMFT and APRP1/2. Each circle represents mean of results in studied group.

Discussion

Dental caries is the most common infectious disease and affects approximately 36% of the population in the world; it is characterized by demineralization and destruction of dental hard tissues, such as enamel dentin and cement [11,12]. This local pathological process represents the most frequent cause of dental loss in adults [13]. Dental caries is caused by cariogenic bacteria, particularly oral streptococci within the produced on the acquired enamel pellicle of a dentally adherent biofilm (plaque) in the presence of dietary carbohydrates and sucrose in particular [14]. In etiology of dental caries the central role is played by Streptococcus mutans, because it can adhere to the pellicle and to other plaque bacteria [15]. Strains of this bacterial species are highly acidogenic. They produce short-chain acids which can dissolve hard tissues of teeth. Moreover, in the presence of sucrose they are able to form large quantities of water-insoluble extracellular polysaccharides (EPS), which, manifesting adherente properties, are responsible for plaque progress. Development of dental biofilm may reflect also action of mutacins (bacteriocins), produced by strains of *S. mutans* [16,17]. Thus, as indicated by the presented data the developed on dental surface the acquired pellicle, incorporating cariogenic bacteria via specific cell-to-surface interactions, represents an essential factor in initiation of caries-developmental process. It has already been well documented that this pellicle is formed by adsorption of proteins from the fluid surrounding the teeth [1]. Using the extraction procedure APRPs have been demonstrated to provide a major component of the new, in the first hour formed acquired pellicle [7,18]. Nevertheless, from the beginning of its existence a degradation process develops of the adsorbed proline-rich proteins, which may explain the minimum content of APRPs in pellicles more than 24 hours old [1]. Structural studies indicate that APRP-1/2 include two positional isoforms, every involving a single polypeptide chain, which consists of 150 residues [1,19]. Its N-terminal domain consists of 30 residues, including the two phosphoserines but only one proline. In turn, the sequence from residue 31 to the C-domain represents a proline-rich region, including repeats high in proline glycine and glutamine. At present, the unique structure of APRP-1/2 is thought to determine its specific properties. The N-terminal domain ensures coupling of APRP-1/2 to hydroxyapatite and formation of the acquired enamel pellicle. In parallel the C-terminal domain of APRP-1/2 binds to oral bacteria, promoting bacterial colonization of dental surface, which may play a significant role in pathogenesis of caries [9,20].

The conducted studies failed to detect significant differences in levels of APRP-1/2 in unstimulated saliva obtained from patients free of dental caries (group 1) and from persons with very low (group 2) intensity of caries. Therefore, the manifestation of dental caries might be suggested to remain not linked to alterations in APRP-1/2 concentrations in saliva of adult patients. Such suggestion is consistent with earlier studies indicating absence of significant differences between values of salivary APRP in caries-resistant patients and caries-susceptible patients [21]. However, such results were obtained for stimulated saliva using visual radial immunodiffusion assay. In contrast, in our work we employed the highly semsitive and specific ELISA test [22]. Moreover, no patients in studied groups reported an excessive consumption of carbohydrates, all of them manifested similar alimentary habits and oral hygiene procedures which, therefore, could not significantly affect manifestation of dental caries in any of studied groups. However, manifestation and progression of dental caries reflects not only environmental conditioning but also genetic influences, as demonstrated in studies on humans and in animal models. In parallel, 45-64% of caries susceptibility has been shown to be genetically determined [23]. A significant association was reported between human caries and genes coding amelogenin (AMELX), ameloblastin (AMBN) and tuftelin (TUFT) [24]. The genes are of key importance for enamel formation. Such data may explain absence of caries in persons of group 1 and its variable intensity in the remaining groups of patients. Nevertheless, in persons with low (group 3), moderate (group 4) and high (group 5) intensity of dental caries which have demonstrated significantly higher levels of salivary APRP-1/2 than in group 1. The results, presented for the first time in this study, allow to conclude that elevated levels of APRP-1/2 may cooperate in development of dental caries in adults. This conclusion, at least in part may be corroborated by studies pointing to a relationship between development of dental caries on one hand and genetic polymorphism in PRH1 and PRH2 loci [24,25].

In light of presented data it can be concluded that levels of APRP-1/2 in saliva of adult patients may exert an effect on origin and development of dental caries.

Conclusions

Our study demonstrated the possible connections between intensity of dental caries in adults and the concentration of salivary acidic-rich proteins, APRP-1/2. Knowledge on manifestation of quantitative alterations in the salivary components allows to explain their involvement in pathogenesis of dental caries. Future research is essential to more completely characterize function of salivary acidic-rich protein phenotypes in the caries process.

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