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Original paper

SALIVARY CARCINOEMBRYONIC ANTIGEN AS AN INFLAMMATORY MARKER

Snežana Golubović[1], Ljiljana Janković[2], Aris Movsesijan[2], Žanka Bojić[1], Miroslava Janković[1]

[1]Institute for the Application of Nuclear Energy – INEP, Banatska 31b, 11080, Zemun–Belgrade, Yugoslavia
[2]Department of Paradontology and Oral Medicine, University School of Stomatology, Belgrade, Yugoslavia

Summary: In this study we examined concentration of carcinoembryonic antigen (CEA) in paired saliva and serum samples from healthy individuals and patients with periodontal disease. CEA concentration was determined immunoradiometrically using highly specific anti-CEA antibodies. The salivas from periodontally healthy subjects revealed CEA concentrations with median value of 62 mg/L. Distribution of salivary CEA concentrations in patients with periodontal diseases were very broad with median values: 74 μg/L (stage I), 84 mg/L (stage II), 240 μg/L (stage III) and 412 μg/L (necrotizing ulcerative periodontitis-NUP). Analysis of the obtained values indicated statistically significant increase in salivary CEA, in subjects with periodontal diseases. Metronisadole treatment in patients with NUP leads to statistically significant decrease in salivary CEA. The results obtained suggested salivary CEA as a potential marker of the alterations of periodontium.

Key words: CEA, saliva, serum, periodontal disease, protein, immunoradiometric assay.

Introduction

Carcinoembryonic antigen (CEA) was first described in 1965 by Gold and Freedman as an oncofoetal antigen present in colonic tumours and foetal gut (1). So far, a number of CEA-related genes as well as splice variants of individual genes have been identified (2 4). CEA gene family is divided into two main subgroups: CEACAM genes, coding for CEA-related cell adhesion molecules and PSG genes, coding for pregnancy-specific glycoproteins (5). The CEA subgroup members are cell surface associated glycoproteins, showing a complex expression pattern in normal and neoplastic tissues (6, 7). Thus, CEA being a well-known colorectal tumour marker was also detected in different normal adult tissues (8).

Immunochemical techniques and RNA blot analyses revealed the presence of CEA and CEA-related molecules in human submandibular gland, gingival tissue and saliva (9, 10). Saliva is a mucosal fluid produced by secretion of different glands (11). It protects oral cavity against harmful external agents (12). Periodontal disease affects the periodontium and includes both gingivitis and periodontitis. Pathogenesis of chronic gingival inflammation and periodontitis is complex and still not fully recognized (13, 14). The most accepted idea is that putative pathogenic bacteria induce periodontal disease by releasing various proteolytic enzymes and by provoking an immune response that triggers host cells for the expression of degrading enzymes (15). Since CEA possesses homo- and hetero-cell adhesion properties, including bacterial recognition, we suppose that it could play a role in the maintenance of the structure and function of oral epithelium as well as in the related pathological processes (16 18).

The aim of this pilot study was to examine wheather locally produced *i.e.* salivary CEA could be a potential marker of the alterations of periodontium. In this study we report on the results of estimation of CEA concentration in the saliva of healthy individuals and patients with various stages of inflammation of oral tissues. This topic have not been extensivelly studied so far, and simple and non-invasive collection of saliva favored its choice as a clinical specimen in comparison to other traditional diagnostic body fluids.

Address for correspondence
Snežana Golubović
Institute for the Application of Nuclear Energy-INEP,
Banatska 31b,
11080 Zemun-Belgrade, Yugoslavia
e-mail: sneza@inep.co.yu

Materials and Methods

Materials

Monoclonal anti-CEA antibodies, capture (IgG_1, $K_a = 3\times10^{10}$ mol ^{1}×L) and tracer (IgG1, $K_a = 2\times10^{10}$ mol ^{1}×L), were purchased from Medix Biochimica (Kauniainen, Finland). Radioiodine ^{125}I, was from Radioisotope Centre Polatom (Otwock-Swierk, Poland). Carcinoembryonic antigen was from Medix Biotech. Inc. (Foster City, USA). Bovine serum albumine (BSA) was from Sigma (St. Louis, USA). All other chemicals were reagent grade.

Subjects

This study was carried out on a group of consenting patients and healthy volunteers (control group) seen at Department of Paradontology and Oral Medicine, University School of Stomatology, Belgrade, Yugoslavia. The status of periodontal tissue was assessed using the following indices: plaque index (Silnes and L e), calculus surface index (Green), gingival index (L e and Silnes), papillary bleeding index (Cowell) and tooth mobility index as described (19). According to the values of periodontal indices 19 patients with periodontal diseases were divided in three groups (stage I III). In addition, the separate group of 18 patients with diagnosis of necrotizing ulcerative periodontitis was examined before and after treatment (250 mg four times per day; five days) with metronidasole (Orvagil, Galenika).

The control group included 11 subjects without any signs of periodontal disease.

Collection of saliva and serum

Both saliva and blood were taken from control and patients groups. Whole saliva was collected by spitting, without any stimulation. All samples were taken till 11 a.m. Saliva was cleared by centrifugation (11000×g; 20 min) followed by dialysis against physiological saline. Peripheral venous blood was drawn and serum is separated by centrifugation after 30 60 min. The corresponding samples were used immediately or stored at 20 ° C until processed.

Analytical procedures

Carcinoembryonic antigen (CEA) concentration was determined using immunoradiometric assay, IRMA CEA (INEP, Yugoslavia), standardized against the 1st International Reference Preparation of CEA 73/601. All probes were done in triplicate. Samples were tested undiluted and diluted (1/10 and 1/100). The diluent was 0.05 mol/L phosphate buffer saline (pH 7.4) containing 10 g/L BSA. Radioactivity was measured using ISOMEDIC 4/600 gamma counter (ICN, USA).

Protein concentration was determined according to Lowry (20) with bovine serum albumin as a standard. Optical density was measured using double beam spectrophotometer CE 594 (CECIL, England).

Statistical analysis

Kruskal-Wallis one way analysis of variance and Mann-Whitney U test were used to analyze the obtained results. The data for each group were averaged and mean, median and standard deviations were calculated. Differences between groups were considered as statistically significant at $p < 0.05$.

Results

CEA concentrations of the examined saliva samples are presented in *Table I*. The salivas from periodontally healthy subjects revealed CEA concentrations with a median value of 62 μg/L in the range from 23 102 μg/L. Generally, salivary CEA concentrations did not exceed 102 μg/L. On the contrary, distribution of salivary CEA concentrations in patients with periodontal diseases were very broad with median values: 74 μg/L (stage I), 84 μg/L (stage II), 240 μg/L (stage III) and 412 μg/L (NUP group). Marked individual variations were noticed in each group tested, especially in the group of patients with necrotizing ulcerative periodontitis (NUP). In spite of this, the scatter diagram indicates that most of the patients (29 of 37) have no overlap with the control range. Analysis of the

Table I Salivary CEA and protein concentrations in healthy subjects and periodontal patients

Subjects	Salivary CEA (μg/L)*			Protein concentrations (g/L)**		
	Median	Range		Median	Range	
Healthy (n=11)	62	23	102	1.07	0.70	1.47
Periodontal I stage (n=6)	74	13	160	0.79	0.60	1.37
Periodontal II stage (n=8)	84	30	2600	0.90	0.66	1.37
Periodontal III stage (n=5)	240	150	1200	0.93	0.70	1.65
NUP (n=18)	412	110	4000	0.93	0.60	2.50

* Difference between groups is statistically significant, $p<0.05$ (Kruskal-Wallis analysis of variance; $H=25.9648$; $\chi > c^2$);
** Difference between groups is not statistically significant, $p>0.05$ (Kruskal-Wallis analysis of variance; $H=1.133$; $\chi < c^2$); (n, number of samples; p, level of significance).

Table II Salivary CEA and serum CEA concentrations in healthy subjects and periodontal patients

	Control	Patients			
Saliva*	Healthy (n=11)	Periodontal I stage (n=6)	Periodontal II stage (n=8)	Periodontal III stage (n=5)	NUP (n=18)
	1.06 ± 0.258	0.84 ± 0.228	0.97 ± 0.240	1.04 ± 0.354	1.13 ± 0.480
Serum	N.D.	N.D.	N.D.	N.D.	N.D.

Values are means ± S.D. Salivary CEA concentrations are expressed as mg CEA/mg total proteins. *Difference between groups is statistically significant, $p<0.05$. N.D. non-detected. CEA concentrations in serum were below assay lowest standard (< 2 μg/L).

Table III Salivary CEA and protein concentrations in NUP patients before and after treatment with metronidasole

NUP patients	Salivary CEA (μg/L)		p**	Protein (mg/mL)		p***
	Median	Range		Median	Range	
I before treatment	410	130 4000	<0.05	0.48	0.60 2.50	>0.05
II after treatment*	200	67 1100		0.21	0.60 1.45	

* Patients were treated with metronidasole as described in Methods (n, number of samples; p, level of significance).
** Difference in salivary CEA concentrations is statistically significant (Man-Whitney U test; $U=39$ $U_{0.05}=45$ $U<U_{0.05}$).
*** Difference in salivary protein concentrations is not statistically significant (Man-Whitney U test; $U=53$, $U>U_{0.05}$).

obtained values by Kruskal-Wallis analysis of variance as well as by Mann-Whitney U test indicated statistically significant ($p<0.05$) increase in salivary CEA concentrations in subjects with periodontal diseases.

In addition to CEA, total salivary proteins were also determined (*Table I*). There were no statistically significant ($p>0.05$) differences in protein concentrations between the examined groups. When CEA concentrations, expressed as mg CEA/mg total protein, were compared, statistically significant increase in patients groups were also observed (*Table II*). In relation to this, it is important to notice that CEA concentrations in sera of all examined subjects were below 2 μg/L, excluding the contribution of serum exudate to the salivary CEA concentration (*Table II*).

The salivary CEA concentrations, before and after the metronisadole treatment, in patients with NUP are presented in *Table III*. Mann-Whitney analysis of variance have not shown statistically significant difference in protein concentration ($p>0.05$) after the treatment. However, it leads to statistically significant ($p<0.05$) decrease in salivary CEA concentrations. The decrease was observed in all examined saliva samples from NUP patients.

Discussion

The results presented in this paper demonstrated statistically significant increase in salivary CEA concentrations in patients with inflammatory periodontal disease in comparison to healthy control group. For accurate determination of CEA and for proper validation of the results obtained, two major issues were carefully addressed. First, the selected capture antibody has no cross-reactivity with human CEA-related molecules, ensuring specific recognition of CEA molecule (10, 21). Second, the control group, used as a reference, included age-matched subjects screened on almost complete absence of gingival inflammation and periodontal breakdown. This was very important in relation to reported large variation in saliva composition between healthy individuals, in general (15).

Thus, salivary CEA concentration correlated with stage of periodontal disease, in contrast to total salivary protein concentration which remains in almost the same range. The examined groups were constituted on the basis of well defined criteria for severity of tissue damage (19). The lowest mean salivary CEA concentration (62 μg/L) was detected in periodontally healthy individuals and the highest mean salivary CEA concentration (904 μg/L) in patients with NUP. Comparison of paired saliva and serum samples indicated that CEA response in patients is locally produced, since in all cases serum CEA concentrations were below cut-off value.

The periodontal tissues are among the most biologically active in the body and the balance between effector-molecule induced tissue breakdown and tissue formation is the essence of periodontal health (23). Pignatelli and co-workers, demonstrated that CEA can function as an accessory cell adhesion molecule, mediating cell-matrix interaction, and they proposed that the detection of high levels of CEA in col-

orectal cancers may be only an epiphenomenon resulting from the disruption of cell-matrix interaction (17). Therefore, one of the hypotheses to explain high salivary CEA level in patients with NUP could be enhanced tissue damage. This might be also related to the results obtained in the group of patients before and after metronidasole treatment. Thus, the administration of metronidasole which acts as anti-inflammatory agent, leads to statistically significant decrease in salivary CEA concentration in all examined subjects.

Finally, the results obtained indicated salivary CEA as potentially useful adjunct for diagnosis and follow-up of patients with periodontal diseases. CEA, such as most salivary molecules is a multifunctional molecule and it may have both protective and detrimental properties (14). The proposed function of CEA, based on *in vitro* studies, such as bacterial ligand (18), suppressive factor to the host immunocompetent cells (23), and innate immunity factor (8), suggested that salivary CEA deserves further studies not only in clinical and diagnostic purposes but also in fundamental terms.

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SALIVARNI KARCINOEMBRIONALNI ANTIGEN KAO INFLAMATORNI MARKER

Snežana Golubović[1], Ljiljana Janković[2], Aris Movsesijan[2], Žanka Bojić[1], Miroslava Janković[1]

[1]Institut za primenu nuklearne energije–INEP, Banatska 31b, 11080, Beograd, Jugoslavija
[2]Klinika za paradontologiju i oralnu medicinu, Stomatološki fakultet, Univerzitet u Beogradu, Jugoslavija

Kratak sadržaj: U ovom radu je uporedno ispitana koncentracija karcinoembrionalnog antigena (CEA) u uzorcima salive i seruma zdravih osoba i osoba sa periodontalnim oboljenjem. Koncentracija CEA je određena imunoradiometrijskom metodom zasnovanoj na upotrebi specifičnih anti-CEA antitela. Medijalna vrednost salivarnog CEA u zdravih osoba bila je 62 μg/L. Medijalne vrednosti salivarnog CEA u pacijenata sa periodontalnim oboljenjem, distribuirane u širokom opsegu koncentracija, bile su: 74 μg/L (I stadijum), 84 μg/L (II stadijum), 240 μg/L (III stadijum) i 412 μg/L (nekrotični ulcerativni periodontitis NUP). Analiza dobijenih vrednosti ukazala je na statistički značajno uvećanje salivarnog CEA u osoba sa periodontalnim oboljenjem. Statistički značajno smanjenje koncentracije salivarnog CEA u pacijenata sa NUP dobijeno je po tretmanu sa metronidazolom. Dobijeni rezultati ukazali su na salivarni CEA kao potencijalni marker periodontalnih promena.

Ključne reči: CEA, saliva, serum, periodontalno oboljenje, protein, imunoradiometrijski test.

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