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FIRST REPORT IN ROMANIA REGARDING SALIVARY IODINE LEVEL AND ITS CORRELATION WITH CARIOUS LESIONS, CHRONIC MARGINAL PERIODONTITIS AND PERIAPICAL PATHOLOGY

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ABSTRACT

Since the beginning of administration of iodine to prevent iodine deficiency, children have less dental cavities; iodine seems to increase the resistance to dental caries, retarding the process and reducing its incidence (in Romania the law regarding universal salt iodization was implemented in January 2003). Given its antioxidant properties, iodide may act as an antimicrobial agent in saliva. In our studied groups (group I, II, III) we found a statistically significant difference between the smokers and non- smokers on each of the above mentioned group. Our results confirm previous studies, showing that smoking had an increasing effect on the concentration of salivary thyocianate and a decreasing effect on the content of iodide in saliva.

Key words: salivary iodine, thiocyanate, smoking, periodontal disease, periapical pathology, carious lesions

INTRODUCTION

Dental caries continue to be the most prevalent chronic disease to affect human population [1]. On the other hand, iodine deficiency disorders (IDD) is arguably the world's most common endocrine disease [2]. Interestingly, since the beginning of administration of iodine to prevent goiter induced by iodine deficiency, children have less dental caries, iodine seems to increase the resistance to dental caries, retarding the process and reducing its incidence (3). The thyroid gland is unique among the endocrine glands for its dependence on an essential micronutrient (iodine) for normal thyroid hormone production. In Romania, the law regarding Universal Salt Iodization was implemented in January 2003, as the main strategy for eliminating iodine deficiency. The natrium iodide symporter (NIS) (the iodide pump) is the plasma membrane glycoprotein that mediates active iodide transport in the thyroid and other tissues, including salivary glands [4]. Human NIS is a 643 amino acid protein and contains 643 transmembrane domains. The molecular characterization of natrium iodide symporter (NIS) began in 1996, when Nancy Carrasco's group isolated the cDNA encoding rat NIS [5]. Although there are similarities between the salivary and thyroid iodine concentrating mechanism (both mechanisms are inhibited by some anions like thiocyanate and perchlorate via competitive inhibition), there are also important differences. The physiological role of iodide secretion in the saliva is a matter of debate [4]. Given to its antioxidant properties, iodide may act as an antimicrobial agent in saliva. A bactericidal/

bacteriostatic effect of iodide is consistent with the presence of an H_2O_2/ peroxidase system in the salivary glands [4]. Cigarette smoking is a major source of thiocyanate in humans [6]. Thiocyanate inhibits competitively the function of NIS in the thyroid and in salivary glands. Tabacco smoking is the main risk factor associated with chronic destructive periodontal disease and the risk is 5 to 20-fold elevated for a smoker compared to a non- smoker [7]. In addition, the outcome of periodontal treatment is less favorable even unfavorable in smokers and treatment failures and relapses of disease are predominantly seen in smokers [7].

SUBJECTS, MATERIALS AND METHODS

One hundred and twenty subjects were enrolled in the study. The groups were selected among the patients presented for treatment in the Department of Endodontics and Parodontology, at the University of Dental Medicine "Carol Davila", Bucharest, during 22.05.2014- 14.05.2015: the control group, consisting of patients with no simple and complicated caries, and no periodontal disease (30 patients, 15 current smokers and 15 non-smokers), group I, consisting of patients with simple carious lesions (30 subjects, 15 smokers and 15 non-smokers), group II, which presented chronic marginal periodontitis (30 patients, 15 smokers and 15 non-smokers) and group III, with periapical pathology (30 subjects, 15 smokers and 15 non-smokers) (Table 1). Salivary samples were collected to measure salivary iodine concentration. The samples were then frozen at -20°C and send for analysis at the National Institute for Mother and Child Care "Alessandrescu - Rusescu", Bucharest. Salivary iodine concentration was determined by digestion with ammonium persulfate followed by Sandell – Kolthoff reaction. The values were expressed in mcg/L. The study was approved by the Local Ethics Committee. An informed consent from the subjects was obtained.

As for the material and method part, was used the following protocol:

Method of determination: ammonium persulfate digestion followed by Sandell - KOLTHOFF reaction .The present method was described for the determination of iodine in urine , but we extended this method for iodine in saliva and blood. Determination of iodine concentration in saliva is based on a complex colorimetric spectrophotometric measurement at 420 nm; complex is formed on reaction Sandell - KOLTHOFF. The sample to be analyzed was treated with ammonium persulfate and incubated at 92-97ºC for 1 hour to release iodine in combination. After cooling, the solution of arsenic acid and then ceric ammonium sulfate were added.The formed product was colorimetrically at 420 nm. The color intensity was inversely proportional to the concentration of the obtained iodine. The samples were received in the laboratory and were processed and prepared for preservation. This process consisted of Merc samples centrifugation for 10-15 minutes centrifugation at 3000 rpm, separating the supernatant in another tube adapters and preservation at -24 ° C freezer until they are worked.

Procedure: introduce a volume of 250µl sample of saliva then add 1 ml 1M ammonium persulfate solution in the analysis tubes (diameter and volume of 13 / 100mm); place in the oven for 1 h at 92-95 ° C; iii) chill to room temperature (23 ± 2 ° C) and then add 3.5 ml Shake arsenic acid. After 15 minutes, add 400µl ceric ammonium sulfate solution every 30 seconds between samples and shaken vigorously; after 30 minutes exactly add of ceric ammonium sulfate in the first tube, read the absorbance at 420nm.

Samples were read sequentially at the same interval of 30 seconds.The same procedure applied for internal standard curve and reference materials. All samples were worked in replicates. For any difference of more than 35 mg / L in the same sample, the determination was repeated.

Special conditions:

Determination of iodine is carried out in a "room free of iodine " which is obtained by its decontamination (work surfaces and pavement) with solution of 5% sodium thiosulfate.
Statistical analysis of data was done using an Anova analysis.

RESULTS AND DISCUSSION

In the salivary glands, NIS is highly expressed in the basolateral membranes of the majority of striated ducts; NIS expression in salivary glands is decreased during inflammation and tumor formation [8] (Figure 1).

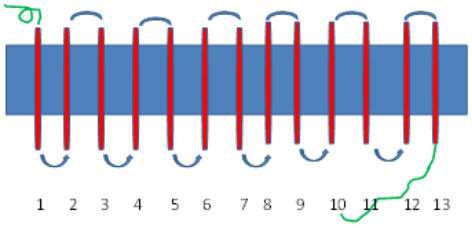

Fig. 1. A schematic representation of the human natrium iodide symporter, composed of 13 transmembrane domains.

Smoking had an increasing effect on the concentration of thiocyanate and a decreasing effect on the content of iodide in saliva [9]. Iodine is the richest in electrons of the elements presently considered essential in the animal and human diets [3]. Inorganic iodide appears to be necessary for all living animal cells, but only the vertebrates have the thyroid gland and its iodinated hormones [3]. Inorganic iodide functions as an antioxidant, since it neutralizes hydrogen peroxide [10]. There have been demonstrated that addition of iodine with thiocyanate increased the fungicidal and bactericidal effect of the lactoperoxydase system [11]. Thiocyanate reacts with hydrogen peroxide (H_20_2) under the catalytic action of peroxidase enzyme producing hypothiocyanite, which has antibacterial properties and is less harmful to human cells than hydrogen peroxide [12]. Heavy smokers can show salivary thiocyanate concentrations as high as 6 mM and levels in non-smokers range from 0.5 to 2 mM, with an average of 1 mM [12].

Our data revealed that in the control group there have been noticed an expected statistically significant difference between the smokers group and the non- smokers group (average mean= 87.51 mcg/l) - Table 1. Moreover, in group 1, there was found a statistically significant difference between smokers and non- smokers group (average mean=207.55 (p= 2.45), whereas in group 2 – 234.69 (p= 1.47) and in group 3- 194.2 (p= 1.31).

Table 1- Mean salivary iodine in control group and study groups (I, II, III).

Groups (no of samples 120)	Non- smokers average (mcg/ l) (60 samples)	Smokers average (mcg/ l) (60 samples)	Mean
Control group	97.41	19.8	87.51 (1.25)
Group I- Simple carious lesions	294.9	120.2	207.55 (2.45)
Group II- chronic marginal periodontitis	279.38	190	234.69 (1.47)
Group III- Apical periodontitis pathology	220.4	168	194.2 (1.31)

Our results confirm previous studies, which revealed that smoking had an increasing effect on the concentration of salivary thyocianate and a decreasing effect on the content of iodide in saliva [9]. As a consistence with other studies, were the smoking represented a major risk factor associated with chronic destructive periodontal disease [7], our results confirmed that the mean average of salivary iodide decreased significantly on group no II (chronic marginal periodontitis), in comparison with group no I (simple carious lesions) and III (apical periodontitis), where the levels were quite elevated.

CONCLUSION

The obtained results demonstrated in the control group, an average mean salivary iodide of 87.51 mcg/l, which can represent an useful measurement for further studies. However, it must be noticed the fact that salivary iodide levels were never performed within Romania territory until so far. In the studied groups, there was an expected statistically significant difference between the smokers and non- smokers groups; moreover, our findings have shown that the mean average of salivary iodide decreased significantly on group no II (chronic marginal periodontitis). In order to validate our current results, there should be performed further studies on larger study groups.

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