Measurement of Salivary Peroxidase Values in Unstimulated and Stimulated Whole Saliva in a Dental Student Population

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Summary

In 28 young, healthy individuals the quantity of salivary peroxidase was determined with the aim of evaluating normal ranges for salivary peroxidase, to determine whether any differences in SP values between unstimulated and stimulated whole saliva exist and to find any possible differences between sexes.

We can conclude that no statistically significant differences between salivary peroxidase values in unstimulated and stimulated whole saliva were found. No statistically significant differences were found between female and male individuals in salivary peroxidase values, either in unstimulated or stimulated whole saliva.

Key words: *salivary peroxidase, saliva.*

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Introduction

Saliva is an essential component required for maintenance of ecologic balance in the oral cavity. Human whole saliva contains two peroxidase enzymes which are considered important mucosal defence factors. Major salivary glands secrete salivary peroxidase which is structurally somewhat different but catalytically quite similar to bovine milk lactoperoxidase (1). Oral polymorphonuclear leucocytes release myeloperoxidase into gingival crevicular fluid and whole saliva in amounts proportional to the degree of gingival inflammation (2). The salivary peroxidase (SP) system is one of the non-immunoglobulin defense systems in saliva. It is composed of the salivary peroxidase enzyme, thiocyanate (SCN) and hydrogen peroxide. Salivary peroxidase can reversibly inhibit bacterial enzyme and transport systems by oxidation of protein sulfhydryl groups (with extended incubation this effect can be made irreversible). Very low concentrations of HOSCN/OSCN have been shown to be effective in elimination of viruses which are transmitted orally, such as herpes simplex 1, respiratory sintitial virus, echovirus type 11 and HIV (3-5). The aim of our study was to determine whether there are any differences betweeen salivary peroxidase values in unstimulated and stimulated whole saliva in young, healthy male and female adults.

Materials and methods

A group of 28 students from the fifth year were recruited for this study. Age range was 23 - 27, mean 25. All the participants had healthy oral mucosas and were generally healthy. Quantification of saliva was determined with two measurements between 8 - 11 AM. The unstimulated whole saliva was obtained by the simple method of spitting the collected saliva into calibrated tubes (0.1 ml) for five minutes. The stimulated whole saliva was obtained after drinking 1% of ascorbic acid solution (1g dissolved in 1dcl of water) and then by spitting into calibrated tubes for the next five minutes. the results were expressed per one minute. Saliva samples were centrifuged (800 turns) during ten minutes and then frozen at -20°C (6). The salivary peroxidase values were determined according to Putter and Becker (7). The reagents used for salivary analysis were 20 mM 2.2 azino-di-(3-etil-benzotiazolin-(6)-sulphonic acid) diammonium acid (ABTS) in 67 mM phosphate buffer pH value 6.0 and 10 mM hydrogen peroxide and peroxidase in quantity of 250 J/kg. The assay was based on 0.5 ml of saliva sample which was diluted with 1.5 ml of phosphate buffer pH value 6.0. Reactive mixture consisted of 2 ml of diluted saliva sample, 0.2 ml of ABTS solution and 0.2 ml of hydrogen peroxide which were mixed in the reactive civette and put into spectrophotometer 405 nm wavelength and a temperature of 25°C. The apsorbance was red after the first and fifth minute with the reagent as a blind trial. The apsorbance difference (delta T = 5 min) was used for reading the activity of peroxidase in saliva from the calibrated curve. The calibrated curve is done from the basic solution of peroxidase (2500 J/ml) in 6 dilutions which contain 5, 10, 15, 20, 30, 50 J/ml. According to the process described above the apsorbance difference is measured for every sample in duplicate and calibrated curve made. Statistical analysis consisted of Student t test.

Results

Figure 1. shows differences in the salivary peroxidase values between unstimulated and stimulated whole saliva.

Table 1. shows ranges for unstimulated and stimulated whole salivary peroxidase values.

Table 2. shows ranges of salivary peroxidase values determined in other studies.

Discussion and conclusion

SP and SCN are normal components of human saliva. The enzyme is synthesized and secreted by acinar cells, whereas SCN is concentrated in the salivary glands from serum and subsequently secreted. H_2O_2 is derived from bacterial and leucocytic metabolism. In saliva SP catalyzes the oxidation of SCN to produce hypothiocyanous acid (HOSCN) and the hypothiocyanate anion (OSCN). This reaction has an important consequence for the host because the HOSCN and OSCN inhibit the growth and metabolism of many species of pathogens. In addition to its antimicrobial properties, the SP system also protects the host cells from hydrogen peroxide toxicity (8, 9).

Previous studies (10-13) have shown that the quantity of salivary peroxidase is increased in stimulated whole saliva in healthy individuals and the authors have concluded that short term increase in SP activity is a result of increased salivary secretion, following gland stimulation. On the other hand, antimicrobial potential of salivary peroxidase is significantly lower in stimulated saliva when compared to unstimulated, indicating its highest activity during sleep (14, 15). The results of this study indicate that the quantity of salivary peroxidase is not statistically significantly changed in stimulated saliva when compared to resting in healthy young individuals. Also no statistically significant differences were found between female and male young adults either in unstimulated or stimulated whole salivary peroxidase values. Laine et al. (16) reported that specific activity of salivary peroxidase increased significantly during the third trimester of pregnancy, thus supporting the concept of oestrogen-dependency of this enzyme.

In literature some information can be found on salivary peroxidase in various systemic diseases and conditions. Ryberg et al. (17) found that salivary peroxidase quantities were decreased in patients with asthma when compared to a control group. Lenander-Lumikari et al. (18) found no differences in the salivary peroxidase values in stimulated whole saliva of patients with asthma and healthy

individuals. A study of Mansson-Rahemtulla et al. (19) showed that patients with leukemia had statistically significantly elevated total peroxidase and SP activity in whole stimulated saliva when compared to controls. Sundh et al. (20) did not find any differences in the salivary peroxidase values in patients suffering from Crohn's disease when compared to healthy controls. Guven et al. (21) reported that elevated salivary peroxidase values were found in patients suffering from diabetes mellitus when compared to a control group. Nederfors et al. (22) did not find any differences in salivary peroxidase values between patients taking antihypertensive drug captopril and controls. Further studies are needed in order to evaluate normal ranges for salivary peroxidase values in unstimulated and stimulated saliva of healthy individuals as well as to determine whether changes in salivary peroxidase values occur in systemic diseases as well as in oral diseases.