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Original Article

Salivary defense system alters in vegetarian

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ARTICLE INFO

Article history:
Received 29 March 2013
Accepted 20 May 2013

Keywords:
Saliva
Antimicrobial activity
Antioxidant enzymes
Vegetarian

ABSTRACT

Purpose: The aim of this research was investigating antimicrobial and enzymatic antioxidant activities in salivary fluids of vegetarians as compared to normal subjects.
Material & Methods: Antimicrobial activity of the saliva samples was evaluated against four clinically important bacteria. The biological activities of three of the main antioxidant enzymes of saliva were measured using appropriate methods of enzyme assay in both groups.
Results: According to the results, saliva obtained from vegetarians showed a reduced inhibitory effect on growth of *Staphylococcus aureus, Klebsiella oxytoca, Pseudomonas aeruginosa* and *Escherichia coli* as compared to those obtained from the non-vegetarian subjects. The activity of salivary peroxidase, catalase and superoxide dismutase showed a statistically marked decrease in vegetarian group.
Conclusions: According to our literature survey, this is the first report on the antibacterial and antioxidant capacity in saliva of vegetarians. Results obtained from the present study have opened a new line of research with the basis of saliva as a research tool.

1. Introduction

Vegetarianism is referred to using plant-based diets excluding meat and including or excluding dairy products, eggs and honey. There are a number of aims including health, religious, political, environmental, cultural, esthetic, economic and ethical reasons that encourage people to become vegetarian. In recent years, scientific research has slightly shifted from concerns about nutritional adequacy to investigating health benefits and disease prevention. It has been shown that at all stages of life, a vegetarian diet is healthful, nutritionally adequate, and provides health benefits in the prevention and treatment of certain diseases. Large-scale studies have shown that mortality from ischemic heart disease was 30% lower among vegetarian men and 20% lower among vegetarian women than in non-vegetarians.[1] It is known that most of necessary nutrients, proteins, and amino acids can be found in vegetables, grains, nuts, soymilk, eggs and dairy.[2] However, as with many long-term non-usual habit, vegetarianism may result in side effects such as reduction in antioxidant and antibacterial activity of various body fluids leading to a decrease in overall defense activity. Saliva is a special body fluid with a rich biochemistry which may be used in various diagnostic investigations.[3] It can protect oral cavity against detritus agents such as microorganisms, toxins and various oxidants.[4] In some special circumstances, the antioxidant capacity and reducing power of saliva is altered.[5] It has been shown that *in vitro* exposure to cigarette smoke could significantly decrease biological activity of some enzymes, both in plasma and in saliva.[6,7] Saliva is secreted by three pair of

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2212-4268/$ – see front matter
http://dx.doi.org/10.1016/j.jobcr.2013.05.004

major salivary glands as well as by hundreds of minor glands located below the mucosal surfaces of the mouth.[3,8] It is composed of complex mixture of substances, similar to other body fluids.[9] Biochemistry of saliva is composed mostly of the locally produced proteins and enzymes together with some other biochemicals including antioxidants, electrolytes, epithelial and immune cells, microorganisms, and bronchial products.[3] Besides, other external substances with varied concentrations are also found in salivary fluid.[10] Having a no invasive type of sampling, the use of saliva as a source of important biomarkers has recently attracted attention of some researchers.[11] It has been shown that biochemical composition can also reflect the causes of some systemic disease.[12] Human saliva can reflect the relationship between oral hygiene and some chronic systemic diseases[13] as well as intensity of exercise.[14]

2. Materials and methods

2.1. Saliva collection, flow rate and storage

Twenty-five female university students aged 22–25 years who had become vegetarian from 4 to 8 years ago together with 25 non-vegetarians volunteered entered into our study. The subjects did not suffer from internal or genetic diseases and they had healthy teeth and gums. They agreed to donate their saliva according to the instruction given to them and signed a consent. They were asked to fill a form about their health background and various aspects of life and history of becoming vegetarian. Their un-stimulated saliva samples (3 ml) were collected in clean and dry sterile pre-weighted tubes after 8 h of fast and rinsing their mouth with distilled water.[14] The time in minutes for collecting one ml of saliva was taken as flow rate. The collected samples were immediately centrifuged at 800× *g* for 10 min at 4 °C to remove squamous cells and cell debris. The resulting supernatant was stored at −18 °C until examination for antimicrobial and enzyme activity.

2.2. Selection of microorganisms and preparation of growth medium

Antimicrobial studies were carried out using four microorganisms which included one Gram-positive: *Staphylococcus aureus* (PTCC 1133) and three Gram-negatives: *Klebsiella oxytoca* (PTCC 1402), *Escherichia coli* (PTCC 1553), and *Pseudomonas aeruginosa* (PTCC 1558). All strains were obtained from Persian Type Culture Collection (PTCC) centre in Tehran and stored frozen at −80 °C. The bacterial vials were opened as recommended by PTCC and the strains were grown in nutrient broth followed by incubation at 37 °C for 4 h. Disk diffusion method was used in order to evaluate *in vitro* antibacterial activity of the saliva samples with a few known antibiotics. In practice, broth subcultures were prepared by inoculating, with one single colony from a plate, a test tube containing 5 ml of sterile nutrient broth. The tubes were then incubated at 37 °C for at least 24 h.

The bacterial culture was allowed to reach a concentration of 10^8 CFU/ml. Each suspension was spread on Muller Hinton Agar medium by sterile swabs. Whitman filter paper disks (diameter 6 mm) dipped in saliva samples and two known antibiotics, i.e. gentamicin and ampicillin (Padtan Teb™, Iran) were placed on the agar surface. The diameter of inhibition zone was measured after incubation of all plates at 37 °C for 24 h. The growth inhibition zone is referred to as the diameter of the clear zone surrounding the filter paper disks which also includes the size of disk itself. The inhibition zones (including disk diameter) less than 10 mm were negative. Zone calculation was the average of three measurements. The *t*-test was used with the GraphPad PRISM® software (GraphPad Software, Inc., San Diego, CA, USA) and $p \leq 0.05$ differences were considered as significant.

2.3. Peroxidase assay

The biological activity of enzyme on 4-amino antipyrine was measured spectrophotometrically. The oxidation of 4-amino antipyrine was measured at 25 °C in 3 ml of 0.3 M phosphate buffer, pH 7.4, containing 0.0010 M hydrogen peroxide, 0.002 M 4-amino antipyrine and 0.15 M phenol. Forty μl of saliva samples was then added and the change in absorption at 510 nm (ΔA/min) was recorded. The change in absorption at 510 nm is due to the formation of a chromogen product with a λ_{max} at 510 nm. One unit of activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under standard conditions.

2.4. Catalase assay

Salivary catalase activity was measured using EnzyChromTM Catalase Assay Kit (ECAT-100) designed for direct assay of catalase in serum, saliva and urine. The method was based on degradation of H_2O_2 by catalase using a redox dye. The change in color intensity at 570 nm, proportional to the catalase activity in the sample, was measured spectrophotometrically. One unit of catalase activity was the amount of catalase that decomposes 1 mol of H_2O_2 per min at pH 7.0 and room temperature. The biological activity of the enzyme was expressed as U/ml.

2.5. Superoxide dismutase (SOD) assay

The activity was measured using a special superoxide dismutase kit made by R&D Systems Europe, Ltd. for research purposes, Catalog number: 7500-100-K. In this assay, superoxide ions (O_2^-), generated by xanthine oxidase (XOD) conversion of xanthine to uric acid and hydrogen peroxide, converts NBT to NBT-diformazan, which absorbs light at 560 nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in a saliva sample. One unit of SOD inhibits the rate of increase in absorbance at 550 nm by 50% under the conditions of the assay. The percent inhibition of the saliva sample correlated with SOD activity using an SOD standard curve.

2.6. Statistical analysis

Each assay was repeated at least in duplicate and the results were presented as mean ± SD values. Statistical difference