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Assessment and comparison of phagocytic function and viability of polymorphonuclear leukocytes in saliva of smokers and non-smokers

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ABSTRACT

OBJECTIVES: Tobacco use is one of the most important public health problems worldwide. It is also linked to impairment of normal immunologic surveillance and defence mechanism of polymorphonuclear leukocytes. Tobacco smoke and its components have been seen to affect the phagocytic ability and viability of polymorphonuclear leukocytes suggesting the pathogenesis of tobacco induced oral diseases. Aim of this study was to assess and compare the phagocytic function and viability of polymorphonuclear leukocytes in saliva of smokers and non smokers.

DESIGN: The study comprised of 35 smokers and 35 non-smokers, age matched. Saliva was collected by rinsing method and the polymorphonuclear leukocytes were separated. Phagocytic activity was determined by using latex spheres as targets. Cell viability was measured using trypan blue stain.

RESULTS: Salivary polymorphonuclear leukocytes in smokers showed significant reduction in the phagocytic activity by ingesting few latex spheres when compared to the non-smokers. The viability of these cells in saliva of smokers was significantly reduced.

CONCLUSIONS: The present study revealed reduced phagocytic activity and viability of salivary polymorphonuclear leukocytes in smokers compared to non-smokers. These findings indicate that smokers are more prone to gingival, periodontal and other oral diseases. Thus indicating that the health care professionals should encourage smoking cessation as an aid in preventing oral diseases.

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Abbreviations: PMNs, Polymorphonuclear leukocytes; HBSS, Hanks balanced salt solution.
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1. Introduction

Tobacco has been variously hailed as a gift from the gods, a miraculous cure-all for life's physical illness, a solace to the lonely soldier or sailor, a filthy habit, a corrupting addiction and the greatest disease producing product known to man.[1] Tobacco smoking is one of the most common deleterious habits worldwide. It is considered as a psychologically motivated and socially conditioned habit rather than addiction.[2] The death toll from tobacco consumption is now 5 million people a year; if present consumption patterns continue, the number of deaths will nearly double, reaching close to 10 million by the year 2020. This higher burden is rapidly shifting to developing countries. In the developing world, tobacco consumption is rising by 3–4% per year. Tobacco leaf is the main source which is used in various forms like smoking, chewing, snuffing etc. A burning cigarette is a chemical cocktail containing more than 4000 harmful chemicals such as carbon monoxide, polycyclic hydrocarbons, beta naphthylamine, nitrosamine, nicotine etc. which have adverse effects on the human body. Smoking has been directly linked to diseases of cardiovascular, pulmonary and gastrointestinal system.[3] Tobacco use has been identified as a potential risk factor for gingival, periodontal disease, acute necrotizing ulcerative gingivitis, candidiasis, caries, halitosis, pre-cancerous lesion, oral cancer etc.

PMNs constitute the first line of defence against all forms of injury and microbial challenge throughout the body. In the resting uninfected host, production and elimination of PMNs are balanced resulting in fairly constant concentration of these cells in blood. When infection occurs, chemotactic agents are generated, that result in migration of PMNs to the site of infection and activation of their defensive function. Salivary PMNs contribute to a major part in the local immunologic mechanism in the oral cavity involved in defence against periodontal and oral diseases.[4] PMNs in saliva are derived from gingival crevicular fluid or secreted in whole saliva. On an average 1 million PMNs per minute enter the oral cavity. These cells protect the gingiva against microbial invasion via processes like chemotaxis, phagocytosis, bacterial killing etc.[5] Any impairment in these functions would predispose an individual to various oral diseases. An increased prevalence of gingival, periodontal disease and other oral diseases have been noted in chronic smokers. This would probably suggest the role of tobacco and its water soluble compounds in the pathogenesis of various oral diseases. An alteration in defensive property of PMNs by agents like nicotine affects the phagocytic function and promotes bacterial colonization in the oral cavity. The negative effect of smoking on PMNs was first described by Eichel and Sharick who reported reduced function and mobility of these cells in smokers. But these proposals are still inconclusive.[6]

In view of these facts, the present study was undertaken to assess and compare the phagocytic function and cell viability of salivary PMNs in smokers and non-smokers.

The objectives of this study were assessment of phagocytic function of PMNs in smokers and in non-smokers, assessment of cell viability of PMNs in smokers and in non-smokers and comparison of the phagocytic function and cell viability of PMNs between smokers with that of non smokers.

2. Materials and methods

Patients reporting to the outpatient Department of Oral Medicine, Diagnosis and Radiology at KLE's VK Institute of Dental Sciences, Belgaum, India were included in this study. The study was independently reviewed and approved by the ethical board of the university.

2.1. Subjects

70 males between the age group of 19–35 years were included and divide into 2 groups –

Group I: 35 smokers and Group II: 35 non-smokers.

Group I comprised of subjects with a history of smoking at least 1 pack of cigarette/day since at least one year. Subjects with any systemic diseases like diabetes, hypertension, asthma, epilepsy, Down's syndrome, oral diseases like leukoplakia, oral submucous fibrosis etc., subjects on any systemic or topical medications like steroids, sulfonamides, quinidine etc. and subjects with habit of chewing tobacco or betel nut were excluded. Group II comprised of subjects with no history of smoking.

The study was conducted with an understanding and written consent of each subject. Saliva samples were collected from each subject in both the groups.

2.2. Method of salivary sample collection and separation of PMNs

Unstimulated whole saliva was collected. Subjects rinsed their mouth with water 5 min before the collection of the sample and then with 5 ml of Hank's balanced salt solution (HBSS) for 30 s. This was collected into a sterile container. The procedure was repeated once again for each subject. The samples were processed immediately. The consecutive rinses were combined and collected saliva sample was centrifuged for 10 min at 200 rpm. The sediment was washed once by resuspension in HBSS and recentrifugated. The resulting sample consisted of PMNs, epithelial cells and bacteria.

2.3. Phagocytic function

PMNs phagocytic function was assessed using latex spheres of 0.81 μm. The source of latex spheres were from Sigma–Aldrich Chemicals Private Limited, Bangalore. The sample consisting of PMNs, epithelial cells and oral bacteria were suspended using a vortex mixer in 1 ml mixture of a pooled human serum with 1 mg/ml dextrose and latex spheres of 0.81 μm diameter. The mixture of PMNs and latex spheres were incubated in 3 ml open test tubes at 37 °C and high humidity for 45 min. Samples were taken from the mixture before and after incubation to make small preparations stained with Giemsa. The final preparations were studied under optical microscope at 1000 magnification. Latex spheres within the boundaries of 100 PMNs in each preparation were counted. The number of spheres taken up per cell was calculated as the difference in