Relationship of Glutathione Status with Oral Pre-cancer and Cancer

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Abstract
Oral cancer accounts for the majority of the cancer in South East Asian region and especially in the Indian subcontinent. If diagnosed at an early stage the disease is reported to have a better prognosis. Sialochemistry involving antioxidants has recently gained importance for oral cancer risk assessment because of its non-invasive nature. The aim of this study was to estimate the levels of glutathione in saliva of patients with oral cancer, oral leukoplakia and healthy controls. Three study groups comprising of, 25 oral cancer patients, 25 oral leukoplakia patients and 25 healthy controls were involved in the study. Saliva sample collected from the patients were evaluated by Beutler's method. The data obtained were analyzed using the one way ANOVA test. There was a significant difference between the levels of salivary glutathione in between oral cancer, oral leukoplakia groups when compared to healthy controls. The levels of salivary glutathione were lower in oral cancer when compared to oral leukoplakia but the difference was not statistically significant. The results suggest that salivary gluthione level estimation could be used for determine the risk of oral cancer as a non-invasive modality.

Key words: *Saliva, glutathione, oral cancer, leukoplakia.*

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Introduction

Oral cancer (OC) is the most common cancer in South Asia and accounts for 40% of all malignancies in the Indian Subcontinent [1]. Several oral lesions such as leukoplakia (OL), erythroplakia and lichen planus carry an increased risk for malignant transformation to OC in the oral cavity [2]. Smoking and alcohol intake are the best defined and extensively researched risk factors for OC [3]. DNA damage, occurring as the result of chemical attack originating from these agents, in large part by products of oxidative metabolism and in particular by reactive oxygen species (ROS), is probably the most frequent potentially mutagenic spontaneous event [4]. The scavenging of the ROS is done by enzymatic and non-enzymatic antioxidants [5]. The enzymatic antioxidant includes Superoxide Dismutase, catalase, and glutathione peroxidase. The non-enzymatic anti-oxidants include lipid soluble vitamins, vitamin E, vitamin A, water soluble vitamin C and glutathione [5].

Glutathione (GSH) acts as both, a nucleophile and a reductant, and can therefore react with electrophilic or oxidizing species before the latter interact with more critical cellular constituents such as nucleic acids and proteins [6]. Glutathione directly scavenges free radicals, sometimes act as a substrate for glutathione peroxidases and glutathione S-transferases during the detoxification of ROS [7,8]. Recently studies have been carried out on salivary glutathione levels in patients with diabetes, dental caries and head and neck squamous cell carcinoma [3,9,10]. The aim of our study was to evaluate the levels of salivary glutathione in oral leukoplakia and oral squamous cell carcinoma patients.

Materials and Methods

The study involved 75 subjects with age range of 20 to 65 years reporting to the Department of Oral Medicine and Radiology. Twenty-five clinically diagnosed and histopathologically confirmed cases of oral leukoplakia formed the first group (OL). Of the 25 cases twenty three cases were with moderate dysplasia and two cases with severe dysplasia. Twenty-five histopathologically confirmed of oral cancer (OC) cases were enrolled into the second group. Twenty-five age and sex matched healthy controls formed the third group (HC). Each of the study groups had five female subjects. Patients with diabetes mellitus and patients with history of previous malignancy were also excluded from the study. Unstimulated saliva samples were collected from each of the patients by the spit method in the calibrated test tube. Care was taken to see that the volunteers did not consume food, smoke or chew gum at least

one hour before the saliva collection procedure. Salivary GSH levels, which were determined by the method of Beutler.[11] 100µL of filtered saliva was diluted with 1 mL of distilled water. 500 µL the mixture was taken and to this 2mL of phosphate solution and 250 µL of DTNB [5,5-Dithiobis (2 nitrobenzoic acid)] solution was simultaneously a blank is maintained containing 200 µL of distilled water, 300 µL of precipitating solution, 2 mL of phosphate solution and 250 µL of DTNB. The intensity of yellow color formed was spectrophotometrically read at 412nm against blank. The optical densities obtained were plotted against the standard graph. The concentration of glutathione was calculated graphically and multiplied with respective dilution factors and the total glutathione in the sample was expressed as µg/mL. The data obtained were subjected to statistical analysis using SPSS version 17 software. One-way ANOVA was used to compare the data among the groups and the differences were considered statically significant if P values were 0.05 or less.

Results

The mean salivary glutathione in Group OL was 0.52 ± 0.21 µg/mL. Whereas in group OC the mean salivary glutathione level was 0.47 ± 0.23 µg/mL, the mean salivary glutathione level of the group HC was 0.94 ± 0.36 µg/mL. The mean salivary glutathione levels of the male subjects in the study groups OL, OC and HC were 0.54 ± 0.27 µg/mL, 0.47± 0.28 µg/mL and 0.97 ± 0.19 µg/mL respectively (Figure 1). Whereas the mean salivary glutathione levels of the female subjects in study groups OL, OC and HC were 0.51 ± 0.07 µg/mL, 0.46 ± 0.19 µg/mL and 0.94 ± 0.23 µg/mL respectively. When the mean salivary glutathione level of group OL was compared to group HC a statistically significant difference (p=0.001) was observed (Figure 2). The mean salivary glutathione level of group OC was higher than group OL but the difference was not statistically significant (p=0.1). The mean salivary glutathione level of OC group was significantly lower (p=0.001) than the HC group (Figure 3).

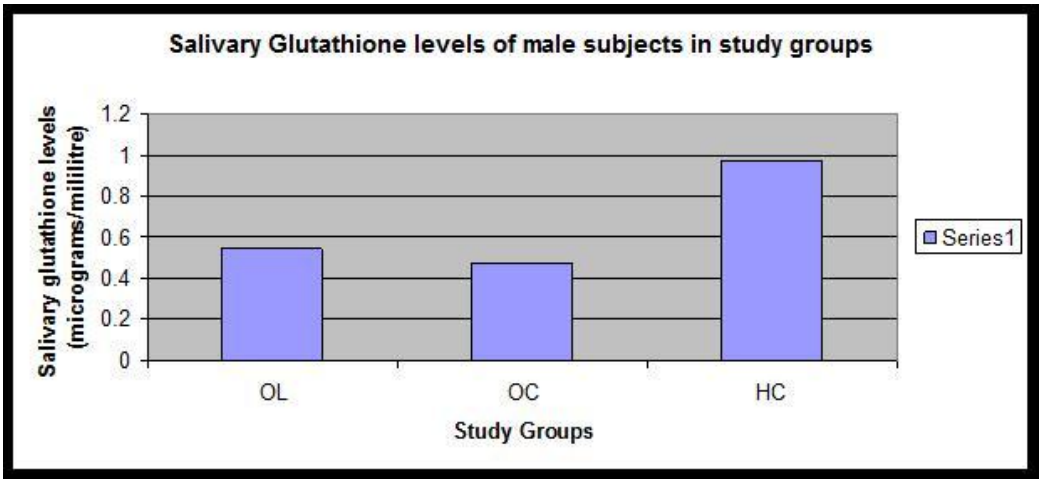

Figure 1. Salivary glutathione levels of male subjects in study groups.

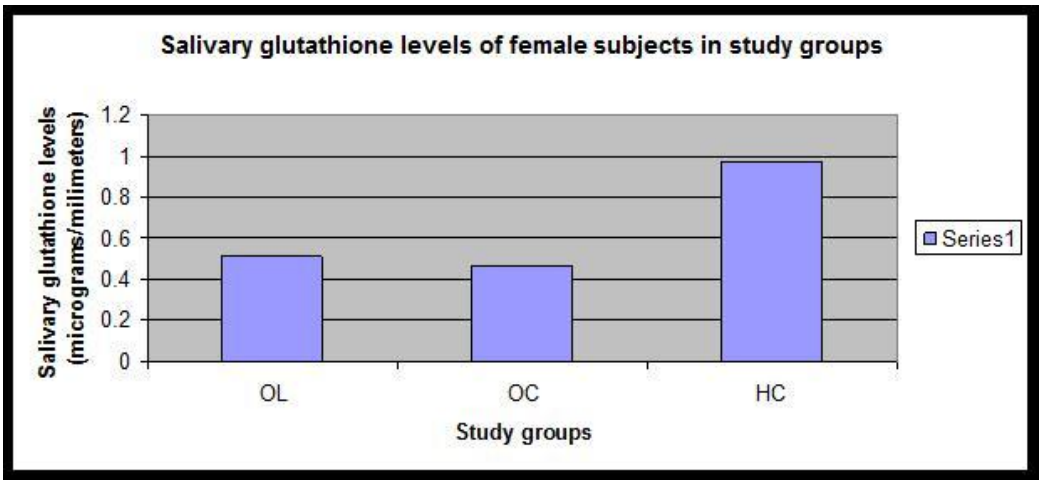

Figure 2. Salivary glutathione levels of female subjects in study groups.

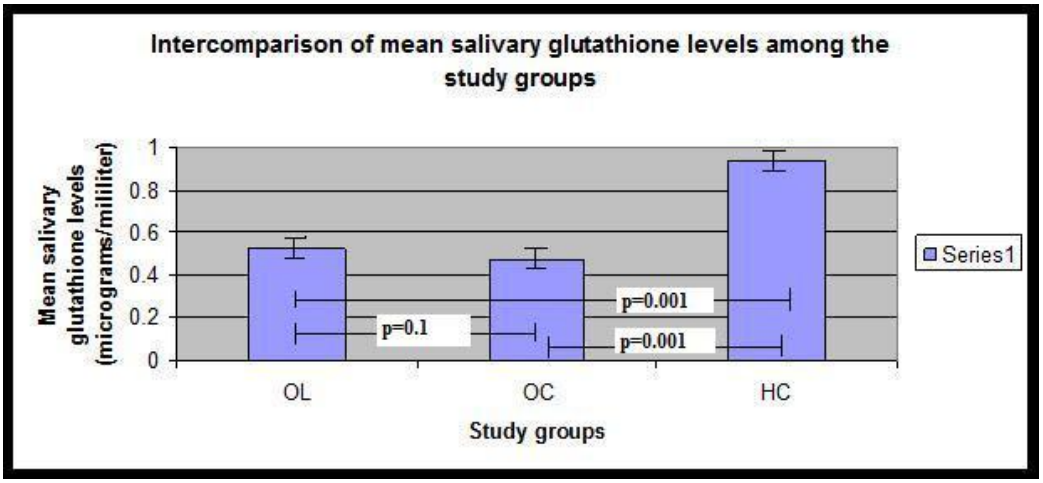

Figure 3. Intercomparison of mean salivary glutathione levels among the study groups.

Discussion

Glutathione (GSH) is a major intracellular antioxidant and hence plays a major role in cancer prevention [12]. GSH acts as first line of defense against oxidative stress [13]. Few studies have shown a reduced occurrence of polycyclic aromatic hydrocarbon induced oral cancer in experimental animals fed with diet rich in glutathione [14,15]. GSH detoxifies carcinogens by Phase II carcinogens and also regulates immune system by mitogenic response and lymphocytic proliferation [16,17]. Several serum studies have revealed decrease in the levels of plasma GSH levels in oral cancer [18]. In one Indian study the levels of plasma glutathione consistently reduced in the advanced stages of oral cancer when compared to initial stages [19]. Utilization of the glutathione by tumor tissues or by excessive oxidation in circulation was the probable reason for the depletion of gluthione in plasma [19]. Recently few studies have been carried out on salivary glutathione levels in smokers, dental caries and oral cancer [3,5,20]. In our study we evaluated salivary glutathione in oral cancer and pre-cancer patients. We found reduced glutathione levels in both oral cancer and pre-cancer study groups. This depletion could be due to an imbalance in the free-radical and antioxidant homeostasis in the body fluids that occurs during carcinogenesis. Although statistically insignificant the salivary glutathione levels in oral cancer patients were higher than patients with leukoplakia. This results obtained from this study suggests that salivary glutathione could be used as non-invasive screening tool for individuals with risk of oral cancer.

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