

# Serum Glutathione Levels in Oral Leukoplakia and Oral Squamous Cell Carcinoma- A Clinicopathological Study

Shishir Ram Shetty<sup>1\*</sup>, Subhas Babu<sup>1</sup>, Suchetha Kumari<sup>2</sup>, Pushparaja Shetty<sup>3</sup>, Vijay R<sup>4</sup>, Arvind Karikal<sup>1</sup>

<sup>1</sup>Department of Oral Medicine and Radiology, AB Shetty memorial Institute of Dental Science, Nitte University, Mangalore, India

<sup>2</sup>Department of Biochemistry, KS Hegde Medical Academy, Nitte University, Mangalore, India

<sup>3</sup>Department of Oral Pathology, AB Shetty memorial Institute of Dental Science, Nitte University, Mangalore, India

<sup>4</sup>Central research laboratory, Nitte University, Mangalore, India

\*Corresponding author: drshishirshettyomr@yahoo.com

Received December 30, 2013; Revised February 01, 2013; Accepted February 25, 2013

**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. Recently studies have been conducted to determine the levels of serum antioxidants for risk assessment of oral cancer. The aim of this study was to estimate the levels of glutathione in serum 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. Serum sample collected from the patients by venipuncture 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 serum glutathione in between oral cancer, oral leukoplakia groups when compared to healthy controls. The levels of serum glutathione were lower in oral cancer when compared to oral leukoplakia but the difference was not statistically significant. The results suggest that serum glutathione level estimation could be used to determine the risk of oral cancer.

**Keywords:** serum, glutathione, oral cancer, leukoplakia

## 1. 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 detrimental effects of certain agents, in large part by the products of oxidative metabolism and in particular by reactive oxygen species (ROS), are probably the most potential spontaneous mutagenic events [4]. The scavenging of the ROS in physiological system such as blood and tissues is carried out by enzymatic and non-enzymatic antioxidants [5]. The enzymatic antioxidant includes superoxide dismutase, catalase, 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, and also serves as a substrate for glutathione peroxidases and glutathione S-transferases during the detoxification of

ROS [7,8]. Recently studies have been carried out on 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 serum glutathione in oral leukoplakia and oral squamous cell carcinoma patients.

## 2. 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). 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. 5ml of venous blood was collected from the antecubital vein by venipuncture. Serum GSH levels, were determined by the method of Beutler [11]. 100µL of filtered serum was diluted with 1mL of distilled water. 500µL of the diluted serum was taken and to this 2mL of phosphate solution and 250µL of DTNB [5,5-Dithiobis (2 nitrobenzoic acid)] reagent were added. Simultaneously (use capital letter) a blank was maintained containing 200µL of distilled water, 300µL of precipitating solution,

2mL of phosphate solution and 250 $\mu$ L of DTNB. The intensity of yellow colour formed was spectrophotometrically read at 412nm against blank using "Cytronics 106"(wavelength 340-960nm). 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 mmol/L. 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 statistically significant if P values were 0.05 or less.

### 3. Results

The mean serum glutathione in group OL was  $0.104 \pm 0.22$ mmol/L. Whereas in group OC the mean serum glutathione level was  $0.94 \pm 0.27$ mmol/L. The mean serum glutathione level of the group HC was  $1.88 \pm 0.36$ mmol/L. The mean serum glutathione levels of the male subjects in study groups OL, OC and HC were  $0.94 \pm 0.28$ mmol/L,  $0.72 \pm 0.28$ mmol/L and  $1.97 \pm 0.17$ mmol/L respectively (Figure 1). Whereas the mean serum glutathione levels of the female subjects in study groups OL, OC and HC were  $0.91 \pm 0.07$ mmol/L,  $0.86 \pm 0.19$  mmol/L and  $1.74 \pm 0.27$ mmol/L respectively (Figure 2). The serum glutathione levels of female subjects were lower than male subjects but not statistically significant. When the mean serum glutathione level of group OL was compared to group HC a statistically significant difference ( $p=0.001$ ) was observed. The mean serum glutathione level of group OC was higher than group OL but the difference was not statistically significant ( $p=0.1$ ). The mean serum glutathione level of OC group was significantly lower ( $p=0.001$ ) than the HC group (Figure 3).

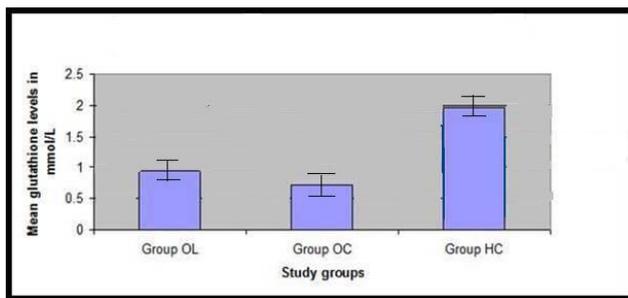


Figure 1. Mean serum glutathione levels in male study subjects

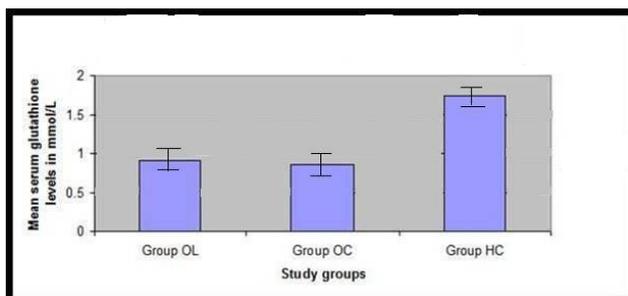


Figure 2. Mean serum glutathione levels in female study subjects

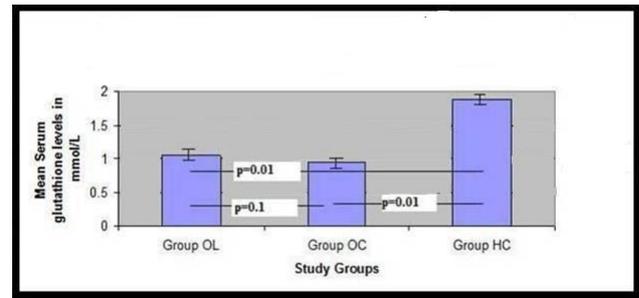


Figure 3. Intercomparison of mean serum glutathione levels in the study groups

### 4. 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 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 were the probable reason for the depletion of glutathione in plasma [19]. Recently few studies have been carried out on salivary glutathione levels in smokers and in patients with dental caries and oral cancer [3,5,20]. These studies showed alterations in glutathione levels in study groups when compared to controls. In our study we evaluated serum glutathione in oral cancer and precancer patients. We found reduced glutathione levels in both oral cancer and precancer 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 serum glutathione levels in oral cancer patients were higher than patients with leukoplakia. The results obtained from this study suggests that the altered levels of serum glutathione could be used as an ancillary screening tool for individuals with risk of oral cancer.

### References

- [1] Parkin DM, Pisani P, Ferlay J. Estimates of worldwide incidence of 25 major cancers in 1990. *Int J Cancer*. 1999; 80:827-841.
- [2] Hirshberg A, Calderon S, Kaplan I. Update review on prevention and early diagnosis in oral cancer. *Refuat Hapeh Vehashinayim*. 2002;19(3):38-48, 89.
- [3] Almadori G, Bussu F, Galli J, Limongelli A, Persichilli S, Zappacosta B, Angelo Minucci A, Paludetti G, Giardina B. Salivary glutathione and uric acid levels in patients with head and neck squamous cell carcinoma. *Head Neck* 29: 648-654, 2007.
- [4] Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis* 2000;21:361-370.
- [5] Bathi RJ, Rao R, Muthalik. GST null phenotype and antioxidants; risk indicators for oral precancer and cancer. *Indian J Dent Res*; 20(3): 298-302.

- [6] Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 1999;31 :273-300.
- [7] Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol* 2003;66:1499-1503.
- [8] Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clin Chim Acta* 2003;333:19-39.
- [9] Öztürk LK, Furuncuoğlu H, Atala MH, Uluköylü O, Akyüz S, Yarat A. Association between dental-oral health in young adults and salivary glutathione, lipid peroxidation and sialic acid levels and carbonic anhydrase activity *Braz J Med Biol Res*, 2008; 41: 956-95.
- [10] Gümüş P, Buduneli N, Çetinkalp S, Hawkins SI, Renaud D, Kinane DF, Scott DA Salivary Antioxidants in Patients With Type 1 or 2 Diabetes and Inflammatory Periodontal Disease: A Case-Control Study. *J Periodontol*, 2009; 80(9):1440-1446.
- [11] Beutler E. Glutathione in red blood cell metabolism. In: Beutler E (Editor), *A manual of biochemical methods*. 2nd edn. New York: Grune and Stratton; 1975. p 112-114.
- [12] Locigno R, Castronovo V: Reduced glutathione system: role in cancer development, prevention and treatment (review). *Int J Oncol* 2001; 19: 221-236.
- [13] Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 1999; 27: 916-921.
- [14] Trickler D, Shklar G, and Schwartz J: Inhibition of oral carcinogenesis by glutathione. *Nutr Cancer* 1993; 20: 139-144.
- [15] Schwartz JL and Shklar G: Glutathione inhibits experimental oral carcinogenesis, p53 expression, and angiogenesis. *Nutr Cancer* 1996; 26: 229-236.
- [16] Hamilos DL and Wedner HJ: The role of glutathione in lymphocyte activation: comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1 one by nuclear size transformation. *J Immunol* 1985; 135: 2740-2747.
- [17] Wu G, Fang YZ, Yang S, Lupton JR, and Turner ND: Glutathione metabolism and its implications for health. *J Nutr* 2004; 134: 489-492.
- [18] Richie JP, Kleinman W, Marina P, Abraham P, Wynder EL, Muscat JE. Blood Iron, Glutathione, and Micronutrient Levels and the Risk of Oral Cancer. *Nutr Cancer* 2008; 60: 474-482.
- [19] Manoharan S, Kolanjiappan K, Suresh K, Panjamurthy K. Lipid peroxidation & antioxidants status in patients with oral squamous cell carcinoma. *Indian J Med Res* 2005; 122: 529-534.
- [20] Sathishkumar T, Shanmugam S, Rameshkumar S, Rajavelan G, Haridoss. Characterization of Salivary Glutathione reductase in Normal Individuals and its Implications on Smokers. *Researcher* 2010; 2: 74-81.