Original Article

Evaluation of serum levels of oxidized and reduced glutathione and total antioxidant capacity in patients with head and neck squamous cell carcinoma

ABSTRACT

Background and Aim: Squamous cell carcinoma (SCC) is the most common type of oral cancer, and it is important for it to be diagnosed in early stages. Researchers are interested in exploring the possibility of using biomarkers in the diagnosis of SCC in early stages. One of the detectable biomarkers in the serum is glutathione. Glutathione includes two forms: reduced form (or GSH) and oxidized form (or GSSG). The GSH/GSSG ratio tends to decrease in severe oxidative stress. The aim of this study was to assess the serum levels of GSH and GSSG as well as GSH/GSSG as total antioxidant capacity in patients with head and neck SCC (HNSCC) and to subsequently compare them with healthy controls.

Materials and Methods: Twenty HNSCC patients as well as twenty healthy controls were included in the study. A blood sample of 5 ml was obtained from both the case and control groups. GSH, GSSG, and total antioxidant capacity were measured spectrophotometrically.

Results: No significant difference in the level of GSSG was observed in the patients from the case and control groups (P = 0.796), whereas the level of GSH and GSH/GSSG was significantly lower in the case group (P = 0.002, P = 0.011, respectively). There was no significant relationship between the level of GSH, GSSG, and total antioxidant capacity, on the one hand, and the stage and grade of the tumor, on the other hand.

Conclusion: Since the levels of GSH and GSH/GSSG were significantly lower in the case group, GSH/GSSG could be used as a prognostic factor for the early diagnosis of HNSCC.

KEY WORDS: Glutathione, head and neck squamous cell carcinoma, oxidative stress, total antioxidant capacity

INTRODUCTION

Oral cancer, the sixth most common form of malignancy, has generated great concern in the world.[1] Squamous cell carcinoma (SCC) accounts for more than 90% of oral cancers and though asymptomatic in early stages, it becomes symptomatic while advancing. Thus, diagnosis in early stages is crucial for a better prognosis.[1,2] In recent years, researchers have been interested in developing early diagnosis methods for oral cancers based on biomarkers in the serum.[3] Serum is a biologic environment that shows variations in the level of biomarkers.[4] One of the detectable biomarkers in the serum is glutathione, which is an intracellular peptide with various functions such as detoxification, antioxidant defense, and regulation of cellular proliferation.[5] Glutathione includes two forms: a reduced form (or GSH) and an oxidized form (or GSSG). The GSH/GSSG ratio tends to decrease in severe oxidative stress and the accumulation of GSSG, which leads to decreased body defense against free radicals.[6] Oxidative stress is associated with the increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defense.[7] This phenomenon has an etiologic role in the pathogenesis of many cancers such as lung cancer, colon cancer, bone marrow cancer, and oral cancer.[8] The aim of the present study was to address the following questions: (1) Is there any significant difference in the level of GSH, GSSG, and GSH/GSSG concentration between the case and control groups? (2) Is there any significant difference in the level of GSH, GSSG, and GSH/GSSG concentration between the different stages of the disease? (3) Is there any significant difference in the level of GSH, GSSG, and GSH/GSSG concentration between the different grades of the tumor? (4) Is there any significant relationship between the level of GSH, GSSG, and total antioxidant capacity, on the one hand, and the stage and grade of the tumor, on the other hand?

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ratio (as total antioxidant capacity) between patients suffering from head and neck SCC (HNSCC) and healthy controls? (2) Is there any relationship between GSH, GSSG, and the GSH/GSSG ratio, on the one hand, and the stage and grade of HNSCC, on the other hand?

MATERIALS AND METHODS

The Institutional Ethical Committee of Mashhad University of Medical Sciences approved this cross-sectional study.

Twenty new histopathologically proven HNSCC patients who had not undergone any treatment were selected; in addition, twenty healthy controls voluntarily participated in this study. Written informed consent was obtained from all the participants after explaining the purpose of the study. The patients were selected from Omid hospital, Mashhad, Iran, during a period ranging from October 2012 to April 2013. The patients in the case group suffered from SCC in at least one of the following areas in the oral cavity: tongue, floor of the mouth, larynx, and other areas. Twenty case group patients including five Stage I, seven Stage II, and eight Stage III were evaluated. Furthermore, the patients were divided into three groups: grade I (n = 2), Grade II (n = 16), and Grade III (n = 2). Blood samples (5 ml) were collected from patients of both the groups. The collected samples were then subjected to centrifugation at 2000 rpm for 15 min to segregate plasma and erythrocytes.

Almost all biological samples used for GSH measurement contain a large amount of proteins. It is necessary to remove as much protein as possible from the sample to avoid interferences due to particulates on the assay. Thus, segregated plasma was then deproteinated by metaphosphoric acid and triethanolamine according to the manufacturer's instructions (Glutathione Assay Kit and Antioxidant Assay Kit, Cayman Chemical Co., Michigan, USA). Deproteinized samples were then stored at −80°C until analysis. All of the assays were done by mentioned kits using the method of spectrophotometry.[9]

The exclusion criteria for enrollment of patients involved previously treated cases of oral cancer, a history of daily alcohol abuse, patients with liver or kidney disorders, and any oral lesion in the healthy controls.[10]

Statistical analysis

All of the statistical analyses were performed by SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) using Student’s t-test, Mann–Whitney U-test, and Spearman’s correlation test. In all the tests, P < 0.05 was considered to be statistically significant.

RESULTS

The study included twenty HNSCC cases with an average age of 49 (±11.44). There were nine males and 11 females among the patients, and 19 males and one female among the healthy controls. The most frequent sites of the tumor were tongue (n = 6), larynx (n = 6), followed by floor of the mouth (n = 5) and other areas (n = 3).

There were significant differences in the level of total glutathione (µM), GSH (µM), and GSH/GSSG ratio between the case group patients and the healthy controls (P < 0.001, P = 0.002 and P = 0.011, respectively). However, the level of GSSG (µM) showed no significant difference between the case group patients and the healthy controls (P = 0.796) [Table 1].

Furthermore, in the case group, the Spearman’s correlation test proved that there is no significant correlation between the level of total glutathione, GSH, GSSG, and GSH/GSSG ratio and the stage or grade of tumor [Tables 2 and 3].

DISCUSSION

SCC essentially occurs at the gene level and finally leads to DNA damage that may result in the activation of carcinogens,

**Table 1: Comparison of serum levels of total glutathione, oxidized glutathione, reduced glutathione, and reduced glutathione/oxidized glutathione between the normal controls and head and neck squamous cell carcinoma patients**

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Study groups (mean±SD)</th>
<th>Test results (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case group</td>
<td>Control group</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>6.75±1.51</td>
<td>8.92±2.02</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.10±0.65</td>
<td>5.22±1.92</td>
</tr>
<tr>
<td>GSH</td>
<td>1.64±1.29</td>
<td>3.69±2.34</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>0.322±0.24</td>
<td>0.92±0.94</td>
</tr>
</tbody>
</table>

GSH=Reduced glutathione, GSSG=Oxidized glutathione, SD=Standard deviation

**Table 2: Comparison of serum levels of total glutathione, oxidized glutathione, reduced glutathione, and reduced glutathione/oxidized glutathione among clinical stages of head and neck squamous cell carcinoma patients**

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Stage (mean±SD)</th>
<th>Test results (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>7.44±1.98</td>
<td>6.23±0.88</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.58±0.60</td>
<td>4.73±0.28</td>
</tr>
<tr>
<td>GSH</td>
<td>1.85±1.85</td>
<td>1.50±0.97</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>0.33±0.32</td>
<td>0.32±0.21</td>
</tr>
</tbody>
</table>

GSH=Reduced glutathione, GSSG=Oxidized glutathione, SD=Standard deviation

**Table 3: Comparison of serum levels of total glutathione, oxidized glutathione, reduced glutathione, and reduced glutathione/oxidized glutathione among histopathologic grades of head and neck squamous cell carcinoma patients**

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Grade (mean±SD)</th>
<th>Test results (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>6.33±1.09</td>
<td>6.63±1.56</td>
</tr>
<tr>
<td>GSSG</td>
<td>4.47±0.18</td>
<td>5.18±0.07</td>
</tr>
<tr>
<td>GSH</td>
<td>1.85±1.27</td>
<td>1.45±1.31</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>0.42±0.301</td>
<td>0.27±0.24</td>
</tr>
</tbody>
</table>

GSH=Reduced glutathione, GSSG=Oxidized glutathione, SD=Standard deviation
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overproduction of free radicals, inactivation of enzyme systems, and alteration in the cellular antioxidant defense system. It has been observed that whenever the level of the cellular antioxidant systems goes down or when the oxidants reach abnormally high levels, oxidative damage to the cells occur, finally leading to several pathological conditions, including cancers.[13] In recent years, researchers have been trying to develop ways of diagnosing oral cancer based on biomarkers in the serum including glutathione.[15] GSH detoxifies cells from free radicals and so plays an important role in preventing oxidative damage.[11]

This is the first study to compare the GSH/GSSG ratio (as total antioxidant capacity) in the serum of patients suffering from HNSCC with healthy controls. Moreover, it is important to note that we have evaluated the link between the level of GSH, GSSG, and the GSH/GSSG ratio with tumor grade for the first time. Our analysis revealed no statistically significant difference.

In a case–control study by Srivastava et al., the status of oxidative stress and antioxidant enzymes in the plasma of the patients with oral cancer was compared with the healthy controls of age-, sex-, and habit-matched individuals. Significantly enhanced oxidant with a decrease in antioxidants was observed in the blood samples of oral SCC patients in comparison with the healthy controls. Moreover, the decrease in antioxidant enzymes showed no significant relation with the stage of the tumor in the patients.[11] Our findings are compatible with these results. Karaman et al. evaluated patients with recent histopathologically proven laryngeal cancer (n = 29) and the same age- and sex-matched healthy individuals (n = 21). Serum biomarkers related to oxidative stress were measured using enzyme-linked immunosorbent assay in fasting blood samples of participants. In line with our results, they also found no statistically significant differences in the levels of biomarkers in relation to tumor staging. There was a statistically significant difference in malondialdehyde levels only in Stage II laryngeal cancer. They concluded that in patients with laryngeal SCC, the total antioxidant capacity is impaired in favor of lipid peroxidation and DNA damage.[12]

In Sharma et al.’s study, a significantly lower level of GSH was observed in patients with newly diagnosed tongue SCC (Stage III/IV), compared to control individuals. Early stages of tongue SCC were not evaluated in their study since all of their patients were in advanced stages.[13]

Prabhu and Bhat have evaluated serum total glutathione-s-transferase levels in patients with various stages of oral SCC. They showed that there was a significant increase in total serum glutathione-s-transferase levels in patients with the oral cancer Stage IV compared to Stage II and Stage III patients. They concluded that alterations in total serum glutathione-s-transferase levels may have a role in cancer progression.[14] whereas we showed no significant relation between the serum levels of GSSG and GSH and the clinical stage of tumor in HNSCC patients, which is consistent with the above-mentioned studies.[11‑13]

Therefore, it seems that the levels of GSH and GSSG will not alter during disease progression. It could be argued that the measurement of these biomarkers in the serum is not useful for evaluating disease recurrence in follow-up visits.

CONCLUSION

The present study found no significant relation between the level of GSH, GSSG, and the GSH/GSSG ratio, on the one hand, and the stage/grade of tumor in HNSCC patients, on the other hand. Thus, measuring these biomarkers in the serum may not be useful for evaluating the recurrence of disease in follow-up visits.

In contrast, there was a significant difference in the level of GSH and the GSH/GSSG ratio in the case group patients as compared with the healthy controls. If further studies with a larger sample size confirm these results, GSH/GSSG may be confidently used to diagnose HNSCC in early stages, which would ultimately result in better prognosis.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

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