Indicators of oxidative stress in thyroid cancer

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Ferric reducing antioxidant power (FRAP), myeloperoxidase (MPO) activity and the levels of protein thiols and carbonyls were estimated in the blood samples of thyroid cancer patients (n = 20) before and after thyroidectomy, as well as in healthy controls (n = 10) to study the extent of damage caused by tumor tissue proliferation-induced oxidative stress and to ascertain that oxidative stress levels drop, when there was no proliferation. A significant decrease (p < 0.001) in the levels of serum protein thiols and FRAP as well as a significant increase (p < 0.001) in the levels of protein carbonyls and MPO activity in the blood of thyroid cancer patients before surgery was observed as compared to healthy controls. All the parameters studied also showed a significant difference (p < 0.001) in their respective levels in thyroid cancer patients, pre- and post-thyroidectomy. These findings present the role of oxidative stress as a pathological implication of thyroid cancer.

Keywords: Thyroid cancer, Carboxyl, Thiols, Myeloperoxidase, FRAP, Free radical, Oxidative damage.

The higher eukaryotic aerobic organisms cannot exist without oxygen, yet oxygen is inherently dangerous to their existence. Reactive oxygen species (ROS) in the form of superoxide anion radical, hydrogen peroxide (H2O2) and the extremely reactive hydroxyl radical are common products in an aerobic environment and appear to be responsible for oxygen toxicity1,5. Living organisms have evolved to generate or mobilise from their surroundings a variety of water and lipid-soluble antioxidant compounds. Additionally, a series of antioxidant enzymes are synthesized to intercept and inactivate reactive oxygen intermediates; but, oxidative damage is an inescapable outcome of aerobic life.

Imbalance between free radicals and antioxidants in favour of free radicals has been considered to have carcinogenic potential4 and to promote invasiveness5. Oxidative stress is common in the thyroid tissue during utilization of H2O2 for thyroxine synthesis and when a tumour is proliferating actively6, but H2O2 through its mutagenic effect has been implicated in carcinogenesis in other tissues7. Thus, the hallmark of thyroid physiology-H2O2 production during hormone synthesis is very likely to be the cause of frequent mutagenesis in the thyroid gland8. There is evidence implicating oxidant/antioxidant imbalance in thyroid cancer9,10 and hyperthyroidism11.

In the present study, the role of oxidative stress in thyroid cancer has been investigated by evaluating protein thiols and carbonyls, myeloperoxidase (MPO) activity and antioxidant power (FRAP) in the blood samples of thyroid cancer patients before and after the thyroidectomy operation, and compared with the levels in healthy controls.

Materials and Methods

Chemicals

2, 4, 6-Tris-(2-pyridyl)-s-triazine (TPTZ), 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), 2, 4-dinitrophenylhydrazine (DNPH) and 4-aminoantipyrine were obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other reagents and chemicals used were of analytical grade.

Subjects

Twenty subjects diagnosed with thyroid cancer and consequently scheduled to undergo thyroidectomy in the Department of Surgery, Kasturba Medical College, Manipal, India were selected for the study. Ten healthy subjects, age- and sex-matched were taken as controls and informed consent was obtained from all the subjects involved in the study.

Blood samples were drawn under aseptic conditions into 2 ml plain and 4 ml K2EDTA vacutainers from the antecubital vein of each subject, before and on the 20th day after surgery and processed for serum and plasma, respectively.

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Abbreviations: DNPH, 2, 4-dinitrophenylhydrazine; DTNB, 5, 5'-dithio-bis-(2-nitrobenzoic acid); FRAP, ferric reducing antioxidant power; H2O2, hydrogen peroxide; MPO, myeloperoxidase; ROS, reactive oxygen species; TPTZ, 2, 4, 6-tris (2-pyridyl)-s-triazine
FRAP assay
This method uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily present oxidant in stoichiometric excess is a method of assessing "total antioxidant power". 3000 µl of FRAP reagent was added to 100 µl of plasma and absorbance was measured after incubation at room temperature for 6 min at 593 nm. Ferrous sulphate (FeSO₄·7H₂O) standards (100-1000 µM) were processed in the same manner and FRAP value of the sample (µM) was obtained from the standard curve.

Myeloperoxidase (MPO) activity estimation
Leucocytes from anti-coagulated blood were separated by hypotonic lysis of erythrocytes. The activity of myeloperoxidase (EC 1.11.1.7) was estimated using 4-aminoantipyrine as the hydrogen donor. The increase in absorbance at 510 nm was measured for 5 min at intervals of 1 min. One unit of MPO was defined as the amount of enzyme that catalyzed the transformation of 1 µmol of H₂O₂ per min at pH 6.1 and 25°C.

Protein content was estimated by the method of Lowry et al. using bovine serum albumin as standard.

Serum protein thiol estimation
Serum protein thiols were measured by a spectrophotometric method using DTNB. Absorbance was measured after incubation at room temperature for 5 min at 412 nm. The protein thiol concentration in serum was determined from the glutathione standard curve and expressed in µmol/l for serum protein.

Protein carbonyl estimation
This method employs DNPH, which reacts with the protein carbonyls, forming a Schiff base to produce the corresponding hydrazone, which is analyzed spectrophotometrically. The carbonyl content (nmol/mg) was calculated from peak absorption (355-390 nm) using an absorption coefficient ε of 0.022 nmol/ml and protein content.

Statistical analysis
The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical package for social sciences (SPSS-16). Student’s ‘t’ test was used to compare the mean values of estimated parameters in patients before thyroidectomy with that of healthy controls, while paired samples ‘t’ test was used to compare the values of pre- and post-thyroidectomy patients. A p value of <0.05 was considered statistically significant in both the cases.

Results and Discussion
Mean ± S.D (yrs) of the age of healthy controls (n = 10) and cancer patients (n = 20) were 48.70 ± 8.6 and 48.65 ± 6.6 respectively and they were sex-matched as well. A significant decrease (p<0.001) in the levels of serum protein thiols and FRAP and a significant increase (p<0.001) in MPO activity and the levels of protein carbonyls in the blood of thyroid cancer patients before surgery was observed, as compared to those of healthy controls. All the parameters also showed a significant difference (p<0.001) in their respective levels in the blood of thyroid cancer patients, pre- and post-thyroidectomy (Table 1).

Table 1—Ferric reducing antioxidant power (FRAP) and myeloperoxidase (MPO) activity and the levels of protein thiols and carbonyls of healthy controls and thyroid cancer patients before and after thyroidectomy

<table>
<thead>
<tr>
<th>Oxidant/antioxidant parameters</th>
<th>Healthy controls</th>
<th>Pre-thyroidectomy</th>
<th>Post-thyroidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP (µM)</td>
<td>486.00 ± 76.62</td>
<td>284.5 ± 39.93</td>
<td>430.00 ± 49.63</td>
</tr>
<tr>
<td>MPO (units/mg)</td>
<td>0.607 ± 0.14</td>
<td>1.74 ± 0.35</td>
<td>0.901 ± 0.234</td>
</tr>
<tr>
<td>Serum protein thiol (µmol/l)</td>
<td>377.87 ± 37.39</td>
<td>244.34 ± 27.0</td>
<td>287.02 ± 29.47</td>
</tr>
<tr>
<td>Protein carbonyl (nmol/mg)</td>
<td>4.32 ± 0.24</td>
<td>6.21 ± 0.63</td>
<td>4.90 ± 0.341</td>
</tr>
</tbody>
</table>

*p<0.05 compared to control; **p<0.05 compared to pre-thyroidectomy levels
proliferating tissues like the thyroid gland could be due to oxidative DNA damage caused by the process of thyroid hormone synthesis which involves generation of free radicals and reactive oxygen. Studies also show that ROS may be necessary for survival, appropriate gene expression and Ras/Raf-mediated cell differentiation in the medullary thyroid carcinoma cell model and suggest that ROS may also be required for these functions in other cell types both in vitro and in vivo.

Most of the protein associated thiol groups in serum are found on albumin. These thiol groups are oxidized by electron-deficient free radicals while acting as antioxidants and FRAP is a method of assessing “antioxidant power.” ROS have been implicated as an important cause of oxidative modification of proteins which may lead to their rapid degradation. Among the various oxidative modifications of amino acids in proteins, carbonyl formation may be an early marker for protein oxidation. The levels of protein thiols and carbonyls indicate the degree of oxidative modification of proteins due to the availability of \( \cdot \mathrm{OH}, \ \mathrm{O}_2^- \) or its protonated form (HO\(_2\))

MPO generates ROS endogenously by functioning as an antimicrobial enzyme, catalyzing \( \mathrm{H}_2\mathrm{O}_2 \)-dependent oxidation of chloride ion (Cl\(^-\)) to generate hypochlorous acid (HOCl), a potent oxidizing agent. MPO-catalyzed chlorination producing hypochlorous acid (HOCl) depends on the relative concentrations of chloride ion (Cl\(^-\)) and \( \mathrm{H}_2\mathrm{O}_2 \). MPO is present in neutrophils, monocytes and some reactive microglial macrophages, which invade inflamed tissues. Chronic inflammation, leading to neoplastic transformation is a well-established clinical phenomenon. Marked decrease in the concentration of protein thiol groups (P-SH) in serum and FRAP and drastic increase in the levels of protein carbonyls and MPO activity in the present study suggested that there existed an imbalance between free radicals and antioxidants in favour of radicals leading to oxidative stress in thyroid cancer.

The free radical levels and antioxidant activities did not return to normal levels 20 days after thyroidectomy, despite an increase in the levels of protein thiols and FRAP and decrease in the levels of protein carbonyls and MPO activity. These levels may or may not change with longer duration of time. Further studies are required to examine longer time intervals such as 3 or 6 months after the operation which may determine treatment implications, such as antioxidant therapy or the duration of any post-operative intervention.

References

15. Ellman G L (1959) Arch Biochem Biophys 82, 70-77