Original Article

Lipid peroxidation and antioxidants status in patients with papillary thyroid carcinoma in India

Namasivayam Senthil M Phil and Shanmugam Manoharan PhD

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India

The levels of lipid peroxidation products (TBARS), non-enzymatic antioxidants and enzymatic antioxidants activity were investigated in plasma and erythrocytes of twenty clinically diagnosed stage II papillary thyroid cancer patients and an equal number of age and sex matched healthy subjects. An increase in the levels of lipid peroxidation products, decrease in non-enzymatic antioxidants levels and enzymatic antioxidant activities in plasma and erythrocytes were detected in papillary thyroid cancer patients as compared to healthy subjects. Impairment in antioxidant defence mechanisms are responsible for enhanced lipid peroxidation observed in plasma and erythrocytes of papillary thyroid cancer patients.

Keywords: papillary thyroid cancer, lipid peroxidation, antioxidants, India

Introduction

Thyroid cancer, the most common endocrine cancer, is a cancerous tumour or growth located within the thyroid gland. The annual incidence rate of thyroid carcinoma varies from 0.5 to 10 cases per 100,000 in different parts of the world.[1] Thyroid cancer accounts for 64% of deaths attributable to malignant endocrine neoplasms, more than all other endocrine cancers combined.[2] Papillary carcinoma and follicular carcinoma are the most common types respectively accounting for about 70% and 15% of cases.[3] In the USA, about 18,000 new cases of thyroid cancer and 1,200 deaths are reported to occur every year.[4] The pattern of thyroid cancer in India is different from that seen in Western countries. In Bombay, North India, the thyroid cancer incidence was found to be at the lowest level in both sexes and it is about three times more frequent among women than men.[5] In Chennai, South India, thyroid carcinoma constitutes about 1-2% of all cancers.[6] Thyroid cancer occurs two to three times more frequently in women than in men.

Free radicals are highly reactive species generated in vivo as by-products of normal metabolism. Although free radicals are utilized by the immune system to kill microbes, free radicals are toxic when generated in excess. Free radicals can damage proteins, lipids, carbohydrates, and nucleic acids. Plasma membranes are critical targets of free radical reactions. Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Lipid peroxidation has been implicated in the pathogenesis of a variety of diseases including cancer.[7,8] The continuous production of oxidants, are however, balanced by equivalent synthesis of antioxidants. Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors and metal-chelating agents. The antioxidant defence system includes antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and small molecule antioxidants (vitamin E, vitamin C and reduced glutathione).[9,10] Studies have demonstrated altered levels of lipid peroxides and antioxidants in tumour tissues of thyroid cancer patients.[11,12] However, the altered pattern of lipid peroxidation products and antioxidants have not been well documented in plasma and erythrocytes of papillary thyroid cancer patients. Hence, the present study was undertaken to analyse the levels of lipid peroxidation products and antioxidants in plasma and erythrocytes of papillary thyroid cancer patients.

Methods and Patients

Twenty clinically diagnosed stage II papillary thyroid carcinoma (tumour size >1cm) patients from Raja Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, India, who had not undergone any previous treatment for their tumours were chosen for the study. An equal number of age and gender matched healthy subjects were also investigated. The patients and healthy subjects were from both genders ranging in age from 40-60 years.

Correspondence address: Dr S Manoharan, Dept. Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar-608 002, Tamilnadu. India
Tel: +91-4144-238343; Fax: +91-4144-238145
Email: manshisak@yahoo.com
Accepted 25 June 2004

Table 1. Blood picture of healthy subjects and papillary thyroid cancer patients

Parameters	Healthy subjects	Papillary thyroid cancer patients
RBC count (10^6 cells/μL)	5.35 ± 0.53	4.83 ± 0.48*
WBC count (10^3 cells/μL)	11.3 ± 1.3	10.08 ± 1.35*
Haemoglobin (g/dL)	14.8 ± 1.35	11.01 ± 1.12*

Values are expressed as mean ± S.D; $N = 20$; * Significantly different from healthy subjects, $P < 0.001$

Table 2. Plasma, erythrocytes and erythrocyte membranes TBARS levels in healthy subjects and papillary thyroid cancer patients

Parameter	Normal subjects	Papillary thyroid cancer patients
Plasma TBARS (nmol/ml)	2.89 ± 0.30	6.57 ± 0.7*
Erythrocytes TBARS (pmol/mg Hb)	3.20 ± 0.32	6.63 ± 0.6*
Erythrocyte membranes TBARS (nmol/mg protein)	0.37 ± 0.03	1.07 ± 0.06*

Values are expressed as mean ± SD; $N = 20$; *Significantly different from healthy subjects $P < 0.001$

The healthy subjects were free from any systemic diseases. The red and white blood cells and blood haemoglobin levels were significantly reduced in papillary thyroid cancer patients as compared to healthy subjects (Table 1).

Blood samples were collected by venous arm puncture into heparinised tubes and the plasma was separated by centrifugation at 3000 rpm for 15 minutes. After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. Known volumes of erythrocytes were lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 10,000 rpm for 15 minutes at 20° C. The erythrocyte membranes were isolated according to the procedure of Dodge *et. al.,*[13] with a change in buffer according to Quist.[14] The erythrocytes remaining after the removal of plasma were washed three times with 310mM isotonic tris- HCl buffer (pH 7.4). Haemolysis was performed by pipetting out the washed erythrocytes suspension into polypropylene centrifuge tubes which contained 20mM tris- HCl buffer (pH 7.2). The erythrocyte membranes were sedimented in a high-speed centrifuge at 10,000 rpm for 40 minutes. The supernatant was decanted and erythrocyte membrane pellet was made up to known volume by using 0.2 M isotonic tris- HCl buffer (pH 7.4). Aliquots from these preparations were used for the estimation of thiobarbituric acid reactive substances (TBARS) and vitamin E.

Lipid peroxidation was estimated as evidenced by the formation of TBARS. Lipid peroxides in plasma were assayed by the method of Yagi.[15] Plasma was deproteinised with phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90°C for 1 hour. Absorbance of the pink coloured complex formed was measured at 535nm. TBARS in erythrocytes and erythrocyte membranes was estimated by the method of Donnan.[16] Absorbance of the pink chromogen formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxides was read at 535 nm.

Vitamin E was estimated by the method of Desai.[17] The method involves the reduction of ferric ions to ferrous ions by the tocopherol and the formation of a pink coloured complex with bathophenanthroline orthophosphoric acid. Absorbance of the stable chromophore was measured at 536nm. Vitamin C level was estimated by the method of Omaye *et al.*[18] The dehydroascorbic acid formed by the oxidation of ascorbic acid by copper, forms a coloured product on treatment with 2,4 dinitro phenyl hydrazene, which was measured at an absorbance of 525nm. Reduced glutathione was measured at an absorbance of 412nm according to the method of Beutler and Kelley.[19] The method was based on the development of yellow colour, when 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) was added to compound containing sulphydryl groups.

The activity of glutathione peroxidase was estimated according to the method of Rotruck *et al.*[20] A known amount of hemolysate preparation was allowed to react with H_2O_2 in the presence of reduced glutathione for a specified time period, and the remaining reduced glutathione content was measured at an absorbance of 412nm according to the method of Beutler and Kelley.[19] Superoxide dismutase activity was assayed at 520nm by the method of Kakkar *et al.*[21] The assay was based on the inhibition of NADH – Phenazine methosulphate nitroblue tetrazolium formation. The activity of catalase was assayed by the method of Sinha[22] based on the utilization of H_2O_2 by the enzyme. The colour developed was read at 620nm.

Statistical analysis

The values are expressed as mean ± SD. Statistical comparisons were done by Student's t-test. The null hypothesis was rejected for $P<0.05$. The relationship between lipid peroxidation and antioxidants in plasma and erythrocytes were determined using Karl Pearson's correlation coefficient.

Results

Table 2 shows the levels of TBARS in plasma, erythrocytes and erythrocyte membranes of healthy subjects and papillary thyroid cancer patients. The TBARS levels were significantly increased in thyroid cancer patients as compared to healthy subjects. Table 3 shows the levels of dietary antioxidants (vitamin C, and E) in plasma and erythrocyte membranes of healthy subjects and papillary thyroid cancer patients. The vitamin C and E levels were significantly decreased in plasma and vitamin E was decreased in erythrocyte mem-branes of thyroid cancer patients as compared to healthy subjects. Table 4 shows the level of reduced glutathione and activity of glutathione peroxidase, in plasma and erythrocytes of healthy

subjects and papillary thyroid cancer patients. The level of glutathione and glutathione peroxidase activity was decreased in thyroid cancer patients as compared to healthy subjects. Table 5 shows the activities of superoxide dismutase and catalase in erythrocyte lysate of healthy subjects and papillary thyroid cancer patients. The superoxide dismutase and catalase activities were significantly decreased in thyroid cancer patients as compared to healthy subjects. Table 6 shows Karl Pearson's correlation coefficient for lipid peroxidation and antioxidants in plasma, erythrocyte lysate and erythrocyte membranes in papillary thyroid cancer patients. Statistically significant negative correlation was observed between lipid peroxidation against vitamin E, SOD, and CAT in patients with papillary thyroid cancer. Although negative correlation was observed between lipid peroxidation against vitamin C, reduced glutathione and glutathione peroxidase in papillary thyroid cancer patients, the values were statistically insignificant.

Discussion

In the present study, we observed an increase in lipid peroxidation products (TBARS) and decline in antioxidants status in plasma, erythrocytes and erythrocyte membranes of papillary thyroid cancer patients as compared to healthy subjects. Durak *et al.*,[23] have reported an increase in malondialdehyde (MDA) levels and decrease in the activities of enzymatic antioxidants as compared to non-cancerous tissues. Sadani and Nadkarni[12] have reported that the higher production of reactive oxygen species is responsible for the elevated lipid peroxidation in adenomas and carcinoma of thyroid tissues. Mano *et al.*,[11] have reported an increase in lipid peroxidation and disturbed antioxidant enzymes in thyroid tumour tissues of patients with papillary carcinoma. They suggested that the lipid peroxides are not completely scavenged in papillary carcinoma tissues and therefore these substances affect some role in cell function of thyroid tissues.

The reactive oxygen species play a crucial role in the pathogenesis of tissue damage in many pathological conditions via a peroxidation of membrane phospholipids. Gutteridge[24] has focused lipid peroxidation and antioxidants as biomarkers of tissue damage. Lipid peroxides that are generated at the site of tissue injury could be transferred through circulation to other organs and tissues to provoke damage by propagating lipid peroxidation. Elevated levels of lipid peroxides have been reported in the erythrocytes and erythrocyte membranes of various cancer patients.[25,26] Our results lend credence to these observations. Hence, the observed increase in plasma TBARS in papillary thyroid cancer patients may be related to over production of lipid peroxides in erythrocytes, erythrocyte membranes or tumour tissues itself with consequent leakage into plasma.

Antioxidants have been reported to protect cell against cancer. Antioxidants play an important role in scavenging lipid peroxides that are generated at the site of tumours. The antioxidant capacity is determined by dynamic interactions between nonenzymatic antioxidants and antioxidative enzymes.[27] α-Tocopherol is known to have the greatest biological activity of the various stereoisomers of the vitamin E. In vivo α tocopherol is the most abundant lipid soluble antioxidant and acts as an important inhibitor of membrane lipid peroxidation.[28] Mano *et al.*,[29] reported an increase in vitamin E concentration in thyroid tumor tissues as compared to normal tissues. Vitamin C, the most important antioxidant in the plasma, scavenges a variety of oxidants. Dumitrescu *et al.*,[30] have reported a decrease in plasma MDA level after one month of vitamin C administration in patients with differentiated thyroid cancer who have undergone surgery and are being treated with Iodine.[131] Hence, the decreased levels of vitamin E and C observed in the present study can be correlated to elevated plasma lipid peroxidation or utilization of these antioxidants by tumour tissues to scavenge excess lipid peroxides that are generated in the tumour tissues.

Glutathione peroxidase, superoxide dismutase and catalase are most important enzymes of the cell antioxidant defense system. Glutathione peroxidase, catalyses the decomposition of H_2O_2 to H_2O and reduces organic

Table 3. Plasma and erythrocyte membranes dietary antioxidant levels in healthy subjects and papillary thyroid cancer patients

Parameter	Normal subjects	Papillary thyroid cancer patients
Plasma		
vitamin C (mg/dl)	1.56 ± 0.15	0.64 ± 0.06*
vitamin E (mg/dl)	1.41 ± 0.13	0.85 ± 0.08*
Erythrocyte membranes		
vitamin E (μg/mg protein)	2.08 ± 0.19	1.58 ± 0.14*

Values are expressed as mean ± SD; $N = 20$; * Significantly different from healthy subjects $P < 0.001$

Table 4. Levels of reduced glutathione and activity of glutathione peroxidase, in plasma and erythrocyte lysate of healthy subjects and thyroid papillary cancer patients

Parameter	Normal subjects	Papillary thyroid cancer patients
Plasma GSH (mg/dl)	48.7 ± 3.76	38.2 ± 3.02*
Erythrocytes GSH (mg/ dl)	52.6 ± 4.1	39.3 ± 2.6*
Plasma GPx (U/L)	222.68 ± 28.8	186.13 ± 8.44*
Erythrocyte lysate GPx (U/g Hb)	34.06 ± 3.5	26.6 ± 2.48*

Values are expressed as mean ± SD; $N = 20$; * Significantly different from healthy subjects $P < 0.001$

Table 5. Activities of superoxide dismutase and catalase in erythrocyte lysate of healthy subjects and papillary thyroid cancer patients

Parameter Erythrocyte lysate	Normal subjects	Papillary thyroid cancer patients
SOD (U/mg Hb)	2.14 ± 0.24	1.64 ± 0.16*
CAT (U/mg Hb)	5.86 ± 0.58	4.42 ± 0.38*

Values are expressed as mean ± SD; $N = 20$l; * Significantly different from healthy subjects $P < 0.001$

Table 6. Karl Pearson's correlation coefficient for lipid peroxidation and antioxidants in papillary thyroid cancer patients

Parameters	Papillary thyroid cancer patients
Plasma	
TBARS-Vitamin E	- 0.546*
TBARS-Vitamin C	- 0.339^{NS}
TBARS-Glutathione	- 0.238 ^{NS}
TBARS-Glutathione peroxidase	- 0.120 ^{NS}
Erythrocyte lysate	
TBARS-Glutathione	- 0.226 ^{NS}
TBARS- Glutathione peroxidase	- 0.172 ^{NS}
TBARS-Superoxide dismutase	- 0.537*
TBARS-Catalase	- 0.454*
Erythrocyte membranes	
TBARS-Vitamin E	- 0.465*

*Values are statistically significant at $P < 0.05$; ^{NS}Not significant

peroxide to corresponding alcohols. The decrease in glutathione peroxidase activity has been reported in thyroid cancer tissues as compared to normal tissues.[31] Reduced glutathione, an important intracellular antioxidant, acts both as cofactor for glutathione peroxidase and as a direct active scavenger to remove reactive species such as hydroxyl radical, peroxynitrite, carbon centered radicals and singlet oxygen. Reduced glutathione also play a very effective role in the protection of erythrocyte from oxidative damage.[32] Lower levels of reduced glutathione in plasma and erythrocytes have been reported in various pathological conditions including cancer.[33] Hence the decreased activities of plasma and erythrocytes glutathione peroxidase in papillary thyroid cancer patients may be due to lowered level of reduced glutathione observed in plasma and erythrocytes.

Decreased activities of catalase and superoxide dismutase have been demonstrated in various pathological diseases including cancer.[34] Decreased activity of superoxide dismutase and unaltered activity of catalase has been reported in thyroid tumour tissues.[35] Lowered activities of these enzymes observed in erythrocytes of thyroid cancer patients can be related to enhanced lipid peroxidation observed in the erythrocytes of papillary thyroid cancer patients. Hence, we feel that significantly impaired enzymatic and non enzymatic antioxidant defence systems are responsible for the elevated lipid peroxidation products in plasma and erythrocytes of papillary thyroid cancer patients.

References

1. Dal Maso L, La Vecchia C, Franceschi S, Preston-Martin S, Ron E, Levi F, Mack W, Mark SD, McTiernan A., Kolonel L, Mabuchi K, Jin F, Wingren G, Galanti MR, Hallquist A, Glattre E, Lund E, Linos D, Negri E. A pooled analysis of thyroid cancer studies. Cancer Causes Control 2000; 11: 137-144.
2. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics. CA. Cancer J Clin 1999; 49: 8-31.
3. Ortapamuk H, Arican P, Alp A. Parapharyngeal lymph node involvement in papillary thyroid carcinoma. Clin Nucl Med 2003; 28**:** 947-948.
4. Wu XC, Chen VW, Steele B, Roffers S, Klotz JB, Correa CN Carozza SE. Cancer incidence in adolescents and young adults in the United States, 1992-1997. J Adolesc Health 2003; 32: 405-415.
5. Rao RS, Parikh DM, Mistry RC, Rao SR. Evidence-based protocols for the management of well-differentiated carcinomas of the thyroid. Asian J Surg 2002; 25: 319-324.
6. Dorairajan N, Pandiarajan R, Yuvaraja S. A descriptive study of papillary thyroid carcinoma in a teaching hospital in Chennai, India. Asian J Surg 2002; 25:300-303.
7. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant theraphy. Lancet 1994; 1: 1396-1397.
8. Ray G, Akthar Hussain S. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol 2002; 40: 1213-1232.
9. Bray TM. Antioxidant and oxidative stress in health and disease. Soc Exp Biol Med 1999; 23: 195-198.
10. Gey KF. Vitamin E plus C and interacting co-nutrients required for optimal health. Biofactors 1998; 7: 113-174.
11. Mano T, Shinohara R, Iwase K. Changes in free radical scanvengers and lipid peroxide in thyroid glands of various thyroid disorders. Horm Metab Res 1997; 29: 351-354.
12. Sadani GR, Nadkarni GD. Radiation Medicine Centre (BARC), Tata Memorial Centre Annexe, Parel, Bombay, India. Role of tissue antioxidant defence in thyroid cancers. Cancer Lett 1996; 109: 231-215.
13. Dodge JF, Mitchell G, Hanhan DJ. The preparation and chemical characterization of haemoglobin free ghosts of human red blood cells. Arch Biochem Biophys 1968; 110: 119-130.
14. Quist EH. Regulation of erythrocyte membrane shape by calcium ion. Biochem Biophys Res Commun 1980; 92: 631-637.
15. Yagi K. Lipid peroxides and human disease. Chem Phys Lipids 1978; 45: 337-351
16. Donan SK. Thiobarbituric acid test applied to tissues from rats treated in various ways. J Biol Chem 1950; 182: 415-419.
17. Desai FD. Vitamin E analysis and methods for human tissue. In: Feischer S, Packer L. eds. Methods Enzymol. New York: Academic Press, 1984; 105: 138-145.
18. Omaye ST, Turbull TD, Sauberlich HE. Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCromic DB, Wright DL, eds. Methods Enzymol. Academic Press, 1979; 62: 3-11.
19. Beutler E, Kelley BM. The effect of sodium nitrate on RBC glutathione. Experienta 1963: 96-97.
20. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hockstra WG. Selenium biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.
21. Kakkar P, Das B, Visvanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984; 21: 130-132.
22. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 17: 389-394.
23. Durak I, Bayram F, Kavutcu M, Canbolat O, Ozturk HS. Impaired enzymatic antioxidant defense mechanism in cancerous human thyroid tissues. J Endocrinol Invest 1996; 19: 312-315.
24. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41: 1819-1828.
25. Manoharan S, Shreeram S, Nagini S. Life style can induce lipid peroxidation and compromise of antioxidant defense mechanisms in the erythrocytes of oral cancer patients. Med Sci Res 1996; 24: 397-400.

26. Ray G, Batra S, Shukla NK, Des S, Raina V, Ashok S, Husain SA. Lipid peroxidation and antioxidant status in breast cancer. Breast Cancer Res Treat 2000; 59: 163-170.
27. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxigology. Toxicology 2000; 153: 83-104.
28. McCay PB. Vitamin E interactions with free radicals and ascorbate. Annu Rev Nutr 1985; 5: 323.
29. Mano T, Iwase K, Hayashi R, Hayakawa N, Uchimura K, Makino M, Nagata M, Sawai Y, Oda N, Hamada M, Aono T, Nakai A, Nagasaka A, Itoh M. Vitamin E and coenzyme Q concentrations in the thyroid tissues of patients with various thyroid disorders. Am J Med Sci 1998; 315: 230-232.
30. Dumitrescu C, Belgun M, Olinescu R, Lianu L, Bartoc C. Effect of vitamin C administration on the ratio between the pro- and antioxidative factors. Rom J Endocrinol 1993; 31: 81-84.
31. Hasegawa Y, Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Kuma K, Amino N. Decreased expression of glutathione peroxidase mRNA in thyroid anaplastic carcinoma. Cancer Lett 2002; 182: 69-74.
32. Banerjee KK, Marimuthu P, Chaudhri RN. Glutathione and lipid peroxidation status. Indian J Public Health 1998; 42: 20-23.
33. Gerad MD, Chaudrie J. Metabolism and antioxidant functions of glutathione. Pathol Biol 1996; 44: 77-83.
34. Oberly LW, Oberley TD. The role of superoxide dismutase and the gene amplification in carcinogenesis. J Ther Biol 1984; 106: 403.
35. Felt V, Cerny K, Klimova H, Kremenova J, Rehak F. Enzyme changes in thyroid tissue with reference to thyroid cancer. Acta Univ Carol Med Praha 1976; 22: 435-442.