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Oxidative Stress in Anti Thyroperoxidase Antibody Positive Hypothyroid Patients

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ABSTRACT

Hypothyroidism is among the most common endocrine disorders in India. One of the most frequent causes of hypothyroidism is the autoimmune thyroid disease characterized by elevated Anti-thyroperoxidase Antibody (TPO Ab). We reported earlier the presence of Oxidative Stress (OS) in hypothyroid patients. In this study, we compared the level of OS in TPO Ab positive hypothyroid patients with TPO Ab negative hypothyroid patients which earlier has not been studied. We recruited untreated hypothyroid patients consecutively from the out patient department. We analyzed their TPO Ab titer, various biochemical and oxidative stress parameters and ultra-sensitive C reactive protein. The thiobarbituric acid reactive substances, an indicator of oxidative stress was significantly higher in TPO Ab positive hypothyroid patients compared to TPO Ab negative cases. TPO Ab titter positively correlated with the OS parameters and extent of low grade inflammation in TPO Ab positive hypothyroid patients. Oxidative stress was found to be more in TPO Ab positive untreated hypothyroid patients in our study indicating that autoimmunity could be one of the mechanisms contributing to the elevated OS level in hypothyroidism.

Key words: Oxidative stress, hypothyroidism, thyroperoxidase antibody

INTRODUCTION

Autoimmune diseases are characterized by body's immune responses targeting its own tissues, causing prolonged inflammation and subsequent tissue destruction. A healthy immune system recognizes, identifies, remembers, attacks and destroys foreign antigens and cancer cells. A defective immune system, on the other hand, inflicts chaos in host body by directing antibodies against its own tissues. Any disease in which cytotoxic cells are directed against self-antigens in the body's tissues is considered autoimmune in nature. Hashimoto's thyroiditis which is an autoimmune thyroid disease, is one of the most frequent causes of hypothyroidism which is among the most common endocrine disorders in India (Kochupillai, 2000).

Oxidative Stress (OS) has been implicated in a number of diseases such as cardiovascular diseases, renal diseases, diabetes, inflammatory problems etc. (Krinsky, 1992). In the previous study, we found increased level of OS in untreated hypothyroid patients (Nanda *et al*., 2007, 2008). Hashimoto's thyroiditis which is a common cause of hypothyroidism, is characterized by elevated

Anti-thyroperoxidase Antibody (TPO Ab). There exists a good correlation between the degree of lymphocytic infiltration of the thyroid gland and the presence of TPO antibodies (Portmann *et al.*, 1985). Association of oxidative stress with various autoimmune diseases has been reported (Kumagai *et al.*, 2003; Kumagai, 2003). However, the association of thyroid antibodies and OS in the pathophysiology of hypothyroidism is not fully understood. Hence, in the present study, we have analyzed the link between anti TPO antibodies and OS in hypothyroid patients.

MATERIALS AND METHODS

Subjects: The present study comprises of sixty seven (55 females and 12 males) newly diagnosed patients with primary hypothyroidism (TSH level more than 10 $\mu IU\ mL^{-1}$). They were recruited consecutively from the out patient department of medicine, Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER) in the study group. Subjects receiving lipid lowering drugs or antioxidant vitamin supplements, pregnant women, women on hormone replacement therapy, alcoholics, smokers, patients with hypertension and patients suffering from diseases other than hypothyroidism were excluded from the study groups. This study was approved by the research council and human ethics committee of JIPMER. Written consent was obtained from all the subjects. The patients were enrolled for the study prior to initiation of therapy.

Blood collection: Overnight fasting blood samples (serum and EDTA whole blood) were collected in separate tubes. EDTA whole blood was immediately used for the estimation of whole blood reduced glutathione. Serum was used for the estimations of glucose and lipid profile on the same day. Remaining serum samples were refrigerated at-50°C till the estimations of thyroid profile, Protein Carbonyls (PCO), Malondialdehyde (MDA) as thiobarbituric acid reactive substance and usCRP were carried out.

Thyroid profile: Total Tri-iodothyronine (T_3) and total tetra-iodothyronine (T_4) were estimated by RIA and TSH was estimated by IRMA using kits procured from BARC (Bhaba Atomic Research Center, Mumbai, India).

Lipid profile: Estimation of serum lipid profile was carried out by using various commercial kits. Total Cholesterol (TC) and Triglycerides (TG) were estimated by enzymatic methods using kits from Biocon (Germany) and Accurex (Mumbai, India), respectively. LDL and Very Low Density Lipoprotein Cholesterol (VLDL C) were precipitated using the phosphor-tungstate magnesium acetate reagent from Agappe diagnostics (Thane, India). HDLC was estimated in the supernatant while LDL C was calculated using the Fridewald formula (Friedewald *et al.*, 1972).

Glucose: Serum glucose was estimated using glucose oxidase kits obtained from Dr. Reddy's lab (Hyderabad, India) using an autoanalyzer (550 express plus, Bayer's Diagnostics, USA).

Antioxidants: Activity of antioxidants such as reduced glutathione (GSH), Glutathione Peroxidase (GPx), Glutathione S Transferase (GST) and Catalase were measured by standard methods described previously (Nanda *et al.*, 2008).

Lipid peroxide and protein carbonyl: Malondialdehyde, the end product of lipid peroxidation was estimated by thiobarbituric acid method modified by K Satoh (Satoh, 1978) and Protein Carbonyl (PCO) was estimated by the method of Levine (Levine *et al.*, 1994).

Anti-thyroperoxidase antibody and us CRP: Anti-thyroperoxidase Antibody (TPO) titer was estimated by ELISA kit (Varelisa, Pharmacia and Upjohn, Germany) following the manufacturer's instruction. It is based on noncompetitive enzyme immuno assay for the quantitative estimation of TPO. The cut off for positive TPO antibody titer is 100.

Quantitative assay of ultrasensitive CRP was performed by a turbidimetric immunoassay kit adapted to autoanalyser (Aptec Diagnostics, Belgium). The ultrasensitive assay for CRP (usCRP) is analogous to high sensitive CRP (hsCRP) assay.

Statistical analyses: All parameters were expressed as Mean±SD. Significance of the differences between control and test groups were analyzed by student's t test. For parameters without normal distribution, we used Mann Whitney-U test for analyzing the significance of differences. The correlations were assessed by Pearson correlation and shown in Table 2. All the statistical analyses were performed using the SPSS software (SPSS Inc, Chicago, USA).

RESULTS AND DISCUSSION

In present study, out of sixty seven cases, thirty nine were TPO positive and twenty eight were TPO negative. When compared between TPO positive and TPO negative cases, MDA was fond significantly higher in the former group indicating increased OS in autoimmune cases of hypothyroidism (Table 1). There was no difference in the level of protein carbonyls, antioxidants and us CRP between the two groups.

Table 1: Comparison of the anthropometric and biochemical and oxidative stress parameters between anti-TPO antibody positive and anti-TPO antibody negative cases of untreated hypothyroidism

Parameters	TPO Negative (n = 28)	TPO Positive (n = 39)
Male: Female	11:17	3:36
Age	32±9	37±12
Body weight (kg)	68.25±8.69	66.42±10.51
BMI (kg m^{-2})	28.12±3.73	27.93±4.22
T3 (ng dL^{-1})	64.64±31.86	62.47±29.92
T4 (µg dL^{-1})	4.23±2.54	3.56±2.03
TSH (mIU mL^{-1})	59.81±36.82	67.75±35.53
Anti-TPO Ab (IU mL^{-1})	24.57±24.41	926.00±739.40###
Serum MDA (µM L^{-1})	2.73±1.08	3.62±1.56*
Serum Protein carbonyls (nmoL mg^{-1} protein)	2.09±0.86	2.55±1.07
Serum usCRP (mg L^{-1})	0.44±0.17	0.47±0.19

All parameters analysed by student's unpaired student 't' test, except TSH and anti-TPO Ab which were analysed by Mann whitney 'U' test; *$p<0.05$ and ### $p<0.001$

Table 2: Pearson correlation of anti-TPO antibody with various parameters in TPO positive cases of hypothyroid patients (n = 39)

Parameters	r	p
Age	0.354	0.032
T4	-0.431	0.009
TSH	0.292	0.079
MDA	0.362	0.028
PCO	0.401	0.014
GSH	-0.195	0.247
usCRP	0.437	0.018

Protein carbonylation is an important biochemical modification which affects the structure and function of proteins. The body's antioxidant defense system protects against such derangements. Autoimmune response is closely associated with the generation of free radicals (Kurien and Scofield, 2008).

Altered antioxidant status was reported in Hashimoto cases in only one previous study (Gerenova and Gadjeva, 2007) in comparison to healthy controls. However, they did not compare the OS level between TPO Ab positive and negative cases. In the present study, we found significantly higher OS in autoimmune hypothyroidism compared to non autoimmune hypothyroidism (Table 1). To the best of our knowledge this comparison has not been reported by any other study till date. In previous studies, patients with various thyroid functional states of autoimmune diseases concluded that oxidative stress occurs at ATD in spite of their altered thyroid status (Tsotsonava *et al*., 2007a, b). In these two studies, only 10 and 19 patients with autoimmune hypothyroidism were included respectively. They found a positive correlation between lipid peroxy radicals and FT4 and advocated inclusion of antioxidants for chronic autoimmune thyroiditis cases. Our present findings corroborates with these reports. In this study, the sample size is larger than the previous reports (39 cases). In this study, there was a direct correlation of TPO Ab titer with various parameters of OS and inflammation which was not reported earlier.

However, the marginal difference in MDA in TPO Ab positive cases compared to non autoimmune hypothyroidism may indicate that the contribution of autoimmunity towards the genesis of OS might not be substantial. Nevertheless, increase in anti TPO Ab titer significantly correlated with the advancement of age and decreased levels of T4, oxidative stress parameters such as MDA, PCO and increase in inflammatory markers like usCRP. Hence, the association of autoimmunity and OS in the pathophysiology and complications of hypothyroidism could be substantial in the long run.

Absence of significant correlation of anti-TPO Ab with TSH may indicate that degree of autoimmunity is not directly linked to the severity of the dysfunction. OS is known to increase with age. Also, incidence of hypothyroidism increases with age. As in the present study, anti-TPO Ab was positively correlated with age, we presume that presence of TPO antibody especially in aged hypothyroid patients could be an important factor in accelerated OS in hypothyroidism. The association of TPO Ab with serum us-CRP, MDA and PCO suggests that inflammation and OS are exacerbated in TPO positive hypothyroid cases due to additional autoimmune assaults.

Hypothyroidism is a treatable endocrine dysfunction. Hence, most often its potential complications are not given due attention. However, it is a disease that requires life long drug supplementation with careful monitoring of thyroid profile to avoid iatrogenic side effects especially on heart. Often the drug therapy takes long to normalize the thyroid profile (Libby, 2005; Mishkel and Crowther, 1977). Flynn *et al*. (2006) reported that despite treatment in primary hypothyroidism, patients are still at increased risk of morbidity associated with various circulatory disease, ischemic heart disease, dysrhythmias and cerebrovascular diseases. Hence, from the present study, keeping in view the increase in OS parameters, judicious inclusion of antioxidant therapy may be recommended to hypothyroid patients especially in anti TPO Ab positive hypothyroid patients. In the later group of patients the antioxidant treatment may be continued even after attainment of euthyroidism till significant decrease in their TPO in titer.

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REFERENCES

Flynn, R.W., T.M. Macdonald, R.T. Jung, A.D. Morris and G.P. Leesse, 2006. Mortality and vascular outcome in the thyroid population. J. Clin. Endocrin. Metab., 91: 2159-2164.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Gerenova, J. and V. Gadjeva, 2007. Oxidative stress and antioxidant enzyme activities in patients with Hashimoto's thyroiditis. Comp. Clin. Path., 16: 259-264.

Kochupillai, N., 2000. Clinical endocrinology in India. Curr. Sci., 79: 1061-1067.

Krinsky, N.I., 1992. Mechanism of action of biological anti-oxidants. Proc. Soc. Exp. Biol. Med., 200: 248-254.

Kumagai, S., 2003. Oxidative stress and autoimmune diseases. J. Japan. Soc. Int. Med., 92: 1096-1103.

Kumagai, S., T. Jikimoto and J. Saegusa, 2003. Pathological roles of oxidative stress in autoimmune diseases. Rinsho. Byori., 51: 126-132.

Kurien, B.T. and R.H. Scofield, 2008. Autoimmunity and oxidatively modified antigens. Autoimmun. Rev., 7: 567-573.

Levine, R.L., J.A. Williams, E.R. Stadtman and E. Shacter, 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol., 233: 346-357.

Libby, P., 2005. The Pathogenesis of Atherosclerosis. In: Harrison's Principles of Internal Medicine, Kasper, D.L., E. Braunwald, A.S. Fauci, S.L. Hauser, D.L. Longo and J.L. Jameson (Eds.). 16th Edn., McGraw-Hill, New York, pp: 1425-1426.

Mishkel, M.A. and S.M. Crowther, 1977. Hypothyroidism an important cause of reversible hyperlipidemia. Clin. Chim. Acta, 74: 139-151.

Nanda, N., Z. Bobby, A. Hamide, B.C. Koner and M.G. Sridhar., 2007. Association between oxidative stress and coronary lipid risk factors in hypothyroid women is independent of body mass index. Metabolism, 56: 1350-1355.

Nanda, N., Z. Bobby and A. Hamide, 2008. Association of thyroid stimulating hormone and coronary lipid risk factors with lipid peroxidation in hypothyroidism. Clin. Chem. Lab. Med., 46: 674-679.

Portmann, L., N. Hamada, G. Heinrich and L.J. de Groot, 1985. Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti microsomal antibody. J. Clin. Endocrinol. Metab., 61: 1001-1003.

Satoh, K., 1978. Serum lipid peroxide in Cardio Vascular Disease, determined by a new colorimetric method. Clin. Chim. Acta., 90: 37-43.

Tsotsonava, T., D. Virsaladze, K. Khitarishvili, T. Sanikidze and D. Tananashvili, 2007a. Comparative analysis of blood redox parameters according thyroid function of patients with autoimmune thyroid diseases. Georg. Med. News, 146: 32-34.

Tsotsonava, T.A., D.K. Virsaladze, D.K. Khitarishvili and D.E. Tananashvili, 2007b. The status of redox system in patients with chronic autoimmune thyroiditis. Georg. Med. News, 145: 55-58.