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Relationship between Thyroid Autoantibodies, Imbalance of Some Essential Trace Elements in the Pathology of Simple Nontoxic Goitre

Nancy A. Mbachu[1*] A.O. Afolabi[2] J.I. Anetor[3]
1.Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi campus
2.Department of Surgery, University College Hospital, Ibadan
3.Department of Chemical Pathology, University College Hospital, Ibadan

Abstract
Autoimmunity and certain micronutrients have been implicated in the pathogenesis of thyroid disorders such as simple nontoxic goitre (SNTG). Alterations in certain trace elements and autoimmune parameters may arise from an indeterminate autoimmune mechanism. We sought to evaluate the association and interactions between trace elements and autoantibodies in simple nontoxic goitre. All consenting participants based on clinical and thyroid function tests were recruited for this case-control study. Subjects comprised of 37 patients and 44 controls, from the Surgery Outpatient and Endocrinology Clinics of the University College Hospital and the Lagos University Teaching Hospital, respectively. Anthropometric indices were measured, serum trace element were determined using atomic absorption spectrophotometer (AAS), thyroid autoantibodies and thyroid function tests were performed in serum using Enzyme linked immunosorbent assay (ELISA). Thyroperoxidase and thyroglobulin antibodies were significantly elevated in SNTG compared with controls, suggesting immune alteration. The free triiodothyronine (FT_3) and free thyroxine (FT_4) were significantly increased in SNTG (3.72±0.02 and 14.9±0.21 pmol/L) compared with controls (3.63±0.12. pmol/L and 13.12±0.12 pmol/L). The level of TSH was significantly increased in SNTG compared with controls. Antithyroglobulin level was positive in 14.0% (130.93±35.02 kiu/L) SNTG. Significantly reduced levels of Cu (11.92±0.32, µmol/L), Fe (20.13±0.52µmol/L) and Se (0.53±0.01µmol/L) relative to the controls (16.12±0.22, 28.73±0.23 and 3.82±0.03µmol/l) were found in SNTG. Simple nontoxic goitre appear to be associated with imbalance in certain essential trace elements and presence of autoantibodies which probably are among the key mechanisms involved in the pathology of thyroid disorders. Early detection of these abnormalities may be useful in the better understanding and improved management of thyroid disorders.
Keywords: Autoimmunity, trace elements, Iodine, nontoxic goitre, thyroid peroxidase

Introduction

Thyroid disorders are metabolic diseases of unclear etiology where autoimmunity has been identified as a major cause (Swain *et* al., 2005). Predisposing genetic factors seem to be evident, but environmental factors such as infections, smoking, drugs and nutrition, especially a deficient or an increased iodine supply, as well as an imbalance in micronutrient status, presence of autoantibodies and an increase in age (Liel and Barchana) have also been linked to autoimmune disease (AID). Simple nontoxic goitre refers to any thyroid gland enlargement not associated with hyper or hypothyroidism and does not result from inflammation or neoplasia (Kumar *et al*., 1999; Ojule and Osotimehin, 1998). It constitutes about 84.5% of all cases of goitre with a female to male ratio of 10:1 occurring in all age groups with a peak age incidence of 31-40 years. It is often seen at puberty, in pregnancy and during lactation. Previous report indicates that SNTG is the commonest form of goitres in endemic area of South-Western Nigeria (Vanderpump, 2011).

Thyroid disruption may be caused by a variety of mechanisms, as different elements interfere with the hypothalamic–pituitary–thyroid (H-P-T) axis at various levels. Established mechanisms of action may involve the thyroid peroxidase enzyme, sodium–iodide symporter, receptors for thyroid hormones (THs) or TSH, transport proteins or cellular uptake mechanisms. Even small changes in thyroid homeostasis may adversely affect human health, and especially fetal neurological development may be vulnerable (Boas *et al*., 2006). Indeed, iodine deficiency is regarded as the most common cause of thyroid disorders worldwide (Vanderpump *et al*., 1996) including other micronutrients which seem to play a role.

Thyroid nodules are very common, and although the majority are benign, approximately 5% may harbour malignancy (Bonnema *et al*., 2003). The etiology of which is multifactorial, encompasses the spectrum from the incidental asymptomatic small solitary nodule to the large intrathoracic goitre, causing pressure symptoms as well as cosmetic complaints (Fast *et al*., 2009). Despite iodization programmes, simple goitre still constitutes a major diagnostic and therapeutic challenge (Hegedus *et al*., 2003). According to current thinking, they are caused by an interaction between genetic susceptibility and environmental triggers; iodine deficiency being the most important (Hegedus *et al*., 2003). The natural history of a nodular goitre is that of gradually increasing size with development of multiple nodules. However, the natural history with respect to growth and function varies and is difficult to

predict in a given patient because no specific growth parameters exist (AACE, 2006). Symptoms are typically those of local pressure (dysphagia, globules sensation, cough or dyspnoea) or cosmetic complaints and are difficult to evaluate objectively. It is mandatory to rule out malignancy, for which purpose fine-needle aspiration biopsy is the golden standard (Bonnema *et al*., 2004). There is no simple relationship between goitre size and symptoms (Laurberg *et al*., 2010). Both low and high levels of iodine intake associated with an increase in the risk of disease in a population (Enig, 2010). Optimally, iodine intake of a population should be kept within a relatively narrow interval where iodine deficiency disorders are prevented, but not higher (Enig, 2010). Minerals that are essential to life are the source of metals and other inorganic elements involved in the most fundamental processes. For example, oxygen is utilized in the human body with the help of metal complexes. The human skeleton is composed of calcium and phosphorus and traces of other metals. The regulation of body-fluid volume and acid-base balance requires sodium, potassium, magnesium, and calcium. Hormones contain iodine, sulfur, and zinc. Dynamic equilibrium exists for the various mineral nutrients as well as mechanisms whereby a system can adjust to varying amounts of these minerals in diet. In forms usually found in foods, most mineral nutrients are not toxic when ingested orally (Laurberg *et al*., 1998). The exact causes of nontoxic goitre are not known. In general, goitre may be caused by too much or too little thyroid hormones. There is often normal thyroid function with a nontoxic goitre. Some possible causes of nontoxic goitre include: heredity (family history of goitres), regular use of medications such as lithium, propylthiouracil, phenylbutazone, or aminoglutethimide, Regular intake of substances (goitrogens) that inhibit production of thyroid hormone-common goitrogens include foods such as cabbage, turnips, brussel sprouts, seaweed, and millet (Doufas *et al*., 1999). Over 2 billion individuals are at risk of goitre worldwide (Bruntland, 2002). The alteration of balance between certain essential (Copper, Iron, Selenium, Zinc) trace elements (Al-Sayer *et al*., 2004), toxicants and autoantibodies has a strong role in the pathogenesis of thyroid disease but this has received little attention. There is an increasing prevalence especially in women (Laurberg *et al*., 2005) indicating gender and hormonal influence, reaching a peak of 21% in women and 16% in men over 74 yr of age (Canaris *et al*., 2000). In addition associated immune disorders occurring in some thyroid diseases increase thyroid stimulation or suppress antibodies and anti-thyroid cell antibodies, which leads to other clinical manifestations (Bjoro *et al*., 2000; Ojule and Osotimehin, 1998). In Nigeria, although several studies have been conducted on thyroid disorders (Okosieme *et al*., 2007; Bahn and Castro, 2011) data are limited and the underlying pathological mechanisms, the possible contribution of imbalance between some key essential trace elements and autoantibodies has received little attention (Osredkar and Sustar, 2011); this imbalance was therefore investigated. The study aimed to evaluate the possible interaction or relationship of alteration in essential trace element status and autoantibodies in individuals with simple nontoxic goitre.

Materials and Methods

Eighty one (37 SNTG; 44 controls) consenting age-matched female subjects of 22 to 65 years were consecutively enrolled based on clinical symptoms and thyroid function tests from the University College Hospital, Ibadan and the Lagos University Teaching Hospital, Lagos, Nigeria. They were divided into two groups: Simple Non-Toxic Goitre (SNTG, n=37 and controls (n=44). Socio-demographic characteristics and anthropometric indices were obtained using a structured questionnaire. Spot urine samples were collected for determination of iodine status (IOD) using colorimetric method (Jooste and Strydom, 2010). Blood (10ml) was collected and the plasma was used for determination of Thyroid Function Tests (TFTs) and trace elements. The TFTs: Total Triiodothyronine (T_3), Total Thyroxine (T_4), Free Triiodothyronine (FT_3), Free Thyroxine (FT_4), Thyroid Stimulating Hormone (TSH), thyroglobulin, autoantibodies (Antithyroid Peroxidase, TPOAb and Antithyroglobulin, TgAb) were determined using Enzyme - Linked Immunosorbent Assay (ELISA). Trace elements were determined by Atomic Absorption Spectrophotometer (AAS).

Statistical Analysis

Data were analysed using Statistical package of the Social Science (SPSS) software version 17.0 and presented as mean ± standard error of mean (SEM). For quantitative variables, paired Student's t-test was used to test the significant difference between the mean values. Pearson's correlation technique was used for the comparison of the strength of association among variables. Chi square test was used for the determination between non quantitative variables. Significant values were considered at $p < 0.05$.

Results

Table 1: Age, Anthropometric indices of SNTG female subjects and controls

Indices	Group (SNTG) n=37	Group (Controls) n=44	p-value
Age (years)	44.51± 1.93	43.65± 1.69	0.737
Weight (kg)	72.06± 2.12	67.40±1.30	0.066
Height (m)	1.64± 0.01	1.61± 0.01	0.007*
SBP (mmHg)	1.62±0.15	1.00± 0.01	0.000*
DBP (mmHg)	1.24± 0.11	1.00± 0.03	0.034*
BMI (kg/m^2)	26.84± 0.73	26.48± 0.70	0.722

Values are reported as mean± SEM (standard error of mean).
Mean values are significantly different ($p<0.05$);
*Significant at $p < 0.05$

Anthropometric and blood pressure measurements revealed a significant increase in the systolic and diastolic blood pressure in SNTG patients (Table1).

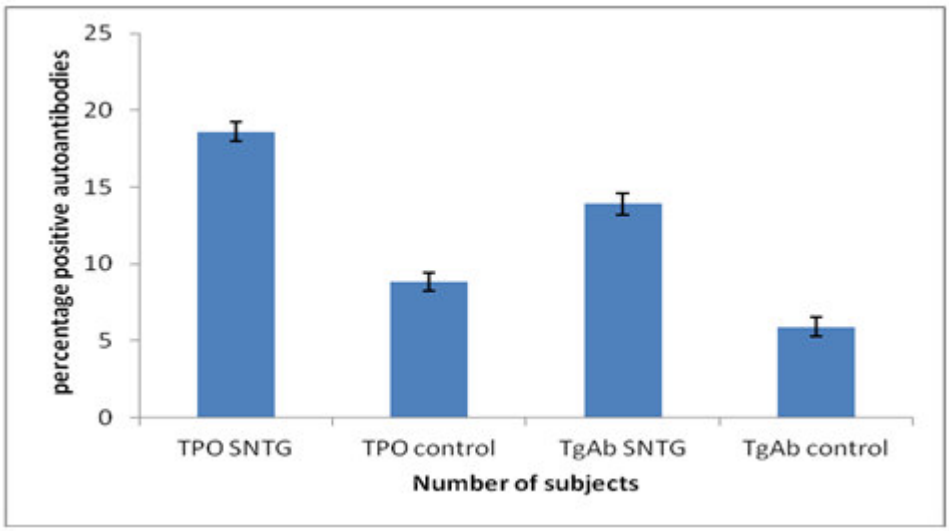

Figure.1 Percentage of subjects with positive TPOAb and TgAb in Simple nontoxic goitre (SNTG) and Controls

Among subjects with Simple nontoxic goitre, 18.6% and 13.9% were positive for TPOAb and TgAb comparedwith controls (8.8% and 5.9%), respectively (Fig.1). The levels of autoantibodies, TPO and Tg as well as urinary iodine demonstrate that there was a high significant increase between the mean levels in SNTG patients compared with controls ($p<0.05$).

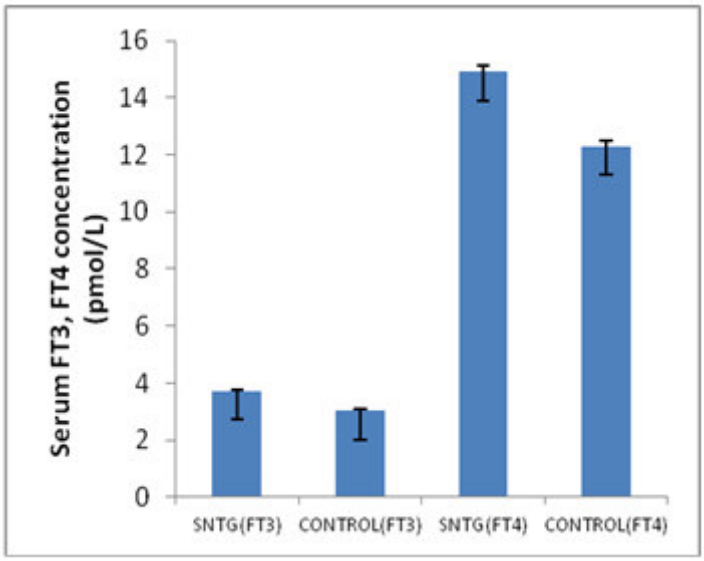

Figure 2: Thyroid function tests (T_3, T_4, FT_3 and FT4) of SNTG female subjects and controls

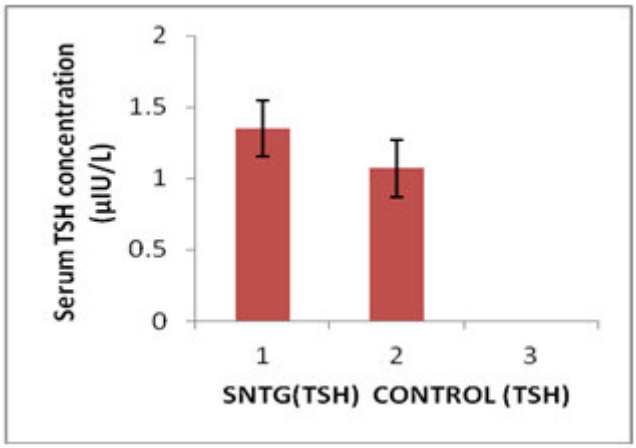

Figure 3: Thyroid function tests (TSH) of Simple nontoxic (SNTG) female subjects and controls.

Indices of thyroid function, the FT_3, FT_4 and TSH displayed significant differences in SNTG (3.72 ± 0.02pmol/L, 14.93±0.21 pmol/L and 1.35±0.04 µIU/L) compared with controls (3.63±0.1 pmol/L, 13.11±0.12pmol/L and 1.07 ± 0.04µIU/L) (Fig. 2 and 3).

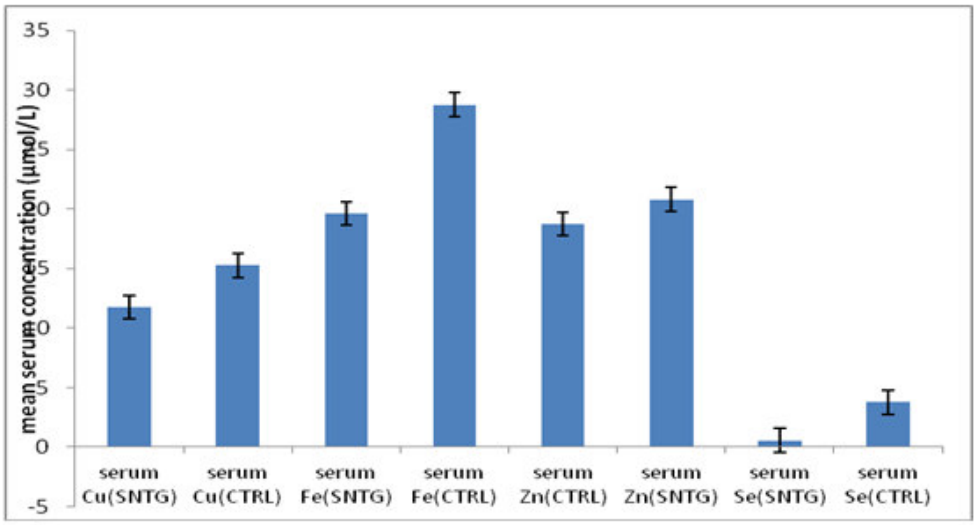

Figure 4: Serum concentrations of trace elements in SNTG and controls.

Significantly reduced levels of Cu (11.76±0.27µmol/L), Fe (19.64±0.54µmol/L), Zn (18.74±0.32µmol/L) and Se (0.52±0.01µmol/L) relative to the controls (15.27±0.48µmol/l; Fe (28.76±0.33 µmol/l; Zn (20.82±0.33 µmol/L) and Se (3.76±0.07 µmol/L) were found in SNTG respectively (Fig, 4). Negative correlations were obtained for FT_4 and TSH (r = - 0.568); FT_3 and TPO (r = - 0.2); TgAb and Zn (r = - 0.3) while positive correlations were found between TPO and IOD (r = 0.3).

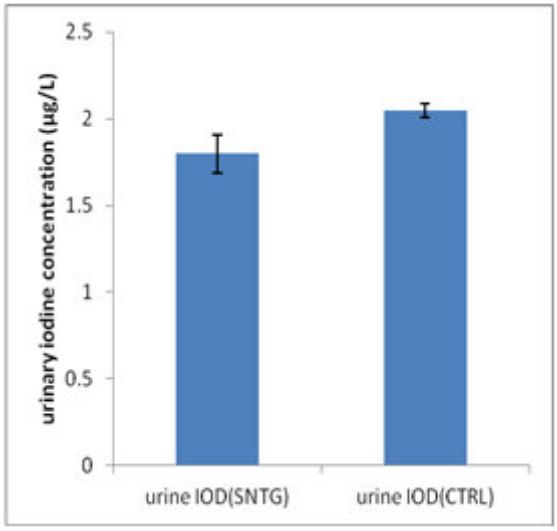

Figure 5: Urinary iodine concentrations of SNTG and controls

Some of the SNTG patients had insufficient urinary iodine concentration while the control subjects all had adequate urinary iodine concentration.

Discussion

The aetiology and or mechanism of simple non toxic goitre remains incompletely elucidated. Data from this study indicate that Thyroid utoantibodies and imbalance between essential and toxic trace elements may have a major contribution to the thyroid disorders; since thyroid diseases are predominantly autoimmune in nature. The significantly elevated autoantibodies in SNTG may suggest an immune alteration (Swain *et* al., 2005).

Thyroid autoantibodies are an invaluable tool in the management of thyroid disorders (Bahn and Castro, 2011). However, antibody testing is not widely available in routine clinical practice in Africa, and few studies have measured thyroid antibodies in African patients. The occurrence of thyroid autoimmunity, and hence the utility of antibody testing in African patients with thyroid disease, is therefore largely unknown. The paucity of data on the alteration of the balance between certain essential and toxic trace elements which has a strong role in the pathogenesis of autoimmune thyroid disease has stimulated the interest in this study. Thyroid autoimmunity is uncommon in iodine-deficient areas but becomes more prevalent with improvements in iodine nutrition (Doufas *et al*., 1999; and Pedersen *et al*., 2006). The majority of participants in this study were resident in Ibadan and Lagos, cities with improving salt iodization, diversified diets, and coastal access to sea foods. The male to female ratio of thyroid disease patients in this study was found to be 1 to 7.16 (6 males to 43 females) strongly indicating hormonal influences. Consequently, varying amounts of these elements may have been attributed to their enrichment in the diet of the inhabitants of polluted areas (Larsen, 2003). Although significant observations have been made on genetic and environmental factors underlying the development of autoimmune thyroid disease, few data are available about the mechanisms by which they interact. The most interesting findings in this field come from research on molecular mimicry between microbial antigens and thyroid autoantigens (Enig, 2010). The molecular mimicry model postulates that, in predisposed subjects, a microbial antigen could trigger autoimmunity because of its structural similarity to an autoantigen of the host, and is a paradigmatic example of the multifactorial interaction of several genes and environmental factors to cause autoimmune diseases, including thyroid diseases.

In the present study, autoantibody positivity and titre were found to be highly significant in SNTG as well as the controls. This may be due to autoimmune involvement. The prevalence rates of TPOAb and TgAb were more frequent in females and increased with age (48-57 years) as a result of hormonal influence. This is in agreement with the findings of Njemini *et al*., 2002, who reported 8-27% and 5-20% of TPOAb and TgAb respectively, but disagree with the findings of Bahn and Castro, 2011, who reported 7% and 4% for TPOAb and TgAb, respectively of healthy adult controls. Of these control groups, four were positive for TPOAb alone, while eight showed both TgAb and TPOAb activity. None was positive for TgAb without being positive for TPOAb. In Western populations, the prevalence of TgAb and/or TPOAb was estimated at about 10% of the general population Hawa *et al*., 2006, but the few available studies in Africans report a much lower prevalence (0–2.7%) in healthy individuals (Hollowell *et al*., 2002 and Li *et al*., 2008). This seemingly reduced tendency for thyroid autoimmunity in black populations is supported by studies of Van den Briel *et al*., 2001, in which thyroid antibodies is less prevalent in African Americans than in Caucasian Americans (Van den Briel *et al*., 2001). The discrepancy between the findings in this study and other African studies may in part be due to differences in methodology (Some of the studies in Africans measured thyroid antibodies using agglutination methods, which are less sensitive

than the more recent chemiluminiscent, RIA and ELISA techniques (used in this study) as well as genetic factors (crucial for the development) re-gional and temporal differences between the groups studied. The prevalence of autoantibodies were significantly higher among females TPO Ab (4.61%) and Tg Ab (3.07%) than males (4.19% and 1.83%), although there was a slight decrease in antibodies in participants older than 58 years and maybe due to hormonal influence, this is similar to the findings of Williams, 2005. Anti-peroxidase and thyroglobulin antibodies can also be present in apparently healthy people, as reported in this study and other previous studies. It is difficult to predict if healthy people with positive autoantibodies will develop thyroid disease (Derumeaux, 2003); it is important to assay along with other thyroid function tests in a routine laboratory. In the present study, there was a negative correlation of iodine with thyroglobulin but positive with autoantibodies (TPO,Tg). The mechanism behind this may be explained by the fact that thyroglobulin combined with high iodine increases its antigenicity and promotes lymphocyte proliferation; this is partly in agreement with the works of Williams, 2005 and Hawk *et al*., 2004. The significantly high level of thyroid hormones T_3, T_4, FT_4, FT_3 and simultaneously low concentration of TSH values in this study maybe due to the mechanism of the HPT-axis and the establishment of a disorder.

From the present study, Fe, Cu, Zn & Se were significantly decreased in SNTG, this may be due to hematopoietic effects leading to increased erythropoiesis (Hazim *et al*., 2006). This is similar to the findings of Zimmermann, 2002 and Zimmermann *et al*., 2007. Iron deficiency hinders the production of thyroid hormone by reducing activity of heme-dependent thyroid peroxidase. Zinc was highly reduced in SNTG, marked alterations in zinc homeostasis were observed in patients with thyroid diseases. Iron deficiency impairs thyroid hormone synthesis reducing the activity of heme-dependent thyroid peroxidase (Zimmermann, 2009); iron is critical for thyroid function, thus iron-deficiency anaemia is often an important factor in hypothyroidism (Lonnerdal, 2000). Iron deficiency has adverse effects on thyroid metabolism, for example it decreases circulating thyroid hormone concentrations and blunts the efficacy of iodine prophylaxis (Kralik *et al*., 1996).

The level of Cu was markedly reduced in patients with SNTG, Copper is known to be critical for normal brain function, and abnormal copper metabolism has been associated with some disorders involving the auditory system (Mooij and Drexhage, 1993). Copper has like zinc been implicated in abnormal thyroid function (Schwartz and Reis, 2000), elements with similar chemical and physical properties interact antagonistically by for instance competing for the same binding sites on transport proteins and enzymes. An example is the antagonism between Cu and Zn which may lead to Cu deficiency and secondary to the Fe deficiency (Zimmermann and Köhrle, 2002). Copper is an integral component of tyrosinase; an enzyme involved in tyrosine metabolism. Tyrosine, an amino acid, important in thyroid hormone biosynthesis (Kucharzewski *et al*., 2002) may be inappropriately metabolised.

Selenium levels was markedly reduced in SNTG, **t**his perhaps is as a result of increased inflammatory activity arising from decreased activity of selenium containing antioxidative enzymes such as glutathione peroxidise (GPx) (Kucharzewski *et al*., 2003). This is consistent with the findings of several investigators (Gartner *et al.*, 2002; Derumeaux *et al*; 2003; Duntas *et al*., 2003; Gartner and Gasnier, 2003). Increasing dietary selenium or administration of selenomethionine has also been reported to diminish TPO antibody levels (Xiaochun *et al*., 2011 and Papanastasiou *et al*., 2007). In support of these observations, several randomized double - blind studies have shown that selenium supplementation can reduce the titre of thyroid autoantibodies (Papanastasiou *et al*., 2007; Xiaochun *et al*., 2011)**.**

A randomized study by (Xiaochun et al., 2011) in 70 women with autoimmune thyroiditis, demonstrated that thyroperoxidase antibody (TPO Ab) concentrations fell significantly in subjects on selenium for three months. Iodine is essential for thyroid function; urinary iodine is the most accurate index for determination a recent iodine intake. A few subjects insufficient urinary iodine concentration indicating a moderate iodine deficiency while most subjects had a adequate iodine intake indicating optimal iodine intake. Worldwide, more people are exposed to more than adequate iodine intake levels with median urinary iodine excretion (MUI 200–300µg/l) or excessive iodine intake levels (MUI >300µg/l) (Vanderpas, 2006). An adverse effect resulting from iodine prophylaxis may be the induction of thyroid autoimmunity (Rasmussen et al., 2002) as substantiated by experimental (Andersen et al., 2005) and epidemiological studies (Vermiglio et al., 2004). Many thyroid processes are inhibited when iodine intake becomes high, and the frequency of apoptosis of follicular cells becomes higher (Enig, 2010). The level of iodine deficiency at which there may be risk of intellectual impairment is not fully established. Brain damage is not directly caused by lack of iodine, but indirectly due to insufficient synthesis of thyroid hormones by the pregnant woman, the foetus and the infant. Thus, the risk would depend not only on the level of iodine supply (and the susceptibility of the brain to such damage), but also on the capability of the thyroid of the mother and child to synthesise hormone at a given level of iodine intake. As discussed above, deficiency of other nutrients and intake of goitrogens that interfere with enzymes or transporters used in the process of thyroid hormone synthesis may worsen the effects of low iodine intake. One such goitrogen is thiocyanate that may be in the diet or generated in the liver from cyanide in tobacco smoke (Lauberg et al., 2009).

We sought to investigate the interaction between autoantibodies and trace elements in simple nontoxic goitre which are comparable with the hypothesis that toxic and altered levels of essential trace elements and

autoantibodies provoke autoimmune disease leading to the increases in thyroid function indices thereby predisposing patients to thyroid disorders. These increases were more pronounced in subjects with autoimmune thyroid diseases due to the presence of autoantibodies. Increased utilization and/or deficiency of the trace elements resulted to their low levels. The prevalence of AITD may be stated to be on the increase in Nigeria. Antimicrosomal antibody is an important and specific marker for thyroid autoimmunity. An appreciable number of patients with simple non toxic goitre and iodine deficiency goitres were found positive for antithyroid antibodies indicative of coexistent autoimmune phenomenon, which may remain undetected or missed unless they are investigated for antibodies. However, further studies are needed to clarify the mechanisms involved in the development of thyroid autoantibodies

Conclusion

Imbalance in toxic and essential trace elements and the presence of these autoantibodies was associated with the development of simple non-toxic goitre dysfunction. This study also indicated that excessive iodine intake might promote thyroid autoimmunity, a risk factor for autoimmune-prone subjects to develop thyroid disorders.

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