·Review·

Selenium supplementation in thyroid associated ophthalmopathy: an update

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Abstract

• The therapeutic effect of selenium (Se) has already been proven in thyroid disease and thyroid associated ophthalmopathy (TAO). In spite of clear scientific proof of its benefits in TAO, there appears to be no clear agreement among the clinicians regarding its optimum dose, duration of the treatment, efficacy and safety to date. In this review, the author summarises the findings of 135 English language articles published on this subject over the past four decades from 1973 to 2013. The regulation and metabolism of thyroid hormones require a steady supply of Se and recent studies have revealed several possible mechanisms by which Se improves the severity of thyroid disease and TAO. These mechanisms include 1) inhibitory effect of HLA-DR molecule expression on thyrocytes; 2) profound reductions of thyroid stimulating hormone (TSH) receptor antibodies (TSHR-Ab) and TPO antibodies (TPO-Ab); 3) prevention of dysregulation of cell-mediated immunity and B cell function; 4) neutralising reactive oxygen species (ROS) and inhibition of redox control processes required for the activation, differentiation and action of lymphocytes, macrophages, neutrophils, natural killer cells involved in both acute and chronic orbital inflammation in TAO; 5) inhibition of expression of pro-inflammatory cytokines and 6) inhibition of prostaglandin and leukotriene synthesis. An increased oxidative stress has been observed in both acute and chronic phases of thyroid disease with raised tissue concentrations of ROS. The benefits of Se supplementation in individuals with TAO appear to be proportionate to the degree of systemic activity of the thyroid disease. The maximal benefit of Se supplementation is therefore seen in the subjects who are hyperthyroid. Restoration of euthyroidism is one of the main goals in the management of TAO and when anti-thyroid drugs are combined with Se, the patients with Graves´ disease (GD) and autoimmune thyroiditis (AIT) achieved euthyroidism faster than those treated with anti-thyroid drugs alone. Se status of normal adult humans can vary widely and Se supplementation may confer benefit only if serum Se levels are insufficient. The author recommends that serum Se levels of patients with TAO to be assessed prior to and during Se supplementation at regular intervals to avoid potential iatrogenic chronic Se overdose.

• KEYWORDS: selenium; selenoproteins; thyroid associated ophthalmopathy; Graves' orbitopathy

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INTRODUCTION

Selenium (Se^{34}_{79}) is a metalloid (semi-metal) which possesses intermediate properties between a metal and a non-metal and its name is derived from Selene- goddess of the moon. Se is found in soil and water and enters the food chain through the roots of plants and aquatic organisms [1]. It acts as an essential cofactor required to activate several enzyme systems in humans [2]. Se is integrated into the polypeptide chains as the 21st amino acid, selenocysteine and the proteins which contain selenocysteine are called selenoproteins (SPs). The key metabolic function of Se has therefore been attributed to its role in this enzymatic cofactor selenocysteine (SeC) [3-7]. A major milestone of Se biochemistry emerged in 1973 when glutathione peroxidise (GPx), a Se containing mammalian enzyme and two such bacterial enzymes were discovered [8-12]. So far, 25 SPs, encoded by 25 human genes, have been characterised in humans although the functions of some of these SPs have yet to be elucidated[13,14]. The most popular selenoenzymes belong to the GPx family which consists of 8 isoforms[15].

The importance of Se and SPs in health and disease is

gaining increasing recognition [16-19]. The possible therapeutic effect of Se has been studied in several diseases such as hemorrhagic pancreatitis, asthma, cardiovascular disease, stroke, severe sepsis, rheumatoid arthritis and even HIV[20-25]. Several studies have also confirmed Se induced inhibition of thyroid cancer cell growth [26-29]. Impaired expression of SPs observed to be associated with perturbed thyroid hormone levels, indicating the importance of Se for thyroid hormone homeostasis and Se found to be beneficial in thyroid associated ophthalmopathy (TAO) which is the most common extra-thyroidal manifestation of thyroid disease[30,31]. Recent studies on the beneficial effects of Se and these SPs in TAO have evoked exciting discussion.

NATURAL SOURCES OF SELENIUM

Although Se is distributed in soils worldwide, factors such as soil composition, plant species and the physiological condition of the plant, environmental conditions and agricultural practices have a profound influence on the Se content of vegetables, fruit, meat, fish and water [32]. Therefore, the Se content of normal adult humans can vary widely and approximately fifteen percent of world's population is Se deficient [33]. Some parts of the world including Middle-East, India, China and some European countries such as Finland are considerably low in soil Se resulting in Se deficiency in the local population [34-38]. In contrast, in seleniferous areas, a significant proportion of the local people consuming locally grown food may manifest signs of Se toxicity [38,39]. As an example, the lentils, grown in Canadian soils are extremely rich in Se (425-673 μg/kg)[33]. A wide geographical variation may also be observed even in different areas in the same country. For example, in one such study, Se intake in adults in Se deficient areas and seleniferous areas in China were found to be 2.6-5.0 and 1338 μg/d respectively [40,41]. Vegetables such as onions and asparagus grown on seleniferous soil may accumulate up to 17 μg/g of Se. Garlic and brassicas (*e.g.* cabbage, broccoli and mustard) are also rich in Se. Other commonly consumed vegetables and fruits generally contain only low amounts, rarely exceeding 10 μg/kg. Brazil nuts have high levels of protein and also known to have a very high concentrations of Se [42-44]. Reyes *et al* [45] reported that the Se content in fish differs widely and ranged between 0.1 and 5.0 mg/kg in the samples of their study. The Se content of cod and shark has been found to be 1.5 and 2.0 mg/kg, respectively [46]. Some marine fish are relatively high in Se [46,47] and Reyes *et al*[45] found a particularly high concentration of 5.6 mg/kg of Se in Tuna. Se concentration in water originates from atmospheric deposits or soil drainage and sub-soils which are naturally rich in Se and varies considerably in different parts of the world. In drinking tap water, the Se concentration is 1 μg/L[32].

ROLE OF SELENOPROTEINS IN THE BODY

Toxins known as reactive oxygen species (ROS) are formed within the cells from oxygen metabolism under normal physiological conditions. If these ROS toxins are not neutralised; they damage to DNA, cell membranes, and a variety of other cellular structures [1]. This may result in cell death and may also trigger a vicious cycle of tissue inflammation. SPs, which are powerful antioxidant enzymes, mitigate the effects of oxidative stress by elimination of ROS [1]. GPx and thioredoxin reductase (TxR) are the two main seleno-enzyme systems responsible for the reduction of these superoxide production [10,48,49]. Se deficiency leads to reduced production of SPs, including GPx, resulting in the accumulation of H_2O_2 causing tissue inflammation and disease [50]. In addition, SPs play a vital role in the regulation of human immune system and Se deficiency is accompanied by dysregulation of both cell-mediated immunity and B cell function[51]. H_2O_2 is also a by-product of inflammatory cascade along with other peroxidases such as lipid hydroperoxide and phospholipid hydroperoxide [52]. Therefore, in Se-sufficient environment, these hydroperoxide intermediates of the cyclooxygenase and lipoxygenase pathways are neutralised effectively resulting in diminished generation of proinflammatory prostaglandins (PGs) and leukotrienes [53]. This minimise the subsequent tissue injury. In addition to these intra-cellular SPs, the Selenoprotein P (SePP) which is produced in the liver is the major circulating form of Se in plasma and it has a high antioxidant potential [54]. It is able to bind to the endothelium and by this mechanism SePP is recruited to the site of the inflammation [19,55-57]. All the above mechanisms of action of SPs in inflammatory disease may explain the beneficial effects of Se in TAO. In addition to the above properties these enzymes have numerous other biological functions in thyroid hormone metabolism, tumour prevention, immune response, reproduction and muscle function[7,32].

EFFECTS OF SELENIUM DEFICIENCY ON THE THYROID GLAND AND THYROID ASSOCIATED OPHTHALMOPATHY

The regulation and metabolism of endocrine systems require a steady supply of several trace elements such as I, Se, Zn, Cu, iron and vitamin A[58-62]. Environmental Se levels strongly correlate with serum Se levels which have been shown to be significantly lower in patients with thyroid disease and TAO and a higher incidence of TAO is seen in the areas deficient in Se. Low Se levels have been observed even in the new

born infants born to mothers suffering from thyroid disease [63]. Se has been found to be an important co-factor for both physiological function and in autoimmune disease of the thyroid. H_2O_2 is an essential co-substrate for thyroid peroxidase (TPO) enzyme during the oxidation of inorganic Iodine for thyroid hormone synthesis and the number of H_2O_2 molecules produced is proportionate to the intensity of TSH receptor stimulation. However, even in physiological conditions a much higher amounts of H_2O_2 are produced than consumed by the iodination process, potentially exposing the thyroid gland to excessive amount of free radicals in addition to the 'normal' share of a cell [64,65]. SPs such as GPx and TxR neutralise these excess H_2O_2 and they are therefore considered as essential SPs in the thyroid hormone synthesis. In pathological hyperactivity, a large volume of H_2O_2 and ROS are produced and proportionately large quantity of Se are required to protect the thyroid gland from superoxide damage [14,66]. Several other agents such as superoxide dismutase, vitamins C and E also assist H_2O_2 disposal[67].

The two main autoimmune thyroid diseases are Graves' disease (GD) which is the most common cause of thyrotoxicosis, and Hashimoto's thyroiditis (HT) which is the most common cause of hypothyroidism. Ninety percent of patients with TAO have Grave's disease and 10% suffer from Hashimoto's thyroiditis and in the latter the eye signs are often mild. These autoimmune thyroid diseases are caused by abnormal immune response to self-thyroid antigens and the key role is played by T lymphocytes when antigen recognition is mediated by receptors on the cell surface (T cell receptor, TC-R). This breaks the tolerance by deficit of suppressor T cells and aberrant expression of DR region of HLA (HLA-DR), absent on normal thyroid cells. The contemporary expression of HLA-DR on thyroid follicular cells and auto-antigens triggers the autoimmune reaction by antibody-dependent, complement-mediated, direct or indirect cytotoxicity [68]. Se has a dose-dependent inhibitory effect on the expression of HLA-DR molecules of thyrocytes induced by interferon-γ and this may explain one of the mechanisms of beneficial effect of Se in reducing the severity of autoimmune thyroid disease [69,70].

In GD, the loss of tolerance of T cells to the thyroid-stimulating hormone receptors (TSHR), *via* yet unknown mechanisms, ignites an autoimmune process. This first step of the disease process of GD is considered to be precipitated by environmental factors of an HLA related organ-specific defect in suppressor T-lymphocyte function. The TSHR is internalized and presented by antigen-presenting cells to helper T cells. This results in an excessive secretion of TSH receptor antibodies (TSHR-Ab) by activated B cells. These antibodies bind to the TSHR on the thyrocytes and fibroblasts of the orbit, where they initiate the ocular changes. This antigen-antibody reaction on the thyrocytes mimic the action of TSH but with a 'long-acting' effect resulting in an unregulated growth and the function of thyroid follicular cells leading to the excessive production of thyroid hormones [68,71]. This also stimulates H_2O_2 production and subjects the thyroid gland to extremely high levels of H_2O_2 requiring a constant, much higher supply of SPs in order to neutralise the excess H_2O_2 to minimise tissue injury. In a population based study Pedersen *et al* [72] demonstrated significantly lower concentrations of serum Se in GD compared to normal subjects. Xu *et al* [73] investigated the effect of Se on the thyroid glands of patients subjected to excessive iodine intake and found that supplemental Se could alleviate toxic effect of excessive iodine on thyroid as well. Although thyroid hormone synthesis is compartmentalized to the lumen of the follicles and both the DUOX enzymes and TPO are localized to the apical membrane of the thyrocytes, H_2O_2 can freely diffuse into the cytoplasm and nucleus, where it may lead to aberrant oxidation and iodination of proteins and lipids trigger apoptosis and induce DNA damage. Therefore, H_2O_2 induced tissue damage may liberate thyroid hormone stored in the colloid in the follicle lumen into the circulation further worsening the severity of hyperthyroidism. In severe Se deficiency, peroxide cleavage within the thyroid cells is diminished [51] and nutritional Se deficiency therefore leads to an increased rate of thyroid cell necrosis and invasion of macrophages and further increase in thyroid hormone levels in blood due to liberation of stored thyroid hormones [74-76]. Like Iodine, Se also influence on the size of the thyroid gland. Rasmussen *et al* [77] showed an inverse relationship between the Serum Se concentration and the volume of the thyroid gland. Se deficiency can also exacerbate the effects of iodine deficiency and the same is true for vitamin A or iron deficiency[60,78].

TAO in GD is caused by inflammation of extra-ocular muscles and orbital adipose tissue. Serum TSHR-Ab is present in 70%-100% patients with Graves' disease and in 1%-2% of normal individuals [79-81]. In addition to thyrocytes, TSH receptors are also expressed in the orbital fibroblasts and preadipocytes and when bound by TSHR-Ab triggers a chronic inflammatory cascade resulting in swelling of the orbital tissues in TAO. Kloprogge and Frauman [82] reported positive TSH receptors even within normal human muscle fibres, using 3G4 and 3B12 antibodies. Using a similar technique Boschi *et al* [83] compared orbital tissues from 30

patients with TAO with 24 patients with non-thyroid orbital inflammation or strabismus and demonstrated significantly high TSH receptor expression in elongated fibroblast-like cells located between the muscle cells in all TAO biopsies. Therefore, the ophthalmic manifestations of GD are the product of a close interaction between orbital fibroblasts and T-cell lymphocytes[84]. Various classes of immunomodulators (*e.g.* HLA antigens, CTLA-4, cytokines) mediate this interaction[84]. Polymorphisms in immunomodulator genes can alter the interaction between T-cells and orbital fibroblasts and impact disease susceptibility and progression[84]. Growing evidence supports that the Se-containing enzymes and their antioxidant capacity somehow modify the autoimmune mechanism[52]. These SPs have diverse effects on the immune system, either stimulating or inhibiting the immunological response in order to regulate inflammation. Similar effects of Se on extraocular muscles and other inflamed orbital tissue may explain the beneficial effect of Se in TAO [85]. Although anti-thyroid peroxidase (TPO-Ab) antibodies are most commonly associated with Hashimoto's thyroiditis and TSHR-Ab are most commonly associated with Graves' disease, there is an overlap[79,80]. TPO-Abs are specific for the autoantigen TPO and present in approximately 90% of Hashimoto's thyroiditis, 75% of Graves' disease and 10%-20% of nodular goitre or thyroid carcinoma. Also, 10%-15% of normal individuals can have high level TPO-Ab titres [79,86]. In addition, the patients with Hashimoto disease have a lower GPx activity than healthy subjects[87].

There is clear evidence that the benefits of Se supplementation are greater when it is commenced earlier in the disease process in patients with autoimmune thyroiditis (AIT) [88]. Karanikas *et al* [89] suggested that the variable benefit of Se supplementation in individuals with AIT may be explained by the disease activity and the degree of inflmmation. Toulis *et al* [90] reported a significant lowering of TPO-Ab titers in patients with Hashimoto's thyroiditis in response to Se supplementation for 3mo. In a blinded placebo-controlled prospective study undertaken by Bhuyan *et al* [74] the mean anti-TPO antibody concentration dropped by 49.5% in the group treated with a daily dose of 200 μg (2.53 μmoL) of oral sodium selenite for 3mo compared to 10.1% reduction in the control group. In a similar study conducted in a Se depleted area of Bavaria in southern Germany, Gärtner *et al* [51] showed a 36% reduction in TPO-Ab titres in the Se-treated group. A subgroup analysis of those patients with TPO-Ab greater than 1200 IU/mL revealed a mean 40% reduction in the Se-treated patients compared with a 10% increase in TPO-Ab in the placebo group [51]. The significantly higher response to oral sodium selenite was noted in hyperthyroid patients compared to euthyroid or hypothyroid patients in this study as well. In the subgroup analysis of their patients, Bhuyan *et al* [74] noted a reduction in the TPO-Ab titre up to 64.42% in their subclinical hyperthyroid group of patients. The reduction in the TPO-Ab titre in the euthyroid, hypothyroid, and subclinical hypothyroid groups were still significant and were 41.13%, 47.18%, and 42.64% respectively [74]. In another prospective placebo-controlled prospective study including 132 patients with autoimmune thyroiditis, Balázs and Fehér [91] demonstrated a decreased inflammatory activity parallel to the reduction of TPO-Ab titres in response to Se supplementation. An inverse correlation was found between antioxidant capacity and level of TPO-Ab. This observation raises the suspicion that Se deficiency by itself might be responsible for the precipitation of thyroid disease. In addition, several studies have seen an improvement in mood and/or general well-being in these patients. Zagrodzki and Ratajczak [58] observed a sharp fall of Se and GPx3 with a marked increase in TPO-Ab promptly after withdrawal of Se supplementation. In contrast, in two similar studies Anastasilakis *et al* [92] failed to demonstrate a significant benefit of Se on serum thyroid auto-antibody levels or lymphocyte infiltration of the thyroid gland in Hashimoto's thyroiditis and Bonfig *et al* [93] observed that Se supplementation did not decrease TPO-Ab concentration in children and adolescents.

The GD is characterised by the presence of increased oxidative stress in both acute and chronic phase of the disease [94,95]. The pathogenesis of TAO in GD substantially lies on the presence of an inflammatory-cell infiltrate predominantly composed of activated T cells producing cytokines (mainly IL-1, TNF-α, IFN-γ) which, in turn, activate orbital fibroblast secretion of glycosaminoglycans, further inducing orbital fibrosis and oedema [96]. In a retrospective study investigating 83 patients with GD, Wertenbruch *et al* [97] demonstrated a significant low concentrations of TSHR-Ab levels in patients who had a high serum Se levels with remission of GD. Corroborating this evidence is the finding that patients with GD, when treated with a mixture of antioxidants, including Se combined with anti-thyroid drugs, achieved euthyroidism faster than those treated with anti-thyroid drugs alone[98,99].

Evidence suggests that Se deficiency affects both the cell-mediated and humoral immunity, which are linked to inflammatory processes involving the production of ROS and redox control processes [7]. ROS production increases

expression of proinflammatory cytokines through up-regulation of nuclear factor-kappa B (NF-κ B) activity[100]. Lymphocytes, macrophages, and especially neutrophils require ROS and proinflammatory molecules for activation, differentiation, and phagocytosis [7]. This may be another mechanism by which Se exerts its beneficial effects in TAO. In addition, high Se levels are associated with fewer natural killer cells [100,101]. Confirming the above hypothesis Xue *et al* [102] demonstrated a significant difference between the severities of lymphocytic infiltration in thyroids of Se treated and untreated mice with AIT.

GPx and TxR decrease free radical formation and reduce H_2O_2 and lipid and phospholipid hydroperoxides. The key enzymes of prostaglandin and leukotriene synthesis require a certain peroxide tone to become active. In fact, they are product-activated [10]. Accordingly, GPx plus reduced glutathione prevent any arachidonate utilization by cyclo-oxygenase, 5-lipoxygenase, and 15-lipoxygenase [10]. In Se-sufficient environment, the hydroperoxide intermediates of the cyclooxygenase and lipoxygenase pathways are therefore reduced and lead to diminished production of proinflammatory PGs and leukotrienes [53]. In addition, both GPx and TxR modulate the respiratory burst and reduce superoxide production [10]. This may be considered as another beneficial effect of SPs in TAO where SPs neutralise the ROS released during autoimmune process and reduce the production of proinflammatory PGs and leukotrienes. In addition, there is evidence to support the beneficial effects of Se on the psychological well-being of patients with TAO[90].

CAUSES OF SELENIUM DEFICIENCY

The Se deficiency is mostly caused by low dietary intake (see above) or poor intestinal absorption. Rarely Se and SP deficiency can be genetically inherited [103]. Individuals with inherited defects in selenocysteine insertion sequence (SECIS) binding protein 2 display a syndrome of selenoprotein-related defects including abnormal thyroid hormone metabolism [103]. Selenocysteine incorporation sequence binding protein 2 (SBP2) represents a key trans-acting factor for the co-translational insertion of selenocysteine into SPs [104]. In individuals with SBP2 deficiency due to mutations in the SBP2 gene the dietary Se intake is obviously not the limiting factor in the individuals when regular daily Se intake is provided [104]. The total serum Se concentrations in such individuals with selenoprotein biosynthesis defects respond to selenomethionine supplementation [104].

METHODS TO ASSESS SELENIUM DEFICIENCY

There are several methods for evaluating Se in humans. With current laboratory facilities Se can be measured in plasma, serum, or even in tissues such as kidney and liver [32]. It can also be measured in urine, hair and nails [32]. The plasma Se level represents the amount of circulating SPs and selenoenzymes [105]. Assessment of GPx activity in erythrocytes is another measure of Se status of an individual and this can be assessed by an indirect technique[32]. Tiran *et al* [106] described a procedure for determination of Se by hydride generation atomic absorption spectroscopy (AAS) in whole blood, serum and urine. It employs sulfuric acid, H_2O_2 and vanadium (V) sulfuric acid reagent solution. This method uses no explosive reagents and can be performed at a constant temperature of 100℃ and it gives rapid reading. Therefore, it is easily applicable in a routine clinical laboratory for a large amount of samples. Se levels in serum can be assessed by a commercially available atomic absorption spectrophotometer such as Perkin-Elmer model 3100 (Perkin-Elmer Corp., Norwalk, CT, USA) in combination with an MHS-FIAS-200 flow injection hydride generation system and an AS-90 auto-sampler [107]. Jacobson and Lockitch[108] also described a development of a direct method for determination of Se in serum by graphite-furnace atomic absorption spectrophotometry with deuterium background correction. There are several methods to determine the GPx activity in plasma and whole blood. In one such method GPx activity can be determined in plasma and whole blood using a modification of the method of described by Paglia DE and Valentine WN using tertiary butyl hydroperoxide [109,110]. An automated analyser such as Cobas Fara Autoanalyser (Hoffman-La Roche, Basle, Switzerland) can be used for this purpose[107].

SELENIUM SUPPLEMENTATION

Blood Se concentrations in residents in a several European locations found to be below the concentration required for optimal plasma GPx activity in humans [111]. Schrauzer and White [112] estimated that typical daily intake of Se per person to range from 90 to168 μg/d based on a 30d study. Se intake in Europe is lower than in the United States and in many countries it is below the UK reference nutrient intake of 75 mg/d[113]. The highest intakes were observed in individuals subsisting on diets rich in whole wheat grain cereal products and seafood such as crab, other shellfish and fish [113]. The Se concentrations in whole blood correlate with the dietary Se intakes directly [112]. To provide a sufficiently wide margin of safety, the reference dose (RfD) for Se from all nutritional sources for a 70-kg human has been set at 350 mg/d [114], corresponding to 5 mg Se/kg body weight/day. The RfD is the amount of safe total intake of Se by an adult who subsists

on a normal diet and is taking an additional 200 mg Se a day in the form of a nutritional supplement [114]. SPs are classified as essential and nonessential and the essential SPs decrease more slowly than non-essentaial SPs in Se deficiency and their levels recover more rapidly on Se re-supplementation than nonessential SPs[115-117].Thomson *et al* [118,119] demonstrated a significant increase in plasma Se and whole blood GPx activity with a daily supplementation of 100 μg L-selenomethionine for 12wk. No changes were found in the concentrations of TSH, free T3, free T4, or thyroglobulin concentrations, apart from a nonsignificant increase in free T3 in this cohort of patients in response to Se supplements[118,120]. Several other studies demonstrated the absence of significant influence of Se on the free T3, Free T4, TSH levels [120-122]. In the meantime, some randomized controlled trials in healthy human adults have shown a statistically-significant decrease in serum T4 after Se supplementation[123,124].

According to Gärtner *et al* [51] it took 3mo to achieve a significant reduction of the mean TPO-Ab concentration when supplemented with 200 μg (2.53 μmoL) of oral sodium selenite. Twenty five percent of their patients showed a complete normalization of both TPO-Ab concentrations as well as thyroid ultrasound echogenicity with Se supplementation [51]. Nacamulli *et al* [125] demonstrated that dietary supplementation with physiological doses of Se for 12mo to be effective in reducing both TPO-Ab and thyroglobulin autoantibodies (Tg-Ab). Combs *et al* [120] indicated that, a period of 6mo is required to reach a new steady state plasma Se concentration in healthy subjects when supplemented with 200 μg Se per day as SeMet. Se supplementation found to be significantly associated with thyroid volume regression in autoimmune thyroiditis [77,126]. Marcocci *et al* [127] recommends a 6-mo course of Se in patients with mild Graves' orbitopathy. To date, there is no robust scientific evidence recommending the optimal duration of Se supplementation in TAO.

SELENIUM OVERDOSE AND TOXICITY

Se supplements have become a popular treatment option universally in the management of TAO and thyroid disease in general. In the absence of robust evidence to support the optimum duration of Se supplementation, the clinicians adopt drug regimens of personal preference rather than a universally agreed protocol. The Se status of the individual is not often investigated prior to or during the period of Se supplementation. Although the need for Se in human nutrition is well recognized, like other trace elements Se has long been recognised for its toxicity [32,128]. Chronic Se overdose, or selenosis, often presents with nail and hair changes and alopecia [129,130]. Nail changes are the most common sign of chronic Se poisoning and they become brittle, and white spots and longitudinal streaks appear on the surface. As chronic poisoning becomes more severe, breaks in the nail occur and the nail can be lost; nails may grow back deformed and be lost repeatedly. Fragile nails and similar changes are obviously not specific for selenosis, and other causes include fungal infection, psoriasis, and arsenic exposure and zinc deficiency. When examining a patient with raised serum Se, the absence of characteristic nail changes suggests that the raised blood Se is due to recent intake rather than due to chronic poisoning. Therefore, the examination of nails should be included in the examination of the patients on Se supplements for overdose. Other features may include nausea, vomiting, diarrhoea, fatigue, and skin lesions. Musculoskeletal disorders such as stiff gait and lameness can occur due to alteration of the cartilages [9]. Peripheral paresthesias can be present, along with hyperreflexia and pain in the extremities. As selenosis progresses, decreased cognitive function, weakness, paralysis, and death may occur. Prevention of further exposure is the most important aspect of the treatment and conservative management is recommended. Chelation is not recommended since animal studies suggest it may increase toxicity[128,131].

DISCUSSION

Se is an essential mineral with several important protective functions in TAO. Se deficiency has shown to be a key environmental factor which together with the genetic variants thought to precipitates autoimmune thyroid disease in several parts of the world deficient in soil Se [132]. It protects the thyrocytes from superoxide induced tissue damage and has several modifying effects on the thyroid autoantibodies which are thought to trigger the ophthalmic manifestations. In addition, Selenoproteins have anti-inflammatory properties. They lower hydroperoxides within tissues and inhibit the production of inflammatory prostaglandins and leukotrienes. Therefore, it is postulated that even mild Se deficiency may contribute to the development and maintenance of autoimmune thyroid diseases and TAO [51]. Dickson and Tomlinson [133] discovered the highest tissue concentration of Se in the human body in the glandular tissue of the thyroid indicating the importance of Se in physiology of thyroid. In Graves' disease, Se supplementation results in euthyroidism being achieved more rapidly with a beneficial effect on mild inflammatory orbitopathy[134]. Marcocci *et al* [135] carried out a randomized, double-blind, placebo-controlled trial to determine the effect of Se or pentoxifylline (an anti-inflammatory agent) in 159 patients who had mild signs or

symptoms of GO of less than 18 months' duration. At the 6-month evaluation, treatment with Se, but not with pentoxifylline, was associated with an improved quality of life (P <0.001) and less eye involvement (P =0.01) and slowed the progression of Graves' orbitopathy (P =0.01), as compared with placebo[135]. Based on this literature review Se appears to have a beneficial in reducing the extraocular muscle and orbital adipose tissue inflammation and Se may exert these beneficial effects by reducing the TPO-Ab and TSHR-Ab concentrations, regulation of immune mechanisms and inhibiting orbital inflammation[74,91,97,98,102,135].

Although Se supplementation appears to be beneficial in TAO it will confer a benefit only if intake of a nutrient is inadequate. Supplementation of people who already have adequate intake with additional Se might increase their risk of type-2 diabetes [111,134]. There is a wide geographical variation of availability of Se in food. The crucial factor that needs to be emphasised with regard to the health effects of Se is that whereas additional Se intake may benefit people with low status, those with adequate-to-high status might be affected adversely and should not take Se supplements [111]. Therefore, it is important to assess the Se status of the individual patients prior to Se supplementation and at regular intervals while on treatment.

CONCLUSION

Se is a unique trace element in its structural incorporation into proteins and it is essential for optimal endocrine and immune function and moderating the inflammatory response. On the other hand, thyroid autoimmune disease, a multifactorial organ-specific autoimmune disorder, is marking a constant increase worldwide and it is thought to be caused by multiple environmental factors triggering autoimmune response in genetically susceptible individuals, though the exact mechanisms linking environmental factors to thyroid autoimmunity are not as yet well understood. Nevertheless, there is increasing evidence that nutritive and environmental factors are the main determinants in the present-day distribution of this disease and its ophthalmic manifestations. Even mild Se deficiency thought to contribute to the development and maintenance of autoimmune thyroid diseases and TAO. The patients with thyroid diseases such as GD and even thyroid cancer appear to have low levels of serum Se levels compared to the age matched controls. Several studies have shown that the Se substitution could have a significant impact on inflammatory activity in thyroid-specific autoimmune disease and a significant improvement its ophthalmic manifestations. There appears to be several mechanisms by which Se reduce the severity of TAO. These include inhibitory effect of HLA-DR molecule expression on thyrocytes, the reduction of serum TPO-Ab/TSHR-Ab concentrations, influence on the cell mediated/humoral immune pathways, anti inflammatory and anti-oxident properties of Se. These beneficial effects of Se explain why the efficacy of Se substitution is proportionate to the inflammatory activity of autoimmune thyroid disease and TAO.

Whilst it seems reasonable to recommend Se substitution to reduce the severity of TAO and autoimmune thyroid disease the Se status of the individual patients should be taken into account prior to prescribing Se supplements to avoid chronic iatrogenic overdose. In current practice, the laboratory measurements of Se are not routine in TAO, but the author propose that early assessment of Se status should become mandatory prior to Se supplementation to determine the dose and duration of supplementation of this vital micronutrient.

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