

Level Of Interleukin 6, Malondialdehyde And Calcium In Hypo And Hyper Thyroidism

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ABSTRACT

INTRODUCTION:Thyroid hormones are essential throughout life for the regulation of development, calorogenesis, and metabolic rate. They operate on almost all organs in the body, including the gut and the visceral, so disturbances in the function of the thyroid may have gastrointestinal manifestations and concomitant effects on the immune system on body tissues. It is possible that cytokines play an important role in thyroid autoimmune disease. IL-6 is a 26 KD protein that is produced by fibroblasts, endothelial cells, monocytes, and T lymphocytes. Typically, hyperthyroidism can cause limited serum calcium elevation. In up to 20 percent of patients with hyperthyroidism, asymptomatic serum calcium elevation has been reported and is due to increased bone resorption and subsequent release of calcium from the bone into the bloodstream. There are well-known effects of thyroid hormones on mitochondrial oxygen intake. Total antioxidant status (TAS) provides information on all antioxidants in the organism, while Malondialdehyde (MDA) is a marker of lipid peroxidation that is used to measure lipid peroxidation due to increased oxidative stress.

METHODS:The study was carried out at SMHRC, Maharashtra India during the period of August 2019 to May 2020. We enrolled 150 patients (50 hyperthyroidism Female, 50 hypothyroidism Female and 50 normal females) aged 25-50 years. The control subjects and patient groups were not taking any medication that affect thyroid metabolism. Serum IL-6 was measured by an enzyme-linked immunosorbent assay. MDA was estimated by TBA method.

Results: In hyperthyroid women's cells, the development of IL-6 by blood mononuclear cells was higher. Substantial increased levels of IL-6 were observed in untreated hyperthyroid patients relative to healthy controls ($p < 0.001$). Hyperthyroidism may be associated with hypercalcemia symptoms. Compared to controls, MDA was elevated in hypothyroid patients.

Conclusion: We found that serum IL-6 increased in hyperthyroidism patients, but reduced in hypothyroidism patients, and normal in control group. The correlation of hypercalcemia with hyperthyroidism should be known to the clinician. These findings indicate an increased malondialdehyde in hypothyroid states, which can be clarified by both the inadequate rise in

antioxidant level and the altered lipid metabolism in these cases. This may promote early diagnosis and effective intervention.

Keywords: Hyperthyroidism, hypothyroidism, IL-6, Hypercalcemia and MDA

INTRODUCTION

For normal skeletal development, thyroid hormones are required. Their abundance, however, can result in bone loss, especially in adulthood. Osteoporosis[1] and increased fracture rate[2] are caused by hyperthyroidism. Thyroid hormones affect the functional or developmental behaviour of bone marrow cells and even cells in secondary lymphoid tissues. Reports suggest that there is impaired production of B cells in extreme hypothyroidism due to thyrotropin receptor (TSHR) defects. [3,4]

It is possible that cytokines play an important role in thyroid autoimmune disease. These molecules, developed by thyroid follicular cells, are important for the growth and differentiation of T and B cells [5,6].

IL-6 is produced in a variety of tissues, such as bone [7], the thyroid, and blood mononuclear cells. These mononuclear cells also express thyroid hormone receptors. It is a pleiotropic cytokine exerting multiple biologic activities on different types of target cells including induction of B cell differentiation [8], stimulation of myeloma, hybridoma and plasmacytoma growth, activation of T cell thymocytes [9], induction of acute phase proteins, stimulation of hemopoietic precursor cell growth and differentiation, induction of myelomonocytic differentiation [10], pyrogenic action, inhibition of cell growth and induction of ACTH synthesis. [11]

Primary hyperparathyroidism and malignancy are by far the most common causes of hypercalcemia in our clinical practise. The most common cause of asymptomatic hypercalcemia is primary hyperparathyroidism and is seen in outpatients, while hypercalcemia secondary to malignancy is seen in hospitalised patients and is typically symptomatic. [12]

By oxidising cellular macromolecules such as carbohydrates, lipids and proteins, mitochondria are the main development site of free oxygen radicals, which can trigger organ dysfunction. Oxidative stress can result from either overproduction or failure of the antioxidant defence systems of these organisms. Thyroid hormones have well-documented effects on mitochondrial oxygen use, however there is uncertainty about how hypothyroidism affects oxidative stress, and less is known regarding oxidative stress in subclinical hypothyroidism. There is uncertainty about the mechanism of increased oxidative stress in hypothyroidism. While most studies have not indicated this, it is suspected that an ineffective antioxidant defence mechanism is a factor. [13]

In this research, we aimed to establish how hyper and hypothyroid patients are affected by cytokines IL-6, Malondialdehyde (MDA) and calcium levels as compared to healthy controls.

METHODS

A population-based retrospective cohort study was conducted in 150 patients (50 female hyperthyroidism, 50 female hypothyroidism and 50 regular females) aged 25-50 years who were admitted to SMHRC, Nagpur, from August 2019 to May 2020. The JNMC & ABVRH's Institutional Review Board accepted this report. Until registration, all participants issued written informed consent.

STUDY POPULATION

The study population consisted of 50 hyperthyroid (age: 25–50 yrs.; 35 pre- and 15 postmenopausal) and 50 hypothyroid (age: 25–5 yrs.; 30 pre and 20 postmenopausal) Caucasian women before antithyroid or replacement therapy, respectively, as well as 50 healthy euthyroid controls (age: 25–50 yrs.; 25 pre- and 25 postmenopausal). Hypothyroidism was caused by autoimmune thyroiditis in 5 patients, radioisotope treatment in 2 patients and surgery in 3 patients. Subjects in all groups were free of any diseases or were not taking any medication known to affect calcium metabolism. Informed consent was obtained from all subjects.

BIOCHEMICAL MEASUREMENTS

3ml blood sample were collected in plain vial, serum separated from the blood sample and were used for the estimation of serum IL-6 by an ELISA Method.[14] The serum was used for estimation of MDA measured by using UV-VIS spectrophotometer.[15] Calcium was estimated by Arsenazo method.[16]

RESULT

Compared to healthy controls, substantially elevated levels of IL-6 were observed in hyperthyroid patients (p<0.001). On the contrary, in hypothyroid patients, comparatively decreased IL-6 levels were found. About (p<0.01). In addition, in control subjects, a positive association between serum IL-6 levels was also observed. IL-6, however, has not shown a correlation with T3, T4 and TSH levels.

In patients with hyperthyroidism, serum calcium was found to be higher than in patients with hypothyroidism, with euthyroid goitre, with subclinical hypothyroidism, and in healthy individuals. 35/150 individuals had serum calcium levels < 2.1 mmol / L, including 31 patients with hypoparathyroidism after strumectomy and 5 patients with primary hypoparathyroidism. 75/150 individuals reported serum calcium of > 2.6 mmol / L, which was due to primary hyperparathyroidism in 20 cases. Of 60 individuals with S-Ca > 2.6 mmol / L and no other cause for hypercalcemia, 45 were found to be hyperthyroid.

Compared to hyperthyroid patients, MDA was elevated in hypothyroid patients, but there was no substantial difference in MDA between the hyperthyroid and control groups. There was a major correlation between MDA and the hypothyroid community. There was no correlation between MDA and fT3, fT4, and TSH levels.

Table no.1 – shows serum IL6, Malonaaldihyde and calcium levels in mean ±SD

	Hyperthyroid (n = 50)	Hypothyroid (n = 50)	Control (n = 50)
IL-6 (pg/ml)	11.1±2.3*	8.2±2.4	10.6±2.2
Malonaaldihyde μ mol/L)	5.8 ± 2.6	6.3 ± 2.4*	6.9±3.5
Calcium mmol/L	14.5±4.4*	9.9±3.2#	11.6±4.3

*p<0.001- significant

Compared to healthy controls, substantially elevated levels of IL-6 were observed in hyperthyroid patients (p<0.001). Compared with hyperthyroid patients (p<0.001) and the control group, MDA was significantly elevated in hypothyroid patients. In patients with hyperthyroidism, serum calcium was found to be substantially higher than in those with hypothyroidism.

DISCUSSION

Thyroid hormones are the most significant factors affecting the basal metabolic rate during normal physiological states by altering the intake of mitochondrial oxygen, the key free radical development site.[17] Thus, it is not surprising that changes in thyroid hormone levels affect the generation of mitochondrial free-radical hormones.[18] Thyroid hormones have been shown to influence synthesis and degradation. Overt hypothyroidism is supposed to delay free radical generation because of the associated lower metabolic rate, as shown in several studies [19] In comparison, several studies have shown increased oxidative stress in overt hypothyroidism.[20] The role of cytokines in pathophysiology has been shown by many in vivo models to demonstrate that a given inflammatory stimulus results not in the generation of a single cytokine, but rather in a complex cytokine release cascade [21]. We have shown that in hyperthyroid women, IL-6 levels are increased in circulation independently of the aetiology of thyroid hyperfunction. Serum IL-6 was not substantially diminished by hypothyroidism. Increased serum

IL-6 in hyperthyroidism could derive from several sources, including the thyroid gland, mononuclear blood cells, and bone tissue. [22]

In a small group of patients with GD, Weetmannel al. (23) observed increased intrathyroidal but not serum IL-6 levels. Celiket al. [13] registered enhanced serum IL-6 levels in both GD and toxic multinodular goitre, which returned to normal with propyl thiouracil treatment after euthyroidism was restored. Increased intrathyroid development of IL-6 in thyroid hyperfunction is therefore one cause of high serum IL-6 levels.

In hyperthyroid patients, Bartalena et al [24] observed elevated serum IL-6 concentrations. Similar to that reported in hyperthyroid disease, our findings of an increase in serum IL-6 levels [25] again indicate that a similar immunological effector mechanism could be involved in our patients, as in hyperthyroid disease. Thus, one cause of elevated serum IL-6 levels is increased intrathyroid development of IL-6 in hyperthyroid patients.

In hypothyroid patients, we observed that MDA was elevated, which may be an indirect suggestion of increased oxidative stress in these pathological states. Pereira et al . found lipid peroxidation decreased in hypothyroid rat lymphoid tissues[26] In comparison, in hypothyroid patients, Dimitriuet et al. found increased lipid peroxidation.[27]

Our findings are close to those of the Konukogluet al. study in which hypothyroid patients find increased oxidative stress.[28]Konukogluet al. found that levels of antioxidant plasma protein thiol decreased and stabilised with thyroxine therapy in hypothyroid patients, indicating that insufficiency of the antioxidant protection system could be a leading factor in increased oxidation. Tissue hypoxia may be accompanied by a rise in oxidative stress and the resulting lipid peroxidation.

Symptoms of hypercalcemia may be associated with hyperthyroidism. Thyrotoxicosis hypercalcemia is usually moderate, with calcium concentrations rarely reaching 4 mmol[29]. As confirmed by low parathyroid hormone (PTH) levels and hypercalcemia resolution with thyroid disease therapy, our case indicates hypercalcemia merely due to thyrotoxicosis. In conjunction with hyperthyroidism, our research reported simultaneous primary hyperparathyroidism.

Albert Shiehand Dorothy Santos Martinez addressed a similar case at the annual meeting of the Endocrine Society[30]. A similar case of vitamin deficiency[31] was reported by Korytnaya E and colleagues. The appearance of our patient, however, was rare as apathic, without any adrenergic symptoms. Just one case study in the literature indicated that symptoms of hypercalcemia were present[32]. A linear association between serum calcium and thyroid hormone levels that is more pronounced in people over 60 years of age[33] has been observed. Our patients were 50 years old and had a mild level of thyroid hormone hypercalcemia[34-35].

CONCLUSION

In conclusion, the current findings indicate that IL-6 levels are significantly elevated in hyperthyroid patients, decreased in hypothyroid patients and normalised in the control group. In order to validate these findings, more in vivo and in vitro studies would be needed. These results indicate an increased oxidative stress in hypothyroid and subclinical hypothyroidism states, which can be clarified in these cases by both the inadequate increase in antioxidant status and the altered lipid metabolism. Clinicians should be conscious that hypercalcemia is associated with hyperthyroidism. This will make early detection and suitable intervention simpler.

REFERENCES

1. Toh SH, Claunch BC, Brown PH. 1985 Effect of hyperthyroidism and its treatment on bone mineral content. *Arch Int Med.* 145:883– 886.
2. Fraser SA, Anderson JB, Smith DA, Wilson GM. 1971 Osteoporosis and fractures following thyrotoxicosis. *Lancet.* 1:981–983.
3. Foster MP, Montecino-Rodriguez E, Dorshkind K. Proliferation of bone marrow pro-B cells is dependent on stimulation by the pituitary/thyroid axis. *J Immunol,* 1999; 163:5883–5889.
4. Dorshkind K, Horseman ND. The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: Insights from genetic models of hormone and hormone receptor deficiency. *Endocrine Rev.,* 2005; 21:292–312

5. Mzezewa, S., Setati, M., Netshiongolwe, T., & Sinoamadi, V. (2020). Prevalence of breast cancer in reduction mammoplasty specimens, in women of African origin: preliminary histology results at Mankweng and Polokwane hospitals. *Journal of Medical Research and Health Sciences*, 3(10), 1109-1113. <https://doi.org/10.15520/jmrhs.v3i10.264>
6. Watson PF, Pickerill AP, Davies R, Weetman AP. Semi-quantitative analysis of interleukin -1a ,interleukin -6 and interleukin-8 mRNA expression by human thyrocytes . *J Mol Endocrinol* 15: 11-21, 1995.
7. Grubeck-Loebenstein B, Buchan G, Chantry D. Analysis of intrathyroidal cytokine production in thyroid autoimmune disease. Thyroid follicular cells produce interleukin-1a and interleukin-6. *ClinExpImmunol* 77: 324-330, 1989.
8. Ahmed, M. S., Naznin, N., & Alam, M. J. (2020). Adaptation of Apraxia Battery for Assessing the Patient with Apraxia. *Journal of Medical Research and Health Sciences*, 3(10), 1114-1123. <https://doi.org/10.15520/jmrhs.v3i10.263>
9. Girasole G, Jilka RL, Passeri G. 1992 17b-estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. *J Clin Invest.* 89:883– 891.
10. Weetmann AP, Bright-Thomas R, Freeman M. 1990 regulation of interleukin- 6 release by human thyrocytes. *J Endocrinol.* 127:357–361.
11. Lotz M, Jirik F, Kabouridis P, Tsokuas C, Hirano T , Kishimoto T, Carson DA. B cells stimulating factor 2 / interleukin 6 is a costimulant for human thymocytes and T lymphocytes. *J Epx Med* 167: 1253, 1988.
12. Chiu C-P, Lee F. IL-6 is a differentiation factor for M1 and WEHI-3B myeloid leukemia cells. *J Immunol* 142: 1909, 1989.
13. Woloski BMRNJ, Smith EM, Meyer WJ III, Fuller GM, Blalock JE. Corticotropin-releasing activity of monokines. *Science* 230: 1035, 1985.
14. Mundy GR (1990) Incidence and pathophysiology of hypercalcemia. *Calcif Tissue Int* 46: 3-10.
15. Oziol, L., Faure, P., Bertrand, N. et al. (2003) Inhibition of vitro macrophage induced low density lipoprotein oxidation by thyroid compounds. *Journal of Endocrinology*, 177, 137–146.
16. Fang Chen, David T. Teachey, Edward Pequignot et al. Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy. *J Immunol Methods.* 2016 Jul; 434: 1–8.
17. Tin Weitner, Suzana Inić, Jasna Jablan, Mario Gabričević, Ana-Marija Domijan. Spectrophotometric Determination of Malondialdehyde in Urine Suitable for Epidemiological Studies. *Croat. Chem. Acta* 2016, 89(1), 133–139.
18. N.C.Kendrick. Purification of Arsenazo III, a Ca²⁺-sensitive dye. *Analytical Biochemistry* 1976;76(2):487-501
19. Oziol, L., Faure, P., Bertrand, N. et al. (2003) Inhibition of vitro macrophage induced low density lipoprotein oxidation by thyroid compounds. *Journal of Endocrinology*, 177, 137–146.
20. Freeman, B.A. & Crapo, J.D. (1982) Biology of disease, free radicals and tissue injury. *Laboratory Investigation*, 47, 412–426.
21. Swaroop, A. & Ramasarma, T. (1986) Heat exposure and hypothyroid conditions decrease hydrogen peroxide generation in liver mitochondria. *Biochemical Journal*, 226, 403–408
22. Yilmaz, S., Ozan, S., Benzer, F. et al. (2003) Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *CellBiochemistry and Function*, 21, 325–330.
23. Creasey A.A, Stevens P, Kenney J, Allison A.C, Warren K, Catlett R, Hinshaw L and Taylor F.B.Jr. Endotoxin and cytokine profile in plasma of baboons challenged with lethal and sub lethal *Escherichia coli*. *Circ Shock* 33: 84, 1991.
24. Bartalena L, Brogioni S, Grasso L, Martino E. 1993 Increased serum interleukin-6 concentration in patients with subacute thyroiditis: relationship with concomitant changes in serum T4-binding globulin concentration. *J Endocrinol.* 16:213–218.
25. Weetmann AP, Bright-Thomas R, Freeman M. 1990 regulation of interleukin-6 release by human thyrocytes. *J Endocrinol.* 127:357–361.
26. Bartalena L, Grasso L, Brogioni S, Aghini-Lombardi F, Braverman LE, Martino E. Serum interleukin-6 in amiodarone-induced thyrotoxicosis. *J ClinEndocrinolMetab* 78: 423-427, 1994.

27. Watson PF, Pickerill AP, Davies R, Weetmann AP. Analysis of cytokine gene expression in Grave's disease and multinodular goiter. *ClinEndocrinolMetab* 79: 355-360, 1994.
28. Pereira, B., Rosa, L.F., Safi, D.A. et al. (1994) Control of superoxide dismutase, catalase and glutathione peroxidase activities in rat lymphoid organs by thyroid hormones. *Journal of Endocrinology*, 140, 73–77.
29. Mishra, D. R., Sharma, D., Barpande, D. J., Kumari, D. N., & Mehta, D. H. (2020). Anaesthetic Management of Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy (CRS/HIPEC): A True Challenge To an Anaesthetist. *Journal of Current Medical Research and Opinion*, 3(07), 503-507. <https://doi.org/10.15520/jcmro.v3i07.307>
30. Dumitriu, L., Bartoc, R. & Ursu, H. (1988) Significance of high levels of serum malondialdehyde (MDA) and ceruloplasmin (CP) in hyper- and hypothyroidism. *Revue Roumaine d'endocrinologie*, 26,35–38.
31. Konukoglu, D., Ercan, M. & Hatemi, H. (2002) Plasma viscosity in female patients with hypothyroidism: effects of oxidative stress and cholesterol. *Clinical Hemorheology and Microcirculation*, 27, 107–113.
32. Iqbal AA, Burgess EH, Gallina DL, Nanes MS, Cook CB (2003) Hypercalcemia in hyperthyroidism: patterns of serum calcium, parathyroid hormone, and 1,25-dihydroxyvitamin D3 levels during management of thyrotoxicosis. *EndocrPract* 9: 517-521.
33. Shieh A, Martinez DS (2013) "Hypercalcemia in Hyperthyroidism" abstract in endocrine society's 95th annual meeting and Expo.
34. Korytnaya E, Rao NG, Mayrin JV (2011) An unusual case of hypercalcemia associated with graves' disease and vitamin d deficiency. *Clin Med Insights Endocrinol Diabetes* 4: 25-28.
35. Ghose RR, Palmer DJ, Yaqoob M, Haboubi N, Ratcliffe WA (1994) Abdominal symptoms, hypercalcaemia and apathetic hyperthyroidism: treatment with pamidronate. *Br J ClinPract* 48: 163-164.
36. Szabó ZS, Ritzl F (1981) Hypercalcemia in hyperthyroidism. Role of age and goiter type. *KlinWochenschr* 59: 275-279.