DOI: 10.7860/JCDR/2022/56898.16940

Original Article

Physiology Section

Serum Levels of Inflammatory Markers in Newly Diagnosed Hypothyroid Patients before and after Levothyroxine Therapy

SHILPI GOYAL[1], ABHINAV DIXIT[2], NEELAM VANEY[3], SV MADHU[4]

ABSTRACT

Introduction: Cytokines have a great significance in autoimmune thyroid disease. They are produced by thyroid follicular cells and have an indispensable role in T-cell and B-cell growth and differentiation. Tumour necrosis factor-alpha (TNF-α) is a cytokine having numerous immunological and metabolic activities and is an important stimulus for Interleukin-6 (IL-6) production. Interleukins play an important role in immune system function. Their deficiency causes autoimmune diseases or immune deficiency.

Aim: To assess inflammatory markers IL-6 and TNF-α in hypothyroidism before and after attainment of euthyroid state by levothyroxine therapy and to find the correlation between serum levels of TNF-α and IL-6 in hypothyroid state.

Materials and Methods: This quasi-experimental study was conducted in Department of Physiology at University College of Medical Sciences, New Delhi, India, from January 2013 to October 2013. Total 30 newly diagnosed hypothyroid patients of the age group 18-45 years formed case group and 30 age and sex-matched subjects formed the control group, were recruited for the study. The patients were given levothyroxine replacement therapy for three months. The inflammatory biomarkers level was evaluated using solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA), using kits from Diaclone. Paired and Unpaired t-tests, respectively, were used for hypothyroid patients in pre-post state and between two groups at baseline. Serum Thyroid Stimulating Hormone (TSH) levels of hypothyroid patients were correlated with serum levels of inflammatory markers using Pearson's correlation test.

Results: There was no statistical difference in age, sex, Body Mass Index (BMI) and lipid profile in the two groups. Hypothyroidism subjects achieved euthyroidism in three months after obtaining levothyroxine therapy with the mean TSH levels 26.43±10.244 vs 3.4863±0.1963, mean free T3 levels 0.2980±0.9408 vs 1.93±0.5690 and mean free T4 levels 0.2736±0.0973 vs 1.8986±0.2853 in pre-post states, respectively. Serum levels of IL-6 and TNF-α were significantly higher (p-value <0.001) in hypothyroid subjects compared to euthyroid controls. A positive correlation between serum levels of IL-6 (r-value=0.5778, p-value=0.0008) and TNF-α (r-value=0.521, p-value=0.003) with serum TSH levels were found.

Conclusion: The study reported a significant effect of levothyroxine therapy in restoring serum levels of raised inflammatory markers among hypothyroid patients. A decrease in low-grade chronic inflammation after treatment showed some clinical importance as chronic inflammation, is known to be associated with atherosclerosis and cardiac disease.

Keywords: Chronic inflammation, Interleukin 6, Thyroid stimulating hormone, Tumour necrosis factor-α

INTRODUCTION

Thyroid hormones are important in the regulation of innate immune responses [1]. Thyroid hormone disturbances do impact the integrity and working of immune cells, which is reflected in host defense status and associated diseases. Immune disturbances are also responsible for the various thyroid dysfunctions, having autoimmune aetiology. Autoimmune thyroiditis is the most common cause of hypothyroidism. Hashimoto's thyroiditis occurs due to the inflammation caused by autoimmunity. Pieces of evidence have been showing the association of chronic inflammation with cardiovascular events [2,3]. Hashimoto's thyroiditis [4,5], has been associated with an increased risk of atherosclerosis and cardiovascular events along with endothelial dysfunction caused by low-grade inflammation. Studies in the past have shown an association of hypothyroidism with inflammatory markers. Some studies have reported raised levels of proinflammatory markers in the hypothyroid state [6-8] while other have shown reduced levels of inflammatory markers in the hypothyroid state [9]. Inflammatory markers have a role in predicting cardiovascular risk [10]. Interleukin-6 (IL-6), an inflammatory marker is acting as an inducer for the activity of C-Reactive Protein (CRP) [11]. The CRP is a known marker for the diagnosis of cardiac risk [12]. Most of these studies are cross-sectional [6-8,13]. Thus, the present interventional study was conducted to see the effect of levothyroxine therapy on the levels of deranged inflammatory markers.

Moreover, the role of increased cytokine levels in severe Coronavirus Disease 2019 (COVID-19) infection with the worst outcomes is well-known [14]. In the current COVID-19 pandemic, clinicians are concerned about the impact of COVID-19 on pre-existing illnesses or newly developing disease processes. Thyroid disease is also associated with severe COVID-19 infection [15,16]. A recent study reported an association between thyroid dysfunction with clinical severity and poorer prognosis in COVID-19 patients. The authors found significantly higher severe inflammation (IL-6 levels) with low free Triiodothyronine (fT3), levels [17].

Goyal S et al., reported a significant reduction in cognitive skills with hypothyroidism and the role of levothyroxine treatment on the restoration of various cognitive domains by using various paper pencil tests [18] and also by computerised automated stroop task for measuring cognitive inhibition among executive functions [19]. They found a positive correlation between cognitive impairments and serum Thyroid Stimulating Hormone (TSH) values.

As one is aware of the adverse effect of inflammatory markers on cardiovascular events [20] and their association in COVID-19 patients along with thyroid dysfunctions [15,16,21-23]. The present study was planned on the hypothesis of higher levels of inflammatory biomarkers in hypothyroid patients. The present study was done to further confirm the available literature and to see the effect of levothyroxine therapy on the raised inflammatory markers in hypothyroid patients, who were drug-naïve on coming to Outpatient Department (OPD).

Most of the earlier studies have been done in sub-clinical hypothyroid patients [6-8] and they were cross-sectional. The present study was planned to evaluate the serum levels of inflammatory markers IL-6 and TNF-α, and their correlation with serum TSH in newly diagnosed hypothyroid patients and euthyroid controls, and to compare the same after attainment of euthyroid state.

MATERIALS AND METHODS

This quasi-experimental study was conducted at Department of Physiology at University College of Medical Sciences, New Delhi, India, from January 2013 to October 2013 with drug-naïve 30 hypothyroid patients. Written informed consent was taken from all the study subjects and the Institutional Ethics Committee clearance was obtained.

Inclusion criteria: Patients of 18-45 years age group without having a previous medical history of any disease like hypertension, diabetes mellitus, cardiovascular risk factor, deranged lipid profile {Low-Density Lipoprotein (LDL) cholesterol<160 mg/dL and triglycerides <180 mg/dL} [7], or any other systemic inflammatory diseases were included in the study.

- **Case group (n=30):** To see the immediate effect of levothyroxine treatment on inflammatory markers, newly diagnosed hypothyroid patients were taken as study subjects.
- **Control group (n=30):** Healthy euthyroid subjects with age, sex, BMI and lipid profile matched with the patient group were recruited as controls among the patient's relatives and staff.

Exclusion criteria: Pregnant women, subjects with BMI ≥30 kg/m^2, smokers, alcoholics and individuals following any drug regimen were excluded from the study.

Study Procedure

An overnight fasting blood sample was taken. The estimation of serum levels of free T3 (fT3), free T4 (fT4) and TSH of the patients and controls were done by using Radioimmunoassay (RIA) kit from Immunotech Bechmancoulter. Following are the normal hormonal ranges of the laboratory:

- TSH: 0.17-4.0 μIU/mL
- fT4: 0.95-2.23 ng/dL
- fT3: 1.5-5 pg/mL

On centrifuging the blood, obtained serum was stored at -80°C until it was processed for inflammatory markers measurement. Serum IL-6 and TNF-α were detected by solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA), using kits from Diaclone. Biochemical markers were assessed in hypothyroid patients at the time of diagnosis and after the attainment of a euthyroid state, as suggested by the normalisation of thyroid hormones with levothyroxine replacement therapy for three months duration. Controls were assessed only once, due to financial constraints. Serum TSH levels of hypothyroid patients were correlated with serum levels of inflammatory markers.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) version 20.0 was used for data analysis. All the baseline parameters were expressed as mean and standard deviation. Biochemical measures were compared at the initial and final level by paired t-test and between cases and controls at baseline by Unpaired t-test. Pearson's correlation test was used for correlation between serum TSH, IL-6 and TNF-α. A p-value <0.05 was considered significant.

RESULTS

There was no statistical difference in age, sex, BMI and lipid profile in the two groups [Table/Fig-1]. Serum TSH was significantly higher (p-value <0.001) 26.43±10.244 vs 3.18±0.855 μIU/mL while serum fT3 0.2980±0.9408 vs 2.35±0.585 pg/mL and serum fT4 0.2736±0.0973 vs 1.957±0.2796 ng/dL were significantly lower (p-value <0.001) in hypothyroid patients than controls. All patients were euthyroid after three months of levothyroxine replacement therapy. Significant decrease (p-value <0.001) in the TSH level (from 26.43±10.244 to 3.4863±0.1963 μlu/mL) was seen in cases after three months. The fT3 from 0.2980±.09408 to 1.93±0.5690 pg/mL and fT4 from 0.2736±0.0973 to 1.8986±0.2853 ng/dL levels showed significant increase (p-value <0.001) post levothyroxine replacement therapy.

Parameters	Case group (Mean±SD)	Control group (Mean±SD)	p-value
Age (years)	31.67±8.405	31.00±8.004	0.754
Gender (Male/Female)	1/29	1/29	1.00
BMI (kg/m^2)	23.11±3.17	22.91±1.23	0.328
TSH (μIU/mL)	26.43±10.244	3.18±0.855	<0.001*
fT3 (pg/mL)	0.2980±.09408	2.35±0.585	<0.001*
fT4 (ng/dL)	0.2736±0.0973	1.957±0.2796	<0.001*
Total cholesterol (mg/dL)	195.67±16.57	191.33±23	0.085
Triglycerides (mg/dL)	117.23±23.23	114.54±16.21	0.079
High-density lipoprotein (mg/dL)	43.67±12.23	45.32±11.31	0.080
Low-density lipoprotein (mg/dL)	139.06±23.41	136.07±22.67	0.202

[Table/Fig-1]: Baseline characteristic between case group and control group.
*p-value <0.05 was considered as statistically significant

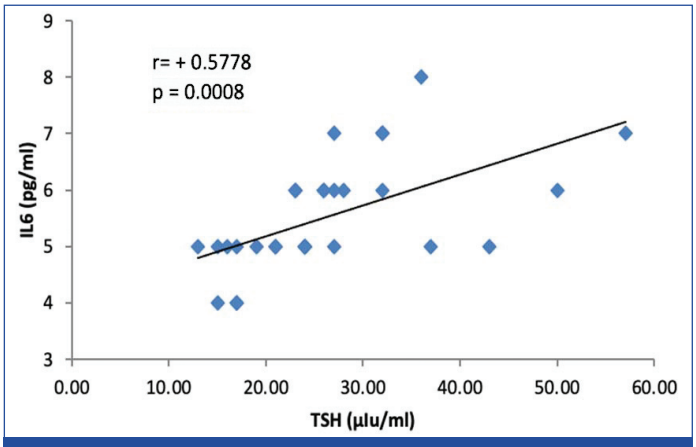
Hypothyroid patients had significantly higher serum IL-6 and TNF-α values than control subjects. Mean serum IL-6 5.53±0.950 vs 4.57±0.734 pg/mL and mean TNF-α levels 15.43±1.568 vs 13.81±1.587 pg/mL were significantly decreased in hypothyroid patients after attaining euthyroidism [Table/Fig-2,3]. Serum TSH values were positively correlated with their serum IL-6 (r-value=0.5778, p-value=0.0008) and TNF α (r-value=0.521, p-value=0.003) values in hypothyroid patients [Table/Fig-4,5].

Groups	IL-6 (pg/mL)		p-value (Paired t-test)
	Before (Mean±SD)	After (Mean±SD)	
Cases	5.53±0.950	4.57±0.734	<0.001*
Controls	4.49±0.839	-	
p-value (Unpaired t-test)	<0.001	-	

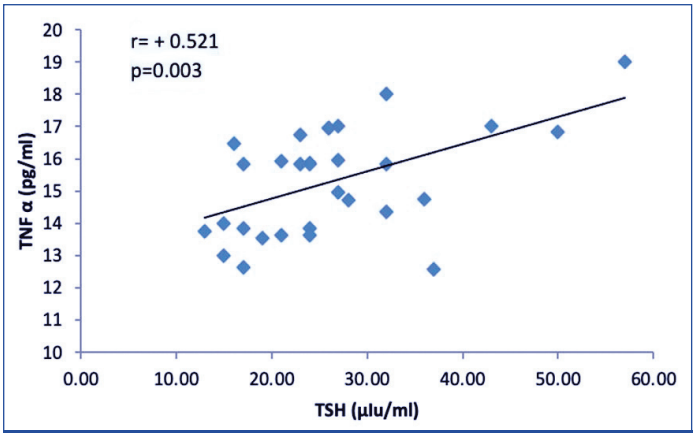
[Table/Fig-2]: Comparison of serum level of IL-6 (pg/mL) between case group and control group.
*p-value <0.05 was considered as statistically significant

Groups	TNF-α (pg/mL)		p-value (Paired t-test)
	Before (Mean±SD)	After (Mean±SD)	
Cases	15.43±1.568	13.81±1.587	0.0006*
Controls	13.2393±1.272	-	
p-value (Unpaired t-test)	<0.001	-	

[Table/Fig-3]: Comparison of serum level of TNF-α (pg/mL) between case group and control group.
*p-value <0.05 was considered as statistically significant



[Table/Fig-4]: Correlation of IL-6 with serum TSH levels.



[Table/Fig-5]: Correlation of TNF-α with serum TSH levels.

DISCUSSION

Present findings have supported the hypothesis of association of hypothyroidism with increased inflammatory markers levels compared to the control group and which can be a reason for cardiovascular risk in hypothyroid patients. In the present study, both case and control groups were age, sex, BMI and lipid profile matched and there was no history of any kind of inflammatory disease, proving that the inflammatory findings in the hypothyroid group was not having any pre-existing pathology. Inflammatory markers levels decreased after levothyroxine therapy in drug-naïve newly diagnosed hypothyroid patients showed the role of early diagnosis and hormone replacement therapy in reversing the future risk of cardiovascular disease, which can arise due to the prolonged hypothyroid state [6].

Previous studies have shown conflicting results regarding hypothyroid state and deranged inflammatory markers [7-9]. This study reported that serum levels of IL-6 and TNF-α were significantly higher in hypothyroid patients than euthyroid controls. This is in concurrence with Taddei S et al., who reported patients with hypothyroidism, were having low-grade chronic inflammation, which was approved by a significant rise in high sensitive CRP and IL-6 [7]. The author suggested the increased risk of atherosclerosis and ischaemic heart disease in hypothyroidism may be due to endothelial dysfunction caused by low-grade chronic inflammation. A significantly higher concentration of IL-6 and TNF-α in sub-clinical hypothyroid patients was supported by Turemen EE et al., [8]. Contrary to present findings, in a study done by Kilziltunc A et al., hyperthyroid patients had increased serum levels of the cytokines IL-6 and TNF-α and hypothyroid patients had decreased levels of IL-6 and TNF-α [9]. Lakatos P et al., found increased IL-6 levels in hyperthyroid women. However, hypothyroidism did not significantly reduce serum IL-6. Serum IL-6 normalised on remission of the disease indicated that it was related to follicular cell damage [13].

The present study found a significant positive correlation between serum levels of TSH and inflammatory markers in hypothyroid patients. Gupta G et al., also reported a significant increase in IL-6 levels in sub-clinical hypothyroid patients than in healthy euthyroid controls and was positively correlated with serum TSH levels [6]. Davies PH et al., found a significant negative correlation between serum total T3 and IL-6 and total T4 and IL-6 [24]. Thyroid-stimulating hormone stimulates the release of IL-6 in adipocytes [25]. IL-6 has a key role in the early stages of inflammation and also plays a modulator in the course of atherogenesis. It helps in speeding up atherogenesis via producing CRP by liver [26]. TNF-α is involved in systemic inflammation and also stimulates the acute phase reaction. It is also an important stimulus for IL-6 production [27]. It acts at each level of the hypothalamic-pituitary-thyroid axis to produce various changes in the axis [28,29]. TNF-α receptors are present in thyroid follicular cells and are implicated in the cytotoxic mechanisms causing thyroid destruction in autoimmune thyroid disease [30].

Patients attained euthyroid status after levothyroxine therapy and there was a significant decrease in the IL-6 and TNF-α levels. These findings were supported by Tayde PS et al., IL-6 and TNF-α were significantly elevated in hypothyroid cases compared to healthy controls and after treatment with levothyroxine, all the markers were reduced significantly [31]. Diez JJ et al., also reported a significantly higher level of TNF-α in patients with hypothyroidism but did not normalise after the normalisation of thyroid functions [30]. Contrary to this, the present study group was newly diagnosed hypothyroid patients, who restored raised inflammatory markers after levothyroxine therapy, proving the role of early treatment in the resumption of normal levels of markers.

Limitation(s)

The inflammatory markers were not tested twice in the control group and the small sample size was the main limitation of this study. Further studies are required with more sample size and other inflammatory markers as well to highlight the mechanistic process of the disease.

CONCLUSION(S)

It was found that the hypothyroid state is associated with raised inflammatory markers. Levothyroxine replacement therapy has a role in reversing the adverse situation of hypothyroidism by restoring raised inflammatory markers. This can be useful to the clinicians as the role of the early diagnosis and treatment on the restoration of raised inflammatory markers and chronic inflammation in newly diagnosed hypothyroid patients. Chronic inflammation seems to be associated with an increased risk of cardiovascular disease so treating the chronic inflammation with levothyroxine in hypothyroidism patients, can be saved from the risk of cardiac diseases in turn.

REFERENCES

[1] Montesinos MDM, Pellizas CG. Thyroid hormone action on innate immunity. Front Endocrinol (Lausanne). 2019;10:350. Doi: 10.3389/fendo.2019.00350.
[2] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135-43. Doi: 10.1161/hc0902.104353.
[3] Ridker PM. High-sensitivity C-reactive protein: Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation. 2001;103(13):1813-18. Doi: 10.1161/01.cir.103.13.1813.
[4] Surks MI, Ocampo E. Subclinical thyroid disease. Am J Med. 1996;100(2):217-23. Doi: 10.1016/s0002-9343(97)89462-4.
[5] Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000;160(4):526-34. Doi: 10.1001/archinte.160.4.526.
[6] Gupta G, Sharma P, Kumar P, Itagappa M. Study on subclinical hypothyroidism and its association with various inflammatory markers. J Clin Diagn Res. 2015;9(11):BC04-06. Doi: 10.7860/JCDR/2015/14640.6806.
[7] Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, et al. low-grade systemic inflammation causes endothelial dysfunction in patients with hashimato's thyroiditis. J Clin Endocrinol Metab. 2006; 91(12):5076-82.
[8] Turemen EE, Cetinarslan B, Sahin T, Canturk Z, Tarkun I. Endothelial dysfunction and low-grade chronic inflammation in subclinical hypothyroidism due to autoimmune thyroiditis. Endocr J. 2011;58(5):349-54.
[9] Kiziltunc A, Başoğlu M, Avci B, Capoğlu I. Serum Il-6 and TNF-α in patients with thyroid disorders. Turk J Med Sci. 1999;29:25-29.
[10] Lowe GD. Circulating inflammatory markers and risks of cardiovascular and noncardiovascular disease. J Thromb Haemost. 2005;3(8):1618-27.
[11] Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499-11.
[12] Ridker PM. C-reactive protein: A simple test to help predict the risk of heart attack and stroke. Circulation. 2003;108(12):e81-85.
[13] Lakatos P, Foldes J, Horvath C, Kiss L, Tatrai A, Takacs I. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. J Clin Endocrinol Metab. 1997;82(1):78-81.
[14] Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis. 2020;95:304e7.
[15] Hariyanto TI, Kurniawan A. Thyroid disease is associated with severe coronavirus disease 2019 (COVID-19) infection. Diabetes Metab Syndr. 2020;14(5):1429-30.
[16] Chen M, Zhou W, Xu W. Thyroid function analysis in 50 patients with COVID-19: A retrospective study. Thyroid. 2021;31(1):08-11. Doi: 10.1089/thy.2020.063.
[17] Dabas A, Singh H, Goswami B, Kumar K, Dubey A, Jhamb U, et al. Thyroid dysfunction in COVID-19. Indian J Endocr Metab. 2021;25(3):198-201.

[18] Goyal S, Dixit A, Vaney N, Madhu S. Cognitive status in hypothyroid patients before and after attainment of euthyroid state. Indian J Physiol Pharmacol. 2018;62:113-19.
[19] Goyal S, Dixit A, Vaney N, Madhu SV. Effect of hypothyroidism on cognitive status: Evidence from Stroop task. Indian J Med Spec. 2020;11:34-39.
[20] Vayá A, Gimenez C, Sarnago A, Alba A, Rubio O, Hernandez-Mijares A, et al. Subclinical hypothyroidism and cardiovascular risk. Clinical Hemorheology and Microcirculation. 2014;58(1):01-07.
[21] Whiting A, Reyes JVM, Ahmad S, Lieber J. Post-COVID-19 Fatigue: A case of infectious hypothyroidism. Cureus. 2021;13(5):e14815. Doi: 10.7759/cureus.14815.
[22] Chen Y, Li X, Dai Y, Zhang J. The association between COVID-19 and thyroxine levels: A meta-analysis. Front Endocrinol (Lausanne). 2022;12:779692. Doi: 10.3389/fendo.2021.779692.
[23] Malik J, Malik A, Javaid M, Zahid T, Ishaq U, Shoaib M, et al. Thyroid function analysis in COVID-19: A retrospective study from a single center. PLoS One. 2021;16(3):e0249421. Doi: 10.1371/journal.pone.0249421.
[24] Davies PH, Black EG, Sheppard MC, Franklyn JA. Relation between serum interleukin-6 and thyroid hormone concentrations in 270 hospital in-patients with non-thyroidal illness. Clin Endocrinol (Oxf). 1996;44(2):199-05.
[25] Auntunes TT, Gagnon A, Bell A, Sorisky A. Thyroid stimulating hormone stimulates interleukin 6 release from 3T3-L1 adipocytes through a cAMP protein kinase A pathway. Obes Res. 2005;13(12):2066-71.
[26] Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868-74.
[27] Jirik FR, Podor TJ, Hirano T, Kishimoto T, Loskutoff DJ, Carson DA, et al. Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol. 1989;142(1):144-47.
[28] Beutler B, Cerami A. Cachectin and tumour necrosis factor as two sides of the same biological coin. Nature. 1986;320(6063):584-88.
[29] Pang XP, Hershman JM, Mirell CJ, Pekary AE. Impairment of hypothalamic-pituitary-thyroid function in rats treated with human recombinant tumor necrosis factor-alpha (cachectin). Endocrinology. 1989;125(1):76-84.
[30] Díez JJ, Hernanz A, Medina S, Bayón C, Iglesias P. Serum concentrations of tumour necrosis factor-alpha (TNF-alpha) and soluble TNF-alpha receptor p55 in patients with hypothyroidism and hyperthyroidism before and after normalization of thyroid function. Clin Endocrinol (Oxf). 2002;57(4):515-21.
[31] Tayde PS, Bhagwat NM, Sharma P, Sharma B, Dalwadi PP, Sonawane A, et al. Hypothyroidism and depression: Are cytokines the link? Indian J Endocrinol Metab. 2017;21(6):886-92.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Physiology, University College of Medical Sciences, New Delhi, India.
2. Professor and Head, Department of Physiology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India.
3. Director, Professor and Head, Department of Physiology, University College of Medical Sciences, New Delhi, India.
4. Director and Professor, Department of Endocrinology, University College of Medical Sciences, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shilpi Goyal,
Assistant Professor, Department of Physiology, University College of Medical Sciences, Dilshad Garden, New Delhi, India.
E-mail: shilpigoyal16@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 13, 2022
- Manual Googling: Jul 07, 2022
- iThenticate Software: Aug 26, 2022 (17%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Apr 06, 2022**
Date of Peer Review: **May 13, 2022**
Date of Acceptance: **Jul 09, 2022**
Date of Publishing: **Sep 01, 2022**