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Association of vitamin D and inflammatory biomarkers with newly diagnosed cases of hypothyroidism- A case-control study

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Abstract

Background: Deranged thyroid hormones and TSH induces the release of inflammatory biomarkers and recent evidence has demonstrated an association between low vitamin D status and autoimmune thyroid diseases, but there is little documented evidence available about the association of vitamin D and inflammatory biomarkers. So, this study was planned to investigate the relationship between vitamin D and inflammatory biomarkers.

Materials and Methods: This case-control study was carried out among 60 study participants (30 cases and 30 controls) in the Department of Biochemistry, F.H Medical College, its associated Hospital from January 2023 to June 2023 The samples were divided in to two groups, cases (hypothyroid patients) and healthy control group. Serum was separated by centrifuging blood at 3000 rpm for 10 mi. Estimation of CRP was done turbidimetric immune assay method. Statistical analysis was done by using the (SPSS) version 20.0. Pearson correlation and Student's t-test was applied, p-value <0.0001 was taken as significance.

Results: The (mean ±SD) CRP values in the subjects having hypothyroid disorder (cases) were 3.12± 0.79 mg/L and in control was 2.01±0.75 mg/L (p-value <0.0001). The weight was found to be higher in cases. The CRP and IL-6 had an insignificant correlation with the BMI (r=0.06, p=0.74) and (r=0.64, p=0.35) and also insignificant correlation was found in between the CRP and the blood pressure.

Conclusion: This study concludes that CRP and IL-6 levels were significantly higher in hypothyroid patients and insignificant correlation was found between anthropometric measurements of hypothyroid patients and inflammatory biomarkers.

Keywords: vitamin D; CRP; IL-6; hypothyroidism; hypothyroid patients

Introduction

It has been reported that around 42 million peoples from India are suffering from various forms of thyroid dysfunctions [1]. Thyroid dysfunctions stand as one of the main endocrine disorders around the world, it's calculable that around 30% to 40% of the patients are directly or indirectly associated with thyroid dysfunctions & affect women 500% more than males may be due to hormone estrogen and the peculiar cyclical pattern of hormonal variations are strong promoters of thyroid disorder in females. Secondly,

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there is a greater prevalence of autoimmune disorders in females as compared to males [2, 3]. Among numerous thyroid disorders in the world i.e., (1) hyperthyroidism (2) hypothyroidism (3) goiter and iodine deficiency disorders (IDD) (4) thyroid cancer and (5) Hashimoto's thyroiditis. Frequency of hypothyroid patients is around 2% [4]. Thyroid hormones and TSH induce proliferation of leukocyte, migration and release of cytokines [5]. In patients having hypothyroidism, the main reason for the inflammatory process could be because of thyroid stimulating hormone (TSH) induced synthesis of tumour necrosis factor (TNF). Other reason which is important for this process of inflammation could be because of cyclooxygenase-2 (COX 2) which increases the production of autocoids that causes the inflammation [6]. The C-reactive protein (CRP) is synthesize in response to infection, injury or different inflammatory stimuli [7] triggers the release of cytokines that successively stimulate the CRP production. Also cytokines like interleukin 6 mediate the inflammatory response, therefore they can serve as potential biomarkers of various diseases of inflammatory nature [8]. Vitamin D a fat-soluble vitamin is recognized for its importance in the bone health. However, recent studies suggest nonskeletal effects also. Low concentrations of vitamin D are related with several types of diseases and inflammatory diseases that are metabolic syndrome, rheumatoid arthritis, type II diabetes mellitus and cardiovascular diseases, infections etc [9].

It makes vitamin D, CRP and IL-6 assessment valuable in the patients suffering from hypothyroidism to estimate the progression and prognosis of the disease and also to establish the risk for the development of various diseases inflammatory in nature in hypothyroid patient. So, this study was planned to investigate the relationship between vitamin D and inflammatory biomarkers.

Materials and methods

This case-control observational study was carried out in the Department of Biochemistry, F.H Medical College, its associated Hospital, Tundla, Uttar Pradesh, India, among 60 participants, over a period of 6 months from January 2023 to June 2023. The Institutional Ethics Committee approved the study (letter Ref. no. FHMC/IEC/R.Cell/2023/034) informed consent was also taken from all the study participants. The sample size of 60 participants constituted 30 cases (hypothyroid patient) and 30 healthy control (euthyroid) is taken, since sample size of about 30 is common across different statistics since it increases the confidence interval of the study population data set significantly and justifies making conclusive statements about the research outcomes.

The study sample were divided into two distinct groups: (i) cases (hypothyroid patient after having laboratory investigations of thyroid hormones (T3, T4) and TSH and (ii) control group (patient attending Outpatient Department (OPD) of medicine not having any sign and symptoms of thyroid dysfunction and diagnosed as euthyroid after laboratory investigations of thyroid profile i.e. T3, T4 and TSH.

Inclusion criteria: For cases: All patients between the age group of 20 and 60 years attending Medicine OPD having signs and symptoms of hypothyroidism (based on laboratory investigation of T3, T4 and TSH) were included as cases.

For controls: Patient attending medicine OPD with no sign and symptoms of hypothyroidism i.e. euthyroid participants (patients having normal thyroid hormone profile based on laboratory investigation of T3, T4 and TSH), study participants between the age group of 20 and 60 years were taken as controls.

Exclusion criteria: Patients on steroids and/or immunosuppressant drugs and patients with chronic renal failure (CRF), liver disorders, skin diseases and other chronic diseases and also pregnant females were excluded.

Data collection

Information regarding the study subject's age, sex, lifestyle, family h/o of diabetes mellitus and other chronic disorders were recorded. Anthropometric measurements like height and weight were also measured. Body mass index (BMI) was measured by dividing weight (kg) by height in (meters^2). Blood pressure was measured with special precaution by trained personnel.

Assay methods

Blood sample (10 mL) was collected from each study subject. Serum was separated by centrifuging blood at 3000 rpm for 10 minutes. A turbidimetric immunoassay for determination of serum levels of CRP was used. Measuring range (0.15 mg/L to 5 mg/L) [10].

Present study followed the risk stratification as recommended by American Heart Association (AHA) [11], i.e. Low:<1.0 mg/L, Average: 1.0--3.0 mg/L, High:>3.0 mg/L, Serum interleukin-6 will be estimated by sandwich enzyme immunoassay (ELISA) technique by diaclone IL-6 ELISA kit having assay range of about 0.064 – 10000 pg/mL [12].

The thyroid hormones assayed for T3, T4 and TSH by enzyme linked florescent assay (ELFA) technique using vidas auto-analyzer. The reference range for T3, T4

and TSH for our laboratory as: T3: 1.23–3.23 nmol/L, T4:59–135 nmol/L & TSH: 0.4–4.2 mIU/L respectively. The patients were categorized into two groups. Those having T3, T4, and TSH levels within the normal reference range were categorized into the euthyroid group (control); subjects having low T3, T4 and high TSH were in the hypothyroid group (cases) [13].

Estimation of serum 25-OH vitamin D levels was done by electrochemiluminescence immunoassay (ECLIA) [11] using rochecobase 411 analyser, serum vitamin D deficiency was defined as level of <=20 ng/ml and insufficiency as a level between >20 ng/ml and <30 ng/ml and normal serum level is defined as >30 ng/ml or=30 ng/ml.

Statistical analysis

All the statistical analysis was done by using the Windows based Statistical Package for Social Sciences (SPSS) version 20.0. To measure the significance of difference between the two groups, Student's t-test was applied. mean±SD (Standard Deviation) was used to represent the results, Pearson's correlation analysis was carried out to find the association between the groups and p-value <0.0001 was taken as significance.

Results

In this study the hypothyroid participants were older and have higher BMI (p-value=0.23) as compare to the euthyroid healthy controls. Cases also had higher diastolic blood pressure (DBP) (p-value <0.0001). The (mean±SD) CRP value in the cases was 3.12±0.79 mg/L and in healthy control subjects, it was 2.01±0.75 mg/L. Table demonstrate that CRP levels were higher among the hypothyroid study subjects (cases) with (p-value <0.05). The (mean±SD) IL-6 value in the cases was 19.68±10.79pg/mL and in healthy control subjects. It was 11.01±9.20pg/mL. Table also demonstrated that IL-6 levels were higher in the hypothyroid study subjects (cases). Blood sugar fasting levels i.e. (mean±SD) were higher in cases as compared to the healthy controls (Table 1).

Table 1: Anthropometric measurement and clinical characteristics of the study subjects.

Parameters	*Cases (n=30)*	*Controls (n=30)*	*t value, p-value*
Age (years)	44.66±7.10	41.63±9.66	1.38, 0.17
Height (cm)	149.06±15.78	152.26±17.65	0.74, 0.46
Weight (kg)	67.76±15.80	63.96±11.34	1.07, 0.28
Body mass index (BMI=kg/m^2)	30.93±7.40	24.64±4.043	1.19, 0.23
Systolic BP (mm of Hg)	126.36±10.63	122.23 ± 7.10	1.77, 0.082
Diastolic BP (mm of Hg)	89.26±9.65	78.56 ± 2.62	5.86,<0.05*
Blood sugar (F)	140.53±45.91	95.23±6.90	5.34,<0.05*
CRP (mg/L)	3.12±0.79	2.01±0.75	5.58, <0.05*
IL-6 (pg/mL)	19.68±10.79	11.01 ± 9.20	2.47, 0.0173

Relationship of TSH with different anthropometric measurements like (Height, weight, BMI), blood pressure (Systolic & diastolic) and with fasting blood sugar was found insignificant with p-value of >0.05 (Table 2).

Table 2: Correlation of TSH with anthropometric measurement in hypothyroid patients (cases).

Parameters	*TSH*	
	r value	*p value*
Age (years)	0.03	0.84
Height (cm)	-0.02	0.88
Weight (kg)	0.08	0.67
Body mass index (BMI=kg/m^2)	0.06	0.74
Systolic BP (mm of Hg)	0.18	0.32
Diastolic BP (mm of Hg)	-0.12	0.50
Blood Sugar (F)	0.13	0.47
p-value <0.05 considered significant		

Relationship of IL-6 & CRP with various anthropometric measurements like (Height, weight, BMI), blood pressure (Systolic and Diastolic) and with fasting blood sugar was found to be insignificant with p-value of >0.05 but significant relation is found between vitamin D & IL-6, CRP (Table 3).

Levels of vitamin D are within the reference range for 9.2% of cases (Patients of hypothyroidism) and around 79 % of healthy controls. Levels are low with respect to the reference range in 90.8% of cases and in 21 % of controls (Table 4).

Discussion

Thyroid dysfunctions, regardless of age or sex, are

Table 3: Correlation of IL-6 & CRP with anthropometric measurement & Vitamin D in hypothyroid patients (cases).

Parameters	IL-6		CRP	
	r value	*p value*	*r value*	*p value*
Age (years)	0.04	0.66	0.07	0.76
Height (cm)	0.11	0.16	0.63	0.20
Weight (kg)	0.06	0.73	0.07	0.90
Body mass index (BMI=kg/m^2)	0.64	0.35	0.14	0.24
Systolic BP (mm of Hg)	0.15	0.14	0.25	0.21
Diastolic BP (mm of Hg)	0.06	0.39	0.78	0.96
Blood dugar (F)	0.14	0.49	0.07	0.14
Vitamin D	0.12	0.04	0.22	0.03
p-value <0.05 considered significant				

Table 4: Distribution of cases and control according to their levels of Vitamin D

Ref. value	*Cases n(%)*	*Control n(%)*
< 30	27 (90.8%)	6 (21%)
≥ 30	3 (9.2%)	24 (79%)
	30 (100%)	30 (100%)

Abbreviations: n: Number

among the most prevalent endocrine disorders. These thyroidal dysfunctions are often range from subclinical asymptomatic thyroid patients to symptomatic thyroid ones [14]. In this study, it was found that the levels of CRP were higher in cases (patients of Hypothyroidism) as compared to healthy controls (euthyroid). This is in accordance with the study conducted by Ahmad et al. [15].

In this study, BMI was higher in cases as compared to control group which is in accordance to the study conducted by Nanda et al. [16], who also reported similar observations in patients of hypothyroidism.

The study was conducted by Czarnywojtek et al. observed that hypothyroid patients had higher levels of CRP as compared to euthyroid (control group) [17]. Similar observations were found in this study also i.e. CRP level are raised in the hypothyroid patients.

A cohort study of around 2,494 subjects in Taiwan between years 2006 to 2008 documented an association between hypothyroidism and higher CRP levels, these results are in accordance with this study [18].

Thyroid disorders, both hypothyroidism and hyperthyroidism may increase the risk of high blood pressure in thyroid patients [19]. Various studies documented positive correlations between subclinical hypothyroidism and elevated blood pressure [20].

Limitations: The sample size of the study is limited, but provided a good insight irrespective of it, a further more light is drawn into the correlation of vitamin D and inflammatory markers with hypothyroidism from a larger sample size study.

Conclusions

The study revealed a notable deficiency in vitamin D levels among hypothyroid subjects compared to the control group. Additionally, a significant correlation was observed between inflammatory markers such as CRP, IL-6 but insignificant relation was observed with anthropometric measurements. The current findings propose a regulatory role of imbalanced thyroid hormone profile on vitamin D levels, potentially resulting in elevated levels of CRP and IL-6. These elevated inflammatory markers may contribute to complications such as cardiovascular disorders. The implications of this study suggest that addressing vitamin D in patients could have a positive impact in reducing inflammatory markers, consequently lowering the risk of cardiovascular diseases. Therefore, treating hypothyroidism along with having eye on vitamin D levels may serve as a proactive measure to mitigate inflammation and its associated risks.

Conflicts of interest

Authors declare no conflicts of interest.

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