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SERUM INFLAMMATORY MARKERS IN SUBCLINICAL HYPOTHYROIDISM

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ABSTRACT

Objective: To investigate the thyroid function, inflammatory markers and blood lipid profile in the subclinical hypothyroidism. **Study design:** Cross sectional study **Place and Duration:** Department of Medicine, Jinnah Postgraduate and Medical College Karachi from December 2016 july 2017. **Materials and Methods:** Cases and controls were selected by inclusion and exclusion criteria through non-probability purposive sampling. SCH was defined as serum TSH level > 6.2 (µIU /ml) with normal free T_4 and T_3. 10 ml venous blood was taken from ante cubital vein. Thyroid hormone profile, Interleukin-6 and C-reactive protein (CRP) were estimated by ELISA assay kit and blood lipids profile by standard methods. Data analysis was performed on SPSS 22.0 at 95% confidence interval (P-value ≤0.05). **Results:** Serum cholesterol, LDLc and triglycerides were raised in SCH cases with low HDLc (P=0.001). Interleukin-6 and CRP in controls and cases were noted as 8.98±0.42 and 18.13±2.87 pg/ml & 2.66±1.31 and 6.41±2.58 ng/ml respectively (P=0.0001). Serum TSH reveals positive association with IL-6 (r=0.951, p=0.0001) and CRP (r=0.626, p=0.0001), negative association was noted with serum T_3 and T_4 (Table 2). **Conclusion:** The present study reports elevated serum interleukin-6 and C-reactive protein and dyslipidemia in subclinical hypothyroidism patients.

KEYWORDS: Interleukin-6, C-reactive protein, Dyslipidemia, Subclinical hypothyroidism.

INTRODUCTION

Subclinical hypothyroidism is a clinical entity defined as low normal thyroid gland functioning with minimal or no symptoms of hypothyroidism.[1] Subclinical hypothyroidism (SC-hypo) is a biochemical diagnosis defined as free T4 in normal range with raised thyroid stimulating hormone (TSH) levels. Patients are usually asymptomatic. Spontaneous normalization of TSH level may be observed on repeat serum TSH. Reduction of secretory functions of thyroid gland instigates the TSH stimulation with raised serum levels.[2] SCH is clinical problem of few or no symptomatic characteristics of hypothyroidism. Clinical symptoms are usually not present in SCH.[3] Pakistan has much prevalence of hypothyroidism, with many cases of SCH.[4] Thyroid dysfunctioning is predominant among female population.[5] Laboratory findings show a raised serum TSH with normal free thyroxin (FT4) and triiodothyronine (T3).[6] Hyperlipidemia and dyslipidemia is a hallmark of hypothyroidism with a tendency of raised serum cholesterol, LDLc, and triglycerides with decreased HDLc. In hypothyroidism, the LDLc receptors on the hepatocyte membrane are reduced with decreased removal of LDLc from circulation resulting in its accumulation.[7] This is just a speculation, as still it is a topic of debate, that the SCH cases have an altered state of dyslipidemia or not. Few previous studies reported no alteration was noted in blood lipids profile in subclinical hypothyroidism cases.[8,9] While other studies reported subclinical hypothyroidism cases are suffering from hyperlipidemia and dyslipidemia.[10] Dyslipidemia is associated with the coronary atherosclerotic disease, hence it is worth to take preventive measures, but this association needs further studies. Pathophysiology of atherosclerosis shows association with inflammatory markers which in the presence of dyslipidemia may accelerate the atherogenesis in future in subclinical hypothyroidism.[11] Interleukin-6 (IL-6) and C-reactive protein CRP) are inflammatory markers which reliable and well known future predictors of cardiovascular risk.[12] CRP is a biochemical tool for diagnosis of coronary artery disease (CAD) risk.[13] IL-6 is a cytokine which induces the activity of CRP, which is an established inflammatory marker.[14] Conflicting results of previous studies have created confusion of such inflammatory markers in SCH from no risk to definite risk.[13,14] Paucity of research on the inflammatory markers and blood lipid profile demands urgent research on the issue to halt atherosclerotic heart disease in the subclinical

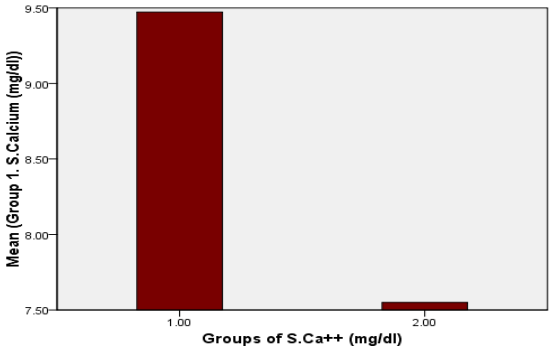
hypothyroidism cases. Hence the present study was planned to analyze the inflammatory markers and blood lipid profile in subclinical hypothyroidism at our tertiary care hospital.

SUBJECTS AND METHODS

The present cross sectional (case control) study was conducted at the Department of Medicine, Department of Medicine, Jinnah Postgraduate and Medical College Karachi from December 2016 july 2017. Sample size was calculated by using Rao-soft sampling calculator (n=200) (Margin of error is 5% with a confidence level of 95%). 100 newly diagnosed subclinical hypothyroidism were included. Subclinical hypothyroidism (SCH), age 20-60 years and both genders were inclusion criteria. Overt hypothyroidism, thyroxine therapy, Diabetes mellitus, systemic hypertensions, pregnancy, smoking, etc were excluded. SCH patients were communicated and interviewed to gain their confidence. They were told about the purpose of study. They were informed about any harm or loss to them. They were asked that they have to give consent for physical examination and blood sampling. The volunteers were asked to come in outpatient department on the day of examination and blood sampling on empty stomach without having breakfast. 10 ml venous blood was taken from ante cubital vein after tourniquet was applied tightly and aseptic measures. Age, blood pressure and body weight were measured and noted on a proforma. Blood was centrifuged to separate the sera. Thyroid hormone profile was estimated by ELISA assay kit. Reference values of thyroid hormones were as T_3 0.8 – 1.1 µg/dl, Thyroxine (T_4) 5 – 13 µg/dl and TSH <9 µU/ml. Subclinical hypothyroidism was defined as serum TSH level > 6.2 (µIU /ml) with normal free T_4 and T_3.[15] Blood lipids were estimated by investigated by CHOD/POD method, GPO-PAP method and CHOD-POD/ Phosphotungstate method respectively. Friedewald's formula16 was used for LDLc estimation.[16] Interleukin-6 (IL-6) and C-reactive protein (CRP) were estimated by commercially available ELISA assay kits. Institutional ethical approval was taken from ethical committee. Consent for was signed by the volunteers or its legal heir. Data was gathered on Performa and typed on Microsoft Excel sheet. Data analysis was performed on SPSS 22.0 (IBM, Incorporation, USA). Continuous variables were analysed by Student`s t-test and presented as Mean ± S.D. Categorical variables were analyzed by Chi square test. Pearson`s correlation was employed for correlation of TSH with T_3, T_4, IL-6 and CRP. 95% confidence interval (P-value ≤0.05) was taken as statistically significant.

RESULTS

The present cross sectional case control study was conducted to analyze the IL-6 and CRP as inflammatory markers in patients with SCH. Table 1 shows the demographic and laboratory findings. Study subjects were of similar age and body weight. Systolic and diastolic BP shows similar values between controls and cases. Serum T_3, T_4, TSH, serum cholesterol, triglycerides, LDLc, HDLc, IL-6 and CRP shows statistically significant differences between groups (P<0.05). Serum TSH shows rise in the SCH cases 10.15±1.85 versus 3.98±1.02 µU/ml in controls. Serum cholesterol, LDLc and triglycerides were raised in SCH cases with low HDLc (P=0.001). Interleukin-6 and CRP in controls and cases were noted as 8.98±0.42 and 18.13±2.87 pg/ml & 2.66±1.31 and 6.41±2.58 ng/ml respectively (P=0.0001). Serum TSH reveals positive association with IL-6 (r=0.951, p=0.0001) and CRP (r=0.626, p=0.0001), negative association was noted with serum T_3 and T_4 (Table 2).



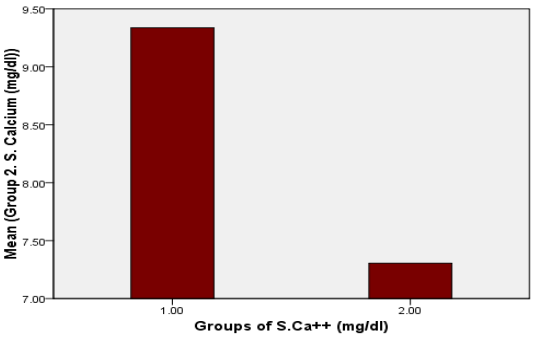
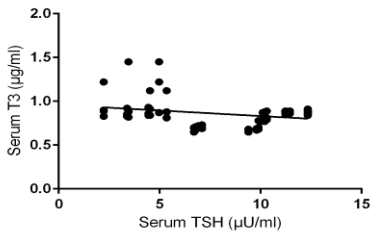


Table 1: Demographic and laboratory findings of study subjects.

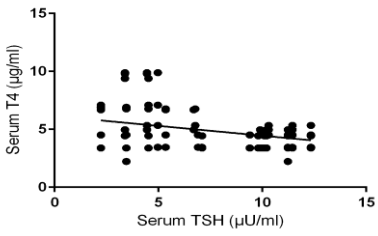
	Controls (n=100)	Cases (n=100)	P-value
Age (years)	46.3±6.34	44.8±10.97	0.306
Body weight (kg)	60.3±9.89	51.4±4.53	0.0108
Systolic BP (mmHg)	126.9±9.96	119.6±3.80	0.043
Diastolic BP(mmHg)	78.06±6.17	78.2±5.55	0.56
Serum T_3 (µg/dl)	0.94±0.19	0.80±0.08	0.003
Serum T_4 (µg/dl)	5.55±2.10	4.36±0.90	0.0001
Serum TSH (µU/ml)	3.98±1.02	10.15±1.85	0.0001
S. Cholesterol (mg/dl)	159.8±17.8	217.73±41.56	0.001
Triglycerides (mg/dl)	189.5±19.6	419.3±57.10	0.0001
LDL-c (mg/dl)	97.0±23.1	190.7±21.51	0.0001
HDL-c (mg/dl)	45.67±5.02	36.19±11.61	0.0001
Interleukin-6 (pg/ml)	8.98±0.42	18.13±2.87	0.0001
CRP (ng/ml)	2.66±1.31	6.41±2.58	0.0001
BP- blood pressure, LDL- low density lipoprotein, HDL- high density lipoprotein, TSH- Thyroid stimulating hormone, CRP- C-reactive protein			

Table 2: Correlation of Serum Thyroid stimulating hormone (TSH).

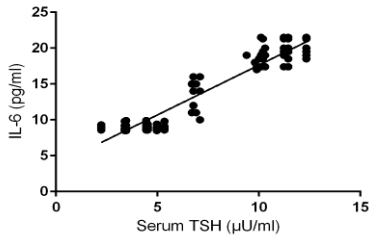
	IL-6 (pg/dl)	CRP (ng/dl)	Serum T_3 (µg/dl)	Serum T_4 (µg/dl)
r-value	0.951**	0.626**	-0.275**	-0.339**
P-value	0.0001	0.0001	0.0001	0.0001
**. Correlation is significant at the 0.01 level (2-tailed).				



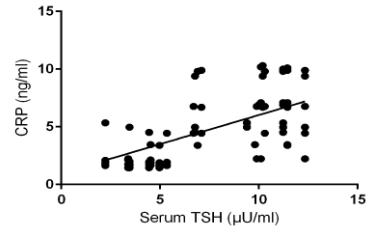
Graph 1. Scatter Plot showing négative corrélation of serum T_3 and TSH



Graph 2. Scatter Plot showing négative corrélation of serum T_4 and TSH



Graph 3. Scatter Plot showing positive corrélation of serum IL-6 and serum TSH



Graph 4. Scatter Plot showing positive corrélation of serum CRP and serum TSH

DISCUSSION

The present study is the first research being reporting on the inflammatory markers and lipids profile in subclinical hypothyroidism at our tertiary care hospital. It was hypothesized that there is no relationship between inflammatory markers and thyroid hormones. The present study found a significant correlation of serum TSH and inflammatory markers in the SCH cases. Present study reports a significant rise in the IL-6 and CRP with dyslipidemia. Inflammatory markers were raised in SCH compared to controls. The CRP is an established predictor of coronary artery disease (CAD.[17] Rise of CRP occurs in the myocardial infarction,[18] rheumatoid arthritis[19] and overt hypothyroidism.[20] In present study; the cases and controls had no history of systemic inflammatory disease, hence the rise in the inflammatory markers was not due to any of overt inflammatory disorders other than the SCH. Previous studies[21,22] have reported conflicting results of dyslipidemia in SCH cases which yet need further studies. In present study, the serum cholesterol, LDLc and triglycerides were raised in SCH cases with low HDLc (P=0.001). The findings are in agreement with previous studies[23,24] which have reported similar results.

Low HDLc as reported by previous study[25]]supports the finding of present study. The CRP is acute phase protein which is raised in various inflammatory conditions.[26,27] As the SCH shows inflammatory response, hence disturbed CRP is a positive finding which may be associated with it.[28] The findings of present study suggest ongoing inflammation in SCH patients which may be due to the thyroid disease itself, and or to the atherosclerosis in the presence of dyslipidemia. The IL-6 and CRP in controls and cases were noted as 8.98±0.42 and 18.13±2.87 pg/ml & 2.66±1.31 and 6.41±2.58 ng/ml respectively (P=0.0001).

Serum TSH reveals positive association with IL-6 (r=0.951, p=0.0001) and CRP (r=0.626, p=0.0001), negative association was noted with serum T_3 and T_4. Previous studies.[29,30] had reported raised CRP concentration in SCH patients which showed positive correlation with serum TSH[31] similar to present study, hence our findings are supported by above studies. Another previous study[32] reported strong association of dyslipidemia, cardiovascular disease and CRP in SCH patients. Xiang et al[33] reported increased risk of CAD in SCH patients with dyslipidemia and raised high sensitivity CRP (hs-CRP). Another previous study[34] reported raised serum CRP levels in subclinical hypothyroidism patients. In present study, the IL-6 was found raised in SCH patients which is supported by previous studies.[35,36] IL-6 is a pro-inflammatory cytokine implicated in the pathogenesis of atherosclerosis through activation of CRP biosynthesis by the liver.[35] The finding of raised serum IL-6 and CRP are consistent with previous study[36] which concluded that the SCH patients had raised CRP and IL-6 levels. A previous study[37] reported that the TSH stimulates the release of IL-6 from the adipocytes. The finding of raised IL-6 of present study is in agreement with previous studies.[37,38] Recent studies reported the subclinical hypothyroidism patients were having raised CRP and IL-6[39,40] which were ameliorated by levothyroxine therapy.[40] The evidence based findings of present study conclude that the subclinical hypothyroid patients should be screened for the inflammatory markers and blood lipid profile in order to prevent atherosclerosis and related morbidities.

CONCLUSION

The present study reported raised serum interleukin-6 and C-reactive protein in subclinical hypothyroidism, with disturbed blood lipids. It is suggested that the subclinical hypothyroidism is associated with dyslipidemia and inflammatory markers. Further studies are recommended and practitioners are advised to scrutinize the subclinical hypothyroidism for preventing the future risk of coronary artery disease.

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