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SUBCLINICAL HYPOTHYROIDISM;

THYROID FUNCTION TEST, C- REACTIVE PROTEIN AND DYSLIPIDEMIA IN SUBCLINICAL HYPOTHYROIDISM PATIENTS, PRESENTING AT SURGICAL WARDS OF A TERTIARY CARE HOSPITAL OF SINDH.

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ABSTRACT… Objectives: Analysis of thyroid function test, C-reactive protein (CRP) and lipids profile in subclinical hypothyroidism (SCH) as indicators of vascular atherosclerotic disease. **Study Design:** Cross sectional (case control) study. **Place and Duration:** Department of Surgery, Liaquat University of Medical and Health Sciences Hospital from January 2016 to July 2017. **Materials and Methods:** Subjects for this study were selected by inclusion and exclusion criteria. Sampling technique was non-probability purposive sampling. A case of SCH was defined as Thyroid stimulating hormone (TSH) > 6.2 (µIU /ml) with normal T_4 and T_3 free levels. 5 ml venous blood was collected from peripheral vein. Blood was centrifuged to extract sera for the detection of serum TSH, T_4 and T_3, and CRP. 12 hours fasting was mandatory for blood lipid detection. Data was analyzed on software SPSS 22.0 (P-value ≤0.05) at 95% confidence interval (P-value ≤0.05). **Results:** Cases showed high TC, TAG and LDLc with low HDLc compared to controls (P<0.05). Serum TSH was elevated in cases compared to controls (P=0.0001). Serum CRP in controls was noted as 2.75±1.12 ng/ml compared to 9.41±1.58 ng/ml in cases (P=0.0001). Pearson`s correlation shows serum CRP was negative correlated with serum T_3 (r= -0.180, P=0.074), and serum T_4 (r= -0.2250, P=0.025), but positive correlated with serum TSH (r= 0.626, P=0.0001). **Conclusion:** Subclinical hypothyroid is associated with dyslipidemia and raised C-reactive protein and both may be interpreted as indicators of vascular atherosclerotic disease.

Key words: C-reactive Protein, Dyslipidemia, Subclinical Hypothyroidism, Thyroid function test.

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INTRODUCTION

Subclinical hypothyroidism (SCH) is a biochemical diagnosis. A case of SCH was defined as Thyroid stimulating hormone (TSH) > 6.2 (µIU /ml) with normal T_4 and T_3 free levels. It is a clinical entity characterized by thyroid gland hypofunctioning in absence of clinical symptoms.[1] Patients of SCH are oftently asymptomatic or rarely may present with minimal symptoms of fatigue and other vague symptoms. Repeated analysis of thyroid function testing sometime may show spontaneous normalization of TSH level and on other occasion gross abnormality is observed. It is reported that the TSH rise occur consequent to a hypofunctioning of thyroid gland.[2] However, clinically the patients are performing routine life activities with few or no complaints at all. Absence of clinical symptoms is characteristic finding of SCH.[3] Currently; some of studies have reported on the SCH[4] that remain undiagnosed in Pakistan. Thyroid hypofunctioning of SCH spectrum is frequently observed in the female population.[5,6] Patients of SCH shows various degree derangements of blood lipids. Dyslipidemia is a hallmark of thyroid gland hypofunctioning. Hyper levels of serum cholesterol, LDLc, and triglycerides with decreased HDLc are noted in hypothyroidism. One postulated mechanism of high LDLc levels is the down regulation of LDL-receptors in the liver, these results in the pooling of LDLc in circulation and poses a tendency of atheroma.[7] As the SCH patients are reported of having hypofunctioning thyroid gland with dyslipidemia, hence there is need of analyzing

and understanding of these biochemical abnormalities in particular to the atherogenic tendency. SCH as a condition of proatherogenic state is a topic of debate. Some of previous studies[8,9] reported no alterations were noted in the blood lipids in SCH. While other studies[10] reported hyperlipidemia and dyslipidemia in SCH.[10] As the dyslipidemia is associated with atherosclerosis, there is need to evaluate the problem. The condition is also associated with presence of inflammatory marker (CRP). A previous study[11] reported dyslipidemia is central to play role in the atherosclerotic heart diseases. Dyslipidemia accelerates the atherogenicity and this is observed in the SCH.[11] CRP is a validated biological marker of inflammatory process.[12] A previous study[13] reported CRP is risk marker of coronary artery disease.[13,14] Conflicting results of previous studies[8,9,10] need further research to probe into the possible risk of atherosclerosis in SCH through studying the C-reactive protein (CRP) and blood lipids. This may help to halt atherosclerosis and associated coronary heart disease.

The present study analyzed the thyroid function test, inflammatory markers and lipids profile in subclinical hypothyroidism (SCH) patients presenting at surgical wards of a tertiary care hospital of Sindh. The present research is a bird's eye view to peep into the problem of dyslipidemia and inflammatory markers as risk of future atherosclerosis in subclinical hypothyroidism. It was hypothesized that there is no association of C-reactive protein and dyslipidemia in subclinical hypothyroidism.

SUBJECTS AND METHODS

A cross sectional case control study was planned at the Department of Surgery, Liaquat University of Medical and Health Sciences Jamshoro from August 2016 to October 2017. Subjects for this study were selected by inclusion and exclusion criteria. Sampling technique was non-probability purposive sampling. Online Rao- Soft sampling calculator was used for sample calculation. One hundred diagnosed cases of SCH were selected according to the inclusion criteria of; age 20-60 years, both genders and serum thyroid stimulating hormone (TSH) > 6.2 (µIU /ml)[15] with normal T_4 and T_3 free levels. Similar number of age and gender matched healthy adults were selected as control. Thyroid disorders of overt hypothyroidism were excluded. Patients with systemic disease such as chronic liver disease, systemic hypertension, chronic kidney disease, and diabetes mellitus were excluded. Pregnant women and smokers were also excluded.

The study protocol was approved by the ethical review committee. Only volunteers were asked enter the study protocol. They were informed about purpose of study. They were informed that they don't have to pay for any blood testing. The volunteers were asked to sign the consent form and to abide by the instructions for blood sampling. They were informed that they are free to leave study protocol at any time without prior information and this will not affect their treatment. Cases and control subjects were interviewed to take their confidence and to understand the purpose of study. They were informed that they have to allow for blood sampling. They were informed that the personal information and blood investigations will be kept confidential and they can get laboratory investigation in person if they need them.

Personal biodata was collected by a pre-assigned medical officer. Volunteers were examined by a consultant surgeon. 10 ml venous blood was collected from peripheral vein. Tourniquet was applied to make the veins engorged with blood. Aseptic measures were applied strictly for the blood sample collection. Blood was centrifuged to extract sera for the detection of serum TSH, T_4 and T_3, C- reactive protein (CRP) and Interleukin-6 (IL-6). For blood lipids, patients were informed to come fasting of at least 12 hours. Elisa assay method was used for thyroid hormone detection. Reference values of kit were serum T_4 (thyroxine) 5 – 13 μg/dl, serum T_3 (tri-iodothyronine) 0.8 – 1.1 μg/dl and serum TSH <9 μU/ml. Subclinical hypothyroidism was defined as cited above.[15] Elisa assay method was employed for the detection of IL-6 (Interleukin-6) and CRP (C-reactive protein). Lipids were estimated by standard methods.[16] Biochemical assay estimation was conducted on

Roche chemistry analyzer. Study protocol was in accordance to the "Helsinki`s declaration" for conducting human research. Microsoft Excel sheet and SPSS were used for statistical analysis and graph designing. Continuous and categorical variable analysis was performed on SPSS by using Student`s t-test and Chi- square test respectively. Continuous data results were presented as mean ± S.D. Categorical data results were presented as frequency and percentage. Correlation of data variables was performed. Data was analyzed on software SPSS 22.0 (P-value ≤0.05) at 95% confidence interval (P-value ≤0.05).

RESULTS

Age of controls and cases was 49.3±8.45 and 48.9±11.71 years respectively (P=0.61). In control and cases the male to female frequency was 37% and 23% & 73% and 77% respectively (P=0.87). Age, gender, body weight, systolic and diastolic blood pressure are shown in Table-I. Control and cases were age and gender matched. Total cholesterol (TC), Triglycerides (TAG), LDLc and HDLc showed revealed statistically significant differences between control and cases (P <0.05). Cases showed high TC, TAG and LDLc with low HDLc compared to controls. Serum T_3 and T_4 showed non-significant findings between control and cases. However, serum TSH was elevated in cases (P=0.0001). Serum CRP in controls was noted as 2.75±1.12 ng/ml compared to 9.41±1.58 ng/ml in cases (P=0.0001). Pearson`s correlation shows serum CRP was negative correlated with serum T_3 (r= -0.180, P=0.074), and serum T_4 (r= -0.2250, P=0.025), but positive correlation with serum TSH (r= 0.626, P=0.0001).

	Controls (n=100)	Cases (n=100)	P-value
Age (years)	49.3±8.45	48.9±11.71	0.61
Male	27 (27%)	23 (23%)	0.87
Female	73 (63%)	77 (77%)	
Body weight (kg)	68.5±8.91	67.5±9.56	0.81
Systolic BP (mmHg)	116.9±10.6	129.6±9.80	0.043
Diastolic BP(mmHg)	75.65±10.15	81.5±12.53	0.048

Table-I. Demographic characteristics of study subjects

	Controls (n=100)	Cases (n=100)	P-value
Total Cholesterol (mg/dl)	149.8±17.8	177.7±31.5	0.0001
Triglycerides (mg/dl)	199.5±19.6	319.5±51.15	0.0001
LDLc (mg/dl)	91.0±13.5	153.7±10.5	0.0001
HDLc (mg/dl)	46.7±9.52	41.9±8.51	0.003
Serum T_3 (µg/dl)	0.931±0.19	0.91±0.18	0.35
Serum T_4 (µg/dl)	4.95±2.51	4.73±1.90	0.87
Serum TSH (µU/ml)	3.78±1.02	11.05±3.85	0.0001
CRP (ng/ml)	2.75±1.12	9.41±1.58	0.0001

Table-II. Biochemical findings of study subjects

	Serum T_3 (µg/dl)	Serum T_4 (µg/dl)	TSH (µU/ml)
r-value	-0.180	-0.225*	0.626**
P-value	0.074	0.025	0.0001

Table-III. Pearson`s correlation of serum C- reactive protein
****. Correlation is significant at the 0.01 level (2-tailed).**

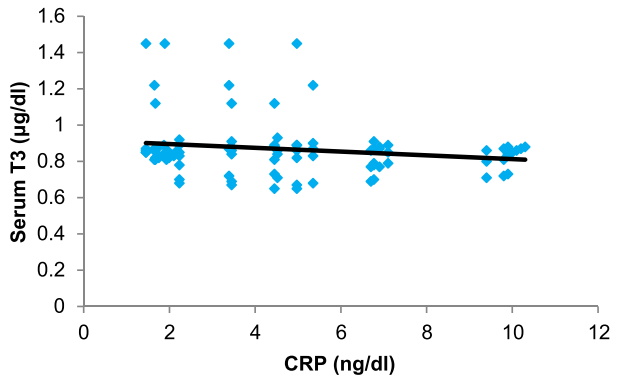

Figure-1. Negative correlation of Serum CRP and T_3

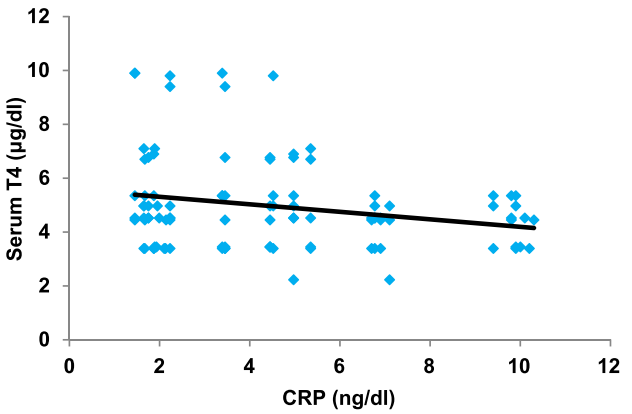

Figure-2. Negative correlation of Serum CRP and T_4

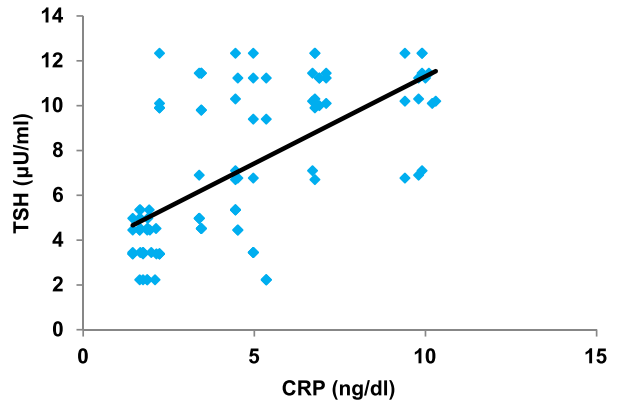

Figure-3. Positive correlation of Serum CRP and TSH

DISCUSSION

The present study is first time reporting on the risk of vascular atherosclerotic disease in subclinical hypothyroidism from our tertiary care hospital. Null hypothesis of no relationship of C-reactive protein and dyslipidemia was rejected and alternative hypothesis was accepted true. Age of controls and cases was 49.3±8.45 and 48.9±11.71 years respectively (P=0.61). In control and cases the male to female frequency was 37% and 23% & 73% and 77% respectively (P=0.87). These findings are in agreement with previous studies.[17-19] Control and cases were age and gender matched. Total cholesterol (TC), Triglycerides (TAG), LDLc and HDLc showed revealed statistically significant differences between control and cases (P <0.05). Cases showed high TC, TAG and LDLc with low HDLc compared to controls. Serum T_3 and T_4 showed non-significant findings between control and cases. However, serum TSH was elevated in cases (P=0.0001). The present study found a significant positive correlation of serum TSH and C-reactive proteins in SCH patients. The CRP is an established indicator of peripheral atherosclerotic vascular disease and coronary artery atherosclerotic disease (CAD).[17] The findings are in agreement with previous studies,[18-20] but contrary to other studies.[21,22] These studies[21,22] yielded conflicting observations of dyslipidemia in SCH that is in contrast to present and previous studies.[18-20] The cases of SCH showed elevated TC, TAG, LDLc and low HDLc and difference was statistically significant compared to controls (P <0.05). Our findings correlate with the previous studies[23,24] as they had reported equivalent results. Low HDLc in SCH cases is supported by previous study[25] that reported equivalent results. The CRP was raised in the SCH cases and it is reported the CRP is an acute phase protein and is closely associated with inflammatory conditions such as the vascular atherosclerotic diseases.[26,27] Finding of raised CRP in SCH patients points towards as proatherosclerotic milieu in these subjects, this finding is in accordance to a previous study.[28] CRP indicates the ongoing inflammatory conditions in SCH cases, this might be because of hypothyroidism itself or because of the ongoing atherosclerotic process by dyslipidemia. In present study, the serum CRP in controls was noted as 2.75±1.12 ng/ml compared to 9.41±1.58 ng/ml in cases (P=0.0001). Pearson`s correlation shows serum CRP was negative correlated with serum T_3 (r= -0.180, P=0.074), and serum T_4 (r= -0.2250, P=0.025), but positive correlation with serum TSH (r= 0.626, P=0.0001). these

finidgns are supported by previous studies.[26-30] Positive correlation of CRP with serum TSH (r= 0.626, P=0.0001) is a worth findings supported by previous studies.[29-31] Association of CRP with dyslipidemia has been reported previously[32-34] in SCH patients. A previous study[33] reported increased risk of atherosclerotic vascular disease in SCH patients as indicated by dyslipidemia and hsCRP. This is in full agreement with the present study. Elevated CRP in SCH patients of present study is concordant to previous study.[34] The results of dyslipidemia and CRP in SCH are supported by recent studies.[35,36] Subclinical hypothyroid patients should be timely screened for the inflammatory marker (C-reactive protein) and dyslipidemia (blood lipids) for future prevention of atherosclerosis related disease of peripheral vasculature and coronary artery morbidities.

CONCLUSION

The present study concludes the subclinical hypothyroidism is associated with dyslipidemia and raised C-reactive protein; both are indicators of vascular atherosclerotic disease. Further studies are recommended to make screening of C-reactive protein and blood lipids justified for the subclinical hypothyroidism for future prevention of atherosclerotic peripheral and coronary artery disease.

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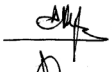
Dream as if you'll **live** forever,
live as if you'll **die** today.

"James Dean"

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Abdul Salam Memon	Sample collection, Materials handling, Collection & compilation of results, Statistical analysis, Data collection, compilation of results, Proof reading.	
2	Bheesham Kumar	Manuscript write up, Proof reading, Collection & compilation of results.	
3	Altaf Ahmed Talpur	Literature review, Manuscript write up, Proof reading, Statistical analysis, Manuscript write up, Proof reading, Correspondence.	
4	Afzal Junejo	Concept, Material handling, results, Statistical analysis, manuscript write up, Literature review, Data collection, compilation of results, Proof reading.