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High Sensitive C-Reactive Protein and Lipid Profile Alteration In Subclinical Hypothyroidism for Cardiovascular Risk Assessment

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ABSTRACT

Background: The purpose of this study was to investigate the association of subclinical hypothyroidism with High sensitive C-reactive protein & lipid profile which can predispose to development of Cardiovascular disease.

Methods: This hospital-based comparative cross-sectional study was conducted for a period of six months. A total of 71 patients with subclinical hypothyroidism & 37 healthy control subjects were enrolled for the study. Thyroid hormones, lipid profile, hs- CRP were measured and lipid variables were used to calculate lipid indices. Student t-test were used to compare means & Spearman's correlation was done to determine the association between variables. ROC curve analysis was used to determine the diagnostic value of tests.

Results: Out of 71 cases & 37 control, majority had female preponderance (71.8% in case & 83.8% in control). The mean values between case & control groups for hs-CRP, AIP, LCI and non-HDL cholesterol were statistically significant. There was positive correlation between TSH and hs-CRP ($r=0.492$, $p<0.001$), AIP and TSH ($r=0.430$, $p<0.001$), LCI and TSH ($r=0.269$, $p=0.005$), TSH and non-HDL cholesterol ($r=0.308$, $p=0.001$) & AI and LDL ($r= 0.712$, $p<0.001$) with weak correlation with statistical significance as per Spearman's correlation. Area under ROC curve for hs-CRP indicated it as a positive biomarker for CVD assessment.

Conclusions: Our findings shows that SCH patients are more at risk of CVD & hs-CRP contributes as a significant marker, thus requiring timely intervention. Lipid indices and AIP must be determined even in patients with a normal lipid profile to improve atherogenic risk.

Keywords: Cardiovascular risk; dyslipidemia; hs-CRP; subclinical hypothyroidism.

INTRODUCTION

The overall prevalence of SCH affects women at a rate of 6-8% and males at 3%.[1] Due to its asymptomatic nature, significant concerns have been raised about its clinical relevance and effective treatment.[2] According to research, the severity of the disease of lipid metabolism increases with increasing levels of thyroid-stimulating hormone (TSH).[3] Chronic inflammation has also shown to damage the thyroid gland which if untreated can cause systemic organ damage.[1] A marker of subclinical inflammation is high sensitive C-reactive protein. Various studies indicate hs-CRP as an important predictor of myocardial infarction & cardiovascular diseases even though the levels of LDL-C are normal or low.[4] This study aims to determine the association of dyslipidemia and inflammatory marker hs-CRP with subclinical hypothyroid patients as a predictor for CVD.

METHODS

This is a hospital based comparative cross-sectional study conducted at the department of Clinical Biochemistry in collaboration with Department of Internal Medicine (endocrinology), TUTH for a period of 6 months i.e., from July 2023 to November 2023. Nonprobability sampling (Purposive sampling) was used to include the patients. Samples of the patients processed in clinical

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biochemistry laboratory with a subclinical hypothyroid profile were incorporated after taking their clinical details. Patients taking medications like thyroxine, steroids and statins were excluded as they could alter the results. Similarly, patients with systemic diseases like diabetes, nephrotic syndrome were also excluded to ensure dyslipidemia if present was solely due to thyroid disease. With type I error of 5% , CI = 1.96 & prevalence of 5% (5), sample size was calculated. A total of 71 cases were included for the estimation of lipid profile and hs-CRP levels along with 37 age and sex matched healthy controls. The research received ethical approval from the Institute of Medicine's (IOM) Institutional Review Committee. [Approval number: 578 (6-11) E2 079/080].

The assay range for subclinical hypothyroid cases were serum
TSH level 4.5 - 10 micro IU/ml[1]
fT3 4.26 -8.1 pmol/L
fT4 10.2 – 28.2 pmol/L
The reference range for lipid profile parameters ATP III classification (mg/dl) were

Variable	Range	Classification
LDL cholesterol	<100	Optimum
	100 – 129	Near or above optimum
	130 – 159	Borderline high
	160 – 189	High
	≥ 190	Very high
Total cholesterol	< 200	Desirable
	200 – 239	Borderline High
	≥ 240	High
HDL cholesterol	<40	Low
	≥ 60	High

hs-CRP values :[6]
Low risk < 1.0 mg/l
Moderate risk : 1 – 3 mg/l
High risk : > 3.0 mg/l

We included the demographic variables like age, geographic location and ethnicity. Biochemical variables included were serum fT3, fT4, TSH, TC, LDL, HDL, TG & hs-CRP were estimated by chemiluminescence immunoassay technique in the Abbott ci4000 autoanalyzer.

Data was entered in MS Excel 2010 and analyzed with Statistical Package for Social Sciences (SPSS version 22.0). The normality of the data was checked using the Kolmogorov Smirnov test. For descriptive statistics, mean, standard deviation, percentage, range was calculated. For parametric variables, students' "t" test, and for non-parametric variables, Chi-square test and Mann-Whitney U-test was used. Correlation between patient age, fT3, fT4, TSH, lipid profile parameters and hs-CRP were determined by Pearson's or Spearman's correlation coefficient. p-value ≤ 0.05 was considered statistically significant. ROC curve analysis was used to determine the diagnostic value of tests.

RESULTS

The total study population of 108 comprised of the age group between 18 and 65+ years. Most of the SCH patients were between 25 to 35 years age group with majority of participants being female (n=82, 75.9%).

Table 1. Age and gender wise distribution of study population.

Age category	Male	Female	Total	Percentage (%)
18-24	1	11	12	11.1
25-35	7	38	45	41.7
36-45	6	12	18	16.7
46-55	2	11	13	12.0
56-65	5	7	12	11.1
65+	5	3	8	7.4
Total	26 (24.1%)	82 (75.9%)	108	100

Comparison of parameters between case and control

The mean and standard deviation parameters of thyroid function test, lipid profile parameters, hs-CRP and lipid indices between case & control are shown in table 2. TSH, TG, hs-CRP, AIP and LCI had statistical significance.

Table 2. Comparison between case and control groups.

	Case	Control	p value
fT3	4.68 ± 0.35	4.64 ± 0.24	0.584$_a$
fT4	11.31 ± 2.02	10.98 ± 1.2	0.303$_a$
TSH	8.5 ± 3.2	2.69 ± 1.31	**<0.001**$_a$
Cholesterol	4.4 ± 1.43	4.08 ± 0.60	0.109$_a$
TG	2.38 ± 1.8	1.2 ± 0.40	**<0.001**$_a$
LDL	2.34 ± 1.01	2.60 ± 0.54	0.242$_a$
HDL	1.04 ± 0.28	1.11 ± 0.30	0.257$_a$
AI	0.29 ± 0.26	0.044 ± 0.17	**<0.001**
Hs-CRP	2.1 [0.9, 4.9]	0.50 [0.40, 0.85]	**<0.001**$_b$
LCI	2.28 ± 0.93	2.4 ± 0.677	0.288
Non-HDL Cholesterol	32.9 ± 58.1	12.28 ± 4.31	**0.004**
	3.36 ± 1.33	2.97 ± 0.70	0.050

a= Student t-test, b= Mann-Whitney U test, p value <0.05 was considered statistically significant

There was positive correlation between TSH and hs-CRP (r=0.492, p <0.001), AIP and TSH (r=0.430, p<0.001), LCI and TSH (r=0.269, p =0.005), TSH and non-HDL cholesterol (r=0.308, p=0.001) and AI and LDL (r= 0.712, p<0.001) with weak r value except AI and LDL with statistical significance.

Table 3. Spearman correlation of study variables.

	r value	p value
TSH and Cholesterol	0.295	0.02
TSH and hs-CRP	0.492	<0.001
TSH and TG	0.434	<0.001
TG and hs-CRP	0.435	<0.001
AIP and TSH	0.430	<0.001
LCI and TSH	0.269	0.005
TSH and non-HDL cholesterol	0.308	0.001
AI and LDL	0.712	<0.001

p value <0.05 was considered statistically significant

Frequency of hs-CRP in case and control

The number of cases with hs-CRP > 1 was 51 whereas there were 2 participants from control group. This indicates SCH can contribute to significant inflammatory process thus contributing to development of cardiovascular disease.

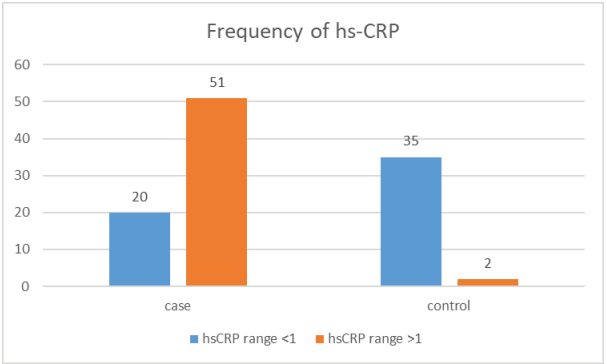

Figure 1. Frequency of hs-CRP in case and control.

Table 4. Cut-off values for hs-CRP and lipid indices.

Biomarker	Area(95% CI)	Optimal cut-off value	Sensitivity	Specificity	PPV	NPV
hs-CRP	0.783 * (0.694-0.873)	0.85 mg/l	77.5%	75.7%	96.23%	63.63%
AIP	0.629 * (0.527-0.731)	0.27	47%	52%	84.37%	61.36%
LCI	0.851 * (0.779-0.923)	15.13	49.3%	50.7%	84.21%	44.28%

***p<0.05**

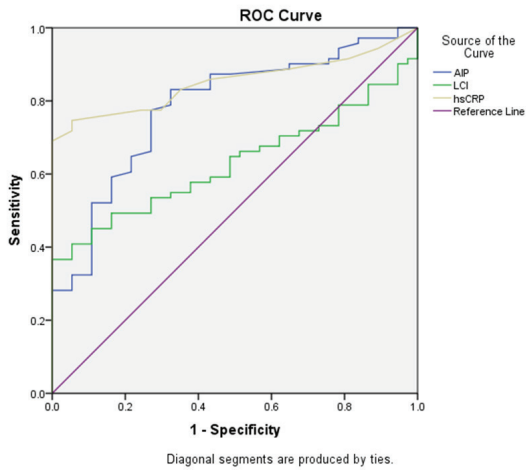

Figure 2. ROC curve for hs-CRP, AIP and LCI

DISCUSSION

Subclinical hypothyroidism (SCH) is a significant thyroid disorder as it is more prevalent in the population and due to its association with CVD.[7] In this hospital-based comparative cross-sectional study in central Nepal, a total of 71 patients were enrolled with subclinical hypothyroidism with 37 age and sex matched controls. This study highlights the significance of screening subclinical hypothyroid patients due to its possible association with cardiovascular risk as depicted by increasing hs-CRP levels, dyslipidemia and lipid indices. Panchal et. al [8] in her study has stated SCH and its association with inflammation via elevated hs-CRP as a possible risk factor for cardiovascular diseases. This maybe due to the progression of SCH to overt hypothyroidism that is associated with positive TPO antibodies. Hossain et. al [9] has also concluded subclinical hypothyroidism and its

association with atherogenic lipid profile. This is due to reduced cholesterol synthesis caused by reduced HMG Co A reductase in thyroid hormone deficiency. The clearance of cholesterol is also decreased due to decreased cholesterol secretion in the bile.[9]

In this study, 75.9% were females indicating that SCH is more prevalent in females & similar findings have been found in studies done by Sharma et. al [10] and Panchal et. al.[8] Majority of participants are female from age group 25-35 indicating that SCH is more prevalent in the reproductive age group. This maybe due to high prevalence of female patients in the study compared to males. This imbalance may also be implicated by estrogen according to studies.[2] Studies have shown SCH and its association with infertility, thus screening and management is an important aspect in this group.[11] Similar findings have been shown with mild subclinical hypothyroidism.[12]

This study also depicts significantly higher levels of TG and hs-CRP among cases compared to control. The differences were statistically significant. Similar findings have been suggested by other studies.[2,10] No association between TC, LDL and HDL among cases and control was found in our study. This may be due to the degree and duration of SCH and TSH levels < 10 (mean TSH 8.5 ± 3.2 in cases) and the differences in population that is being studied. In contrast, Colorado study [13] and Tromso [14] studies have shown SCH and its association with TC and LDL levels. The lipid indices AIP, LCI and non-HDL cholesterol were higher in SCH cases compared to healthy controls. It is similar to other studies[15,16] which is caused by elevated TG and decreased HDL-C. This shows SCH cases are more at CVD risk. Similarly, LCI which is also increased in coronary artery disease is elevated in SCH cases indicating CVD risk. Similar findings suggested by Cai et al. [17]

There was a weak positive correlation between TSH and TC ($r = 0.295$, $p = 0.02$) and similar findings have been suggested by other studies.[9,10] Thyroid hormones when deficient, reduces HMG coA reductase activity and may decrease cholesterol synthesis slightly but since LDL receptors are decreased further, LDL accumulates in serum.[18] Thyroid hormones also plays a role in TG metabolism as it inhibits lipoprotein lipase in deficient state leading to inhibition of TG breakdown and thus its accumulation.[9] In addition to raising LDL-C concentrations, thyroid dysfunction speeds up LDL-C oxidation, so when macrophages consume oxidized LDL-C, they transform to foam cells that are more cardiovascular-friendly and atherogenic disease.[19]

However, TC and TG can be affected by dietary habits & some studies have shown inconsistent results.[20] There was weak positive correlation between TSH & hs-CRP ($r = 0.492$ $p < 0.001$) with statistical significance. Similar findings have been shown by Vyakaranam et. al.[7] However, contrasting findings have also been elucidated by other studies.[21] In our study r value was less than 0.5 as per Spearman's correlation. This maybe due to small sample size and an even smaller control group which can be a limitation of this study.

Figure 1 shows the frequency of hs-CRP levels in case and control. The number of cases with hs-CRP >1 was 51 whereas only 2 in control group, taking cut-off hs-CRP > 1 as risk factor for CVD. This shows the effect of SCH on inflammatory marker like hs-CRP that can lead to development of cardiovascular diseases and acts as a strong predictor for CVD. Similar findings were seen in other studies.[7,8]

Figure 2 illustrates the ROC curve analysis for hs-CRP, AIP and LCI to evaluate CVD risk in SCH. The Area Under the ROC curve for hs-CRP, AIP and LCI were 0.783 (95% CI 0.694-0.873), 0.629 (95% CI 0.527-0.731) and 0.851 (95% CI 0.779-0.923) respectively. It depicts that LCI and hs-CRP were positive biomarkers for CVD risk assessment. The sensitivity, specificity, PPV and NPV for each biomarkers are shown in table 4. However, hs-CRP had the highest sensitivity, specificity and PPV compared to lipid indices thus, indicating its diagnostic superiority compared to other lipid markers.

CONCLUSIONS

Our findings shows that SCH patients are more at risk of CVD and hs-CRP contributes as a significant marker, thus requiring timely intervention. Lipid indices and AIP must be determined even in patients with a normal lipid profile in order to improve the assessment of atherogenic risk.

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Conflict of Interests

The authors disclose no competing interests in this work.

REFERENCES

1. Krishnamurthy H, Ravitej S. Predictive Significance of High Sensitive C-reactive Protein in Subclinical Hypothyroidism: A Prospective Observational Study. J Clin Diagn Res [Internet]. 2022 [cited 2023 Dec 10]; [Article]
2. Jha UC. To Compare the Thyroid Profile, hs-CRP, and Lipid Profile in Newly Diagnosed Hypothyroid Adults to Controls, as well as to Compare the above Parameters in Subclinical and Clinical Hypothyroid Instances. Eur J Mol Clin Med. 2021 Jun 22;8(4):1409-17. [Article]
3. Assessment of the relationship between subclinical hypothyroidism and blood lipid profile: reliable or not? | Lipids in Health and Disease | Full Text [Internet]. [cited 2023 Dec 11]. [Article]
4. The JUPITER Trial | Circulation: Cardiovascular Quality and Outcomes [Internet]. [cited 2023 Dec 11]. [Article]
5. Kc R, Khatiwada S, Deo Mehta K, Pandey P, Lamsal M, Majhi S. Cardiovascular Risk Factors in Subclinical Hypothyroidism: A Case Control Study in Nepalese Population. J Thyroid Res. 2015 Oct 7;2015:e305241.[Article]
6. Kamath DY, Xavier D, Sigamani A, Pais P. High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: An Indian perspective. Indian J Med Res. 2015 Sep;142(3):261-8.[Article]
7. Vyakaranam S, Kondaveedu S, Nori S, Dandge S, Bhongir AV. Study of Serum High-sensitivity C-reactive Protein in Subclinical Hypothyroidism. Indian J Med Biochem. 2018 Jun;22(1):66-70. [Article]
8. Panchal M, Gondaliya UB. A Study of High Sensitive C Reactive Protein in Subclinical Hypothyroidism. Int J Contemp Med Surg Radiol [Internet]. 2019 Jan [cited 2023 May 9];4(1). [Download PDF]
9. Hossain MA, Atiquzzaman -, Sharifuzzaman M, Amin F, Kabir L, Sultana D, et al. Study of Fasting Lipid Profile in Patients with Subclinical Hypothyroidism in a Tertiary Care Hospital in Bangladesh. Faridpur Med Coll J. 2021 Oct 3;16(1):17-20.[Article]
10. Lipid profile and hs-CRP levels in patients with subclinical hypothyroidism | International Journal of Medical Science and Public Health [Internet]. [cited 2023 Dec 11]. Available from: https://www.bibliomed.org/?mno=221745
11. Practice Committee of the American Society for Reproductive Medicine. Subclinical hypothyroidism in the infertile female population: a guideline. Fertil Steril. 2015 Sep;104(3):545-53.[Article]
12. Feldthusen AD, Pedersen PL, Larsen J, Toft Kristensen T, Ellervik C, Kvetny J. Impaired Fertility Associated with Subclinical Hypothyroidism and Thyroid Autoimmunity: The Danish General Suburban Population Study. J Pregnancy. 2015;2015:132718.[Article]
13. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000 Feb 28;160(4):526-34.[Article]
14. Iqbal A, Jorde R, Figenschau Y. Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Tromsø Study. J Intern Med. 2006 Jul;260(1):53-61.[Article]
15. Assessment of Cardiovascular Disease Risk in Females with Subclinical Hypothyroidism [Internet]. [cited 2023 Dec 10]. [Article]
16. James S, Ray L, Ravichandran K, Nanda S. High atherogenic index of plasma in subclinical hypothyroidism: Implications in assessment of cardiovascular disease risk. Indian J Endocrinol Metab. 2016;20(5):656.[Article]
17. Cai G, Shi G, Xue S, Lu W. The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population. Medicine (Baltimore). 2017 Sep;96(37):e8058.[Article]
18. Pearce EN, Wilson PWF, Yang Q, Vasan RS, Braverman LE. Thyroid function and lipid subparticle sizes in patients with short-term hypothyroidism and a population-based cohort. J Clin Endocrinol Metab. 2008 Mar;93(3):888-94.[Article]
19. Karthick N, Dillara K, Poornima KN, Subhasini AS. Dyslipidaemic Changes in Women with Subclinical Hypothyroidism. J Clin Diagn Res JCDR. 2013 Oct;7(10):2122-5.[PMC3843389]
20. Pearce EN. Update in Lipid Alterations in Subclinical Hypothyroidism. J Clin Endocrinol Metab. 2012 Feb 1;97(2):326-33.[Article]
21. Peixoto de Miranda ÉJF, Bittencourt MS, Santos IS, Lotufo PA, Benseñor IM. Thyroid Function and High-Sensitivity C-Reactive Protein in Cross-Sectional Results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): Effect of Adiposity and Insulin Resistance. Eur Thyroid J. 2016 Dec;5(4):240-6.[Article]