Original Article

Association of metabolic risks with subclinical hypothyroidism: A cross-sectional analysis

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ABSTRACT

Objective: To compare lipid parameters, HbA1c, uric acid and albumin creatinine ratio (UACR) among subjects having euthyroidism, Sub-Clinical Hypothyroidism (SCH) and overt hypothyroidism.

Methods: This comparative cross-sectional analysis was carried out from Dec-2015 to Oct-2016 in collaboration between PNS HAFEEZ hospital and department of chemical pathology and endocrinology, Armed Forces Institute of Pathology, Rawalpindi. Biochemical parameters including lipid indices, HbA1c and UACR were compared between euthyroidism (TSH: 0.5 to 4.0 mIU/L, n=163), subclinical hypothyroidism (TSH: 4.0 to 10 mIU/L, n=16) and overt hypothyroidism (TSH:≥ 10.0 mIU/L, n=9).

Results: LDL-cholesterol, non-HDL-cholesterol and UACR results were as: [(Euthyroid: 2.66 ± 0.73), (SCH: 2.68 ± 0.51) and (Overt hypothyroidism: 3.23 ± 0.59), p-value=0.063], [(Euthyroid: 3.49 ± 0.64), (SCH: 3.35 ± 0.59) and (Overt hypothyroidism: 4.01 ± 0.30), p-value=0.033] and [{Euthyroid: 2.48 (95% CI: 1.63-3.33)}, {SCH: 2.27 (95% CI: 0.37-4.90)} and {Overt hypothyroidism: 14.95 (95% CI: 10.71-19.14){, (p-value< 0.001)] Results for total cholesterol, triglycerides and HDL-cholesterol though increased in overt hypothyroid group were not found to be statistically significant.

Conclusion: LDL-cholesterol, non-HDL-cholesterol and UACR increased from euthyroid subjects to overt hypothyroidism group. However, these changes were found to be more subtle in the subclinical hypothyroid subjects than cases with overt hypothyroidism.

KEYWORDS: Subclinical hypothyroidism (SCH), HDL-cholesterol, LDL-cholesterol, nonHDL-cholesterol, UACR.

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INTRODUCTION

Cardiovascular diseases (CVD) remain one of the most prevalent causes of morbidity and mortality in the current era. The causes underlying the increased prevalence of CVD have been researched a lot; however, still cases remains where the actual pathogenesis remains obscure.[1] Overt hypothyroid disease has always been considered to increase CVD related morbidity and mortality.[2] The diagnosis of hypothyroidism is considered with regards to TSH levels, which in cases of primary thyroid pathology becomes elevated with low T4 and T3. However, in routine clinical practice there are more cases with border line elevations of TSH with normal T4 and T3, where the etiology could not be explained alone by non-thyroidal illnesses (NTI), medications, age or simply a variation of normality.[3,4]

Lipid changes in blood especially atherogenic dyslipidemia are considered to actually represent a state of increase atherosclerosis. Thyroid hormones play a significant role in the metabolism of

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cholesterols by maintaining the normal turnover and utilization at the cellular levels.[5] While Overt hypothyroidism has been established to dyslipidemia, subclinical hypothyroidism(SCH) in the setting of normal thyroid hormones can also slow down metabolic pathways to accelerate atherosclerotic lipid deposition within vasculature needs more elaboration. Literature review on SCH provides varying conclusions in terms of association with dyslipidemia. Some large sample studies have identified no such association for dyslipidemia in subjects with SCH[6,7], while others suggested the possibility of such an association.[8] With regards to cardiovascular events, role of lipid metabolism remains instrumental, where the literature review suggests inconsistent evidence. Few studies have found significant dyslipidemia in subjects with SCH[9,10], but studies like Tehran Thyroid Study(TTS) have demonstrated no significant lipid changes in SCH.[11]

Consequent upon these highlighted discrepancies in data we planned to compare various lipid parameters, HbA1c and uric acid and albumin creatinine ratio among subjects with euthyroidism, SCH and overt hypothyroidism.

METHODS

Our study was a comparative cross-sectional analysis which was carried out at the departments of pathology and medicine PNS HAFEEZ in liaison with department of chemical pathology and endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. Based upon non-probability convenience sampling, asymptomatic subjects from medical OPDs were requested to participate in the study. Subjects who volunteered were explained about study requirements and consequences, formally consented, interviewed for presence of any chronic metabolic or other disease, clinically examined for measurements of

Table-I: Gender differences for various evaluated parameters.

Dependent Variable	*Gender*	*N*	*Mean*	*95% Confidence Interval*		*(p-value)**
				Lower Bound	*Upper Bound*	
Age (years)	Male	96	46.87	44.06	49.68	0.045
	Females	92	42.91	40.25	45.56	
Weight (Kg)	Male	96	72.66	69.42	75.90	0.460
	Females	92	70.98	67.92	74.05	
Height (cm)	Male	96	169.08	167.32	170.84	< 0.001
	Females	92	158.24	156.58	159.91	
Waist (cm)	Male	96	90.83	88.41	93.26	0.061
	Females	92	94.03	91.74	96.32	
Hip (cm)	Male	96	97.47	95.17	99.78	0.030
	Females	92	101.00	98.82	103.18	
Total cholesterol (mmol/L)	Male	95	4.42	4.29	4.555	0.861
	Females	92	4.44	4.31	4.564	
Fasting triglycerides (mmol/L)	Male	95	1.59	1.43	1.75	0.308
	Females	92	1.48	1.32	1.63	
HDL-cholesterol (mmol/L)	Male	95	0.92	0.86	0.98	0.005
	Females	91	1.04	0.98	1.10	
LDL-cholesterol (mmol/L)	Male	95	2.67	2.50	2.84	0.716
	Females	92	2.63	2.46	2.79	
Non-HDL-cholesterol (mmol/L)	Male	95	3.49	3.34	3.63	0.439
	Females	92	3.41	3.28	3.54	
Uric acid (umol/L)	Male	96	342.01	325.04	359.05	<0.001
	Females	92	276.12	260.05	292.19	
A1c (%)**	Male	95	5.38	5.18	5.58	<0.001
	Females	90	5.84	5.65	6.03	
ALT (IU/L)	Male	96	32.78	26.49	39.07	0.088
	Females	92	25.25	19.31	31.20	
UACR*** (mg/mmol)	Male	68	2.50	1.21	3.78	0.393
	Females	75	3.26	2.50	4.48	

*Independent sample t-test,**HbA1c (Glycated hemoglobin),***Urine Albumin Creatinine Ratio(UACR).

their anthropometric data, and sampled for 10 ml of blood in various phlebotomy tubes. Subjects who had pregnancy, acute infectious disease process, diabetes, IHD and inappropriate medical fasting were excluded from the study. A total sample considered after these exclusions was 188 subjects.

Total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were analyzed using CHOD-PAP, GPO-PAP, cholesterol esterase end-point methods on clinical chemistry analyzer (Selectra-ProM and AVIDA-1800). Serum TSH was measured using chemiluminescence's technique on Immulite® 1000. Total T4 and Total T3 were measured by competitive ELISA (Human). In case of a TSH result swaying away from 0.5-3.5 mIU/L, free T4 was also measured using chemiluminescence's technique on Immulite® 1000. HbA1c was measured by fast ion-exchange resin separation method, while UACR thru immune-turbidimetric method on ADVIA-1800 Chemistry System.

Based upon the TSH results subjects were classified as having euthyroidism (TSH: 0.5 to 4.0 mIU/L), subclinical hypothyroidism (TSH: 4.0 to 10 mIU/L) and overt hypothyroidism (TSH: ≥ 10.0 mIU/L).

Data analysis: All data were entered into SPSS version-15. Descriptive statistics in terms of mean ± SD were calculated for various parameters for gender differences. 163 subjects were diagnosed to have euthyroidism, while SCH and overt hypothyroidism were labeled in 16 and 9 subjects. Age, lipid indices, HbA1c, UACR and uric acid were compared between the 3 groups by univariate GLM model as the latter 2 groups had sample size <30.

RESULTS

There were 96 males and 92 females in our study. Differences between age, anthropometric indices and biochemical parameters between genders are shown in Table-I. The comparison between age, anthropometric indices, ALT, uric acid and glycated hemoglobin among euthyroid subjects, patients with SCH and overt hypothyroidism is depicted in Table-II. LDL-cholesterol and Non-HDL-cholesterol shows a progressive worsening from euthyroid subjects to subjects having overt hypothyroidism (Table-III). Pearson's correlation was found to be significant only for LDL-cholesterol and urine albumin creatinine ratio (Table-IV).

Table-II: Comparison of various biochemical and anthropometric indices among subjects having euthyroidism, SCH and overt hypothyroidism.

Dependent Variable	*Thyroid groups*	*N*	*Mean ± SD**	*Sig (p-value)*
Age (Years)	TSH 0.5 - 3.99 mIU/L	163	45.52 ± 11.50	0.410
	TSH 4.0 - 10.0 mIU/L	16	42.38 ± 9.16	
	TSH > 10.0 mIU/L	9	48.56 ± 15.72	
BMI	TSH 0.5 - 3.99 mIU/L	163	26.93 ± 5.31	0.070
	TSH 4.0 - 10.0 mIU/L	16	30.09 ± 5.22	
	TSH > 10.0 mIU/L	9	26.63 ± 3.72	
Waist to hip ratio (WHpR)	TSH 0.5 - 3.99 mIU/L	163	0.93 ± 0.092	0.619
	TSH 4.0 - 10.0 mIU/L	16	0.94 ± 0.044	
	TSH > 10.0 mIU/L	9	0.95 ± 0.050	
Waist to height ratio (WHtR)	TSH 0.5 - 3.99 mIU/L	163	0.57 ± 0.071	0.120
	TSH 4.0 - 10.0 mIU/L	16	0.61 ± 0.068	
	TSH > 10.0 mIU/L	9	0.58 ± 0.072	
ALT (IU/L)	TSH 0.5 - 3.99 mIU/L	163	29.61 ± 25.87	0.705
	TSH 4.0 - 10.0 mIU/L	16	24.19 ± 12.68	
	TSH > 10.0 mIU/L	9	30.00 ± 22.16	
UA (umol/L)	TSH 0.5 - 3.99 mIU/L	163	304.17 ± 80.08	0.456
	TSH 4.0 - 10.0 mIU/L	16	279.00 ± 72.03	
	TSH > 10.0 mIU/L	9	293.56 ± 61.94	
A1c (%)	TSH 0.5 - 3.99 mIU/L	160	5.73 ± 0.95	0.508
	TSH 4.0 - 10.0 mIU/L	16	5.31 ± 1.37	
	TSH > 10.0 mIU/L	9	5.93 ± 1.12	

*Mean + standard deviation as calculated by univariate general linear model, **HbA1c (Glycated hemoglobin).

Table-III: Comparison of various biochemical and anthropometric indices among subjects having euthyroidism, SCH and overt hypothyroidism.

Dependent Variable	*Thyroid groups*	*n*	*Mean ± SD*	*Sig (p-value)**
Total cholesterol (mmol/L)	TSH 0.5 - 3.99 mIU/L	162	4.47 ± 0.59	0.068
	TSH 4.0 - 10.0 mIU/L	16	4.36 ± 0.63	
	TSH > 10.0 mIU/L	9	4.90 ± 0.33	
Triglycerides (mmol/L)	TSH 0.5 - 3.99 mIU/L	162	1.57 ± 0.72	0.113
	TSH 4.0 - 10.0 mIU/L	16	1.36 + 0.46	
	TSH > 10.0 mIU/L	9	1.97 ± 0.58	
HDL-cholesterol (mmol/L)	TSH 0.5 - 3.99 mIU/L	162	0.98 ± 0.27	0.347
	TSH 4.0 - 10.0 mIU/L	16	1.02 ± 0.19	
	TSH > 10.0 mIU/L	9	0.86 ± 0.15	
LDL-cholesterol (mmol/L)	TSH 0.5 - 3.99 mIU/L	162	2.66 ± 0.73	0.063
	TSH 4.0 - 10.0 mIU/L	16	2.68 ± 0.51	
	TSH > 10.0 mIU/L	9	3.23 ± 0.59	
Non- HDL-cholesterol (mmol/L)	TSH 0.5 - 3.99 mIU/L	162	3.49 ± 0.64	0.033
	TSH 4.0 - 10.0 mIU/L	16	3.35 ± 0.59	
	TSH > 10.0 mIU/L	9	4.01 ± 0.30	

*Mean ± standard deviation as calculated by univariate general linear model.

UACR provided being having an overall significant model p-value depicted minimal change between euthyroid and SCH group (Fig.1).

DISCUSSION

Our study has shown a worsening pattern for lipid indices from euthyroidism to overt hypothyroidism. However, this pattern was not found to be more tangible in the SCH group except for LDL and non-HDL cholesterol. These results are consistent with the findings of Laway et al., Turham et al., Dunts et al. and the Tromso Study.[12-15] While the Tehran Lipid and Glucose Study (TLGS) and some other researchers did not find significant differences among subjects within SCH and control group.[16-19]

The first question from our data arises as to why the SCH group did not show marked changes in lipids as compared to the overt hypothyroid group. The first possibility could be the graded decline of primary thyroid functioning which at this stage of SCH may not be associated with metabolic complications. Secondly, considering primary thyroid abnormality to initiate metabolic derangements in the presence of normal pancreatic and hepatocyte function would allow compensatory mechanisms to reduce metabolic derangements at this stage. The evidence for this observation comes from the compensatory hyper-stimulation in insulin sensitivity which has been demonstrated by certain researchers.[20,21] This compensatory insulin release could in theory result in slight improvement in lipid parameters like triglycerides and HDL-cholesterol as we have observed in our work. But our study did find increase in LDL and non-HDL-cholesterol which may predict an early decompensation and possible risks for cardiovascular disease. Lastly, it must also be taken into consideration that TSH cut-offs as being employed to diagnose SCH

Table-IV: Person's correlation between TSH levels and lipid parameters, UACR, A1c and uric acid.

Parameter	*N*	*Pearson's correlation Coefficient (r)*	*Sig 2-tailed (p-value)*
Age (Years)	188	0.049	0.503
Total cholesterol (mmol/L)	187	0.137	0.062
Fasting triglycerides (mmol/L)	187	0.049	0.504
HDL-cholesterol (mmol/L)	187	-0.031	186
LDL-cholesterol (mmol/L)	187	0.220	0.003
Non- HDL-cholesterol (mmol/L)	187	0.134	0.066
Uric acid (umol/L)	188	-0.005	0.949
Urine albumin creatinine ration (mg/mol)	143	0.374	<0.001
A1c (%)	185	0.108	0.145
ALT (IU/L)	188	-0.003	0.972

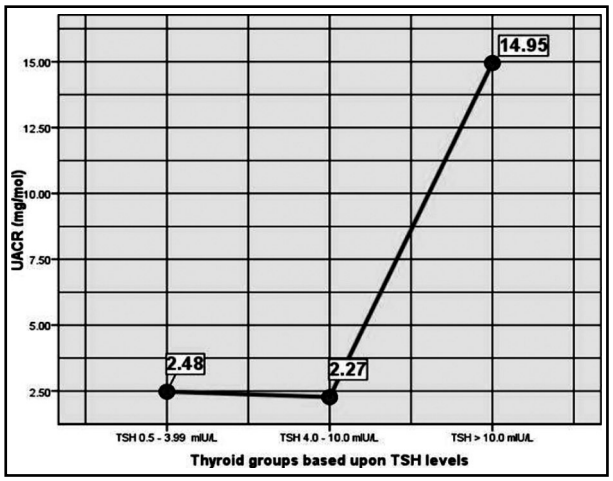

Fig.1: Univariate GLM model with thyroid groups as independent factor and Urine Albumin Creatinine Ratio (UACR) as dependent variable [Euthyroid: 2.48 (95% CI, 1.63-3.33], [SCH: 2.27(95% CI: 0.37-4.90] and [Overt hypothyroidism: 14.95 (95% CI: 10.71-19.14)], (p value<0.001).

have been varying across literature with studies using a higher TSH cut-offs like 15 mIU/L have demonstrated higher dyslipidemias.[14,22] Staub et al. have found worsening of various inflammatory markers including LDL-cholesterol in subjects with TSH greater than12 mIU/L.[23]

Limitations: Firstly, our study remains inclusive of sub-continental subjects who are otherwise considered to be more prone towards metabolic risk,[24] highlighting a multi-factorial risk assessment for CVD risk calculation. Secondly, we do acknowledge the possibility of Type-2 statistical error resulting from decreased sample size. The matter could not be addressed as the sampling was made randomly and multiple exclusions were made due to patients already suffering from IHD, hypertension, diabetes or other chronic disorder. Moreover, the study design being a cross-sectional one was not designed to establish a cause to effect ratio for which we recommend a more appropriate randomized controlled trial.

Clinically important: Our study is clinically important because it indirectly emphasized the importance of the subclinical hypothyroidism where every attempt should be made to find metabolic risks. Also the cross-sectional study provides new avenues for doing randomized controlled trials to further segregate multi-risk clustered Pakistani population considering worsening of thyroid function.

CONCLUSION

Lipid parameters were observed to get deteriorated from euthyroid subjects to patients having overt hypothyroidism. However, these changes were found to be more subtle in the subclinical hypothyroid group than cases with overt hypothyroidism. Out of all evaluated parameters LDL-cholesterol, non-HDL-cholesterol and urine albumin creatinine ratio demonstrated the most significant differences.

REFERENCES

1. Lee MS, Flammer AJ, Li J, Lennon RJ, Singh M, Holmes DR Jr, Rihal CS et al. Time-trend analysis on the Framingham risk score and prevalence of cardiovascular risk factors in patients undergoing percutaneous coronary intervention without prior history of coronary vascular disease over the last 17 years: a study from the Mayo Clinic PCI registry. Clin Cardiol. 2014;37(7):408-416. doi: 10.1002/clc.22274.
2. Zhang M, Sara JD, Matsuzawa Y, Gharib H, Bell MR, Gulati R, et al. Clinical outcomes of patients with hypothyroidism undergoing percutaneous coronary intervention. Eur Heart J. 2016;37(26):2055-2065. doi: 10.1093/eurheartj/ehv737.
3. Luca F, Goichot B, Brue T. Non thyroidal illnesses (NTIS). Ann Endocrinol (Paris). 2010;71(Suppl 1):S13-S24. doi: 10.1016/S0003-4266(10)70003-2.
4. Alkafajei A, Amarin Z, Alazaizeh W, Khader Y, Marji M. Prevalence and risk factors for hypothyroidism in Jordanian women: comparison between different reference ranges. East Mediterr Health J. 2012;18(2):132-136.
5. Mondal S, Raja K, Schweizer U, Mugesh G. Chemistry and Biology in the Biosynthesis and Action of Thyroid Hormones. Angew Chem Int Ed Engl. 2016;55(27):7606-7630. doi: 10.1002/anie.201601116.
6. Sgarbi JA, Matsumura LK, Kasamatsu TS, Ferreira SR, Maciel RM. Subclinical thyroid dysfunctions are independent risk factors for mortality in a 7.5-year follow-up: the Japanese-Brazilian thyroid study. Eur J Endocrinol. 2010;162(3):569-577. doi: 10.1530/EJE-09-0845.
7. Roos A, Zoet-Nugteren SK, Berghout A. Evaluation of cardiac ischaemia in cardiac asymptomatic newly diagnosed untreated patients with primary hypothyroidism. Neth J Med. 2005;63(3):97-102.
8. Balli M, Cetin M, Tasolar H, Uysal OK, Yilmaz M, Durukan M, et al. The relationship between serum thyroid hormone levels, subclinical hypothyroidism, and coronary collateral circulation in patients with stable coronary artery disease. Turk Kardiyol Dern Ars. 2016;44(2):130-136. doi: 10.5543/tkda.2015.00905.
9. Zha K, Zuo C, Wang A, Zhang B, Zhang Y, Wang B, et al. LDL in patients with subclinical hypothyroidism shows increased lipid peroxidation. Lipids Health Dis. 2015;14:95. doi: 10.1186/s12944-015-0092-4.
10. B UU, Mn S, Km S, Prashant A, Doddamani P, Sv S. Effect of insulin resistance in assessing the clinical outcome of clinical and subclinical hypothyroid patients. J Clin Diagn Res. 2015;9(2):OC01–OC04. doi: 10.7860/JCDR/2015/9754.5513.
11. Khazan M, Amouzegar A, Gharibzadeh S, Mehran L, Tohidi M, Azizi F. Prevalence of hypothyroidism in patients with dyslipidemia: Tehran Thyroid Study (TTS). Horm Metab Res. 2014;46(13):980-984. doi: 10.1055/s-0034-1389997.

12. Laway BA, War FA, Shah S, Misgar RA, Kumar Kotwal S. Alteration of lipid parameters in patients with subclinical hypothyroidism. Int J Endocrinol Metab. 2014;12(3):e17496. doi: 10.5812/ijem.17496.
13. Turhan S, Sezer S, Erden G, Guctekin A, Ucar F, Ginis Z, et al. Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism. Ann Saudi Med. 2008;28(2):96-101.
14. Duntas LH, Wartofsky L. Cardiovascular risk and subclinical hypothyroidism: focus on lipids and new emerging risk factors. What is the evidence? Thyroid. 2007;17(11):1075-1084. doi: 10.1089/thy.2007.0116.
15. 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 Tromso Study. J Intern Med. 2006;260(1):53-61. doi: 10.1111/j.1365-2796.2006.01652.x.
16. Alamdari S, Amouzegar A, Tohidi M, Gharibzadeh S, Kheirkhah P, Kheirkhah P, et al. Hypothyroidism and Lipid Levels in a Community Based Study (TTS). Int J Endocrinol Metab. 2015;14(1):e22827. doi: 10.5812/ijem.22827.
17. Vaya A, Gimenez C, Sarnago A, Alba A, Rubio O, Hernandez-Mijares A, et al. Subclinical hypothyroidism and cardiovascular risk. Clin Hemorheol Microcirc. 2014;58(1):1-7. doi: 10.3233/CH-141871.
18. Vierhapper H, Nardi A, Grosser P, Raber W, Gessl A. Low-density lipoprotein cholesterol in subclinical hypothyroidism. Thyroid. 2000;10(11):981-984. doi: 10.1089/thy.2000.10.981.
19. Hueston WJ, Pearson WS. Subclinical hypothyroidism and the risk of hypercholesterolemia. Ann Fam Med. 2004;2(4):351-355.
20. Ahmed OM, Gabar MA, Ali TM. Impacts of the coexistence of diabetes and hypothyroidism on body weight gain, leptin and various metabolic aspects in albino rats. J Diabetes Complications. 2012;26(6):491-500. doi: 10.1016/j.jdiacomp.2012.05.021.
21. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes. 2004;53(Suppl 3):S16-S21.
22. 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-BC06. doi: 10.7860/JCDR/2015/14640.6806.
23. Staub JJ, Althaus BU, Engler H, Ryff AS, Trabucco P, Marquardt K, et al. Spectrum of subclinical and overt hypothyroidism: effect on thyrotropin, prolactin, and thyroid reserve, and metabolic impact on peripheral target tissues. Am J Med. 1992;92(6):631-642.
24. Babusik P, Duris I. Comparison of obesity and its relationship to some metabolic risk factors of atherosclerosis in Arabs and South Asians in Kuwait. Med Princ Pract. 2010;19(4):275-280. doi: 10.1159/000312713.

Authors' Contribution:

SHK: Study design, data collection, analysis, results and discussion.
SMM, NF: Data collection, analysis.
NKN: Data collection, analysis, article design.
NA: Results, analysis and discussion.
AI: Study design, discussion.
SKH: takes the responsibility and is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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