

Role of Insulin, Homeostatic Model Assessment - Insulin Resistance with Lipid Profile Test to Evaluate Cardiovascular Disease Risk in Thyroid Disorder Patients

ABSTRACT

Aims: To find out role of insulin, homeostasis model assessment for insulin resistance (HOMA-IR) with lipid profile test in evaluation of cardiovascular disease risk in thyroid disorder patients.

Study design: an analytical case control study.

Place and Duration of study: Department of Biochemistry, School of Health and Allied Sciences, Pokhara University, between November 2013 and February 2014.

Methodology: The study encompassed of 90 subjects; 30 randomly selected healthy volunteers and 30 Hypothyroid and 30 Hyperthyroid subjects selected from different Hospital of Pokhara. The participants were identified as Hypothyroid and Hyperthyroid relying in serum FT3, FT4 and TSH levels, clinical history and medication. The participants were asked to fill the questionnaire, 5 ml of the venous blood was collected the measurement of thyroid hormones (FT3, FT4 and TSH), serum lipid profile (TC, TG, HDL, LDL, VLDL) and serum Insulin.

Results: The mean value of FT3, FT4, TSH of control subjects were 2.5 ± 0.63 (pg/ml), 1.39 ± 0.28 (ng/dl), 2.82 ± 1.75 (umol/ml), hyperthyroid subjects were 2.18 ± 0.88 (pg/ml), 0.96 ± 0.32 (ng/dl), 24.28 ± 19.8 (umol/ml) and hypothyroid subjects were 4.52 ± 1.72 (pg/ml), 3.36 ± 3.61 (ng/dl), 0.156 ± 0.14 (umol/ml) respectively. The mean value of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein LDL cholesterol and very low density lipoprotein (VLDL) were greatly increased in hypothyroid subjects 240 (mg/dl), 194.7(mg/dl), 49.63, 151.51 and 38.78 (mg/dl) respectively as compared to control 166.59 (mg/dl), 151.07 (mg/dl), 50.23, 86.05 and 30.21(mg/dl) respectively and hyperthyroid subjects 62.13 (mg/dl), 131.61(mg/dl), 45.07, 90.75 and 26.32 (mg/dl) respectively. An increase in serum insulin level is in both hypothyroid 32.78 (uIU/mL) and hyperthyroid 30.08 (uIU/mL). HOMA-IR was significantly associated with TC, TG and LDL. Spearman's correlation analysis of insulin with TC, LDL Cholesterol, TG and VLDL that of HOMA-IR with TC and LDL while insulin with TC /HDL ratio, LDL Cholesterol and TC also HOMA-IR with TC /HDL and LDL Cholesterol shows significant correlation.

Conclusion: Thyroid dysfunction has a great impact on lipid profile as well as a number of other cardiovascular disease (CVD) risk factors. There is a close relation between IR and thyroid hormones. The insulin resistance is present in hypo and hyperthyroid patients and thyroid dysfunctions induce significant changes in lipid profile. Therefore estimation of traditional lipid profile along with some additional parameters like serum insulin, IR helps to assess the thyroid status. Lipid profile test along with insulin and HOMA-IR can also help in the early evaluation of possible risk of CVD in thyroid disorder patients.

Keywords: Thyroid Diseases, Cardiovascular Disease, Homeostasis Model Assessment- Insulin Resistance, Lipid Profile

1. INTRODUCTION

Thyroid hormones have many important biological effects; major function is to control of basal metabolic rate and calorogenesis through increased oxygen consumption in tissue via the effects on membrane transport (cycling of Na⁺/K⁺-ATPase with increased synthesis and consumption of ATP) and enhanced mitochondrial metabolism (stimulation of mitochondrial respiration and oxidative phosphorylation) [1]. Hyperthyroidism is defined as hyper metabolic condition caused by excessive production of thyroid hormones, clinical symptoms include weight loss, rapid or irregular heartbeat, anxiety, irritability, trouble sleeping, trembling in the hands and fingers, increased sweating, increased sensitivity to heat, muscle weakness diagnostic features are increased serum FT3 and FT4 with reduced value of serum TSH while Hypothyroidism is defined as a deficiency in thyroid hormone secretion and function, clinical symptoms ranges from weight gain, increased sensitivity to cold, muscle weakness, joint or muscle pain, depression, fatigue, pale dry skin, puffy face, hoarse voice and diagnostic features are reduced serum FT3 and FT4 with increased serum TSH. Subclinical Hypothyroidism is a milder form characterized by an elevated serum TSH (5 mIU/L-10mIU/L) level but normal serum FT3 without signs and symptoms. Subclinical Hypothyroidism will develop into overt Hypothyroidism. Overt primary hypothyroidism is a condition characterized by an elevated TSH greater than 5mIU/L and appropriate symptoms or TSH of greater than 10mIU/L and reduced serum T4 and T3 levels [2]. Both hypothyroidism and hyperthyroidism have potentially fatal systemic manifestations [3].

Insulin is a 51 amino acids long peptide hormone with molecular weight of 5808 daltons, produced by beta cells of the pancreas and is central to regulating carbohydrate metabolism in body [4]. The homeostasis model assessment for insulin resistance (HOMA-IR) derives estimates of insulin sensitivity from the mathematical modeling of fasting plasma glucose (FBS) and insulin or C-peptide concentrations for assessing β -cell function and insulin resistance [5].

The prevalence of CVD risk in patients with thyroid disorder is significantly higher than that in the general population and indicates a possible interplay between thyroid status and insulin sensitivity [6]. It is estimated world widely more than 200 million people develop thyroid disease and found prevalence in Nepal is 25%. Higher prevalence is observed in subjects with age above 30 years [7]. Females have more thyroid dysfunction than males [8]. Hypothyroidism (8%) and subclinical hypothyroidism (8%) have higher prevalence compared to subclinical hyperthyroidism (6%) and hyperthyroidism (3%) [9]. In our study, it has been hypothesized that thyroid hormone has great impact on lipid metabolism which eventually lead to insulin resistance and CVD in thyroid disorder patients and the aim of our study to find out role of insulin, HOMA-IR with lipid profile test in evaluation of CVD risk in thyroid disorder patients.

2. MATERIAL AND METHODS

Study design: The study population included native residents of Pokhara valley and surrounding areas and those who have migrated from other parts of Nepal. The study was an analytical case control study conducted in School of Health and Allied science, Pokhara University, Nepal from November 2013 to February 2014. The study encompassed of 90 subjects; 30 randomly selected healthy volunteers and 30 Hypothyroid and 30 Hyperthyroid subjects selected from Western Regional Hospital, Pokhara, Diabetic and Endocrinology center, Pokhara, National Reference Laboratory, Pokhara, United Reference Laboratory, Pokhara and Charak Memorial Hospital, Pokhara.

Selection criteria: Thyroid dysfunction is diagnosed by thyroid function tests. Clinical manifestations are often insidious and vary considerably among patients. Routine laboratory screening is considered more significant in establishing thyroid disease. Those individuals with abnormal serum FT3, FT4, TSH and classical symptoms were selected while individuals, taking medication of thyroid disorder, diabetes mellitus, hypertension hyperlipidemia and pregnancy were excluded from the study. Guideline for the diagnosis of thyroid disease was developed for the selection of patients: TSH measurement is the most reliable test to screen for and to diagnose of hypothyroidism, hyperthyroidism and euthyroidism.

- Primary hypothyroidism: subclinical hypothyroidism (FT4) and clinical hypothyroidism (decreased FT4), TSH will be elevated.
- Secondary hypothyroidism: TSH is usually normal, but may be low or even mildly elevated; FT4 must be performed along with TSH.
- Autoimmune mechanism (e.g. Hashimoto thyroiditis): thyroid peroxidase antibodies (TPOAb) test will be performed
- Hyperthyroidism: TSH will be decreased
 - i. if TSH less than 0.1 mU/L, FT4 will be measured - indicated to severity of hyperthyroidism
 - ii. If TSH decreased but FT4 is normal, FT3 will be measured - indicated to out toxicosis.
- FT3 toxicosis (e.g. Graves' disease): TSH receptor antibodies (TRAb) test will be performed

The clinical examination consisted of a personal interview. Recruitment of participants for the investigation was performed by principal investigators.

Collection: The overnight fasted subjects were asked to fill the questionnaire, 5 ml of the venous blood was collected in EDTA vacutainer then, samples were centrifuged at 1500 g for 10 min .The serum was preserved in deep freeze (-44°C) for the measurement of thyroid hormones (FT3, FT4 and TSH) , serum lipid profile(TC,TG,HDL,LDL,VLDL) and serum Insulin.

Ethical Clearance: The research is carried out accommodating all principles of Helsinki declaration, verbal and written consent was taken to individual subject during sample collection and ethical clearance was given by institutional research board, School of Health and Allied Sciences, Pokhara University.

Measurement of serum FT3/ FT4: The FT3 and FT4 measurement use a competitive ELISA method. Competitive ELISA is based on the principle of competitive binding between FT3 / FT4 in a test specimen and T3/T4-peroxidase conjugate for a limited number of binding sites on the anti- T3/T4 (sheep) coated well. Thus the amount of T3/T4- peroxidase conjugate bound to the well is inversely proportional to the concentration of FT3/ FT4 in the specimen. After incubation of specimen and T3/T4 -peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/substrate solution is added and a blue color develops. The intensity of this color, which changes to yellow after stopping reaction, is inversely proportional to the amount of FT3/ FT4 in the specimen.

Calibrators, specimens and controls were recorded carefully on the spread sheet supplied with kit. The required numbers of micro-titer strips were selected and placed firmly in the holder. 50 µl of calibrators, specimens and controls were pipette in the micro-titer wells. Then 100 µl of enzyme conjugate was added in those micro-wells. Micro-titer wells were rocked gently and covered with adhesive tape. Incubation was done for 60 minutes at 20-25 °C and washed for 3 times. 100 µl of substrate was added in each well and incubated for 15

112 minutes at 20-25 °C. 50 µl of stop solution was added to each micro-titer wells and was
113 mixed carefully. Then the absorbance was measured at 450 nm using microplate reader.

114 **Measurement of serum TSH/Insulin:** The TSH and Insulin measurement use a
115 sandwich ELISA method. Sandwich ELISA makes use of highly specific monoclonal anti-
116 TSH/Insulin antibody coated on the surface of the micro-titer wells. In the first incubation
117 step, specimens, calibrators or controls and enzyme conjugated with biotin are mixed to form
118 the sandwich complex which is bound to the surface of the wells by the interaction with
119 immobilized antibody. At the end of the incubation excess enzyme conjugate is washed out.
120 In second incubation step, streptavidin peroxidase enzyme complex binds to biotin anti-
121 TSH/Insulin antibody. Substrate reagent added and color developed after adding stop
122 solution. The intensity of color is directly proportional to the TSH/Insulin concentration in the
123 sample.

124 Calibrators, specimens and controls were recorded carefully on the spread sheet supplied
125 with kit. The required numbers of micro-titer strips were selected and placed firmly in the
126 holder. 25 µl of calibrators, specimens and controls were pipette in the micro-titer wells.
127 Then 25µl of enzyme conjugate was added in those micro-wells. Micro-titer wells were
128 rocked gently and covered with adhesive tape. Incubation was done for 30 minutes at 20-25
129 °C and washed for 3 times with washing solution. 50 µl of enzyme complex was added in
130 each well and incubated at room temperature (RT) for 30 minutes. 50 µl of substrate was
131 added in each well and incubated for 15 minutes at RT. 50 µl of stop solution was added to
132 each micro-titer wells and was mixed carefully. Then the absorbance was measured at 450
133 nm with micro-titer plate reader.

134 **Measurement of serum Triglycerides (TG):** The triglyceride is determined after
135 enzymatic hydrolysis with lipases .The indicator is quinoneimine formed from hydrogen
136 peroxide, 4-amino-antipyrine and 4-cholorophenol under the catalytic influence of
137 peroxidase. The intensity of color produced is proportional to the concentration of triglyceride
138 in the serum.

139 1000ul of working reagent and 10ul of serum was mixed and incubated for 20 min at 37 °C.
140 Absorbance of the sample and standard were measured against the reagent blank at 546nm
141 (520-580nm). The lipid activity level >200 mg/dl was considered abnormal or elevated.

142 **Measurement of serum total cholesterol:** The cholesterol is determined after
143 enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen
144 peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The intensity of
145 color produced is proportional to the concentration of cholesterol in the serum.

146 1000ul of working reagent and 10ul of serum was mixed and incubated for 20 min at 37 °C.
147 Absorbance of the sample and standard were measured against the reagent blank at 546nm
148 (520-580nm). The lipid activity level >250 mg/dl was considered abnormal or elevated.

149 **Measurement of HDL cholesterol:** The chylomicrons, VLDL (very low density
150 lipoproteins) and LDL (low density lipoproteins) are precipitated by the addition of
151 phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid
152 contains the HDL (high density lipoproteins) fractions, which is assayed for HDL cholesterol
153 with the cholesterol test kit.

154 1000 µl of HDL precipitating reagent and 500 µl serum was mixed and incubated for 5
155 minutes and centrifuged for 20 minutes. 1000ul of working cholesterol reagent and 100ul of
156 supernatant was mixed and incubated for 20 min at 37 °C. Absorbance of the sample and

standard were measured against the reagent blank at 546nm (520-580nm). The lipid activity level <20 mg/dl was considered abnormal.

Measurement of LDL and VLDL: Serum LDL and VLDL level is calculated by using Frederickson's formula
VLDL=Triglyceride/5
LDL=Total Cholesterol –HDL-VLDL

Measurement of serum glucose: GOD-POD colorimetric test based on principle where glucose is oxidized to gluconic acid and hydrogen peroxide, so formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator, the absorbance of this complex is proportional to the concentration of glucose in the test.

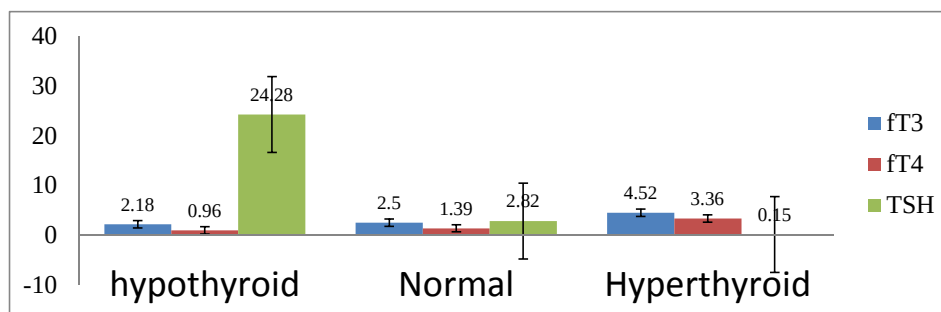
1000ul of working reagent and 10ul of serum was mixed and incubated for 5 min at 37 °C. Absorbance of the sample and standard were measured against the reagent blank at 546nm (520-580nm).

Calculation of HOMA-IR: $HOMA-IR = \text{Glucose} \times \text{Insulin} / 405$

Statistical analysis: All the data were entered into Excel 2007 and analyzed using SPSS for window version 16.0. Data were presented as mean $\pm$ SD. Spearman's correlation coefficient were determined between controls, hypothyroid and hyperthyroid cases. A p-value of <0.05 was used to establish statistical significance and p-value of <0.01. The results were expressed in the forms of tables and bar diagrams

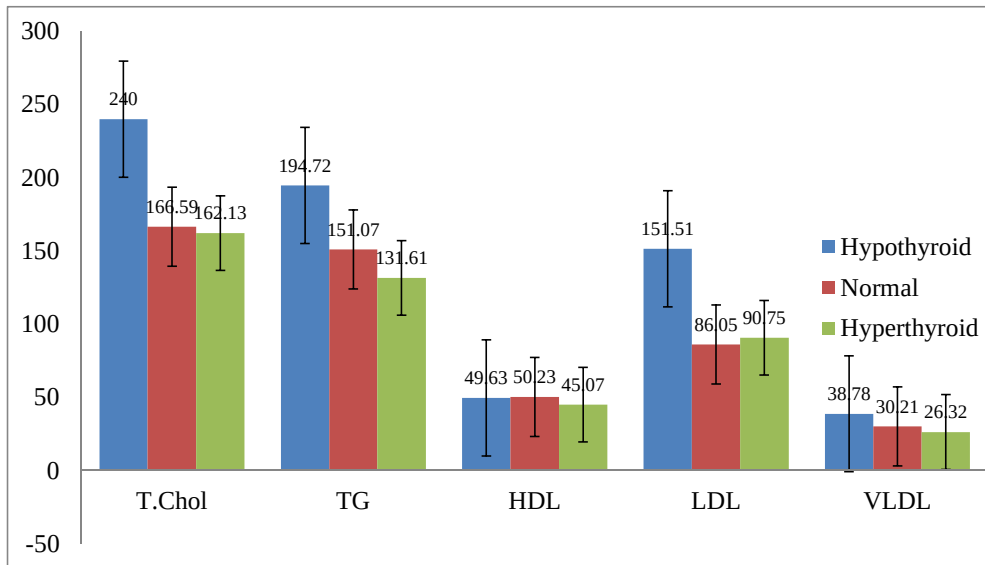
3. RESULTS AND DISCUSSION

The results of the study are presented in figure 1 to 4



Values are given as mean value, mean difference is significant ($p < 0.05$), mean difference is highly significant ($p < 0.0001$). FT3: free triiodothyronine (pg/ml), FT4 : free iodothyroxine (ng/dl) ; TSH : Thyroid Stimulating Hormone (umol/ml)

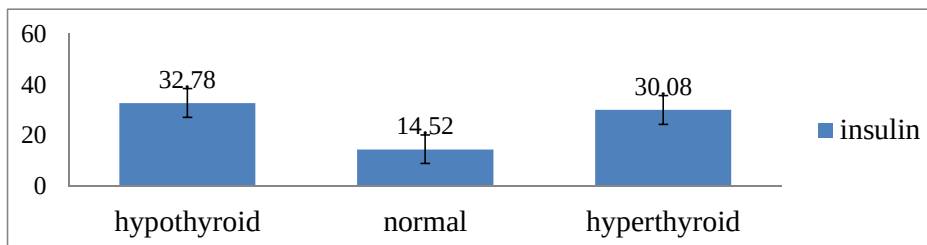
Fig 1: Thyroid profile of hypothyroid, normal and hyperthyroid subjects



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Values are given as mean value, mean difference is significant ($p < 0.05$), mean difference is highly significant ($p < 0.0001$); T.Chol : total cholesterol (mg/dl); TG: triglyceride (mg/dl); HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein

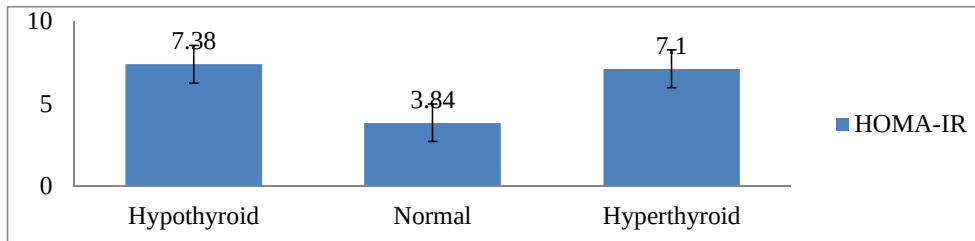
Fig 2: Lipid profile level in hypothyroid, normal and hyperthyroid subjects



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Values are given as mean value, mean difference is significant ($p < 0.05$), mean difference is highly significant ($p < 0.0001$); Insulin (uIU/mL)

Fig 3: Insulin level in hypothyroid, normal and hyperthyroid subjects



Values are given as mean value, mean difference is significant ($p < 0.05$), mean difference is highly significant ($p < 0.0001$)

Fig 4: HOMA –IR in hypothyroid, normal and hyperthyroid subjects

Out of 90 subjects, there were 26 (28.9%) males and 64 females (71.1%) with mean age 47.23 ± 12.86 and 46.06 ± 15.67 respectively. The mean value of FT3, FT4, TSH of control subjects were 2.5 ± 0.63 , 1.39 ± 0.28 , 2.82 ± 1.75 , hypothyroid subjects were (2.18 ± 0.88), (0.96 ± 0.32), (24.28 ± 19.8) and hypothyroid subjects were FT3 (4.52 ± 1.72), FT4 (3.36 ± 3.61), (0.156 ± 0.14) respectively. In hypothyroid subjects the mean FT3 and FT4 were lower in comparison to controls whereas mean TSH was markedly high. In hyperthyroid cases the mean FT3 and FT4 were higher compared to controls and hypothyroid cases. The serum level of TC, TG and LDL cholesterol were greatly increased in hypothyroid subjects as compared to control and hyperthyroid subjects, Similar finding were also observed in 2010 Regmi .A, et al, Kathmandu [10]. Thyroid hormones stimulate the utilization of the lipid substrates. Hyperthyroidism stimulates proliferation of LDL receptors and receptor activity which causes to increase metabolism of cholesterol molecule hence it lowers the serum cholesterol level [11], it also stimulates in the increase in mobilization of the TG and up-regulation of apo-lipoprotein AV (which plays a major role in triglycerides regulation) hence lowers the TG serum level [12] while in Hypothyroidism there is less stimulation of cholesterol receptor in cell which lead to increased TC in serum. It causes fall in HDL and rise in serum LDL. The level of TG significantly increases due to the decreased activity of LPL, which results in a decreased clearance of triglyceride-rich lipoproteins [13]. Purvi Purohit et al in 2012 and B.M.Singh et al in 2010, shows a hyperinsulinemia of the hypothyroid patient was associated with dyslipidemia characterized by raised TC, TG and cholesterol rich lipoproteins.[14, 15]. Our study shows an increase in serum insulin level is in both hypothyroid and hyperthyroid subjects as compare to control. Kapadia B.K,et al in 2013, shows HOMA-IR was significantly associated with TC, TG and LDL [16], consisting with our result where HOMA-IR is increase in both hypothyroid and hyperthyroid subjects compare to control. Hypothyroidism is greatly associated with obesity which in fact causes insulin resistance and then increased serum insulin level. Thyroid dysfunction leads to alterations in glucose and lipid metabolism which is an important risk factor for CVD. The dyslipidemia and insulin resistance should be managed aggressively to reduce the impending risk.

Spearman's correlation analysis of insulin with TC and LDL Cholesterol shows highly significant, TG and VLDL shows significant correlation, HDL cholesterol shows negative correction and TC /HDL shows non-significant

Table 1: Correlation of insulin with lipid profile and lipid ratio in Hypothyroid patients

	r	P -value	Significance
Total Cholesterol	0.606**	0.000	Highly significant

Triglyceride	0.424*	0.020	Significant
HDL Cholesterol	-0.007	0.971	Negative correlation
LDL Cholesterol	0.546**	0.002	Highly significant
VLDL Cholesterol	0.424*	0.020	Significant
T. Cholesterol /HDL	0.248	0.186	Non significant

237 *.Correlation is significant at the 0.05 level.

238 Spearman's correlation analysis of HOMA-IR IR with TC and LDL shows highly significant,
239 HDL cholesterol shows negative correction and TC /HDL ratio and TG shows non-significant

240 **Table 2: Correlation of HOMA-IR with lipid profile and ratio in Hypothyroid patients**

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	r	P-value	Significance
Total Cholesterol	0.602**	0.000	Highly significant
Triglyceride	0.269	0.150	Non significant
HDL Cholesterol	-0.004	0.985	Negative correlation
LDL Cholesterol	0.578**	0.001	Highly significant
VLDL Cholesterol	0.269	0.150	Non significant
T. Cholesterol /HDL	0.299	0.108	Non significant

242 **.Correlation is significant at the 0.05 level

243 Spearman's correlation analysis of insulin with TC /HDL and LDL Cholesterol and shows
244 highly significant, TC shows significant correlation, HDL cholesterol shows negative
245 correction and TG and VLDL shows non-significant correlation.

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247 **Table 3: Correlation of Insulin with lipid profile and ratio in hyperthyroid patients**

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	r	P-value	Significance
Total Cholesterol	0.437*	0.016	Significant
Triglyceride	0.30	0.876	Non significant
HDL Cholesterol	-0.109	0.565	Negative correlation
LDL Cholesterol	0.530**	0.003	Highly significant
VLDL Cholesterol	0.030	0.876	Non significant
T. Cholesterol /HDL	0.493**	0.006	Highly significant

249 *.Correlation is significant at the 0.05 level.

**.Correlation is significant at the 0.01 level.

250 Spearman's correlation analysis of HOMA-IR with TC /HDL and LDL Cholesterol shows
251 significant correlation, HDL cholesterol shows negative correction and TC, TG and VLDL
252 shows non-significant

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254 **Table 4 Correlation of HOMA-IR with lipid profile and ratio in hyperthyroid patients**

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	r	P -value	Statistical significance
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Total Cholesterol	0.334	0.071	Non significant
Triglyceride	0.127	0.503	Non significant
HDL Cholesterol	-0.114	0.550	Negative correlation
LDL Cholesterol	0.437*	0.016	Significant
VLDL Cholesterol	0.127	0.503	Non significant
T. Cholesterol /HDL	0.436*	0.016	Significant

*.Correlation is significant at the 0.05 level. **.Correlation is significant at the 0.01 level.

4. CONCLUSION

The study was a prospective cohort cross sectional study carried out in the community population of Pokhara Valley and its surrounding. Since, there have been no studies in the role of insulin, HOMA-IR and lipid profile test in cardiovascular disease and thyroid disorder in our study area before; we found it likely that our population in this respect represents that of Nepal. Our study clearly revealed that thyroid dysfunction has a great impact on lipid profile as well as a number of other cardiovascular risk factors. A close relation was found between IR and thyroid hormones were observed. This study shows that there is insulin resistance in both hypo and hyperthyroid patients. And thyroid dysfunctions induce significant changes in lipid profile. Thus, we concluded that the estimation of traditional lipid profile along with some additional parameters like serum insulin, IR help assess the thyroid status. Lipid profile test along with insulin and HOMA-IR can also help in the early evaluation of possible risk of CVD in thyroid disorder patients.

5. LIMITATION OF STUDY

Our study involves small sample size with short study duration. Chemiluminescence method is considered most specific and sensitive method for the estimation of thyroid function and insulin, role of Apo A, Apo B and C-peptide has significant role in this study and thyroid peroxidase antibodies (TPOAb) test for detection of autoimmune mechanism (e.g. Hashimoto thyroiditis) and TSH receptor antibodies (TRAb) test for detection of FT3 toxicosis (e.g. Graves' disease) were imperative for this study but due to economical limitation we were unable to estimate

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