

Original Research Article

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 science, 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 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.

Results: 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 hyperthyroid subjects were fT3 (4.52 ± 1.72) , fT4 (3.36 ± 3.61), (0.156 ± 0.14) respectively. The serum level of total cholesterol (TC), triglyceride (TG) and low density lipoprotein LDL cholesterol were greatly increased in hypothyroid subjects as compared to control and hyperthyroid subjects. An increase in serum insulin level is in both hypothyroid and hyperthyroid. 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 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.

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 calorigenesis 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 peoples develops 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

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. The participants were identified as Hypothyroid and Hyperthyroid and healthy controls relying in serum fT3, fT4 and TSH levels, clinical history and medication. The clinical examination consisted of a personal interview. Recruitment of participants for the investigation was performed by principal investigators. 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. The participants 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.

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

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

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. 25 µl of calibrators, specimens and controls were pipette in the micro-titer wells. Then 25µl of enzyme conjugate was added in those micro-wells. Micro-titer wells were rocked gently and covered with adhesive tape. Incubation was done for 30 minutes at 20-25 °C and washed for 3 times with washing solution.50 µl of enzyme complex was added in each well and incubated at room temperature (RT) for 30 minutes. 50 µl of substrate was added in each well and incubated for 15 minutes at RT. 50 µl of stop solution was added to each micro-titer wells and was mixed carefully. Then the absorbance was measured at 450 nm with micro-titer plate reader.

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

115 1000ul of working reagent and 10ul of serum was mixed and incubated for 20 min at 37 °C.
116 Absorbance of the sample and standard were measured against the reagent blank at 546nm
117 (520-580nm). The lipid activity level >200 mg/dl was considered abnormal or elevated.

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

122 1000ul of working reagent and 10ul of serum was mixed and incubated for 20 min at 37 °C.
123 Absorbance of the sample and standard were measured against the reagent blank at 546nm
124 (520-580nm). The lipid activity level >250 mg/dl was considered abnormal or elevated.

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

130 1000 µl of HDL precipitating reagent and 500 µl serum was mixed and incubated for 5
131 minutes and centrifuged for 20 minutes. 1000ul of working cholesterol reagent and 100ul of
132 supernatant was mixed and incubated for 20 min at 37 °C. Absorbance of the sample and
133 standard were measured against the reagent blank at 546nm (520-580nm). The lipid activity
134 level <20 mg/dl was considered abnormal.

135 **Measurement of LDL and VLDL:** Serum LDL and VLDL level is calculated by using
136 Frederickson's formula
137 $VLDL = \text{Triglyceride} / 5$
138 $LDL = \text{Total Cholesterol} - HDL - VLDL$

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

144 1000ul of working reagent and 10ul of serum was mixed and incubated for 5 min at 37 °C.
145 Absorbance of the sample and standard were measured against the reagent blank at 546nm
146 (520-580nm).

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

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

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

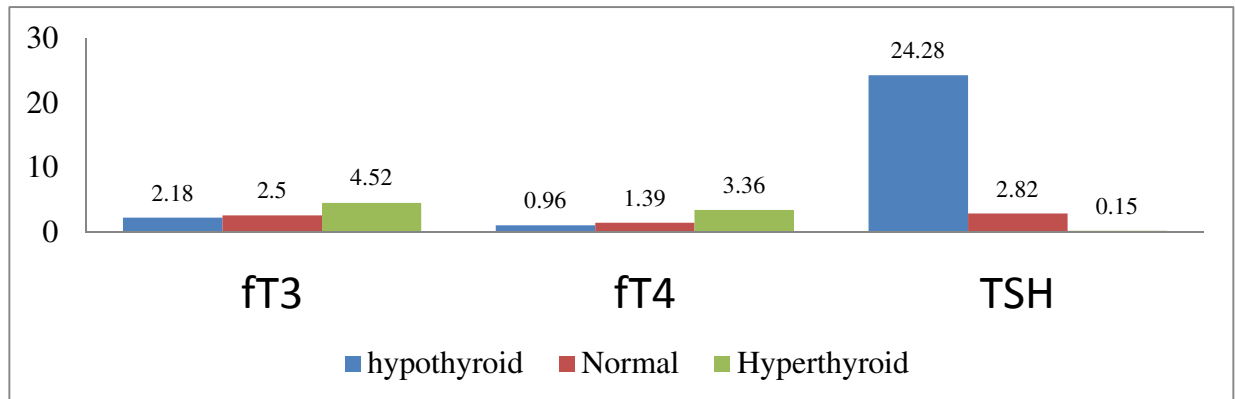


Fig 1: Thyroid profile of hypothyroid, normal and hyperthyroid subjects

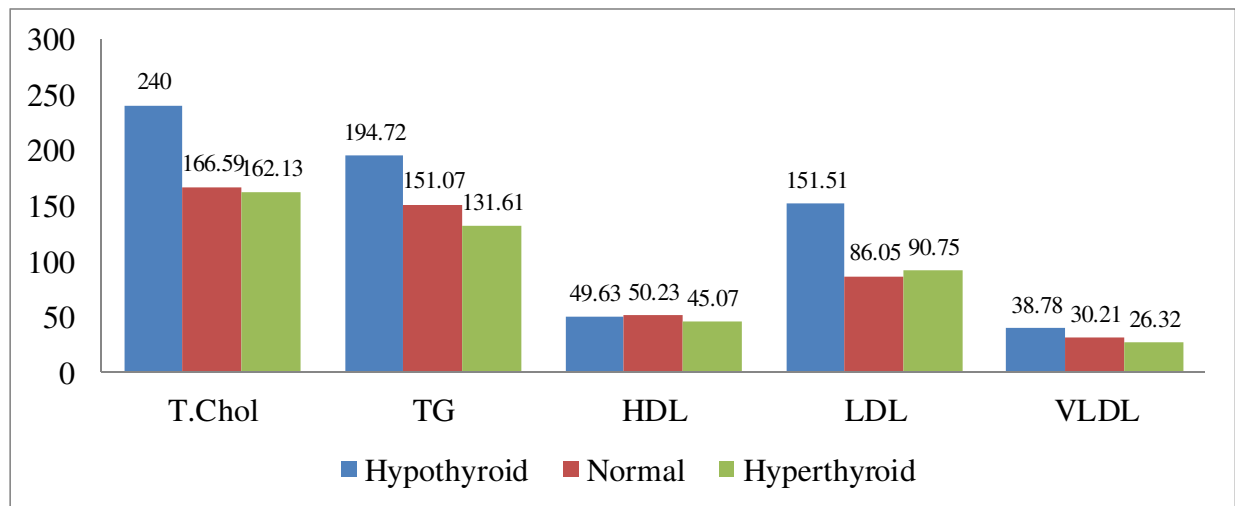


Fig 2: Lipid profile level in hypothyroid, normal and hyperthyroid subjects

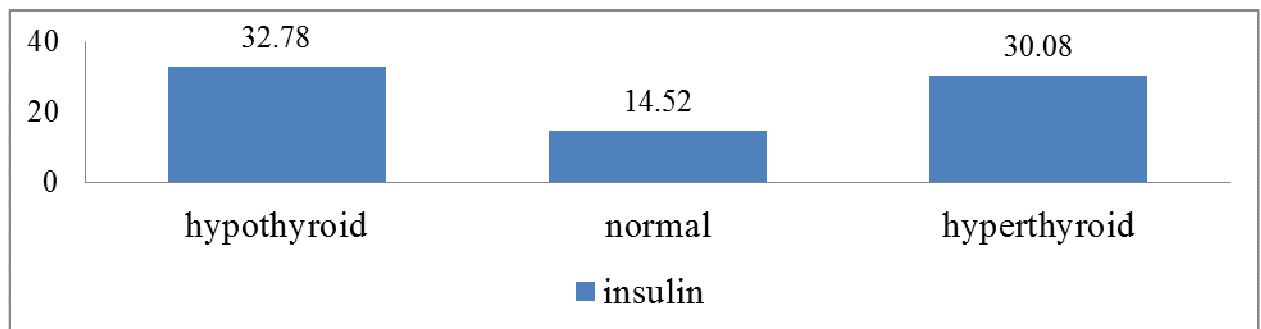
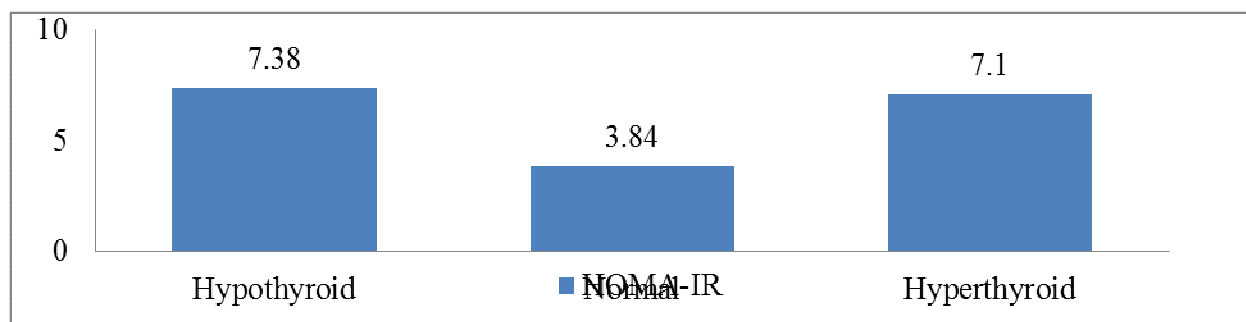


Fig 3: Insulin level in hypothyroid, normal and hyperthyroid subjects

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Fig 3: HOMA –IR in hypothyroid, normal and hyperthyroid subjects

173 The mean value of fT3, fT4 , TSH of control subjects were 2.5 ± 0.63 , 1.39 ± 0.28 , 2.82 ± 1.75 ,
174 hypothyroid subjects were (2.18 ± 0.88), (0.96 ± 0.32), (24.28 ± 19.8) and hypothyroid subjects
175 were fT3 (4.52 ± 1.72) , fT4 (3.36 ± 3.61), (0.156 ± 0.14) respectively. . In hypothyroid subjects
176 the mean fT3 and fT4 were lower in comparison to controls whereas mean TSH was
177 markedly high. In hyperthyroid cases the mean fT3 and fT4 were higher compared to
178 controls and hypothyroid cases. The serum level of TC, TG and LDL cholesterol were greatly
179 increased in hypothyroid subjects as compared to control and hyperthyroid subjects, similar
180 finding were also observed in 2010 Regmi .A, et all, Kathmandu [10]. Thyroid hormones
181 stimulate the utilization of the lipid substrates. Hyperthyroidism stimulates proliferation of
182 LDL receptors and receptor activity which causes increase metabolism of cholesterol
183 molecule hence it lowers the serum cholesterol level [11], it also stimulates in the increase
184 in mobilization of the TG and up-regulation of apo-lipoprotein AV (which plays a major role
185 in triglycerides regulation) hence lowers the TG serum level [12] while in Hypothyroidism
186 there is less stimulation of cholesterol receptor in cell which lead to increased TC in serum.
187 It causes decreased in HDL and increased serum LDL. The level of TG significantly
188 increases due to the decreased activity of LPL, which results in a decreased clearance of
189 triglyceride-rich lipoproteins [13]. Purvi Purohit et all in 2012 and B.M.Singh et all in 2010,
190 shows a hyperinsulenemia of the hypothyroid patient was associated with dyslipidemia
191 characterized by raised TC, TG and cholesterol rich lipoproteins.[14, 15]. Our study shows
192 an increase in serum insulin level is in both hypothyroid and hyperthyroid subjects as
193 compare to control. Kapadia B.K,et al in 2013, shows HOMA-IR was significantly associated
194 with TC, TG and LDL [16], consisting with our result where HOMA-IR is increase in both
195 hypothyroid and hyperthyroid subjects as compare to control. Hypothyroidism is greatly
196 associated with obesity which in fact causes insulin resistance and then increased serum
197 insulin level. Thyroid dysfunction leads to alterations in glucose and lipid metabolism which
198 is an important risk factor for CVD. The dyslipidemia and insulin resistance should be
199 managed aggressively to reduce the impending risk.

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

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

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	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

212 *.Correlation is significant at the 0.05 level.

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

215 **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

217 **.Correlation is significant at the 0.05 level

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

221

222 **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

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

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

228

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

230

	r	P-value	Statistical significance
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

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

233 **4. CONCLUSION**

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

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