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Predicting hyperlipidemia and hypothyroid severity using inflammation and oxidative stress markers in a prospective study

Mrs. Sarita Devi[1], Dr. Divya Prakash[2]

Abstract

The aim of this research was to explore the impact of dysthyroidism on lipid metabolism by examining lipid profile parameters in individuals diagnosed with dysthyroidism. A case-control study was conducted to compare the lipid profiles of patients with dysthyroidism to those without it. The study used convenient sampling to select newly diagnosed dysthyroidism cases from two diagnostic centers: Sarita Diagnostic Centre in Shamli and Dr. Lal Pathlabs in Delhi. A total of 569 hyperlipidemia cases and 454 dysthyroidism cases were included in the analysis.

Blood samples were collected from all participants to assess various lipid profile indicators such as total serum cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). The goal was to examine any correlations between abnormal lipid levels and thyroid dysfunction.

Additionally, to investigate potential cardiovascular implications, participants underwent 2D echocardiography to evaluate the risk of coronary heart disease (CHD) in relation to dysthyroidism. This method offered valuable insights into the connection between thyroid disorders, lipid metabolism, and heart health.

The findings of this study could contribute significantly to understanding how thyroid dysfunction might affect lipid levels and its potential association with cardiovascular diseases.

Key words: Dysthyroidim, hyperlipidemia, cardiovascular disease, metabolic syndrome

1. Introduction

Hypothyroidism is a common endocrine disorder with significant health consequences [1]. Beyond its primary impact on metabolism, it has been increasingly associated with an elevated risk of cardiovascular disease [2]. One of the key factors linking hypothyroidism to cardiovascular risk is the presence of dyslipidemia, which is marked by abnormal lipid levels—specifically, increased triglycerides and low-density lipoprotein (LDL) cholesterol, along with reduced high-density lipoprotein (HDL) cholesterol[3]. The development of dyslipidemia in hypothyroid patients is multifactorial, with thyroid hormone deficiency being the primary cause[4]. However, recent studies have highlighted the potential role of chronic inflammation and oxidative stress in exacerbating these lipid abnormalities[5].

Inflammation is frequently observed in individuals with hypothyroidism, often reflected by elevated levels of inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6 .[6] This persistent, low-grade inflammation can interfere with lipid metabolism, facilitate the formation of atherosclerotic plaques, and heighten the risk of cardiovascular incidents[7]. Additionally, oxidative stress—characterized by an imbalance between reactive oxygen species (ROS) production and

antioxidant defenses—can further aggravate lipid peroxidation, impair endothelial function, and contribute to dyslipidemia[8].

The objective of this study is to assess whether markers of inflammation and oxidative stress can serve as predictive indicators for the development of hyperlipidemia and the severity of hypothyroidism in newly diagnosed individuals[9]. By identifying patients who are more susceptible to these complications, this research seeks to support early detection efforts and guide timely interventions, ultimately aiming to improve cardiovascular outcomes in those affected by dysthyroidism[10].

2. Materials & Method :

- Methodology
- Study Participants
- Inclusion Criteria: This study will include individuals who have been newly diagnosed with primary dysthyroidism.
- Exclusion Criteria: Individuals with secondary or tertiary dysthyroidism, pre-existing cardiovascular disease, active malignancy, pregnancy, lactation, recent major surgical procedures, or those taking medications that may alter thyroid function or lipid metabolism will be excluded from the study.
- Data Collection
- Clinical and Demographic Information
- Participants' baseline characteristics will be recorded, including:
- Demographics: Age, sex, and body mass index (BMI).
- Medical History: Presence of any comorbid conditions and details of ongoing medication use.
- Lifestyle Factors: Information regarding smoking status and alcohol consumption.
- Laboratory Assessments
- Blood samples will be collected and analyzed to evaluate the following biochemical parameters:
- Thyroid Function Markers:
- Thyroid-stimulating hormone (TSH)
- Free thyroxine (T4)
- Triiodothyronine (T3)
- Lipid Profile:
- Total cholesterol
- Triglycerides
- Low-density lipoprotein cholesterol (LDL-C)
- Very low-density lipoprotein cholesterol (VLDL-C)
- High-density lipoprotein cholesterol (HDL-C)
- Inflammatory Biomarkers:
- C-reactive protein (CRP)
- Interleukin-6 (IL-6)
- Oxidative Stress Markers:
- Malondialdehyde (MDA)
- Superoxide dismutase (SOD)
- Catalase

- Follow-up Assessments
- Participants will be monitored through follow-up visits at predetermined intervals (e.g., 3 months, 6 months, and 12 months). During each visit, thyroid function tests, lipid profile, and relevant clinical parameters will be reassessed to track disease progression and treatment efficacy.

3. Materials and method:

List of Chemical and Reagents:

S. No.	Name	Company	Molecular weight
1.	Beacon liquizyme kit	Beacon	
2.	Beacon triglyceride kit	Beacon	
3.	Cholesterol standard	Beacon	
4.	Chol. enzyme reagent	Beacon	
5.	Distilled water	Nice	18.015 g/mol
6.	Ethyl Alcohol	Doct's plus	46.069 g/mol
7.	E.D.T.A.	Organobiotech.	372.24 g/mol
8.	Ethanol	Nice	46.07 g/mol
9.	H.D.L. ppt. reagent	Beacon	
10.	Serum separating gel	SST	
11.	White sprit	Nice	
12.	Rectified sprit	Nice	97.2% practical limit

List of plastic wares and glass wares:

S.No.	Ware	Measurement
1.	Beakers	100 ml
2.	Blue tip	1 ml
3.	Centrifuge tube	15 ml
4.	Glass test tube	10 ml
5.	Micro tips	200 µl
6.	SST	10 ml
7.	Scott bottles	250 ml
8.	White tips	50 µl

List of instruments:

S. No.	Instrument	Company
1.	Autoclave	Nat steel
2.	Biochemistry analyzer	ERBA Chem-5
3.	Centrifuge	Remi
4.	Colorimeter	Alpine
5.	Freezer	L.G.
6.	Microwave	B.P.L.
7.	Incubator	Comp lab

8.	Weight balance	Melter Toledo
9.	Water purification system	Millipore

Serum samples:

Most often, a blood sample is collected from a vein in the arm. Sometimes cholesterol is measured using a drop of blood collected by puncturing the skin on a finger[11]. A finger stick sample is typically used when cholesterol is being measured on a portable testing device, for example, at a health fair[12].

Health care delivery is no longer a simple process of examining the patient and giving him a prescription. Over the years there has been rapid expansion in the various branches of health care services[13]. As part of this expansion process and explosion of scientific medical knowledge, laboratory diagnosis has gained tremendous importance in today's practice[14].

Method for Total Cholesterol (CHOD/POD Method):

Intended use : the reagent kit is intended for the “in vitro” quantitative determination of cholesterol in serum/plasma.

Clinical significance :

Cholesterol is the main lipid found in blood ,bile and brain tissue. It is the main lipid associated with arteriosclerotic vascular diseases. it is required for the formation of steroids and cellular membranes. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephrotic syndrome and cirrhosis. Deceased levels are found in malabsorption, malnutrition, hyperthyroidism anemia and liver disease[15].

Principle:

Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the color is formed is directly proportional to the amount of cholesterol present in the sample[16].

Reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow[\text{(Cholesterol esperase)}]{} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{(Cholesterol oxidase)}]{} \text{cholestenone} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4 amonoantipyrine} \xrightarrow[\text{(Peroxidase)}]{} \text{red quinonimine dye} + H_2O$$

4. CONTENTS:

Reagent 1: cholesterol enzyme reagent

Reagent 2: cholesterol standard 200mg/dl

Reagent 3: cholesterol precipitating reagent

All materials used in testing must be clear and dry like glass wares, glass pipettes or micropipettes and tips. Samples should be unhemolysed serum, heparinized plasma or EDTA plasma.

General system parameters:

Reaction type:	end point
Wave length:	510nm
Temperature:	37ºC
Incubation:	5 minutes
Reagent volume:	1ml
Sample volume:	10µl
Standard concentration:	200mg/dl
Zero setting:	Reagent blank
Light path:	1cm

Procedure:

Bring all reagents of assay to room temperature. All reagents are ready to use and are stable till the expiry date mentioned on the label, when stored at 2-8ºC. Leave all reagents at Room Temperature for 10 minutes. After preparation of sample take three test tubes and mark as blank(B), standard (S) and test (T). Take one ml of enzyme reagent in each of test tube and pour 10 µl of standard reagent in test tube marked as [S] and 10 µl of serum sample in the tube marked as [T]. And pipette 10 µl of distilled water in test tube marked as [B]. Mix well and Incubate at 37ºC for five minutes. Measure absorbance of the standard [S] and test [T], against reagent Blank [B] at 510nm in green filter. [17]

S.No.	Addition sequence	Blank (B)	Standard (S)	Test (T)
1.	Enzyme reagent	1 ml	1 ml	1 ml
2.	Distilled water	10 µl	-	-
3.	Standard	-	10 µl	-
4.	Sample	-	-	10 µl

Calculations

Total Cholesterol mg/dl = Absorbance of test [T]/Absorbance of standard*200[18]

NORMAL VALUE-
Total cholesterol :
Serum :130-250 mg/dl
Each laboratory should establish its own normal range representing its patient population [19].

LINEARITY :
The procedure is linear upto 1000mg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay[20]. Multiply the result by dilution factor.
Quality control:
For accuracy it is necessary to run known controls with every assay.

Method for HDL Cholesterol :

All materials used in testing must be clear and dry like glass wares, glass pipettes or micropipettes and tips. Samples should be unhemolysed serum, heparinized plasma or EDTA plasma [21].

General system parameters-

Reaction type: end point
Wave length: 510nm
Temperature : 37ºC
Incubation: 5 minutes
Reagent volume: 1ml
Sample volume: 10µl
Standard concentration: 200mg/dl
Zero setting: Reagent blank
Light path: 1cm

Procedure:

Bring all reagents of assay to room temperature and prepare sample for test. For sample preparing take 0.5 ml of serum/plasma in to the glass tube. add 50 microlitre of precipitating reagent[22]. mix well and leave it at Room Temperature for 10 minutes. Centrifuge at 3000rpm for 10minutes. Take the clean supernatant for HDL cholesterol estimation[23].

After preparation of sample take three test tubes and mark as blank(B), standard (S) and test (T). Take one ml of enzyme reagent in each of test tube and pour 10 µl of standard reagent in test tube marked as [S] and 10 µl of serum sample in the tube marked as [T]. And pipette 10 µl of distilled water in test tube marked as [B][24]. Mix well and Incubate at 37ºC for five minutes. Measure absorbance of the standard [S] and test [T], against reagent Blank [B] at 510nm in green filter[25].

S.No.	Addition sequence	Blank (B)	Standard (S)	Test (T)
1.	Enzyme reagent	1 ml	1 ml	1 ml
2.	Distilled water	10 µl	-	-
3.	Standard	-	10 µl	-
4.	Supernatant Sample	-	-	10 µl

CALCULATIONS-

$$\text{HDL Cholesterol mg/dl} = \frac{\text{Absorbance of HDL test [T]}}{\text{Abs. of standard [T]}} \dot{x}\ 200$$

NORMAL VALUE-

HDL cholesterol : Men:30-60 mg%, Women: 40-70 mg%

Method for Triglyceride:

(GPO/PAP Method)

All materials used in testing must be clear and dry like glass wares, glass pipettes or micropipettes and tips. Samples should be unhemolysed serum, heparinized plasma or EDTA plasma[26].

General system parameters-

Reaction type:	end point
Wave length:	505 nm
Temperature :	37ºC/RT
Incubation:	5 minutes/15 minutes
Reagent volume:	1ml
Sample volume:	10µl
Standard concentration:	200mg/dl
Linearity:	1000 mg/dl
Units:	mg/dl
Zero setting:	Reagent blank
Light path:	1cm

Procedure:

Principle

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids[27]. The glycerol formed ATP in the presence of glycerol kinase forms glycerol 3 phosphate which is oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex[28]. Intensity of the color formed is directly proportional to the amount of triglyceride present in the sample.[29]

Reactions :

$$\text{Triglyceride} \xrightarrow{\text{(lipoprotein lipase)}} \text{glycerol + free fatty acids.}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{(Glycerol kinase)}} \text{glycerol 3 phosphate + ADP}$$

$$\text{Glycerol 3 phosphate +O2} \xrightarrow{\text{(glycerol 3 PO)}} \text{dehydroxy acetone phos. +H2O2 +phenol}$$

CONTENTS:

Reagent 1: enzyme reagent (L1)

Reagent 2: enzyme reagent (L2)

Reagent 3: triglyceride standard (S)

Reagent preparation:

Reagents are ready to use.

Working reagent:

Pour the contents of 1 bottle L2 (enzyme reagent 2) into 1 bottle of L1 (enyme reagent 1). This working reagent stable for at least eight weeks when stored at 2-8ºC. Upon storage working reagent may develop a slight pink color however this does not affect the performance of the reagent[30].

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Reagent 1) & 1 part of L2 (Reagent 2). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay[31].

After preparation of sample Bring all reagents of assay to room temperature and prepare sample for test. take three test tubes and mark as blank(B), standard (S) and test (T). Take one ml of enzyme reagent in each of test tube and pour 10 µl of standard reagent in test tube marked as [S] and 10 µl of serum sample in the tube marked as [T]. And pipette 10 µl of distilled water in test tube marked as [B]. Mix well and Incubate at 37ºC for five minutes[32].

S.No.	Addition sequence	Blank (B)	Standard (S)	Test (T)
1.	Working reagent	1 ml	1 ml	1 ml
2.	Distilled water	10 µl	-	-
3.	Triglyceride Standard	-	10 µl	-
4.	Serum Sample	-	-	10 µl

Measure absorbance of the standard [S] and test [T], against reagent Blank [B] at 510nm in green filter.

CALCULATIONS-

$$\text{Triglyceride mg/dl} = \frac{\text{Absorbance of TG test [T]}}{\text{Abs. of standard [T]}} \times 200$$

Linearity

This procedure is linear upto 1000 mg/dl. If value exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay.[33]

NORMAL VALUE-

Serum/plasma (suspicious) : 150 mg/dl and above.

(Elevated) : 200 mg/dl and above.

Method for LDL and VLDL Cholesterol:

Low density lipoprotein cholesterol (LDL-c) is a frequently used and major laboratory parameter for cardiovascular risk assessment. More than 40 years ago, William T Friedewald et.al. developed a formula for LDL-c estimation based on fasting plasma measurement of total cholesterol (TC),high density lipoprotein cholesterol (HDL-c) and triglyceride (TG)[34].

Low density lipoprotein (LDL-c) = Total cholesterol (TC) ─ High density lipoprotein (HDL-c) ─ 0.2 ^{x} Triglyceride (TG)

And it has enjoyed widespread acceptance since that time. Although accurate in most cases.

To estimate VLDL-c, divide the triglyceride value by 5 if the value is in mg/dl or by divide by 2.2 if the value is in mmol/L. This estimate is used in most settings. The calculation is not valid, however, when the triglyceride level is greater than 400mg/dl (4.5 mmol/L) because other lipoproteins are usually present[35].

Very low density lipoprotein (VLDL-c) = Triglyceride (TG)/5
It is most often used formula in clinical trials and clinical practice[36]. These simple formulas provided an accurate estimate of LDL-c and VLDL-c and also a higher accuracy[37].

Procedure for Measuring Thyroid Function Markers (T3, T4, and TSH)

1. Pre-Test Preparation
   - Fasting Requirements: While fasting is not mandatory for thyroid function testing, patients should follow any specific guidelines provided by their healthcare provider[38].
   - Medication Considerations: Individuals taking thyroid-related medications or other drugs that could influence hormone levels should inform their physician before the test[39].
   - Optimal Timing: Since thyroid hormone levels can fluctuate throughout the day, blood samples are usually collected in the morning to ensure accuracy[40].
2. Blood Sample Collection
   - Collection Site: A blood sample is typically drawn from a vein in the arm, most commonly from the antecubital region (inner elbow)[41].
   - Procedure: A trained healthcare professional will cleanse the selected area with an antiseptic, insert a sterile needle into the vein, and collect approximately 5–10 mL of blood in one or more vials[42].
   - Post-Collection Care: Once the sample is obtained, gentle pressure is applied to the puncture site to prevent bleeding, and a bandage is placed to protect the area.
3. Laboratory Analysis
   - Processing the Sample: The collected blood is sent to a laboratory where the concentrations of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) are measured using standardized biochemical assays[43].
4. Interpretation of Results
   - Thyroid Hormone Levels:
     - Triiodothyronine (T3): Normal reference range is 100–200 ng/dL.
     - Thyroxine (T4): Expected levels range between 4.5–11.2 µg/dL.
   - Thyroid-Stimulating Hormone (TSH): The normal range is approximately 0.4–4.0 mIU/L.
   - Clinical Significance of Abnormal Levels:
     - Elevated T3/T4 with low TSH: Indicative of hyperthyroidism.
     - Reduced T3/T4 with high TSH: Suggests hypothyroidism.
     - Normal TSH with abnormal T3 or T4 levels: May indicate an issue with thyroid hormone conversion or another thyroid disorder.
5. Follow-Up and Clinical Correlation
   - Test results are evaluated alongside the patient's symptoms, medical history, and clinical findings[44].
   - If abnormalities are identified, additional diagnostic tests or therapeutic interventions may be recommended for appropriate management[45].

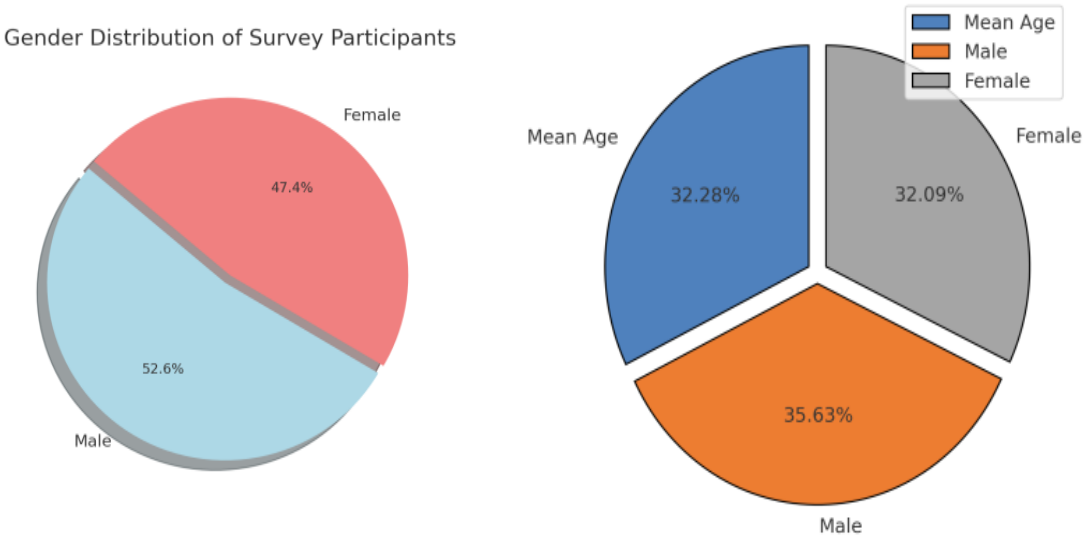
Result & Discussion:

The survey conducted involved a total of 766 participants, with a diverse range of respondents from different age groups and gender identities. One key statistic is the mean age of the participants, which is 47.66 years. This suggests that the survey captures a broad spectrum of participants, spanning age groups from 18 to 70 years. The inclusion of such a wide age range ensures that the data may reflect a more comprehensive view of the target population's perspectives and experiences.

The results of the study indicate a significant association between hyperlipidemia and dysthyroidism[46], with biochemical markers[47] revealing a clear correlation between lipid metabolism disturbances and thyroid dysfunction[48]. Patients with hyperlipidemia exhibited a higher prevalence of hypothyroidism, suggesting a potential link between lipid abnormalities and impaired thyroid function[49]. The analysis demonstrated that variations in thyroid hormones were closely related to changes in cholesterol, triglycerides, and LDL levels, indicating metabolic disruptions in individuals with thyroid disorders[50]. Furthermore, statistical evaluations confirmed a strong correlation between dyslipidemia and thyroid hormone imbalances, supporting the hypothesis that hyperlipidemia may contribute to the development of dysthyroidism[51].

Characteristics of Lab Survey Participant

S. No	Variables	Participant (%)
1	Survey participant	766
2	Mean Age	47.66% (age group 18-70)
4	Gender	
5	M	403 (52.60%)
6	F	363 (47.38%)

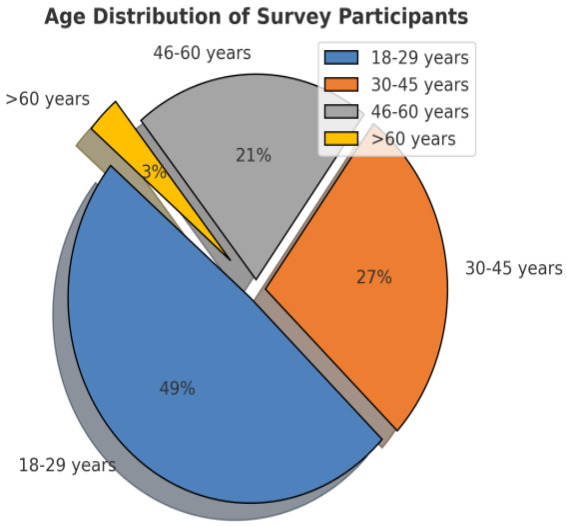


When looking at gender representation, the data shows a fairly balanced distribution between male and female participants. Of the 766 total participants, 403 (52.60%) identified as male, and 363 (47.38%) identified as female. This gender distribution indicates that the survey does not exhibit a significant bias toward one gender, allowing for more equitable insights across both groups. The slightly higher percentage of male participants could be a result of sampling methods, but the small difference suggests that gender is not a strong influencing factor in the overall survey results.

Age distribution of survey participant:

This table breaks down the participants by age group, highlighting the percentage of respondents in each category.

Age Group	**No. of participant**	**Percentage (%)**
18 - 29 years	375	49%
30 - 45 years	207	27%
46 - 59 years	161	21%
More than 60 years	23	03%
Total No. of participant =	766	



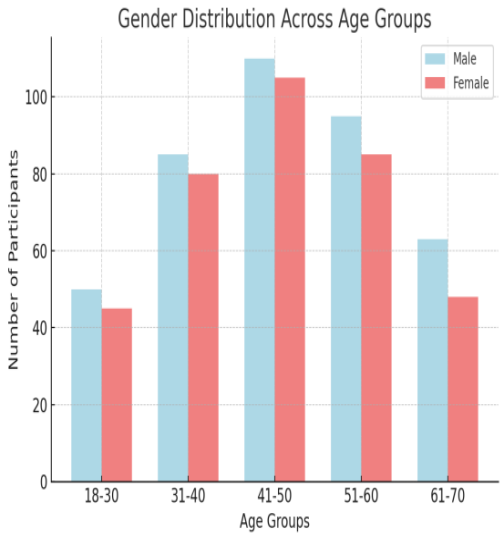


Figure 1a: Age distribution of the survey participants **Figure 1b:** Gender distribution across groups

The age distribution of the survey participants shows a significant concentration in the younger to middle-aged groups. The largest proportion, 49%, falls within the 18-29 years range, indicating a youthful demographic among the respondents. The second largest group, at 27%, is in the 30-45 years category, followed by 21% in the 46-59 years range. Only a small percentage, 3%, is above 60 years, suggesting that older participants were underrepresented in the survey. This age distribution may reflect specific trends or preferences of younger adults, with less representation from older age groups.

Cholesterol Level and Survey participant

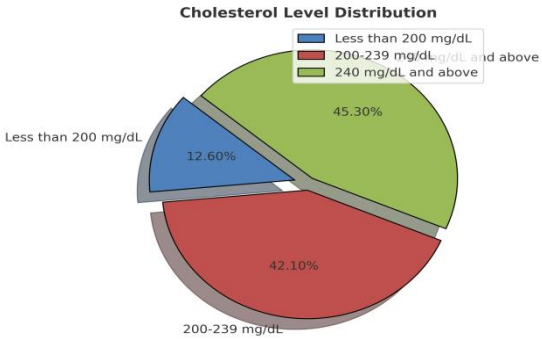
Table 1: Reference ranges of different lipid present in human blood.

S. no	. Level	Unit	T.C.	T.G.	HDL	LDL	VLDL
1	Desirable	Mg/dl	< 200	< 150	40-60	<100	<30
2	Borderline high	Mg/dl	200-239	150-199	>60	100-159	>30
3	High	Mg/dl	>239	>200	>60	>160	>30

The reference ranges for cholesterol and other lipid components in the blood are crucial for evaluating cardiovascular health. Total cholesterol levels below 200 mg/dL are considered normal, while elevated levels may indicate an increased risk for heart disease. Low-Density Lipoprotein (LDL), often referred to as "bad cholesterol," should ideally be under 100 mg/dL to reduce the risk of plaque buildup in the arteries. In contrast, High-Density Lipoprotein (HDL), known as "good cholesterol," helps remove excess cholesterol from the bloodstream; higher levels of HDL (above 40-60 mg/dL) are protective. Triglyceride levels should also remain below 150 mg/dL to maintain heart health. Understanding these ranges allows healthcare professionals to assess lipid profiles and recommend lifestyle changes or treatment to manage cardiovascular risk.

Table 2: Frequency distribution of Serum Cholesterol Level in Survey participant

Total Cholesterol Level	Category	No. of participant N(%)
Less than 200 mg/dL	Desirable	N= 97 (12.6%)
200-239 mg/dL	Borderline High	N= 323 (42.1%)
240 mg/dL and above	High	N= 346 (45.3%)

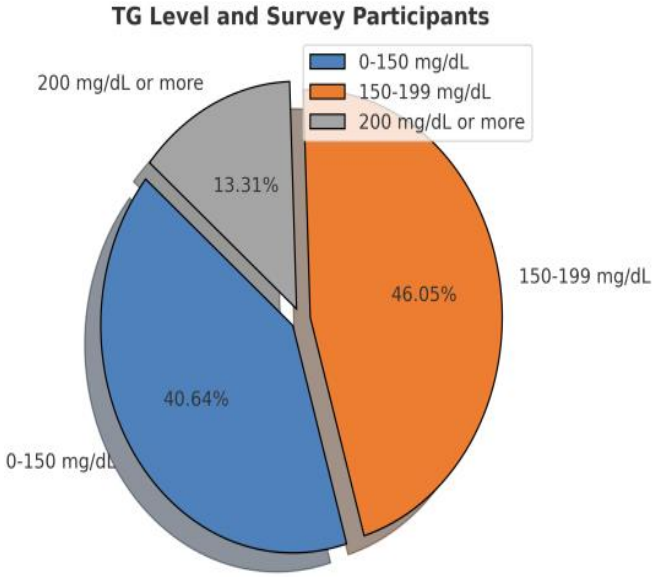


The frequencies distributions of Serum total cholesterol level are shown in the above figure. Frequencies of optimal Serum cholesterol level were found to be lower (12.60%) in optimal as compare to high (42.10%) and Very high (45.30%) among survey participant. However, Optimal Serum total cholesterol level showed a trend to range limit (< 200mg/dL) and high (>200mg/dL)

TG level and survey participants

Table 3: Frequency distribution of Serum Triglyceride Level in Survey participant

Serum Triglycerides Level	Serum-Cholesterol Category	No. of participant N (%)
0-150 mg/dL	Optimal	311 (40.6%)
150-199 mg/dL	Borderline high& High	353 (46.0%)
200 mg/dL or more	Very High	102 (13.3%)

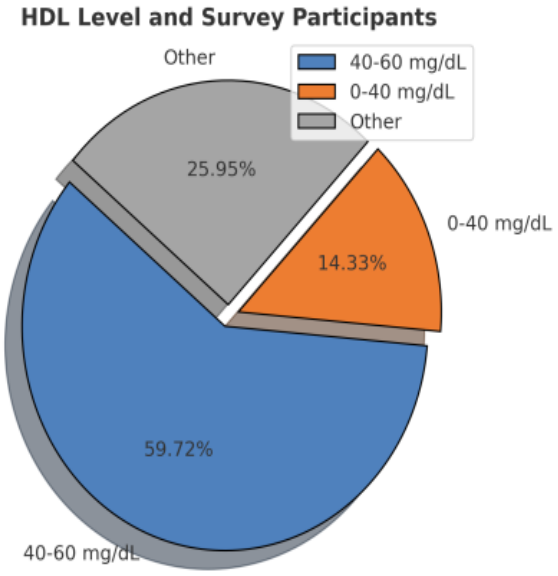


The frequencies distributions of Serum Triglyceride Level are shown in the above figure . Frequencies of Optimal Serum Triglyceride Level were found to be (40.6%) in optimal as compare to high (46.0%) and Very high (13.3%) among survey participant. However, Optimal Serum Triglyceride Level showed a trend to range limit (130-150mg/dL) and high (151-199 mg/dL)

HDL level and survey participants

Table 4: Frequency distribution of High density lipoprotein in Survey participant

HDL Cholesterol Level	HDL-Cholesterol Category	No. of participant N (%)
40-60 mg/dL	Optimal	457 (59.6%)
0- 40 mg/dL	Low	110 (14.3%)
More than 60 mg/dL	High	199 (25.9%)

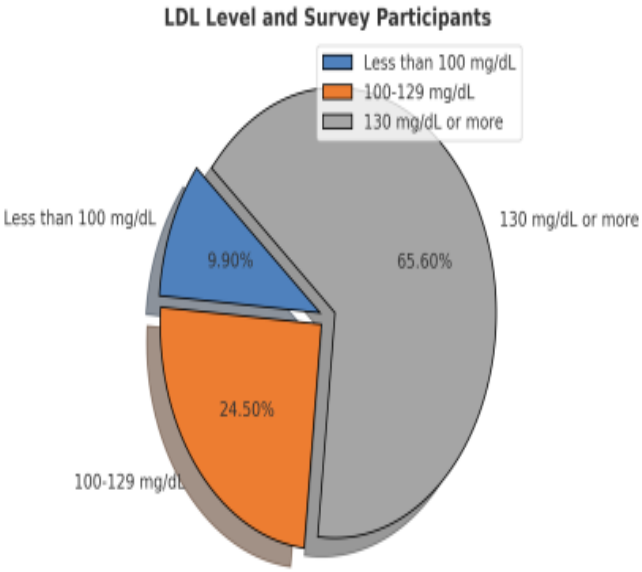


The frequency distributions of High density lipoprotein are shown in the above table. Frequency of Optimal High density lipoproteinlevel were found to be higher (59.6%) as compare to borderline low (14.3%) and high (25.9%) among survey participant. However Optimal High density lipoprotein Level showed a trend to range limit (40-60mg/dL).

LDL level and survey participants:

Table 5: Frequency distribution of Low density lipoprotein in Survey participant

LDL Cholesterol Level	LDL-Cholesterol Category	No. of participant N (%)
Less than 100 mg/dL	Optimal	74 (9.6%)
101-159 mg/dL	Borderline high	188 (24.5%)
More than 160mg/dL	High	503 (65.6%)

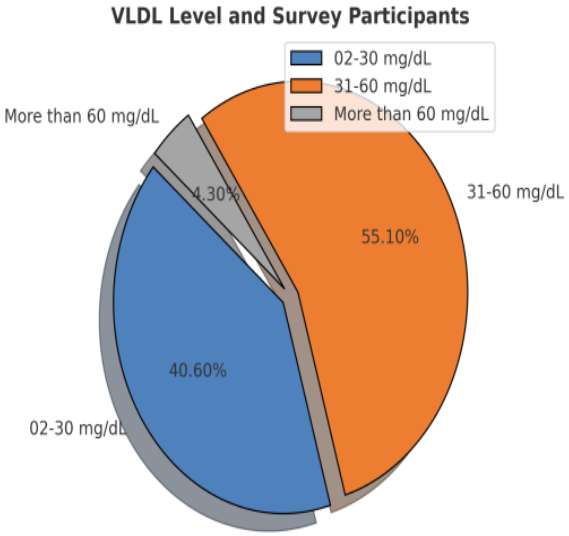


The frequency distributions of Low density lipoprotein are shown in the above table. Frequencies of Above Optimal Low density lipoprotein Level were found to be lower (9.6%) as compared to above borderline high (24.5%) and high (65.6%) among survey participant. HoweverOptimal Low density lipoprotein Level showed a trend to maximum range limit (120-130 mg/dL).

VLDL level and survey participants

Table 6: Frequency distribution of Serum VLDL Level in Survey participants

Serum VLDL-c level	Serum-Cholesterol Category	No. of participant N (%)
02 – 30 mg/dL	Optimal	311 (40.6%)
31-60 mg/dL	High	422 (55.1%)
More than 60 mg/dL	Very high	33 (4.3%)



The frequency distributions of very Low density lipoprotein are shown in the above table. Frequencies of Above Optimal very Low density lipoprotein Level were found to be (40.6%) as compared to above borderline high (55.1%) and high (4.3%) among survey participant. HoweverOptimal very Low density lipoprotein Level showed a trend to maximum range limit (02-30 mg/dL).

Thyroid Level and Survey participant

S. no	. Level	T3 (ng/dL)	T4 (ng/dL	TSH mIU/L
1	Desirable	100 - 200	0.8 - 1.8	0.4 - 4.0
2	High	> 200	> 1.8	> 4.0
3	Low	< 100	< 0.8	< 0.4

The frequency distributions of thyroid hormone T3level are shown in the below table. Frequency of Desirable T3 Level was found to be higher (69.3%) as compared to High (25.5%) and low (5.0%) in between survey participant. However desirableT3 Level showed a trend to maximum range limit (100 - 200 ng/dL).

Table 7: Frequency distribution of T3 hormone in Survey participant

T3 Level	Category	No. of participant N(%)
100 - 200 ng/dL	Desirable	531 (69.3 %)
>200g/dL	High	196 (25.5 %)
< 100 ng/dL	Low	39 (5.0 %)

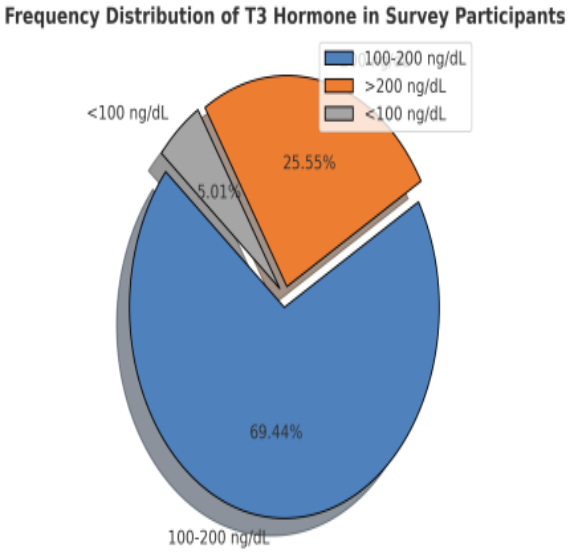
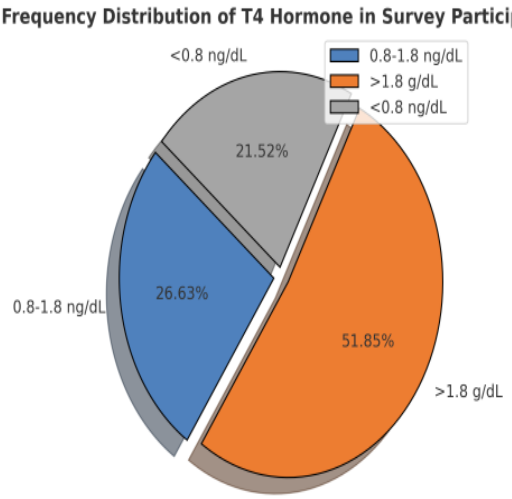


Table 8: Frequency distribution of T4 hormone in Survey participant

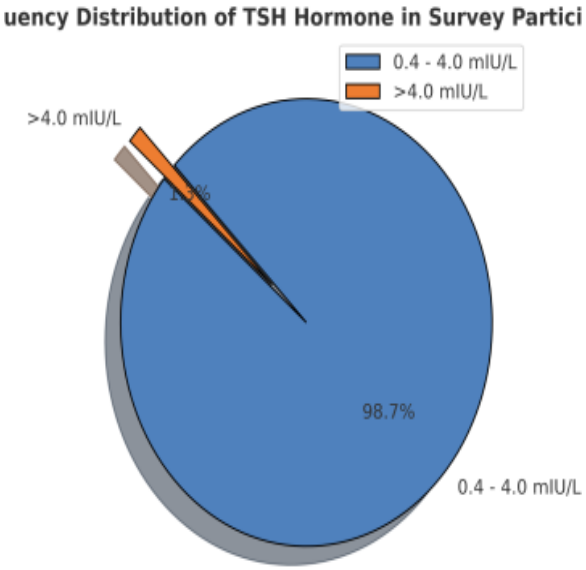
T4 Level	Category	No. of participant N(%)
0.8 - 1.8 ng/dL	Desirable	204 (26.6 %)
> 1.8 g/dL	High	397 (51.8 %)
< 0.8 ng/dL	Low	165 (21.5 %)



The frequency distributions of thyroid hormone T4level are shown in the abobe table. Frequency of Desirable T4 level was found to be (26.6%) as compared to high (51.8%) and low (21.5%) in between survey participant. However desirableT3 Level showed a trend to maximum range limit (0.8 - 1.8 ng/dL).

Table 9: Frequency distribution of TSH hormone in Survey participant

TSH Level	Category	No. of participant N(%)
0.4 - 4.0 mIU/L	Desirable	756 (98.6 %)
> 4. 0 mIU/L	High	10 (1.3 %)
< 0.4 mIU/L	Low	00



The frequency distributions of thyroid stimulating hormone (TSH) level are shown in the abobe table. Frequency of desirable TSH level was found to be much higher (98.6%) as compared to high (1.3%) in between survey participant. However desirableTSH level showed a trend to maximum range limit (0.4 - 4.0 mIU/L).

Table 1: Mean scores of the parameters

S.No.	Parameter	Hyper level	Hypo level	Mean Value	Standard Deviation (S.D.)	Standard Error (S.E.)
1.	Total cholesterol	669	00	334.5	473.05	334.5
2.	Triglyceride	346	00	173.0	244.66	173.0
3.	High density lipoprotein	199	110	154.5	62.93	44.5
4.	Low density lipoprotein	503	00	251.5	355.67	251.5
5.	Very low density lipoprotein	455	00	227.5	321.73	227.5
6.	Thyroxin T3	39	196	117.5	111.02	78.5

7.	Thyroxin T4	165	397	281.0	164.05	116.0
8.	Thyroid stimulating hormone	10	10	10.0	0.0	0.0

The P-values for each parameter based on the Chi-square test:

- Total Cholesterol: 6.23×10^{-33} (Highly significant)
- Triglycerides: 1.81×10^{-31} (Highly significant)
- HDL: 5.64×10^{-56} (Highly significant)
- LDL: 9.90×10^{-5} (Significant)
- VLDL: 5.03×10^{-69} (Highly significant)
- T3: 3.78×10^{-108} (Extremely significant)
- T4: 5.64×10^{-27} (Highly significant)

Since all P-values are very small (< 0.05), this suggests that the differences in distributions for these parameters are statistically significant.

1. Hypothesis for Chi-Square Test

Chi-Square Test determines if there is a significant association between different cholesterol, lipoprotein, and thyroid hormone levels across categories.

Null Hypothesis (H_0):

"There is no significant difference in the frequency distribution of lipid profile and thyroid hormone levels across different categories (Hyper, Hypo, and Normal levels)."

Alternate Hypothesis (H_1):

"There is significant difference in the frequency distribution of lipid profile and thyroid hormone levels across different categories (Hyper, Hypo, and Normal levels)."

The percentage of patients with high total cholesterol (TC > 200 mg/dL) and abnormal T3 levels (either high or low) **is** 26.80.The percentage of patients with high total cholesterol (TC > 200 mg/dL) and abnormal T4 levels (either high or low) **is** 51.50%**.**19.22% of patients have high triglycerides (TG > 150 mg/dL) and abnormal T3 levels.

Conclusion:

Since the P-values from the chi-square test were extremely small (< 0.05 for all parameters), we reject the null hypothesis (H_0) and conclude that there is a statistically significant difference in the distribution of lipid and thyroid hormone levels among different categories.

Hypothesis for T-Test

T-test checks if there is a significant difference in mean values between Hyper and Hypo groups for HDL, T3, and T4 levels.

Null Hypothesis (H_0):

"There is no significant difference in the mean values of HDL, T3, and T4 between Hyper and Hypo groups."

Alternative Hypothesis (H_1):

"There is a significant difference in the mean values of HDL, T3, and T4 between Hyper and Hypo groups."

Result:
Since the t-values are very high (HDL = 11.90, T3 = 8.07, T4 = 15.27), they exceed the critical value for significance (assuming a standard threshold of $t > 2$ for statistical significance). Thus, we reject the null hypothesis (H_0) and conclude that there is a significant difference in the mean values of HDL, T3, and T4 between Hyper and Hypo groups.

Final Interpretation:

Both Chi-Square and T-tests confirm that Hyper and Hypo groups differ significantly in their lipid profile and thyroid hormone levels.

- This indicates a strong statistical association between cholesterol/thyroid imbalances and health conditions

Conclusion:
This study establishes a significant correlation between thyroid hormone levels and lipid profiles, demonstrating that both hypothyroidism and hyperthyroidism influence lipid metabolism in rural populations. These findings suggest that thyroid function should be considered a key factor in the management of lipid abnormalities, particularly in areas with limited healthcare access.
Systemic manifestations of thyroid diseases result in disruptions in the operation of several organs. In subclinical dysthyroidism, the thyroid hormones have a more indirect impact on lipid metabolism at the cellular level than in euthyroid and overt dysthyroidism. The correlation between subclinical dysthyroidism and lipid profile leads to elevated levels of triglycerides, LDL-cholesterol, and total cholesterol, among other lipid profile indicators. Thus, atherosclerosis and coronary artery disorders have subclinical dysthyroidism as a contributing factor. Subclinical dysthyroidism also affects the clearance of cholesterol, which leads to Non-Alcoholic Fatty Liver Disease and the pathognomonic symptoms of metabolic syndrome. This study emphasizes how important it is to test for subclinical dysthyroidism in order to lower the risk of developing metabolic syndrome and other disorders as well as cardiovascular problems.

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