Section : Research Paper

Non-Alcoholic Fatty Liver Disease (Nafld) In Hypothyroidism And It's Correlation With Biochemical Parameters.

Brahmanandam Lingudu[1], Vivekanand Bongi[2], Subrahmanyam KAV[3], Mounika Anitha[4] and Rajesh Moganti[5]

[1]Associate Professor of endocrinology, government medical college, Ananthapuram, Andhra Pradesh India.

[2]Associate Professor of endocrinology, Andhra medical college, Visakhapatnam Andhra Pradesh India

[3]Professor of Endocrinology, Andhra medical college, Visakhapatnam Visakhapatnam Andhra Pradesh India

[4]Assistant Professor of Endocrinology, Andhra Medical College,Visakhapatnam, Andhra Pradesh India

[5]Junior Resident, Department Of Endocrinology,Andhra Medical College, Visakhapatnam, Andhra Pradesh India

Corresponding author email: brahmaendo2k2@gmail.com

ABSTRACT

Background: Hypothyroidism has been identified as an independent risk factor for the development of NAFLD, although the literature is inconsistent and the prevalence of NAFLD in hypothyroidism has not been well studied.

Objectives: To study the prevalence of NAFLD in Hypothyroidism. To correlate hormonal, biochemical parameters and lipid indices in hypothyroidism with severity of NAFLD.

Methodology: Observational, cross-sectional study amongst 100 adults with hypothyroidism aged between 18 to 60 years. Consecutive male and female hypothyroid patients attending the Endocrinology outpatient department were recruited after the inclusion and exclusion criteria were satisfied during the study period. All patients underwent thyroid and liver function testing and were evaluated for NAFLD using ultrasonography and Transient elastography.

Results: The prevalence of NAFLD in hypothyroid patients is 38% (mild - 25 % and moderate - 13%). Waist circumference is significantly associated with the severity of NAFLD (p=0.006). Liver function tests - AST, ALT, and alkaline phosphatase are statistically associated with NAFLD severity (p=0.001). Subcutaneous abdominal adipose tissue (SAT) thickness is also statistically correlated with the NAFLD severity (p=0.05). Fibroscan results correlated significantly with the grades of NAFLD, which were based on USG criteria ($p < 0.001$). FIB-4 index also correlated significantly with the severity of hypothyroidism (p=0.016). There is a

positive correlation between serum TSH level and grade of NAFLD, and it is found to be statistically significant (p<001).

Conclusion: This study found a higher prevalence of NAFLD among adults with hypothyroidism. Higher TSH levels may be a risk factor for the increased incidence of NAFLD in hypothyroidism.

Keywords: NAFLD, Hypothyroidism, Waist circumference, Dyslipidemia, Fibroscan

Introduction

Hepatic steatosis is the accumulation of cholesterol, triglycerides, and phospholipids leading to excess 5-10% liver weight[1.] A condition called non-alcoholic steatohepatitis (NASH) was coined by Ludwig et all, describing lesions on varying degrees of severity in people not exposed to ethanol [(2)]. Non-alcoholic fatty liver disease (NAFLD) exists as a spectrum, from simple steatosis without proof of cell injury, and has shown to be stable over time but can progress to liver cirrhosis. In the later stages, cirrhosis caused by NASH will lose diagnostic fatty infiltration and may resemble cryptogenic cirrhosis[3-5] On the other hand; non-alcoholic steatohepatitis is a clinicopathologic disease that excludes ethanol as the main reason for the disease, focal necrosis, cellular ballooning, fibrosis, and inflammation. Hypothyroidism is more common in patients with non-alcoholic steatohepatitis than in patients with non-alcoholic fatty liver disease, according to the report. The findings of the study remain important after considering other factors such as the patient's age and presence of other health conditions like diabetes, hypertension, and dyslipidaemia, but have nothing to do with gender.[8] Hence, we aim to conduct a study to assess the prevalence of NAFLD in hypothyroid patients and to correlate with various metabolic and hormonal parameters.

Materials and Methods

It was an Observational, Cross-sectional study. Adult Hypothyroid patients attending outpatient and inpatient departments of Endocrinology and in King George Hospital,Visakhapatnam conducted between Dec 2020 to Nov 2021. 100 Hypothyroid patients meeting the criteria for the present study.

Inclusion Criteria:

1. Subjects with preexisting hypothyroidism, newly detected hypothyroidism (both subclinical and overt) presenting to the endocrinology department.
2. Age: 18-60 years
3. Patients who gave valid consent are included in the study

Exclusion Criteria:

1. Previous history of chronic liver disease/Cirrhosis

2. Suffering from conditions that can occur with known liver enzyme abnormalities;
3. Using medication that are risk factors for NAFLD such as corticosteroids, tamoxifen, amiodarone, diltiazem, protease inhibitors (ARVs);
4. Daily consumption of ethanol ≥ 20 grams;
5. Failure to agree in participating in the study.
6. Evidence of biliary tract obstruction by USG
7. Diabetes
8. Chronic kidney disease
9. BMI: > 30 kg/m^2

Methodology

Information will be collected through prepared proforma from each patient after giving consent, who is attending out-patient and in-patient Department of Endocrinology, KGH.

The diagnosis of hypothyroidism is defined as follows:

- **Overt hypothyroidism** is defined as high serum TSH concentrations and low serum free T4 concentrations.
- **Subclinical hypothyroidism** is defined as high serum TSH and normal serum free T4 concentrations.

All patients will be screened for non-alcohol fatty liver disease (NAFLD).

The participants were subjected to anthropometry and detailed clinical examination. Height, weight, circumference of the waist and hip, and blood pressure were measured in all the subjects using a standard methodology and apparatus. Body Mass Index was calculated with weight in kilograms divided by height in m^2. The subject's overnight fasting blood samples were taken to measure their hormones and biochemical profiles. Enzymatic colorimetric method with glucose oxidase was used to measure fasting plasma glucose (FPG) and 2-hr post 75 gm glucose (2hr-PPG). Free T4, TSH, liver function tests, lipid profile, and fasting insulin level was estimated.

A single experienced investigator conducted a Trans-abdominal ultrasound system (PHILIPS HD7). Estimation of adipose tissue depots was done. SAT or Subcutaneous Abdominal Adipose Tissue Thickness pertains to the depth starting from the cutaneous boundary to the linea alba. VAT or Visceral Adipose Tissue pertains to the depth from linea alba's posterior surface to the lumbar vertebra's corpus.

Calculations-

HOMA-IR was used as insulin resistance marker and calculated as FPG in mg/dl X fasting insulin in mu/L)/405. Hormone measurements were done using Abbott Architect Plus I 2000 SR.

Statistical Analysis-

The statistical analysis was performed using SPSS for windows version 22.0 software (Mac, and Linux). The findings were present in number and percentage analyzed by frequency, percent, and Chi‑ squared test. Chi‑ squared test was used to find the association among variables. The critical value of P indicating the probability of significant difference was taken as <0.05 for comparison.

Results-

Table 1: Baseline details of study participants

Parameter	**Value**	**SD**
Mean age (in years)	44.93	10.75
Mean BMI (in kg/m^2)	25.40	2.22
Mean WC (in cm)	82.70	5.39
Mean WHR	0.931	0.66
Mean HOMA IR	3.47	2.95
Mean AST (IU/L)	45.92	20.92
Mean ALT (IU/L)	44.22	21.79
Mean SAT (cm)	21.884	7.18
Mean TSH (uIU/mL)	9.46	3.75
Mean Ferritin (ng/mL)	82.18	40.43
Mean Fibroscan (kpa) scores (kPa)	6.395	1.39

A total of 100 subjects with subclinical and overt hypothyroidism were enrolled in the study. Out of 100 subjects 64 were females and 36 were males.

Table 2: Comparison of mean age among study population.

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
Age	Absent	62	45.37	10.608	2.387	.097
	Mild	25	46.84	11.198		
	Moderate	13	39.15	9.290		
	Total	100	44.93	10.753		

Table 2 shows that there is no significant difference in the mean age group across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test).

Table 3: Sex Distribution in Case Groups

			Sex		Total
			MALE	FEMALE	
NAFLD	Absent	Count	23	39	62
		%	37.1%	62.9%	100.0%
	Mild	Count	11	14	25
		%	44.0%	56.0%	100.0%
	Moderate	Count	2	11	13
		%	15.4%	84.6%	100.0%
Total		Count	36	64	100
		%	36.0%	64.0%	100.0%

Sex distribution among the study population shows that 36 (36%) patients are male whereas 64 (64%) are females. Among the males out of total 36 patients, 23 (23%) are without NAFLD, 11 (11%) are with mild and 2 (2%) are with moderate NAFLD. Among the females out of total 64 patients, 39 (39%) are without NAFLD, 14 (14%) are with mild and 11 (11%) are with moderate NAFLD stages. On the whole female patients outnumbered males in the study population. When it occurred in females it was more severe (NAFLD) when compared to males.

Table 4: Presence of (absent, mild, moderate) NAFLD in 2 study groups (Overt and sub clinical hypothyroidism)

			Hypothyroid		Total
			Overt	SCH	
NAFLD	Absent	Count	12	50	62
		%	19.4%	80.6%	100.0%
	Mild	Count	11	14	25
		%	44.0%	56.0%	100.0%
	Moderate	Count	10	3	13
		%	76.9%	23.1%	100.0%
Total		Count	33	67	100
		%	33.0%	67.0%	100.0%

As per table 4 subclinical and Overt hypothyroidism – the distribution of NAFLD in patients of subclinical group – absent in 50 (80.6%), mild in 14 (56 %) and moderate in 3 (23.1 %). Whereas the distribution of NAFLD in patients of overt hypothyroidism group – absent in 12 (19.4%), mild in 11 (44 %) and moderate in 10 (76.9 %). Among the patients without NAFLD – subclinical are more than overt and in the moderate group overt outnumbered the subclinical group.

Table 5: Comparison of Waist Circumference among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
WC	Absent	62	83.73	4.778	5.397	.006
	Mild	25	79.76	6.809		
	Moderate	13	83.46	2.757		
	Total	100	82.70	5.398		

Table 5 significant difference in the mean Waist circumference across the three subgroups of NAFLD among hypothyroidism in the total study population, male and female patients, respectively (using the ANOVA test).

Table 6: Comparison of Body mass index (BMI) among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
BMI	Absent	62	24.0719	1.97784	2.021	.138
	Mild	25	24.8296	2.71491		
	Moderate	13	26.2008	2.14535		
	Total	100	25.4081	2.22558		

Table 6 show no significant difference in the mean BMI across the three subgroups of NAFLD among Hypothyroidism in the total study population, male and female patients, respectively.

Table 7: Comparison of mean fasting insulin and HOMA-IR among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
INSULIN	Absent	62	15.1979	14.11011	1.035	.359
	Mild	25	19.3936	10.08718		
	Moderate	13	15.5477	6.47221		
	Total	100	16.2923	12.47682		
HOMA IR	Absent	62	3.12956	3.472421	.362	.697
	Mild	25	3.21332	1.993084		
	Moderate	13	3.73077	1.423361		
	Total	100	3.47566	2.950010		

As per table 7 shows that there is no significant difference in the mean fasting insulin level across the three subgroups of NAFLD among Hypothyroidism in absent, mild and moderate NAFLD sub groups. HOMA-IR - Table 9 shows that there is no significant difference in the mean HOMA-IR across the three subgroups of NAFLD among Hypothyroidism.

Table 8: Comparison of Lipid parameters among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
TRIGLYCERIDE	Absent	62	85.03	26.664	.184	.832
	Mild	25	82.88	23.891		

	Moderate	13	80.77	18.700		
	Total	100	83.94	24.924		
TOTAL CHOLESTEROL	Absent	62	152.18	17.498	.172	.842
	Mild	25	149.80	17.623		
	Moderate	13	150.85	17.165		
	Total	100	151.41	17.342		
LDL-C	Absent	62	81.95	27.895	1.702	.188
	Mild	25	73.36	23.322		
	Moderate	13	89.23	25.137		
	Total	100	80.75	26.684		
HDL-C	Absent	62	47.35	6.526	.957	.388
	Mild	25	47.12	6.099		
	Moderate	13	44.69	5.907		
	Total	100	46.95	6.346		
VLDL	Absent	62	42.10	12.254	1.336	.268
	Mild	25	39.44	10.251		
	Moderate	13	46.23	15.073		
	Total	100	41.97	12.229		

Table 8 shows that there is no significant difference in the mean cholesterol level across the three subgroups of NAFLD among Hypothyroidism (using the ANOVA test). There is no significant difference in the mean triglyceride level across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test). There is no significant difference in the mean HDL level across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test). LDL that there is no significant difference in the mean LDL level across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test).

Table 9: Comparison of mean SAT and VAT among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
SAT	Absent	62	20.315	6.6620	5.520	.005

	Mild	25	23.160	7.6631		
	Moderate	13	26.915	6.2883		
	Total	100	21.884	7.1871		
VAT	Absent	62	4.3424	2.16070	1.079	.344
	Mild	25	5.1960	3.29045		
	Moderate	13	4.9100	2.75946		
	Total	100	4.6296	2.56268		

Table 9 shows that there is significant difference in the mean SAT thickness across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test). The post hoc Benferroni shows that there is statistically significant difference (P value <0.05) in mean SAT thickness when compared among sub groups of absent and moderate NAFLD.

Table 10: Comparison of mean of Fibroscan(liver stiffness - Kpa) among study population

Variable	NAFLD	N	Mean	Std. Deviation	F VALUE	P VALUE
Fibro scan	Absent	62	5.805	.9373	23.404	.001
	Mild	25	7.076*	1.1282		
	Moderate	13	7.900*	1.9506		
	Total	100	6.395	1.3942		

Table 10 shows that liver stiffness values in absent NAFLD subgroup is <6 kpa and in mild and moderate NAFLD sub groups is >6 kpa indicating mild to moderate fibrosis (range 6-10kpa for mild to moderate fibrosis). There is significant difference in the mean liver stiffness values across the three subgroups of NAFLD among Hypothyroidism (using ANOVA test).

Discussion

The present study evaluated the clinical, hormonal, ultrasonographic, and biochemical parameters of 100 patients with hypothyroidism. The study population consisted of patients with both overt and subclinical hypothyroidism. Because of the proven association of NAFLD with hypothyroidism, we have calculated the prevalence of NAFLD in hypothyroidism and divided them into three subgroups - absent, mild, and moderate NAFLD (based on ultrasound evidence of fatty liver)[7].

Females outnumbered males in both the NAFLD stages (mild and moderate) and the total study population, which is in line with other studies like Pagadala et.al[8,9], the association between

NAFLD and thyroid dysfunction has been characterized by a relatively small sample and gender imbalance showing female preponderance. The mean age for male patients was 45.58 yr, and for females were 45.09 yr, which was fairly, correlated.

The mean age group across three subgroups, i.e., absent, mild, and moderate NAFLD among hypothyroidism, were 45.37 ± 10.6 years, 46.84 ± 11.1 years, and 39.15 ± 9.2 years, respectively. A study demonstrated that the prevalence of NAFLD increases with age[10]. Still, in our study, most NAFLD cases, i.e., 56.6%, were found in the age group 40-59 yrs. (p=0.97) which is statistically insignificant[10]

Based on the meta-analysis done[11], the risk of NAFLD was related to BMI in hypothyroidism patients (MD = 3.39, p= < .001). The probable reason for this may be due to limitations of BMI, which are most evident in cases of lean but metabolically unhealthy individuals who, despite a normal BMI, have increased central adiposity and a predisposition to cardiometabolic complications, including NAFLD[12]. This finding is in line with[13]whose results showed WC was the strongest independent predictor of NAFLD risk.

Measurement of the waist-to-hip ratio provides no advantage over waist circumference alone and is infrequently used by clinicians. It is not recommended as part of the routine obesity evaluation by the American Heart Association (AHA)/American College of Cardiology (ACC)/The Obesity Society (TOS) guideline. However, it was in the previous guidelines[14].

Observational studies revealed that 30% were shown to have a significant increase in total cholesterol and LDL levels among participants with overt hypothyroidism, and 90% had dyslipidemia. Treating the condition with levothyroxine could reverse the alterations in lipid, except in patients with hyperlipidemia[15]. We observed in our study population the following: high LDL (≥130 mg/dl) in 20% of patients, low HDL (≤50 mg/dl in females and ≤40 mg/dl in males) in 87.5%, and hypertriglyceridemia (TG≥150 mg/dl) in 25% of the study population.

The effect of subclinical hypothyroidism on lipid indices is not obvious, and there have been inconsistent results in clinical studies. Some observational studies showed no difference in lipid levels among patients with subclinical hypothyroidism and matched controls[16]. In contrast, others have a noticeable increase in total cholesterol, LDL-C levels, and triglycerides in subclinical hypothyroidism[17]. Possible confounding factors are smoking and insulin resistance, as such, because an increase in high cholesterol levels during hypothyroidism.

The results of our study revealed that the mean FIB-4 index was significantly associated with the severity of NAFLD Using a lower cut-off value of 1.45, a FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4-6, which includes early bridging fibrosis to cirrhosis). In contrast, a FIB-4 >3.25 had 97% specificity and a positive predictive value of 65% for advanced fibrosis. They revealed that the FIB-4 score could risk-stratify patients for morbidity and mortality related to liver conditions, with comparable performance to liver biopsy [18].

Conclusion

This study found a higher prevalence of NAFLD - 38 % among adults with hypothyroidism. Waist circumference (WC) and Subcutaneous abdominal adipose tissue (SAT) were significantly correlated with the severity of NAFLD. Liver function tests - AST, ALT, and alkaline phosphatase are statistically associated with NAFLD grades. Testing for thyroid hormones in assessing NAFLD is important as hypothyroidism is a modifiable risk factor. Further studies are needed on whether or not treating hypothyroidism can improve the progression and outcome of NAFLD.

References

1. Zelman S. The liver in obesity. Arch.Intern.Med.1958;90:141-156.
2. Ludwig J ,Viggiano TR,McGill DB, Ott Bj. Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease.mayo clin.proc.1980;55:434-438.
3. Powell EE, Cooksey WG,Hanson r et al. The natural history of non-alcoholic steatohepatitis: a follow up study of forty two patients for upto 21 years. Hepatology 1990;11:74-80.
4. PoonawalaA,NairSP,Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis; A case control study. Hepatology 2000; 32: 689-692.
5. Matteoni CA, YounossiZM ,Gramlich T et al. Nonalcoholic fatty liver disease .A sprectum of clinical and pathological severity. Gastroenterology 1999; 116; 1413-1419.
6. Pagadala, M.R., Zein, C.O., Dasarathy, S. et al. Prevalence of Hypothyroidism in Nonalcoholic Fatty Liver Disease. Dig Dis Sci 57, 528–534 (2012).
7. Lonardo A., Ballestri S., Mantovani A., Nascimbeni F., Lugari S., Targher G. Pathogenesis of hypothyroidism-induced NAFLD: Evidence for a distinct disease entity? Dig. Liver Dis. 2019; 51:462–470.
8. Liangpunsakul, S.; Chalasani, N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? J. Clin. Gastroenterol. 2003, 37, 340–343.
9. Pagadala, M.R.; Zein, C.O.; Dasarathy, S.; Yerian, L.M.; Lopez, R.; McCullough, A.J. Prevalence of hypothyroidism in nonalcoholic fatty liver disease. Dig. Dis. Sci. 2012, 57, 528–534.
10. Neeland IJ, Ayers CR, Rohatgi AK, Turer AT, Berry JD, Das SR, Vega GL, Khera A, McGuire DK, Grundy SM, de Lemos JA. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. Obesity. 2013 Sep;21(9):E439-47.
11. Bruinstroop E, Dalan R, Cao Y, Bee YM, Chandran K, Cho LW, Soh SB, Teo EK, Toh SA, Leow MK, Sinha RA. Low-dose levothyroxine reduces intrahepatic lipid content in patients with type 2 diabetes mellitus and NAFLD. The Journal of Clinical Endocrinology & Metabolism. 2018 Jul;103(7):2698-706.

12. Burhans MS, Hagman DK, Kuzma JN, Schmidt KA, Kratz M. Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Comprehensive Physiology. 2018 Dec 13;9(1):1.
13. Ergelen R, Yilmaz Y, Asedov R, Celikel C, Akin H, Bugdayci O, Altun E, Tuney D. Comparison of Doppler ultrasound and transient elastography in the diagnosis of significant fibrosis in patients with nonalcoholic steatohepatitis. Abdominal Radiology. 2016 Aug;41(8):1505-10.
14. Siddiqui MS, Vuppalanchi R, Van Natta ML, Hallinan E, Kowdley KV, Abdelmalek M, Neuschwander-Tetri BA, Loomba R, Dasarathy S, Brandman D, Doo E. Vibration-controlled transient elastography to assess fibrosis and steatosis in patients with nonalcoholic fatty liver disease. Clinical Gastroenterology and Hepatology. 2019 Jan 1;17(1):156-63.
15. Sharma P. Assessment of CRP level in patients with meningitis- A clinical study. J Adv Med Dent Scie Res 2018;6(12):68-71.
16. Du, SX., Lu, LL., Geng, N. et al. Association of serum ferritin with non-alcoholic fatty liver disease: a meta-analysis. Lipids Health Dis 16, 228 (2017).
17. Sachdeva A, Singh V, Malik I, Roy PS, Madaan H, Nair R. Association between serum ferritin and thyroid hormone profile in hypothyroidism. Int J Med Sci Public Health 2015;4:863-865
18. Lee J, Vali Y, Boursier J, Spijker R, Anstee QM, Bossuyt PM, Zafarmand MH. Prognostic accuracy of FIB- 4, NAFLD fibrosis score and APRI for NAFLD- related events: A systematic review. Liver International. 2021 Feb;41(2):261-70.