Study on Association of Hypothyroidism in Patients with Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is more common in subjects with obesity and diabetes mellitus (DM). Hypothyroidism is a risk factor for development of NAFLD. The study was done to assess the association of hypothyroidism with NAFLD and severity of NAFLD.

Methods: The study was carried out on 50 NAFLD patients. Among them 25 patients had no hypothyroidism and 25 patients had hypothyroidism. Histological diagnosis of NAFLD (non-NASH & NASH), based on NAFLD activity score (NAS) was done. Stages of fibrosis was evaluated by separate fibrosis score. Association of TSH with non-NASH, NASH and stages of fibrosis score were analyzed.

Results: Mean NAS, lobular inflammation and hepatocellular ballooning were significantly increased in high TSH group ($p < 0.05$). Majority 15 (60.0%) patients were non NASH in normal TSH group and majority 18 (72.0%) patients were NASH in high TSH group. The difference was statistically significant ($p = 0.022$) between the two groups. Positive correlation was found in between TSH level and NAS. Mean difference was statistically significant. Negative correlation between TSH level and stages of fibrosis was found which was not statistically significant.

Conclusions: There is significant positive association between NAFLD activity score and level of TSH and negative association between hepatic fibrosis score and serum TSH level.

Key words: hypothyroidism, non alcoholic fatty liver disease.

Abbreviations:

NAFLD - Nonalcoholic fatty liver disease;
DM - diabetes mellitus;
HCC - hepatocellular carcinoma.

INTRODUCTION

Macrovesicular fat accumulation in more than 5% of hepatocytes in the presence of less than 20 gm alcohol ingestion per day is called nonalcoholic fatty liver disease (NAFLD) (1).

NAFLD is one of the most prevalent liver diseases in Western countries, with a prevalence of 20%-30% in general population (2). The prevalence of NAFLD in India above 20 years age was 18.9% and increasing secondary to an increase in burden of diabetes mellitus (DM), metabolic syndrome and changing in lifestyle

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(3). NAFLD is seen in all age groups, prevalence peaks in the fourth decade in men and sixth decade in women. Recently in a study, it revealed that NAFLD is more prevalent in female among Bangladeshi population and prevalence of NASH was 42.4% in NAFLD which is much higher (4). NAFLD is more common in subject with obesity and diabetes mellitus (DM), it also occurs in lean and non-diabetic subject. NASH was found in 18.5% of obese patients, compare to 2.7% of lean patients (5).

Diagnosis of NAFLD is based on clinical history and examination with liver biopsy or imaging (Ultrasonography, computed tomography, proton magnetic resonance spectroscopy). The clinical consequence of NAFLD include nonalcoholic fatty liver (NAFL), nonalcoholic Steatohepatitis (NASH), NASH cirrhosis (6). Simple steatosis proved to be a benign form of NAFLD with minimal risk of progression. Instead, NASH progression to cirrhosis in up to 20% of patients and can subsequently determine liver failure or hepatocellular carcinoma (HCC) (7).

The pathological committee of the NASH clinical research network designed and validated a histological feature scoring system that addresses the spectrum of lesions of NAFLD and proposed NAFLD Activity Score (NAS) for use of clinical trial.

The scoring system comprises steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2) and a separate fibrosis staging (0-4).

The proposed NAS is the sum of steatosis, lobular inflammation and hepatocellular ballooning score. NAS of ≥ 5 correlated with diagnosis of NASH and biopsy with scoring of 3-4 suggestive of borderline NASH and less than 3 were diagnosed as non-NASH fatty liver⁸. Other pathological findings such as Mallory denk bodies or perisinusoidal fibrosis can make the diagnosis even stronger. Stages F0, F1, F2 were considered early fibrosis and Stage F3, F4 were considered to be advanced fibrosis (8).

Thyroid hormone plays an important role in the regulation of diverse metabolic processes. Decreased thyroid hormone concentrations may promote hyperlipidemia and obesity, thus contributing to NAFLD (9,10). Furthermore several animal studies showed that thyroid replacement therapy effectively decreased hepatic steatosis (11).

There is no proven cause-effect relationship between the two conditions. In Indian cohort, patients with NAFLD had higher serum TSH level and lower free thyroxine level than control subjects (12,13). In Germany cohort, there was a confirm association

between both subclinical and clinical hypothyroidism and hepatic steatosis (14).

Hypothyroidism and NAFLD

Thyroid hormone plays an important role in hepatic lipid metabolism, increasing hepatic lipogenesis and enhancing beta oxidation. All this action mediated by thyroid hormone receptor - beta which is mainly expressed in liver, also present in brain and kidneys (15,16). Thyroid hormone receptor-alfa is ubiquitously expressed. Decreased in thyroid hormone concentration in serum promote hyperlipidemia and obesity, thus contributing to NAFLD.

Furthermore several animal studies showed that thyroid hormone receptor agonist reduced hepatic steatosis (11). This finding suggested a link between hypothyroidism and NAFLD.

MATERIAL AND METHOD

It was an observational cross sectional study, carried out from July 2016 to August 2017, done in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

We included 50 patients of NAFLD from 18 to 60 years of age considering inclusion and exclusion criteria; 50% of patients had normal TSH and 50% of patients had high TSH. Those with significant alcohol intake (more than 30 gm/day in case of male and more than 20 gm/day in case of female), those having history of taking drugs that may cause fatty liver (i.e. tamoxifen, valproate, amiodarone, methotrexate), pregnancy, decompensated cirrhosis of liver, and co-morbid conditions (chronic obstructive pulmonary disease, chronic kidney disease, cardiac failure etc) were excluded from the study.

Before commencement, ethical clearance for the study was taken from the Institutional Review Board (IRB) of BSMMU. Patients attending outpatient department of Hepatology, BSMMU with sonological evidence of fatty liver was assessed by history taking and physical examination. Those given informed written consent, were finally included the study. Amongst the included patients, 25 were euthyroid, and 25 were hypothyroid.

After inclusion, blood sample from each case was drawn and biochemical investigations done. Before liver biopsy, we assessed coagulation and bleeding profile with informed written consent. All liver biopsy were done on in-patient basis. The percutaneous liver biopsy technique was applied in all cases. Liver biopsy specimens were preserved in 10% formol saline and

sent for histological evaluation. Patients were closely monitored for 24 hours following liver biopsy. Liver biopsy specimens were analyzed by an experienced pathologist who was blind to the clinical and biochemical results of the patients. The diagnosis of severity of NAFLD was based on the criteria of Brunt et al. 1999 (17), as modified by Kleiner et al. 2005 (8).

Metabolic syndrome defined according to Asian criteria and three of five listed criteria was considered: waist circumference ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg/dl (1.7 mmol/L), serum high density lipoprotein (HDL) cholesterol < 50 mg/dl (1.3 mmol/L) for women and < 40 mg/dl (1 mmol/L) for men, elevated blood pressure (systolic blood pressure ≥ 130 and or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and plasma glucose ≥ 100 mg /dL (5.6 mmol/l) or drug treatment for diabetes. BMI (kg/m^2) was measured and categorized into: Underweight < 18.5 , normal 18.5 – 22.9, overweight ≥ 23 – 24.9, obese 1 25 -29.9, obese 2 ≥ 30 .

In the histological scoring system, the degree of

disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of scores for steatosis (0 -3), lobular inflammation (0 -3), and hepatocyte ballooning (0 -2); therefore, the score ranged from 0 to 8. A NAS of 5 or more is diagnosed as “definitive NASH”, a NAS of 2 or less as “non-NASH”, and a NAS of 3 or 4 as “borderline NASH”. Diagnosis other than NASH was considered to be NNFL. The hepatic fibrosis staging was as following: 0 = no fibrosis only, 1 = zone 3 fibrosis (perisinusoidal or periportal), 2 = zone 3 and portal/periportal fibrosis, 3 = Bridging fibrosis and 4 = cirrhosis.

The statistical analysis was carried out using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). P value of < 0.05 was taken as significant.

RESULTS

The results are mentioned in *tables 1, 2, 3, 4* and *fig. 1*.

Table 1 - Demographic characteristics of the study patients (n=50)

Demographic characteristics	Normal TSH (n=25)		High TSH (n=25)		P value
	n	%	n	%	
Age (years)					
≤30	6	24.0	4	16.0	
31-40	9	36.0	13	52.0	
41-50	8	32.0	7	28.0	
>50	2	8.0	1	4.0	
Mean±SD	39.36±9.50		37.64±7.55		^a 0.482 ^{ns}
Sex					
Male	4	16.0	2	8.0	^b 0.384 ^{ns}
Female	21	84.0	23	92.0	
Occupational status					
Housewife	17	78.0	22	88.0	^b 0.121 ^{ns}
Businessman	2	8.0	2	8.0	
Others	6	24.0	1	4.0	

ns = not significant, ^aP value reached from unpaired t-test, ^bP value reached from Chi square test

Table 2 - Distribution of the study population by liver histology (n=50)

Liver Histology	Normal TSH (n=25)	High TSH (n=25)	P value
	Mean ±SD	Mean ±SD	
NAS score	3.96±1.67	5.24±1.36	0.004 ^s
Steatosis	1.40±0.91	1.88±0.78	0.051 ^{ns}
Lobular inflammation	1.28±0.45	1.68±0.47	0.003 ^s
Hepatocellular ballooning	1.28±0.61	1.60±0.50	0.048 ^s
Fibrosis score	1.36±0.75	1.40±0.70	0.846 ^{ns}

s=significant; ns=not significant; P value reached from unpaired t-test

Table 3 - Association between serum TSH level with NAFLD activity score (NAS) (n=50)

NASH	Normal TSH (n=25)		High TSH (n=25)		P value
	n	%	n	%	
0-4 (Non NASH)	15	60.0	7	28.0	0.022 ^s
5-8 (NASH)	10	40.0	18	72.0	

s=significant; P value reached from Chi square test

Table 4 - Association between serum TSH level with fibrosis score (NAS) (n=50)

Fibrosis score	Normal TSH (n=25)		High TSH (n=25)		P value
	n	%	n	%	
0-2 (Non fibrosis)	24	96.0	24	96.0	1.000 ^{ns}
3-4 (fibrosis)	1	4.0	1	4.0	

ns=not significant; P value reached from Chi square test

DISCUSSIONS

This observational cross sectional study was carried out among 50 patients with NAFLD. The purpose of the study was to assess the association of hypothyroidism in patients with NAFLD, and also with the severity of NAFLD.

Figure 1 - Receiver-operator characteristic curves of the serum TSH for prediction NASH. The cut off value >5.7 mIU/L, with 64.3% sensitivity and 68.2% specificity for prediction of NASH. Area under ROC curve was 0.691 established the fair accuracy of diagnostic test

Majority patients belonged to age 31-40 years in both groups, which was 9 (36.0%) in normal TSH group and 13 (52.0%) in high TSH group. The mean age was found 39.6 ± 9.50 years in normal TSH group and 37.64 ± 7.55 years in high TSH group. The mean age difference was not statistically significant ($p > 0.05$) between the groups. Female was predominant in both groups, which was 21 (84.0%) in normal TSH group and 23 (92.0%) in high TSH group. Male-female ratio was 1:5 in normal TSH group and 1:11 in high TSH group. The difference was not statistically significant ($p > 0.05$) between the groups. These observations are consistent with the study result done in 2015 by Parikh et al where they observed that, the mean age was found 42 ± 1.4 years hypothyroidism in NAFLD group and 44 ± 2.3 years non hypothyroidism group; male female ratio was 1.6:1 in hypothyroidism and 1.8:1 in non hypothyroidism group (12).

It was observed that in both groups, majority patients were asymptomatic, 12 (48.0%) in normal TSH group and 12 (48.0%) in high TSH group. Upper abdominal heaviness was 7 (28.0%) in normal TSH group and 7 (28.0%) in high TSH group. So, TSH level had no impact on producing the symptom of upper abdominal heaviness. Nausea was 5 (20.0%) and 4 (16.0%) in normal and high TSH respectively. The difference was not statistically significant ($P > 0.05$) between the groups. The study by Sweet et al. (18) found persistent nausea, vomiting, diarrhoea and abdominal pain in hypothyroidism. Our findings are not similar with those study symptoms.

Regarding clinical findings of the study population,

the mean weight, height, BMI, waist circumference, systolic BP and diastolic BP were not statistically significant ($p>0.05$) between the groups. Similar findings showed in Parikh et al. (12) where the mean weight, height, BMI, waist circumference, systolic BP and diastolic BP were not statistically significant ($p>0.05$) between the groups. This result was consistent with the current study.

Regarding component of metabolic syndrome of the patients, it was observed that presence of dyslipidaemia and T2DM was statistically significant ($p<0.05$). Dyslipidaemia higher in normal TSH level than high TSH level (76.0% vs 48.0%). T2DM was more in high TSH group 11 (44.0%) than normal TSH group 4 (20.0%). Obesity was found in both two groups which was 21 (84.0%) in normal TSH and 20 (80.0%) in high group. The association between thyroid dysfunction and metabolic syndrome may support the link between hypothyroidism and NAFLD. Xu et al in a study in 2011 (19) found significant correlation between thyroid dysfunction and metabolic syndrome parameter indirectly support the relationship between thyroid dysfunction and NAFLD. The current study found same correlation like the study result of Xu et al (19).

It was observed in liver histology that mean NAS score, lobular inflammation and hepatocellular ballooning were significantly increased in high TSH group ($p<0.05$). Similar result was found in Parikh et al. (12) where steatosis with ballooning was seen in 17 patients with hypothyroidism as compared to 7 patients with non-hypothyroid group.

Regarding NAS, majority 15 (60.0%) patients were non NASH (NAS 0-4) in normal TSH group and 18 (72.0%) patients were NASH (NAS 5-8) in high TSH group. The difference was statistically significant ($p=0.022$). Our findings are consistent with the data reported by Carulli et al. (20) and Pagadala et al. (24) suggested that the TSH concentration is associated with the severity of hepatic steatosis. These strengthen the notion of association between hypothyroidism and NAFLD.

Regarding fibrosis score of the patients, it was observed that in both normal and high TSH group majority patients were early fibrosis (Fibrosis score 0-2) had statistically no difference (96.0% vs 96.0%). Similar finding found in study by Parikh et al. (12) where there was no difference in the histological features of the two groups.

As per the study, the receiver-operator characteristic (ROC), constructed by using serum TSH, gave a cut off value >5.7 mIU/L, with 64.3% sensitivity and 68.2% specificity for prediction of NASH.

In present study, positive correlation was found in NAS and serum TSH ($r=0.292$; $p=0.039$) which was statistically significant. Correlation coefficient (r) value showed weak association in between TSH and NAS.

In this present study, there was negative correlation to fibrosis score with serum TSH level ($r=0.049$; $p=0.737$), but not significant between these two groups. Correlation coefficient (r) value showed negligible association in between TSH and fibrosis score.

CONCLUSIONS

There is a positive association between NAFLD activity score and level of TSH and no association between hepatic fibrosis score and serum TSH level. High serum TSH concentration is a risk factor for development of NASH. Serum TSH may be a predictor to differentiate non-NASH and NASH but may not be a predictor to differentiate different stages of liver fibrosis.

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Conflict of interest

The study was done in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

We all the authors state that we do not have any conflict of interest to report.

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