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INTERLEUKIN 10, THYROID STATUS AND FERRITIN ARE NON-INVASIVE PROGNOSTIC BIOMARKERS FOR DIAGNOSIS OF FATTY LIVER DISEASE IN CHILDREN

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AAM designed the study, wrote the protocol and interpreted the data. Authors KKEG, NSK and RAK anchored the field study, gathered the initial data and performed preliminary data analysis. Authors GMEM, AMA and GEH managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is one cause of a fatty liver, occurring due to deposition of fat (steatosis) in the liver. NAFLD in fact covers a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis. Survival is lower in patients with NASH based on the findings from long-term longitudinal studies. It is therefore imperative to distinguish simple steatosis from NASH in order to provide risk stratification and intervention slowing down disease progression for patients with the latter condition.
Aim of the Study: The present study aimed to find relevant non-invasive prognostic markers which might help minimize the frequency of liver biopsies to evaluate disease progression.
Patients and Methods: This case control study enrolled 50 child patients with NAFLD diagnosed by liver enzymes and hepatic ultrasonography. They were 30 males and 20 females (mean age 11±2.68 years). The control group included 50 healthy children, they were 19 males and 31 females (mean age 9.48±3.22 years).
Results: In this study, higher BMI, mean fasting plasma glucose, total cholesterol, LDL, triglycerides, CRP, total bilirubin, serum ALT, AST, GGT, iron, Ferritin, IL10 and APRI score along with lower HDL, albumin, TSH, free T3 and free T4, were all associated with NAFLD. While, Patients with grade 2 NAFLD had lower TSH, IL10 and higher Iron, Ferritin than grade 1 NAFLD patients.

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Conclusion: Decreased TSH, free T3 and free T4 and IL10 along with increased iron and Ferritin may all serve as non-invasive prognostic biomarkers for diagnosis of NAFLD grades.

Keywords: NAFLD; NASH; IL10; ferritin.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one cause of a fatty liver, occurring due to deposition of fat (steatosis) in the liver, not by chronic intake of alcohol in excess [1]. NAFLD in fact covers a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis [2]. Simple steatosis without fibrosis or inflammation has a benign clinical course in most but not in all cases without excess mortality [3,4].

Survival is lower in patients with NASH based on the findings from long-term longitudinal studies. It is therefore imperative to distinguish simple steatosis from NASH in order to provide risk stratification and intervention slowing down disease progression for patients with the latter condition. Liver biopsy remains the gold standard for making the diagnosis of NASH. However, this procedure is invasive, costly, and is associated with rare but potential complications and sampling errors; hence it is not suitable as a screening tool [5]. Imaging studies such as ultrasonography, computed tomography (CT), and magnetic resonance imaging have been used to diagnose NAFLD. These modalities have the advantages of being noninvasive and can be repetitively performed over a period of time. Nevertheless, none of them have sufficient sensitivity and specificity for staging the disease and cannot distinguish between simple steatosis and NASH with or without fibrosis [6]. The exact pathogenesis of NAFLD is not clear, however, several risk factors have been proposed, including advanced age, obesity, insulin resistance, hyperlipidemia, high serum Ferritin and thyroid dysfunction [7,8].

On the other hand, alcoholic steatohepatitis (the most inflamed condition in NAFLDs, which more frequently evolves towards chronic and serious liver diseases) is characterized by a marked activation of inflammatory cells and the up¬regulation of several soluble inflammatory mediators. Among several mediators, cytokines and chemokines might play a pivotal active role in NAFLD and are considered as potential therapeutic targets. IL-10 is considered as an anti-inflammatory cytokine that regulates the inflammation in several organs and tissues in physiological or pathological situations. It inhibits T cell-, monocyte-, and macrophage-mediated functions. In the liver, IL-10 has been detected in several cells, including hepatocytes, stellate cells, and Knuppfer cells, but only few studies have been performed to investigate the role of endogenous IL-10 in the progression of NAFLD. Emerging evidence suggests that the severity of NAFLD may associate with decreased concentration of mediators with anti-inflammatory actions, such as interleukin (IL) 10, [9]. Therefore, understanding the pathophysiology, risk factors and new treatment options of NAFLD/NASH should be among the priorities in the field of hepatology.

1.1 Aim of the Study

The present study aimed to find relevant non-invasive prognostic markers which might help minimize the frequency of liver biopsies to evaluate disease progression.

1.2 Patients

This case control study enrolled 50 child patients with NAFLD who were attending the El Sahel Hospital and Faculty of Medicine, Minoufia University. They were 30 males and 20 females and mean ages were 11±2.68 years. The control group included 50 age and sex matched healthy children, they were 19 males and 31 females, and mean ages were 9.48±3.22 years. Patients who had hypothyroidism, anemia, acute or chronic liver disease and who used corticosteroids were excluded from this study.

Written informed consent was taken from all participants in this study.

All patients and controls were subjected to: Full history taking, thorough clinical examination and laboratory investigations. The heights were measured by the same examiner. Weight was determined in underwear by using a calibrated electronic scale. BMI was calculated as weight (kg) / height (m^2). Obesity was defined as BMI $\geq 95^{th}$ percentile.

Laboratory investigations including: liver and renal function tests (ALT, AST, total bilirubin, albumin, GGT, Alkaline phosphatase and creatinine serum levels), total cholesterol and triglycerides, C-reactive protein, serum iron, serum ferritin, thyroid function tests (TSH, Free T3 and Free T4), IL10. Abdominal ultrasonography was performed to all cases.

The diagnosis of NAFLD was based on liver hyperechogenicity on ultrasound with or without elevated alanine aminotransferase (ALT). NAFLD was diagnosed when at least two of the following five abnormal ultrasonographic findings were present: (a) diffusely increased echogenicity ("bright") of the liver compared with the kidney, (b) unclear display of intra-hepatic lacuna structure, (c) slight-to-moderate hepatomegaly, (d) intrahepatic vessels undetectable, but with normal distribution of blood flow and (e) deep attenuation of the ultrasound signal, with the right hepatic lobe and diaphragm not seen. Mild NAFLD was diagnosed if point (a) plus any one of points (b) through (d) were present. Moderate NAFLD was diagnosed if point (a) plus any two of points (b) through (d) were present. Severe NAFLD was diagnosed if points (a) and (e) plus any two of points (b) through (d) were present (10).

2. METHODS

Eight ml of morning fasting blood samples were collected, then 2 ml of each sample were delivered into EDETA containing tubes for Complete blood count (CBC), using coulter counter model Beckman 750 Int, U.S.A., meanwhile, while 1ml was delivered into sodium fluoride-containing tubes for enzymatic colorimetric determination of blood glucose [11].

The remaining 5 ml was delivered into serum separator tubes and allowed to clot for 30 minute at room temperature. Then, serum was separated by centrifugation at 3000 rpm for 10 minute. Sera were divided into aliquots and kept in tightly closed aliquots at–20°C until analysis of the following;

- Measurement of liver and renal function tests (ALT, AST, total bilirubin, albumin, GGT, Alkaline phosphatase and creatinine levels using autoanalyzer SYNCHRON CX9ALX - Beckman Coulter Inc., CA, USA). AST to platelet ratio index (APRI) was calculated, for predicting significant fibrosis and cirrhosis [12] and the aspartate aminotransferase to alanine aminotransferase ratio (AAR) for fibrosis assessment [13].
- Determination of total cholesterol and triglycerides (TG) was done using colorimetric enzymatic method. HDLc was measured by the precipitation of chylomicrons, VLDL, and LDL by adding phosphotungstic acid and magnesium ions to the samples. Centrifugation leaves only the HDL in the supernatant, their cholesterol content is determined enzymatically [14]. LDLc was calculated when triglyceride concentration was less than 400 mg/dl by the formula of Friedewald et al. [15].
- Latex agglutination slide test for semiquantitive determination of C-reactive protein using Human kit, Germany [16].
- Quantitative colometeric determination of Iron using kits provided by Stanbio Laboratory 1261 North Main Street Boerne, Texas [17].
- Measurement of serum ferritin level by Enzyme Linked Immune Sorbent Assay (ELISA) technique (ELISA; Ramco Laboratories Inc, Stafford, Texas, USA) on Microplate reader (Bio-Rad 680 Hercules, California, USA) [18].
- Thyroid function tests (TSH, Free T3 and Free T4). Quantitative measurement of TSH in serum was done by using the immulite 1000 analyzer using a solid phase two site chemiluminescent immunometric assay capable of measuring TSH at low concentration [19]. Quantitative measurement of non-protein bound thyroxine (free T3 and free T4) was done by using immulite 1000 using direct one step hormone analogue immunoassay [20].
- Determination of IL10 levels using commercial ELISA kits (Immunodiagnostic Systems Limited, Bolden, UK) according to the manufacturer's recommendations [21].

2.1 Statistical Analyses

Statistical analyses were carried out with SPSS 18.0 software for Windows. Non-parametric correlation analyses were assessed using Spearman Rank Correlation. The 95% confidence interval (CI) and Odds ratio (OR) were used to estimate the risk for developing NAFLD. The criterion for significance was set at $P< 0.05$ for all the tests.

3. RESULTS

Baseline characteristics of controls and NAFLD patients are outlined in Table 1. BMI was significantly ($P< 0.0001$) higher in patients with NAFLD (33.2±6.02) compared to those without NAFLD (25.8±4.84). Mean fasting plasma glucose, total cholesterol, LDL, and triglycerides were significantly ($P<0.0001$) higher in patients with NAFLD while HDL was significantly lower in NAFLD patients than controls. Hemoglobin levels were not significantly changed in NAFLD patients compared to controls (Table 2).

Higher serum ALT and AST, GGT, total bilirubin and lower albumin were also significantly associated with NAFLD. TSH was highly significantly ($P<0. 001$) decreased in NAFLD patients (0.75 IU/L) compared to controls (3.95 IU/L), in addition, free T3 and free T4 were significantly ($P<0. 05$) lower in NAFLD patients (3.9 and 1 pmole/L, respectively) compared

to matching controls (4.2 and 1.25 pmole/L, respectively) (Table 2). NAFLD patients were also associated with significantly (P<0. 001) higher iron levels (205 µg/dl) compared to controls (9 µg/dl). The same was true for Ferritin levels, which were significantly (P<0. 001) higher in NAFLD patients (200 ng/mg) compared to controls (123 ng/mg). IL10 levels were also highly significantly lower (P<0. 001) in NAFLD patients (160 pg/ml) compared to controls (221 pg/ml).

Comparing CBC indices in both groups revealed no significant difference in either WBCs or INR, however, while platelet count was highly significantly (P<0. 001) decreased in NAFLD patients (200 / µL) compared to controls (250 / µL). It was also observed that APRI was highly significantly (P<0. 001) increased in NAFLD patients (0.27) compared to controls (0.12). The same was true for CRP which was significantly higher (P<0. 001) in NAFLD [15] than controls (4.8).

Non-parametric correlation analysis of thyroid stimulating hormone (TSH) level, Ferritin, and Interleukin 10 with anthropometric and biochemical features are listed in Tables 3, 4 and 5, respectively.

Table 1. Demographic and anthropometric characteristics of the studied groups

Parameters	Control group (n=50)	NAFLD group (n=50)	P value
	Mean±SD	Mean±SD	
M/F	19/31	30/20	0.033
Age (year)	9.48±3.22	11.0±2.68	0.006
BMI (Kg/m²)	25.8±4.84	33.2±6.02	<0.0001

NAFLD: Non-alcoholic fatty liver disease,
BMI: Body mass index

Table 2. Biochemical parameters of control and NAFLD groups

Parameters	Control	NAFLD	P value	t test
	N:50	N:50		
	Median	Median		
	(Min-Max)	(Min-Max)		
Fasting plasma glucose (mg/dl)	98 (85-160)	150 (90-220)	<0.0001	8.551
T.cholesterol (mg/dl)	165 (100-256)	190 (160-270)	<0.0001	6.558
HDLcholesterol (mg/dl)	42 (22-60)	35 (20-60)	0.0002	3.888
LDLcholesterol (mg/dl)	105 (89-134)	124 (99-202)	<0.0001	0.311
Triglycerides (mg/dl)	148 (121-245)	190 (87-300)	<0.0001	4.54
WBCs	7000 (3500-11200)	8500 (4500-10000)	0.0723	1.816
Platelet count (10^3/µL)	250 (142-420)	200 (142-320)	0.0006	3.547
INR	1.17 (1.0-1.7)	1.20 (1.0-1.7)	0.498	0.679
Hb(g/dl)	12.0 (9.0-15.0)	13.0 (9.0-15.0)	0.778	0.283
CRP(mg/l)	4.8 (0.2-18)	15 (1.0-56)	<0.0001	7.09
T. Bilirubin (mg/dl)	0.7 (0.4-1.0)	1.0 (0.5-3.6)	<0.0001	5.75
AST (U/l)	28 (12-58)	60 (25-180)	<0.0001	8.48
ALT (U/l)	28 (18-41)	60 (25-210)	<0.0001	7.89
GGT(U/l)	33 (24-59)	57 (15-105)	<0.0001	6.87
AAR	1.06 (0.41-2.16)	1.04 (0.43-2.25)	0.7293	0.347
APRI	0.12 (0.04-0.25)	0.27 (0.13-0.86)	<0.0001	8.76
Albumin (g/dl)	3.9 (3.5-4.2)	3.6 (2.1-4.2)	0.0001	4.14
Alkaline phosphatase (U/l)	186 (125-320)	199 (160-490)	0.0038	3.04
Creatinine (mg/dl)	1.0 (0.7-1.4)	0.9 (0.2-1.5)	0.0016	3.35
TSH(IU/l)	3.95 (1.3-14)	0.75 (0.4-10)	0.0008	3.56
Iron(µg/dl)	93 (60-345)	205 (80-321)	<0.0001	4.82
Ferritin(ng/mg)	123 (60-300)	200 (40-345)	0.0001	4.13
IL10(pg/ml)	221 (100-630)	160 (100-560)	<0.0001	4.49
F T3(Pmol/L)	4.2 (2.80-7.80)	3.9 (3.50-4.20)	<0.0001	5.083
FT4(Pmol/L)	1.25 (0.70-2.90)	1.00 (0.70-4.10)	0.026	2.256

Values are presented as median (minimum-maximum). Statistical t test with Welch correction. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein, AAR: AST/ALT ratio, TSH: Thyroid stimulating hormone; CRP: C-reactive protein, APRI: AST to platelet ratio index, INR: International normalized ratio; IL10: Interleukin10. FT3: Free triiodothyronine. FT4: Free thyroxine

Table 3. Non-parametric correlation analysis of thyroid stimulating hormone (TSH) level with anthropometric and biochemical features (spearman rank correlation)

Parameters	(95% confidence interval)	R	P
Age (year)	Rang = (-0.3311) - (0.0657)	-0.138	0.17
BMI (Kg/m²)	Rang = (-0.4831) - (-01167)	-0.311	0.0016
Fasting plasma glucose (mg/dl)	Rang = (-0.6197) - (-0.3046)	-0.4773	<0.0001
T.cholesterol (mg/dl)	Rang = (-0.4491) - (-0.0736)	-0.2716	0.0063
HDLcholesterol (mg/dl)	Rang = (0.0081) - (0.3952)	0.21	0.0361
LDLcholesterol (mg/dl)	Rang = (-0.4044) - (-0.0189)	-0.22	0.028
Triglycerides (mg/dl)	Rang = (-0.4329) - (-0.0536)	-0.253	0.0111
Platelet count (10³/µL)	Rang = (-0.0825) - (0.3160)	0.1216	0.2281
INR	Rang = (-0.2551) - (0.1479)	-0.0559	0.5805
Hb(g/dl)	Rang = (-0.0471) - (0.3476)	0.1565	0.12
CRP(mg/l)	Rang = (-0.6034) - (0.2808)	-0.457	<0.0001
AST (U/l)	Rang = (-0.6078) - (-0.2873)	-0.4625	<0.0001
ALT (U/l)	Rang = (-0.5001) - (-0.1387)	-0.332	0.0008
AAR	Rang = (-0.3176) - (-0.0807)	-0.1234	0.2214
APRI	Rang = (-0.5744) - (-0.2395)	-0.421	<0.0001
GGT(U/l)	Rang = (-0.3728) - (0.0182)	-0.185	0.066
Albumin (g/dl)	Rang = (-0.2212) - (0.1829)	-0.02	0.8434
Iron (µg/dl)	Rang = (-0.3907) - (-0.0028)	-0.205	0.041
Ferritin (ng/mg)	Rang = (-0.4217) - (-0.0399)	-0.24	0.0161
IL10(pg/ml)	Rang= (-0.3772) - (0.0131))	0.1896	0.0589
FT3 (Pmol/L)	Rang = (0.0776) - (0.4524)	0.2754	0.006
FT4 (Pmol/L)	Rang = (-0.1305) - (0.2717)	0.0736	0.4667

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein, AAR: AST/ALT ratio, TSH: Thyroid stimulating hormone; CRP: C-reactive protein, APRI: AST to platelet ratio index, INR: International normalized ratio; IL10: Interleukin10. FT3: Free triiodothyronine. FT4: Free thyroxine

Table 4. Non-parametric correlation analysis of ferritin level with anthropometric and biochemical features (Spearman rank correlation)

Parameters	(95% confidence interval)	R	P
Age (year)	Rang = (-0.1962) - (0.2080)	0.00618	0.9513
BMI (Kg/m²)	Rang = (-0.0175) - (0.3734)	0.1852	0.065
Fasting glucose (mg/dl)	Rang = (0.1257) - (0.4901)	0.3196	0.0012
T.cholesterol (mg/dl)	Rang = (-0.0711) - (0.3263)	0.1329	0.1873
HDLcholesterol (mg/dl)	Rang = (-0.3570) - (0.0364)	-0.1669	0.097
LDLcholesterol (mg/dl)	Rang = (-0.1350) - (0.2674)	0.069	0.4953
Triglycerides (mg/dl)	Rang = (-0.0691) - (0.3280)	0.1349	0.1809
Platelet count (10³/µL)	Rang = (-0.1456) - (0.2228)	0.0399	0.665
INR	Rang = (-0.1308) - (0.2371)	0.055	0.5508
Hb(g/dl)	Rang = (-0.2255) - (0.1785)	-0.0246	0.8085
CRP(mg/l)	Rang = (-0.0858) - (0.3130)	0.1184	0.2408
AST (U/l)	Rang = (0.0144) - (0.4006)	0.2159	0.031
ALT (U/l)	Rang = (-0.0386) - (0.3551)	0.1648	0.1014
APRI	Rang = (-0.0161) - (0.3746)	0.1867	0.063
AAR	Rang = (-0.1102) - (0.2906)	0.0939	0.3523
GGT(U/l)	Rang = (-0.1048) - (0.2956)	0.0994	0.3251
Alb g/dl	Rang = (-0.1570) - (0.2464)	0.0466	0.6454
IRON (µg/dl)	Rang = (-0.0279) - (0.3644)	0.1752	0.0812
TSH(IU/l)	Rang = (-0.4217) - (-0.0399)	-0.2401	0.0161
IL10(pg/ml)	Rang = (-0.1320) – (0.2702)	-0.0720	0.4764

Table 5. Non-parametric correlation analysis of interleukin10 with anthropometric and biochemical features (spearman rank correlation)

Parameters	(95% confidence interval)	r	P
Age (year)	Rang =(-0.0688)-(0.3283)	0.1352	0.1799
BMI (Kg/m²)	Rang =(0.1477)-(0.5070)	-0.3397	0.0005
Fasting glucose (mg/dl)	Rang= (0.1759) - (0.5282)	-0.365	0.0002
T.cholesterol (mg/dl)	Rang= (0.1141) - (0.4812)	-0.3091	0.0018
HDLcholesterol (mg/dl)	Rang = (-0.3327) - (0.0639)	0.14	0.1649
LDLcholesterol (mg/dl)	Rang = (0.1072) - (0.4757)	-0.3027	0.0022
Triglycerides (mg/dl)	Rang = (-0.0349) - (0.3583)	-0.1684	0.094
Platelet count (10^3/µL)	Rang = (-0.3927) - (0.0051)	0.2070	0.0388
INR	Rang = (-0.0828) - (0.3157)	0.1214	0.2290
Hb(g/dl)	Rang = (-0.0639) - (0.3327)	0.1400	0.1648
CRP(mg/l)	Rang = (0.2021) - (0.5475)	-0.3883	<0.0001
AST (U/l)	Rang = (0.1693) - (0.5232)	-0.3591	0.0002
ALT (U/l)	Rang = (-0.1808) - (0.2232)	-0.0221	0.8274
AAR	Rang = (-0.2125) - (0.1917)	-0.0109	0.9146
APRI	Rang = (0.1929) – (0.5408)	0.3802	<0.0001
GGT(U/l)	Rang = (0.0785) - (0.4531)	0.2762	0.0054
Alb g/dl	Rang = (-0.3491) - (0.0454)	0.1582	0.116
IRON (µg/dl)	Rang = (0.0098) - (0.3967)	-0.2115	0.0346
TSH(IU/l)	Rang = (-0.3772) - (0.0131)	0.1896	0.0589
Ferritin (ng/mg)	Rang = (-0.1320) - (0.2702)	-0.07203	0.4764

Table 6. Correlation between grade 1 and 2 of NAFLD and each of TSH, Iron, Ferritin and IL10 levels

Studied parameters	Grading		Mann-whitney test (U)	P value
	Grade I (n=27)	Grade II (n=23)		
TSH				
Mean±SD	3.93±3.73	0.69±0.16	3.01	0.003
Range	0.40–10.00	0.50–0.90		S
Iron:				
Mean±SD	162.78±65.78	246.13±44.48	4.52	<0.001
Range	80.00–300.00	199.00–331.00		HS
Serum ferritin (ng/ml):				
Mean±SD	167.52±72.53	270.04±55.29	4.75	<0.001
Range	40.00–344.00	190.00–345.00		HS
IL 10:				
Mean±SD	359.52±152.58	206.67±52.88	4.28	<0.001
Range	191.00–630.00	100.00–340.00		HS

4. DISCUSSION

NAFLD is a health problem affecting Egyptian community. The disease is so dangerous because it is what the National Institutes of Health refers to as a ‘‘silent disease’’. Non- alcoholic fatty liver disease develops over a long period of time, but many people experience few, if any, symptoms until the condition worsens to non-alcoholic steatohepatitis (NASH) or cirrhosis. This disease must not be ignored specially in our country; Egypt is considered a highest endemic area for prevalence of HCV infection. Fatty liver disease was found to be a component of metabolic syndrome, especially in adults this was evidenced by the significant correlations between liver size and BMI; SBP;DBP; waist circumference; hip circumference; SFT; VFT; fasting blood glucose; IR; and lipids which are components of the metabolic syndrome. Fatty liver is not only associated with overt obesity but also just overweight children can have enlarged liver [22].

In the present study, children with NAFLD showed higher BMI, mean fasting plasma glucose, total cholesterol, LDL, triglycerides, CRP, total bilirubin, serum ALT and AST, GGT, and APRI score along with lower HDL and albumin.

In accordance with this study, Palekar et al. [23], stated that patients with female gender, age ≥ 50 years, BMI ≥ 30 kg/m2, aspartate aminotransferase (AST) ≥ 45 U/L, AST/ALT ratio ≥ 0.8 are more likely

to have NASH than simple steatosis. While, Gholam et al. 2007 was able to predict NASH in a severely obese cohort (BMI ≥ 40 kg/m2) by simply using two markers, AST and the presence of diabetes.

Matched with the present study with significant increase in BMI in children with NAFLD, Meanwhile, the study of Ishibashi et al. [24] showing an association between abdominal obesity and NAFLD. While, the study of Rocha et al. [25] showed that waist circumference has been used as a measure of abdominal obesity.

The thyroid gland is significantly involved in energy homeostasis, lipid and carbohydrate metabolism, regulation of body weight and adipogenesis [26,27]. In a clinical setting, subclinical hypothyroidism has been associated with metabolic syndrome, cardiovascular mortality and disturbance of lipid metabolism [7]. In recent years, growing body of evidence has led to speculation on the association between NAFLD/NASH and thyroid dysfunction [1]. In the present study, TSH, free T3 and free T4 were lower in children with NAFLD than control and lower in patients with grade 2 than grade 1 NAFLD.

This is in accordance with the study of Pagadala et al. [28], who stated that hypothyroidism is more prevalent in patients with NAFLD when compared to healthy controls and may occur in more severe pathologic form of NAFLD.

In contrast to the present study, Xu et al. [29] and Carulli et al. [30], have shown higher free T4 and TSH respectively in NAFLD patients than healthy control.

Moreover, in the past few years, hepatic iron overload and its correlation with chronic liver disease have been considered (El gouhari et al. [31]). with progress in understanding iron metabolism in patients with hereditary hemochromatosis at the molecular level, accumulating evidence suggests a link between altered iron metabolism and NAFLD. In the last decade, many studies have found a relationship between hepatic iron and NASH or its progress [32].

The present study show a significant increase in iron and ferritin levels in children with NAFLD than control with more increase in patients with grade 2 than grade 1.

This result is in accordance with the study of Kowdley et al. [33], who revealed that elevated serum ferritin was associated with advanced hepatic fibrosis and greater iron accumulation in the body. The patients with an increased serum ferritin also had higher serum transaminases and gamma-glutamyl transferase, and a lower platelet count.

Cytokines are soluble molecules that are involved in intercellular communication, IL-10 is considered as an anti-inflammatory cytokine that regulates the inflammation in several organs. In the liver, it inhibits T cell-, monocyte-, and macrophage-mediated functions [34].

In the present study, serum IL10 levels significantly decreased in NAFLD children than control. On the other hand, in comparison of IL10 according to grade of NAFLD, serum IL10 was significantly decrease in grade 2 than grade1.

This is in accordance with the recent study by Paredes-Turrubiarte et al. [9], who found that IL-10 levels decreased in accordance with the severity of NAFLD, which supports a role for systemic inflammatory mediators in promoting steatosis progression.

The current result is also in agreement with the study of den Boer et al. [35], who suggested that endogenous IL-10 was protective against hepatic steatosis. Moreover, Cintra and co-workers [36], observed that the inhibition of IL-10 (either using an anti-IL-10 antibody or an IL-10 antisense oligonucleotide) led to increased expression of pro-inflammatory markers (TNF-α, IL-6, IL-1β, and F4/80) lead to increase hepatic steatosis.

5. CONCLUSION

Decreased TSH, free T3 and free T4 and IL10 along with increased iron and Ferritin may all serve as non-invasive prognostic biomarkers for diagnosis of NAFLD grades.

In conclusion, decreased TSH, free T3 and free T4 and IL10 along with increased iron, ferritin may all serve as non-invasive biomarkers for diagnosis of NAFLD. Further clinical prospective studies are needed to elucidate the role of IL-10 in the development of NAFLD while also establishing its clinical utility in the assessment of morbidly obese patients at higher risk to develop severe steatosis.

ETHICAL APPROVAL

The study protocol was approved by the ethics committee of the Faculty of Medicine, Minoufia University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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