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Research Article

Suboptimal Thyroid Function and Elevated ALT in Children and Adolescents with Severe Obesity: A Single Tertiary Center Experience

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Abstract

Background: Pediatric obesity is a public health issue. Elevated alanine transferase (ALT) may co-exist with suboptimal thyroid function in this population. **Objectives:** The aim of this cross-sectional study was to investigate and compare the association of thyroid function tests, ALT and metabolic profile in Greek children and adolescents with obesity and severe obesity. **Methods:** 279 children with body mass index (BMI) ≥95th percentile, were divided in two groups (obese: 95th≤BMI<99th percentile and severely obese: BMI≥99th percentile). Insulin resistance was defined as homeostatic model assessment of insulin resistance (HOMA-IR) ≥ 3. Screening markers of suboptimal thyroid and liver function were expressed as 0.7 ng/dl<FT4<1ng/dl, ALT>22mg/dl (females) and >26mg/dl (males) respectively. **Results:** Elevated ALT levels were found in children with FT4<11 ng/dl in comparison to peers with FT4≥1 (53.3% vs 25%, p: 0.024). Children with severe obesity were younger (p: 0.003) and had higher ALT levels (p: 0.001). In multivariate logistic regression, severe obesity status, FT4<1 and HOMA-IR ≥3 were predictive of higher ALT (ORs: 2.23, 3.37, 3.21 with p: 0.029, 0.04, 0.007, respectively). **Conclusions:** We suggest that children with severe obesity and insulin resistance are routinely screened for FT4 and ALT as surrogate markers of obesity related thyroid and liver dysfunction.

Keywords: Obesity; Pediatric; ALT; Thyroid; Insulin resistance; Liver steatosis

Introduction

Over the last decade, the rising prevalence and adverse outcomes of hepatic steatosis, to be referred as non-alcoholic fatty liver (NAFL), have been at the epicenter of global public health both in adult and pediatric populations. NAFL is of multifactorial etiology and is associated with obesity, insulin resistance, diabetes type 2, dyslipidaemia and hypertension [1,2]. Surrogate laboratory indices including elevated transaminases, abnormal lipid profile and markers of insulin resistance. Liver steatosis is found on ultrasonographic or magnetic resonance imaging. Progression to NAFL disease (NAFLD) may occur in a number of patients with NAFL [3-5], where hepatic steatosis is complicated by lobular inflammation, hepatocellular injury, and less frequently to non-

alcoholic steatohepatitis (NASH) with ballooning hepatocyte injury and fibrosis, potentially leading to end stage liver disease. Disease pathogenesis, risk and protective factors of event sequence are not completely understood [6].

According to published guidelines by the expert committees of the North American Society of Paediatric Gastroenterology, Hepatology and Nutrition and The American Association for the Study of Liver Diseases, the diagnosis of NAFL and NAFLD largely depends on clinical history, biochemical, imaging and less on histological criteria; these are reserved for more complex cases of NAFLD staging and diagnosis of NASH or cirrhosis, where liver biopsy is warranted [7]. Most patients with suspected NAFL/NAFLD are referred due to incidental radiological finding of hepatic steatosis [8].

Rising prevalence rates of NAFL/NAFLD parallels the sharp rise in childhood obesity over the past decades [9]. Available epidemiologic data from Europe demonstrate that NAFL in childhood and adolescence is a problem of global scale [10]. The central role of obesity as risk factor for pediatric NAFLD is underscored by cohort studies in children and adolescents, where reported prevalence rates range from 10% to 77% [11-16]. The mechanisms of progression of NAFL to NAFLD are incompletely understood.

Paediatric studies focusing on values of surrogate markers of liver function, such as serum alanine aminotransferase (ALT), are scarce in children with obesity and severe obesity; current evidence is largely extrapolated from adult studies [11]. NAFL prevalence is reported to be up to 30% in obese children and adolescents [17], therefore liver enzyme screening is recommended in children and adolescents with body mass index ≥95th percentile [18,19]. Elevated liver enzymes per se are not included in the adult NAFL/NAFLD diagnostic criteria, because individuals with normal ALT may have NAFL/NAFLD or NASH [20,21]. According to the recommendations of the pediatric SAFETY study, elevated ALT yields an estimate of hepatic steatosis similar to a paediatric population-based autopsy NAFLD prevalence, therefore ALT may be used as a screening tool in the general population [12, 22].

Insulin resistance in peripheral tissues is a putative pathogenetic mechanism which contributes to visceral adipose tissue expansion in obesity. Bile acids activate nuclear transcription receptors, such as the farnesoid X receptor, which regulate hepatic gluconeogenesis, glycogen synthesis and insulin resistance; impaired signaling therefore may be implicated in NAFL pathogenesis [23]. Ethnicity, sex, age, genetic, hormonal, metabolic, nutritional and environmental parameters including gut microbiota profiling, have been reported to have diverse effects on the hepatocyte metabolic milieu [24-28].

Thyroid hormone status is a key regulator of energy metabolism, while adverse alterations of body composition, lipid status, cardiac function, arterial blood pressure and various non-traditional cardiovascular risk factors are associated with various degrees of thyroid dysfunction, ranging from subclinical to overt hypothyroidism [16]. According to Marzullo et al. [29], in euthyroid obese subjects, FT4 appears more closely related than TSH levels, to parameters of cardio-metabolic risk. Hypothyroidism may possibly contribute to NAFL development, through its important role in lipid metabolism, fatty acid oxidation, hepatic lipid peroxidation [15,30]. The actual mechanism is presumed to be mediated by hepatokines, which are liver derived molecules, upregulated in hepatic steatosis and linked to metabolic dysfunction by impacting on glucose homeostasis through inter-organ communication [31]. Thyroid hormone receptors crosstalk with nuclear transcription factors in target tissues such as the liver, resulting in under-utilization of fatty acids, esterification and ectopic accumulation of triglycerides. Hypothyroidism is therefore associated with dyslipidaemia due to impaired insulin mediated lipolysis suppression in adipose tissue [32]. It therefore seems plausible to hypothesize a role for thyroid dysfunction in the pathogenesis of NAFL and NAFLD [30]. Furthermore, a potential link between altered thyroid function test and NAFLD has recently been reported in children [33,34].

The purpose of this manuscript is to present and discuss the findings of a single pediatric tertiary center cross-sectional study comparing ALT values in children and adolescents with obesity and severe obesity and their association with thyroid function tests.

Material and Methods

This is a cross-sectional retrospective study of Greek children and adolescents, without known history of liver disease, who attended our obesity clinics accepting nationwide referrals at one of the two major tertiary children's hospitals in Athens. Patient data had been collected over a ten-year period from 1st January 2010 until 31st December 2020. The study protocol was approved by the P. & A. Kyriakou Tertiary Children's Hospital ethics committee (Approval number 11482/29-06-2022) as per standard regional process. Written consent was waivered, because this study was observational and retrospective, data were derived from anonymized password protected clinical database of patients with obesity and severe obesity, who had attended clinic over the aforementioned time period.

For definition of obesity in children older than 2 years of age, BMI and BMI percentiles were used, as defined by the Centre for Disease Control and Prevention (CDC). In detail, obesity is defined as BMI ≥95th percentile and severe obesity as BMI ≥99th percentile for age and sex, according to the expert committee recommendations [35]. Demographic, anthropometric and laboratory data were collected, including age, sex, pubertal

status, BMI, ALT, fasting lipid profile, fasting serum glucose and insulin, thyroxine stimulating hormone (TSH) and free thyroxine (FT4) levels. Insulin resistance was defined as homeostasis model assessment of insulin resistance (HOMA-IR) ≥ 3.0 [36].

Elevated values of ALT ≥ 22 mg/dl for females and ≥ 25.8 mg/dl for males were used as biomarkers of possible liver dysfunction [22]. Suboptimal thyroid function was defined by low normal 0.7 ng/dl <FT4<1ng/dl [37]. Each participant underwent a detailed physical examination including anthropometric measurements and Tanner staging. Standing height was measured to the nearest 0.1 cm with a Harpenden fixed stadiometer and body weight was measured on a SECA balance scale to the nearest 0.1 kilogram (kg) by trained health care providers. BMI was calculated by dividing weight by height in meters squared (kg/m^2). Children with syndromic obesity (such as Prader Willi, Laurence-Moon-Bardet-Biedl syndrome), Cushing's syndrome or primary hypothyroidism were excluded from the analysis. Subjects with systemic conditions, including cystic fibrosis, inflammatory bowel disease, coeliac disease, infectious or autoimmune hepatitis, Wilson's disease, personal history of parenteral nutrition administration, cigarette, drug and alcohol use, family history of hereditary hyperlipidemia and/or premature atherosclerosis were also excluded. All participants were Caucasian.

Fasting blood glucose was measured by glucose oxidase technique (Siemens Advia 1800, USA) and insulin levels were analyzed by direct chemiluminescence technique (Siemens Centaur, USA). Insulin resistance was defined as homeostasis model assessment of insulin resistance (HOMA-IR) ≥ 3.0 [36]. HOMA-IR values were calculated from fasting plasma measurements with the use of the formula [insulin (mU/L) x glucose (mmol/L)/22.5]. Elevated values of ALT ≥ 22 mg/dl for females and ≥ 25.8 mg/dl for males were used as biomarkers of possible liver dysfunction [22]. Suboptimal thyroid function was defined by low normal 0.7 ng/dl <FT4<1ng/dl [37]. Thyroid hormone levels including FT4 and TSH were measured using a direct chemiluminescence technique (ADVIA Centaur XP, USA). The normal values for the respective ranges were between 0.8-5.4 μ IU/ml for TSH, and between 0.7-1.9 ng/dl for FT4.

Two hundred and seventy-nine (279 children) with obesity (47.3%) and severe obesity (56.7%), aged between 4 and 16 years old were included in the analysis (51.3% females), and were divided in two groups, as follows; Group 1 included subjects with obesity (95th percentile ≤BMI ≤99th percentile for age and sex), and Group 2 included subjects with severe obesity (BMI≥99th percentile for age and sex).

Statistical analysis

All continuous variables were tested for normal distribution using Kolmogorov-Smirnov statistic. Since they deviated from normality, they are described as median and Interquartile range (75th – 25th percentile) (IQR), therefore non-parametric tests were used. Categorical data are shown as absolute (N) and relative (%) frequencies. Any differences in the distribution of study participants' categorical characteristics compared across subgroups were evaluated using Pearson's χ^2 test or Fisher's exact test. The comparison of the distribution of continuous variables was performed using Mann-Whitney U statistic. The ALT values (gender dependent) that were described in the Methods section were used as the cut off points in order to construct the surrogate marker of liver dysfunction which was further used as the dependent variable of interest in both univariate and multiple logistic regression analysis. Due to the relatively small number of comparisons, no adjustment for inflation of type I error was applied. All tests were two-sided at a significance level of p<0.05. IBM® SPSS® software version 25.0 was used for the analysis.

Post hoc power analysis showed that in order to detect significantly higher differences in the proportion of the variables of interest (higher than 15%) at a significance level lower than 0.05, at the same time achieving statistical power equal to 70%, 137 subjects would be required for each study (obesity) group.

Results

Sex and pubertal status were comparable between the two groups of participants. The pairwise comparisons revealed that children with severe obesity (Group 2) were much younger (9.9 ± 5.4 *vs*. 11.3 ± 2.6 years old respectively, p: 0.003) and had higher BMI (p: <0.001). ALT median values were higher in children with severe obesity compared to their peers with obesity (21 ± 11 *vs*. 20 ± 8 respectively, p: 0.001). This finding was further supported when implementing ALT cut off values for categorization as 'normal *vs* abnormal liver function' as per Schwimmer et al. Higher percentage of children with severe obesity had elevated ALT, compared to children with obesity (41.9 % *vs.* 26.1% respectively, p=0.008).

		Obesity (N=132)		Severe obesity (N=147)		Total		
		Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**P**
Age (yrs)		11.3	2.6	9.9	5.4	10.7	4.0	0.003
BMI(kg/m^2)		27.5	4.2	31.8	7.5	29.3	6.0	<0.001
TSH(μiu/ml)		2.9	1.9	3.1	2.0	3.0	1.8	0.094
FT4 (ng/dl)		1.3	0.3	1.3	0.2	1.3	0.2	0.643
ALT (mg/dl)		20.0	8.0	21.0	11.0	21.0	9.0	0.001
HOMA-IR		3.0	2.6	3.4	3.4	3.1	2.9	0.328
Total Chol (mg/dl)		164.5	40.0	161.0	36.0	161	38	0.213
HDL(mg/dl)		50.0	13.0	47.5	13.0	48.0	13.0	0.059
LDL (mg/dl)		99.0	38.2	95.0	34.0	96.0	37.0	0.197
TG (mg/dl)		102.5	87.5	95.0	80.0	98	81	0.260
		N	**%**	**N**	**%**	**N**	**%**	**P**
ALT	Normal	88	73.9%	79	58.1%	167	65.5%	0.008
	Abnormal	31	26.1%	57	41.9%	88	34.5%	
Thyroid function	FT4<1	8	9.6%	7	6.9%	15	8.2%	0.504
	FT4≥1	75	90.4%	94	93.1%	169	91.8%	
Sex	Boys	63	47.7%	73	49.7%	136	48.7%	0.747
	Girls	69	52.3%	74	50.3%	143	51.3%	
Puberty status	Pre-pubertal	67	50.8%	86	58.5%	153	54.8%	0.194
	Pubertal	65	49.2%	61	41.5%	126	45.2%	
HOMA-IR	<3	57	43.2%	54	36.7%	111	39.8%	0.272
	≥3	75	56.8%	93	63.3%	168	60.2%	

Table 1: Descriptive statistics of the study population: demographic, clinical and laboratory characteristics.

The results of univariate logistic regression are shown in (Table 2) The odds of having elevated ALT values were 2.05 times higher among children with severe obesity than among children with obesity; the same trend (OR=2.54) was demonstrated in children with HOMA-IR ≥3 when compared to children with HOMA-IR<3 (p:0.008, 0.001 respectively). In addition, the odds of having elevated ALT was almost four times higher among children with fT4<1 than among children with fT4≥1 (p: 0.011). Both LDL<130 and TG<100 reduced significantly and close to 50% the odds of elevated ALT (p=0.037 and p=0.003 respectively).

Explanatory variable	Odds Ratio	[95%Conf.	Interval]	p
Exteme *vs* typical obesity	2.05	1.20	3.49	0.008
Constant	0.17	0.07	0.42	<0.001
Girls *vs* Boys	1.16	0.69	1.95	0.573
Constant	0.42	0.18	0.96	0.040
Tanner 2 *vs* 1	1.13	0.67	1.89	0.653
Constant	0.44	0.20	0.98	0.045
FT4<1 *vs* FT4≥1	4.1	1.38	12.17	0.011
Constant	0.37	0.26	0.514	<0.001
TSH (per unit increase)	1.13	0.96	1.33	0.152
HOMA-IR ≥3 *vs* <3	2.54	1.44	4.46	0.001
Constant	0.11	0.04	0.31	<0.001
Total cholesterol <200 *vs* ≥200	0.49	0.22	1.07	0.075
Constant	2.05	0.45	9.28	0.349
HDL<40 *vs* ≥40	1.87	0.92	3.81	0.084
Constant	0.24	0.10	0.58	0.002
LDL <130 *vs* ≥130	0.45	0.21	0.95	0.037
Constant	2.23	0.54	9.20	0.267
TG <100 *vs* ≥100	0.45	0.26	0.76	0.003
Constant	1.73	0.77	3.93	0.187

Table 2: Univariate logistic regression. Elevated ALT as surrogate marker of liver dysfunction is the dependent variable.

Multiple logistic regression modeling (Table 3) showed that the significant effects of severe obesity, low normal 0.7 ng/dl<FT4<1 ng/dl and HOMA-IR ≥3 on ALT values remained significant even after adjusting for the effect of the explanatory variables such as age, sex, LDL and TG (ORs: 2.23, 3.38, 3.21, p: 0.03, 0.04, 0.007, respectively as shown below).

Explanatory variable	Odds Ratio	[95% Conf.	Interval]	p
Severe *vs* Simple obesity	2.23	1.08	4.61	0.030
fT4<1 *vs* fT4≥1	3.38	1.02	11.12	0.046
HOMA-IR ≥3 *vs* <3	3.21	1.37	7.54	0.007
Age (1-year increase)	1.02	0.90	1.14	0.798
Girls *vs* Boys	0.90	0.44	1.82	0.766
LDL <130 *vs* ≥130	0.67	0.23	2.00	0.472
TG <100 *vs* ≥100	0.73	0.33	1.63	0.444
TSH (per unit increase)	1.00	0.80	1.24	0.988
Constant	0.05	0.00	1.49	0.084

Table 3: Multiple logistic regression analysis. Elevated ALT as surrogate marker of liver dysfunction is the dependent variable.

Discussion

Given the increasing prevalence of obesity and NAFL worldwide, it is essential to understand the inherent characteristics of children with obesity and to identify potential screening tools for risk stratification in this population. This single center cross-sectional study provides evidence that children and adolescents with severe obesity and insulin resistance have low normal FT4 and relatively higher ALT values.

Previous studies have confirmed strong association between insulin resistance and elevated ALT, for instance Shashaj et al showed a rising trend of ALT ≥40, when HOMA-IR increased above the 75th percentile for age and sex in normal weight and obese young Caucasians [38]. Our results reflect other cross-sectional studies, such as the study by Radulescu et al. [39] in a regional population of 2-18 years old children at risk for NAFLD in the United States of America, where elevated ALT was associated with obesity class rather than age. In our study younger patients had higher BMI and higher ALT; this finding could be explained by the shifting and rising trend of obesity presenting in younger ages globally, with Greece having a higher than average prevalence of childhood obesity with 16,7% obesity prevalence in boys and 10,64% in girls, according to the Global Obesity Observatory ranking [40]. It is however possible that age could also have an effect on normal baseline ALT values; in a Swedish cross-sectional pediatric study ALT has been shown to rise with age in obese boys but not girls, where abnormal ALT was defined as >27 U/L for males and >23 U/L for females, similar to the cut-offs implemented in our study

[41]. ALT demonstrated complex trends in different age groups in a large Chinese study of 1394 children aged 2-14 years old, with rising trend in males between 11-14 years of age [42]. No difference in ALT values between sexes was noted in our population.

As far as the absence of significant influence of pubertal status on ALT values is concerned, our findings are consistent with those of a multicenter longitudinal study by Koutny et al. [43]. In over a hundred thousand patient visits in 51 centers over 18 years, no significant ALT variation was reported with regards to Tanner staging in overweight and obese children and adolescents between 2-18 years of age from Germany, Austria and Switzerland.

An important new finding of the current study is that suboptimal thyroid function expressed by the low normal suboptimal values of 0.7 ng/dl<FT4<1 ng/dl, may be combined with significantly elevated ALT values in children with severe not simple obesity. TSH values however had no significant effect on ALT. The effect of low normal 0.7 ng/dl< FT4<1 ng/dl on elevated ALT values was outweighed by the severity of obesity (BMI) and insulin resistance (HOMA-IR), as shown in the multiple logistic regression analysis. Obesity and insulin resistance are presumably the key players in this interplay, pointing to a pathological metabolic milieu affecting various organs, manifesting as elevated ALT and suboptimal FT4 values. The nature of the association between liver and thyroid dysfunction in childhood obesity cannot

be established through this study, however we have shown that obesity related metabolic dysfunction can adversely affect thyroid and liver function tests in the severe end of the spectrum.

In adults, subclinical hypothyroidism has been considered a risk factor for metabolic syndrome and NAFLD. The concept of liver steatosis improvement through subclinical hypothyroidism treatment in adults has been raised [44,45].

Cross-sectional studies in adult populations have reported significant association between subclinical hypothyroidism (defined as TSH ≥ 4.1 m IU/L, FT4 0.7-1.8 ng/dl) and NAFL (diagnosed by ultrasonography and elevated ALT> 33/25 IU/L for males and females respectively, absence of known liver disease and alcohol consumption <20g/day) [46]. In a population based cross-sectional adult study in Germany, Ittermann et al found significant association between FT4 and ultrasonographic hepatic steatosis, but not with TSH and FT3 [47].

In children and adolescents however, findings regarding the association between NAFL and thyroid function remain controversial [34,48]. In our study, both FT4 and TSH levels have been reported, the focus being on FT4, because FT4 levels reflect directly the true thyroid status of the patient, TSH rather an indirect index. ALT levels were implemented as surrogate marker of liver dysfunction as several studies support that NAFL is positively correlated with ALT values which may be used as screening tool.

The strengths of this study include clear and novel hypothesis testing, founded on plausible pathophysiological mechanisms and based on clinical practice observations. The study participants were taken from a representative national obesity clinic register over a ten-year period, which allowed us to capture real world experience over a decade. The study sample calculation was performed post hoc on available data in order to ensure adequate statistical power to detect differences between the two groups. The study design was appropriate and focused on a well characterized population of interest that carries similarities with children and adolescents across the developed world; therefore, our findings are generalizable.

The limitations of this study include single tertiary center experience, inclusion of Caucasians only, retrospective study design with cross-sectional data, rather than multi center prospective longitudinal cohort study where additional thyroid function tests, liver enzyme parameters at various time points, liver ultra-sonographic or magnetic resonance imaging and follow up information over time could also be collected and analyzed sequentially.

In summary our data underline that adolescents with severe obesity, insulin resistance and low normal FT4 as surrogate of suboptimal thyroid function demonstrate significantly higher ALT values in comparison to their euthyroid peers with simple obesity. The current findings therefore underline the importance of thyroid and liver function screening of children and adolescents with severe obesity. Further larger scale studies are needed in order to describe and decipher the nature of the association between thyroid and liver dysfunction. Increased awareness and surveillance are required to promptly recognize and address obesity related comorbidities and their predisposing factors in children and adolescents.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

AK and SLE contributed equally as first authors to the conception of study design, data analysis and interpretation, and drafted and substantively revised the manuscript. FT contributed to critical revision of the manuscript. CM performed the statistical analysis of the study and contributed to data interpretation and critical revision of the manuscript. AE and ED contributed to data acquisition and interpretation. MK contributed to overseeing the laboratory aspect of data collection. EV contributed to the conception of study design, interpretation of data and substantive revision of the manuscript. All listed authors have approved the submitted manuscript and have accepted responsibility for the entire content of this submitted manuscript.

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