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Effect of obesity and NAFLD on leukocyte telomere length and hTERT gene MNS16A VNTR variant

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It is known that telomere length (TL) (evaluated with T/S ratio) is shortened in the presence of obesity. In this study, we aimed to investigate how obesity in adolescents and non-alcoholic liver disease (NAFLD) within the obese group affect TL and the clinical significance of the human telomerase reverse transcriptase (hTERT) gene MNS16A VNTR variant in terms of NAFLD. Adolescents with exogenous obesity and healthy controls (aged 10–19 years) who applied to our adolescent outpatient clinic between May-October 2023 were included in this study. We performed upper abdominal ultrasonography to investigate the presence of NAFLD in adolescents with obesity and divided into two groups: those without hepatosteatosis (obese NAFLD (-)) and those with hepatosteatosis (obese NAFLD (+)). We recorded body weight, height, waist circumference, and blood pressure measurements and measured the T/S ratio (telomere sequence copy number/gene single copy number) by the Quantitative Polymerase Chain Reaction method. The groups were compared using frequentist and Bayesian methods. Eighty-three obese adolescents [63 NAFLD(+) 20 NAFLD(-)] and 69 lean controls were included in the study. Pairwise comparisons revealed that T/S ratio was significantly lower in the obese NAFLD (-) group than the obese NAFLD (+) and the control group ($p = 0.025$, $p = 0.007$, respectively). T/S ratio was lower in the LL allele group than in the other alleles ($p = 0.022$) and slightly higher in the obese group with metabolic syndrome compared to the obese group without metabolic syndrome ($p = 0.072$). hTERT-MNS16A-VNTR gene variant LL allele had a negative correlation with T/S ratio among the obese adolescent group. Patients with LL alleles had higher ALT, GGT, HOMA-IR, and ALT/AST. Diastolic blood pressure had a significant correlation with the T/S ratio. The T/S ratio was shorter in the obese adolescent group compared to healthy ones but was higher in the NAFLD (+) obese compared to the NAFLD (-) obese. ALT level and ALT/AST ratio were higher, T/S ratio was lower in the hTERT MNS16A VNTR variant LL allele group among obese adolescents. In addition, there was a significant correlation between the T/S ratio and diastolic blood pressure in obese adolescents.

Keywords Telomere length, hTERT-MNS16A-VNTR gene, NAFLD, Obese adolescents, Metabolic syndrome

Non-alcoholic fatty liver disease (NAFLD) is a chronic metabolic disease with progressive hepatosteatosis without alcohol abuse or other liver diseases[1,2]. NAFLD causes liver-related morbidities and mortalities, and it has an association with chronic illnesses like hypertension and diabetes mellitus [3,4]. Obesity is associated with an increase in the prevalence and severity of NAFLD cases[5]. Adiposity, defined by body mass index (BMI), is a significant risk factor for NAFLD. Prospective studies in children show a clear association between childhood adiposity and adverse liver disease in adolescence[6].

Telomeres are nucleoprotein complexes that attach to the ends of eukaryotic chromosomes[7] and are of fundamental importance in maintaining genome stability, as the decrease in telomere length (TL) during cell division will initiate cellular senescence[8]. This type of cellular aging is commonly referred to as replicative

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aging and is linked to telomere biology. Telomere shortening limits the replicative capacity and the number of cells participating in tissue regeneration. Therefore, the regenerative potential of an organ depends on the cell population with sufficient telomere reserves necessary for cell proliferation. In chronic diseases associated with continuous tissue renewal, such as cirrhosis, a high regenerative effect occurs on a rapidly proliferating subpopulation of cells, which increases the telomere shortening rate by going through several cycles of cell division[9]. Genetic data indicate that NAFLD is common in patients with telomeropathy. This finding suggests that steatosis may be a consequence of hepatocellular aging or a trigger for the progression of liver disease, as seen in animal models[10,11]. In NAFLD patients, triggering factors such as obesity and insulin resistance also cause chronic liver damage and a regeneration state characterized by telomere shortening and aging in hepatocytes[12]. Obesity is also associated with chronic inflammation mediated by cytokines (and adipocytokines), increased production of reactive oxygen, nitrogen species (pro-oxidation, decreased anti-oxidation), and increased systemic oxidative stress[13,14].

Telomerase which consists of human telomerase reverse transcriptase (hTERT) and a telomere RNA component is the main regulator of telomere length[15,16]. It is an enzyme that protects the telomere ends that shorten with each division by using the RNA it carries in its structure as a template and prevents chromosome shortening. However, since it is in small amounts in somatic cells, telomere shortening and cell senescence continue over time. Telomerase has three subunits: human telomerase RNA (hTERC), human telomerase-related protein (hTEP1) or dyskerin, and human telomerase reverse transcriptase (hTERT). hTERC and hTEP1 constitutively express, so the enzymatic activity of telomerase depends on hTERT transcription[17]. The hTERT gene encodes the catalytic subunit of telomerase. The polymorphic tandem repeat minisatellites of hTERT (MNS16A) were first identified in a study on lung cancer patients. MNS16A, an antisense transcript of the hTERT gene, is located downstream of exon 16 of the TERT gene and upstream of the putative promoter region of an antisense hTERT transcript[18]. The MNS16A variable number tandem repeat (VNTR) polymorphism of the hTERT gene acts as a regulator of hTERT promoter activity[19]. A recent meta-analysis reported that this gene variant predisposes to cancer and that this is variable according to ethnicity (mostly in Asians)[20]. In NAFLD, telomerase activity is impaired with increased free fatty acid uptake at the cellular level and increased intracellular reactive oxygen species (ROS)[21]. However, there is no research yet on the relationship between NAFLD, which predisposes to hepatocellular carcinoma (HCC), and the hTERT-MNS16A-VNTR gene variant. Differentiating the effects of impaired telomerase activity or gene variants is important for NAFLD pathogenesis.

Adolescence is a separate period when obesity is frequently observed and includes NAFLD characteristics that differ from adults. Few studies aim to investigate the relationship between TL and NAFLD in the pediatric population and none in adolescents. In addition, although the relationships between obesity, NAFLD, and TL have been examined, the comparison between groups with and without NAFLD in obese individuals is also critical. Although the relationship between the hTERT VNTR MNS16A gene variant of telomerase, which plays a particular role in TL, and various cancers has been examined, it is necessary to investigate its relationship with NAFLD, which causes shortening in TL and causes HCC development at earlier ages. Therefore, in this study TL and the MNS16A VNTR variant of the hTERT gene in obese adolescents with and without NAFLD were examined, and association with clinical parameters were investigated by comparing it with the healthy adolescent group.

Materials and methods

We included obese and healthy adolescents aged 10–19 who applied to the Adolescent Health Outpatient Clinic of Istanbul University Faculty of Medicine, between May and October, 2023. The obese group consisted of exogenously obese non-syndromic adolescents with a BMI SDS ≥ 2, without additional chronic diseases. The control group included healthy adolescents without acute or chronic diseases, were not under medication, and had a BMI between -1 and $+1$ SDS. An experienced radiologist performed upper abdominal ultrasonography to examine the presence of NAFLD in adolescents with exogenous obesity. According to the ultrasonography results, obese adolescents were allocated into two groups: without hepatosteatosis (obese NAFLD (-)) and with hepatosteatosis (obese NAFLD (+)). We defined hepatosteatosis as grade 1 if there was a slight increase in echogenicity, grade 2 if the hyperechogenic liver appearance prevented the image of the portal vein wall, and grade 3 if the portal vein wall and diaphragm were not visible due to hyperechogenic liver[22]. After a comprehensive physical examination, we recorded body weight, height, waist circumference, and blood pressure. Height was measured with a tape measure fixed to the wall, with bare feet, feet together, and the adolescent's head, back, hips, and heels touching the wall. Body mass index was calculated by dividing body weight (kg) by the square of height (m2). Waist circumference was measured with a non-flexible tape measure from the middle of the distance between the lowest rib and the iliac crest while the participant was upright. Waist circumference and BMI SDS were noted by looking at the reference curves for Turkish children created by Neyzi et al.[23]. Those with a standard deviation of $+2$ SDS were considered obese patients, and between $-1/+1$ SDS were included in the lean control group. An experienced clinician performed arterial blood pressure measurements, and we calculated SDS values[24].

Istanbul University Istanbul Faculty of Medicine Ethics Committee approved this cross-sectional and single-center study (2023/594). Informed consents were obtained from the adolescents and their parents. The study was conducted by adhering the Decleration of Helsinki.

Laboratory analysis

After 12 hours of fasting, participants gave venous blood samples in an EDTA and a dry tube, and the laboratory studied biochemical parameters on the same day. Blood samples taken into EDTA tubes were stored at -20 degrees and given to the study collectively for genetic analysis. The laboratory isolated the Genomic DNA from blood and carried it into EDTA tubes, and used Quantitative Polymerase Chain Reaction (qPCR) with the Human

Relative Telomere Length Quantification qPCR Assay Kit (ELK Biotechnology, Wuhan, China). DNA samples were diluted between 0.5 and 2 ng/ µl. Two qPCR reactions were prepared for each genomic DNA sample, one for telomere sequence copy number and the other for reference gene single copy number. Reference gene single copy (SCR) primer specifically recognizes the 78 bp region on human chromosome 11. Also, the amplified samples developed a 78 bp long band in agarose gel. T/S ratios were calculated in each patient's sample with the 2-ΔΔCt formulation using the reference gene single copy number (S) and the telomere sequence copy number (T)[25]. The hTERT-MNS16A-VNTR variant analysis was performed with the PCR method. DNA samples were amplified under appropriate PCR conditions using F: 5'AGGATTTCTGATCTCTGAAGGGTG-3' and R: 5'-TCTGCCTGAGGAAGGACGTATG-3' primers for the region to be analyzed. Then, the samples were run on gel electrophoresis and genotyped under UV light. The L allele showed bands of 302 bp and 332 bp, and the S allele showed bands of 243 bp and 272 bp[26].

Statistical analysis

We assessed normality with the Kolmogorov-Smirnov test and Q-Q plots and presented data as mean+/-sd or median (interquartile range) by distribution. We used the Mann-Whitney u test for comparing two groups with non-normal distributed continuous data, the Student's t-test for normally distributed data, and the Welch t-test for normally distributed data with non-homogenous variances (variance homogeneity was tested with Levene's test). We used the Pearson test for the assessment of correlations after converting non-normal data to a normal distribution with Ln, square root, or Boxcox transformations and Kendall's tau test to compare groups with nominal data. A p-value < 0.05 was accepted for significance.

We built H1 (correlation) and H0 (independence) hypotheses for Bayesian calculations (stretched beta prior width set at 1) and presented Bayes factors as BF10, including evidence levels (anecdotal, moderate, strong, and very strong). We built a generalized linear model with the correlated variables and presented an estimated marginal means table and graph. We used the JAMOVI 2.3.18 statistical package program with *jsq*, *gamlj*, and *moretests* extension packs.

Results

We included 83 obese patients and 69 lean controls in the study. NAFLD was positive in 75.9% ($n=63$) of the patient group.

As we compared NAFLD – and NAFLD + groups among obese patients; serum ALT was higher in the NAFLD + group by 4 IU, GGT by 4.5, TG by 30.6, LDL by 14, transaminase index by 0.2, and t/s ratio by 268, where blood urea-nitrogen was lower by 1.4. The descriptive features and statistical results are presented in Table 1.

T/S ratio was prominently higher in control group compared with the obese group, but did not reach statistical significance (mean difference 366 units, $p=0.073$, Mann-Whitney u test).

T/S ratio was prominently higher in metabolic syndrome positive group compared with metabolic syndrome negative group among obese patients, but again did not reach statistical significance (mean difference 515 units, $p=0.072$, Mann-Whitney u test).

A total of 33.6% ($n=51$) had LL, 51.3% ($n=78$) had LS, and 14.5% ($n=22$) had SS alleles where 0.7% ($n=1$) did not have hTERT-MNS16A-VNTR gene variant among obese patients.

T/S ratio and htert VNTR gene associations among obese patient group

As we assessed the correlation co-efficients among obese patients regarding T/S ratio and hTERT-MNS16A-VNTR (binomial result as L/L and others), calculations revealed a very strong correlation between T/S ratio and diastolic TA SDS ($r=0.402$, $p<0.001$ (95%CI:0.196–0.574)). There was no significant correlation between gender and T/S ratio ($r=0.034$, BF10: 0.159, moderate evidence for independence), and gender and hTERT-MNS16A-VNTR gene variant ($r=0.151$, BF10:1.084, anecdotal evidence). But hTERT-MNS16A-VNTR gene LL allel had a negative correlation with T/S ratio ($r=0.194$, BF10:4.282, moderate evidence). hTERT-MNS16A-VNTR gene variant with LL allels had correlation with ALT level and ALT/AST index. Patients with LL alleles had higher ALT by 5.7 unit ($p=0.012$, Mann- Whitney u test), higher GGT by 3 unit ($p=0.053$, Mann-Whiyney u test), higher HOMA-IR by 1.6 ($p=0.012$, student's t test), and higher ALT/AST ratio by 0.2 ($p=0.055$, student's t test); which resulted as strong evidence for ALT, ALT/AST ratio, and anecdotal evidence for HOMA-IR and GGT (Bayesian kendall's tau test) (Table 2).

T/S ratio was independent from WC SDS, Systolic Blood Pressure SDS, HOMA-IR, AST, ALT, GGT, Total Bilirubin, Cholesterol, Triglyceride, BUN, Albumin, fT4, TSH, cortisol, ALT/AST index, and TyG levels with moderate evidence.

hTERT-MNS16A-VNTR gene variant was independent from BMI SDS, Systolic Blood Pressure SDS, diastolic Blood Pressure SDS, Total Bilirubin, Cholesterol, Triglyceride, LDL-cholesterol, creatinine, BUN, Albumin, and TyG levels with moderate evidence.

Diastolic blood pressure had significant correlation with T/S ratio (GLM, R2:0.195, LLR X2:24216). The graph (Fig. 1) and estimated marginal means table (Table 3) is presented.

T/S ratio and hTERT-MNS16A-VNTR gene variant associations among the control group

Individuals with hTERT-MNS16A-VNTR gene variant LL alleles had lower GGT by 3.5 units ($p=0.002$, Welch t-test which resulted as very strong evidence in Bayesian Kendall's tau test). There was no statistically significant difference between hTERT-MNS16A-VNTR gene alleles regarding T/S ratio ($p=0.15$, student's t test) among the control group.

	Total obese group ($n=83$)	Obese NAFLD (−) ($n=20$)	Obese NAFLD (+) ($n=63$)	Lean control ($n=69$)	p^*	p^{**}
Age	14.4+/-2.5	13.8+/-2.4	14.6+/-2.6	13.9+/-2.4	0.276	0.88
Female	45.8% ($n=38$)	26.3% ($n=10$)	73.7% ($n=28$)	49.3% ($n=34$)	0.664	0.864
Male	54.2% ($n=45$)	22.2% ($n=10$)	77.8% ($n=35$)	50.7% ($n=35$)		
BMI SDS	2.64+/-0.70	2.54+/-0.59	2.67+/-0.73	0.101+/-1.11	0.482	<0.001
WC SDS	3.43+/-0.66	3.02+/-0.53	3.54+/-0.65	0.80+/-0.66	0.004	<0.001
SBP SDS	0.60+/-0.92	0.41+/-1.07	0.65+/-0.87	-0.15+/-0.94	0.332	0.033
DBP SDS	0.99+/-0.85	0.60+/-0.65	1.09+/-0.87	0.35+/-0.92	0.042	0.294
HOMA-IR	4.23 (2.82–5.84)	3.41 (1.86–4.83)	4.64 (2.85–5.86)	1.96 (1.38–2.49)	0.214	<0.001
AST$^{(U/L)}$	19.8 (16.0-22.8)	19.1 (15.5–20.4)	19.9 (16.2–23.2)	18.6 (15.2–24.2)	0.163	0.594
ALT$^{(U/L)}$	20.0 (15.0-30.1)	17.1 (14.7–20.6)	21.7 (15.3–31.9)	13.1 (10.2–16.6)	0.064	0.046
GGT$^{(U/L)}$	16.0 (12.0-21.5)	12.5 (9.25-14.0)	17.0 (14.0–23.0)	10.0 (9.0–13)	<0.001	0.388
T. Bil.$^{(mg/dL)}$	0.39 (0.26–0.5)	0.42 (0.33–0.59)	0.35 (0.24–0.47)	0.44 (0.35–0.61)	0.084	0.893
Chol.$^{(mg/dL)}$	150.7+/-30.4	142.2+/-22.9	153.4+/-32.1	150.9+/-31.3	0.151	0.256
Trig.$^{(mg/dL)}$	97.1 (68.6128.3)	80.6 (65.0-106.9)	111.2 (74.2-144.8)	72.4 (55.4-112.9)	0.057	0.872
LDL-C$^{(mg/dL)}$	89.0 (73.5–109.0)	77.0 (73.5–91.0)	91.0 (74.0-113.5)	82.0 (75.0–94.0)	0.055	0.391
HDL-C$^{(mg/dL)}$	43.9 (37.4–50.3)	46.0 (40.6–55.3)	43.7 (37.3–48.6)	51.9 (43.8–57.5)	0.047	0.711
Kreatinin$^{(mg/dL)}$	0.61+/-0.13	0.62+/-0.10	0.62+/-0.14	0.59+/-0.14	0.83	0.421
Bun$^{(mg/dL)}$	10.5+/-2.5	11.6+/-1.9	10.2+/-2.6	10.4+/-2.9	0.023	0.075
Albumin$^{(g/dL)}$	4.72+/-0.26	4.70+/-0.23	4.73+/-0.27	4.73+/-0.27	0.535	0.608
fT4$^{(ng/dL)}$	15.3+/-1.8	15.6+/-1.4	15.2+/-1.9	15.7+/-2.5	0.396	0.95
TSH$^{(mIU/L)}$	2.61+/-1.37	2.48+/-1.28	2.65+/-1.40	2.14+/-1.1	0.707	0.258
Cortisol$^{(ng/mL)}$	9.61+/-4.32	8.52+/-4.05	9.87+/-4.42	10.02+/-4.76	0.502	0.558
Alt/Ast Ratio	1.13+/-0.40	0.98+/-0.19	1.18+/-0.44	0.78+/-0.30	0.116	0.001
TyG	8.29+/-0.53	8.11+/-0.40	8.35+/-0.55	8.06+/-0.50	0.076	0.689
T/S Ratio	2034 (1567–3030)	1852 (1035–2337)	2120 (1612–3421)	2470 (1783–3795)	0.021	<0.001
MNS16A-VNTR LL allele %	31.3% ($n=26$)	25% ($n=5$)	33.3% ($n=21$)	36.8% ($n=25$)	0.484	0.329

Table 1. The descriptive status and statistical results of the study group. *BMI SDS* body mass index standard deviation score, *WC* waist circumference. **Comparison between NAFLD + and NAFLD – obese patients. ** Comparison between NAFLD- obese patients and the control. All calculations were performed with students t test after transforming data to normal in case of non-normal distribution.*

T/S ratio and hTERT-MNS16A-VNTR gene variant associations between the NAFLD(+), NAFLD(-), and the control group

T/S ratio was statistically different among 3 groups: control, NAFLD (+) and NAFLD (−) ($p=0.009$, ANOVA using normalized values with BOXCOX formation). Pairwise comparisons revealed that T/S ratio in obese NAFLD (−) group was significantly lower from obese NAFLD (+) by 1198 unit ($p=0.025$) and the control group by 1074 units ($p=0.007$) (Tukey pairwise comparison test) (Fig. 2).

hTERT-MNS16A-VNTR gene variant LL alleles were at 25.0% in NAFLD (−), 33.3% at NAFLD (+), and 36.8% at control groups ($p=0.617$, chi-square test). T/S ratio was lower in LL allel group by 542.6 units compared with the other alleles ($p=0.022$, student's t test) across all groups (obese and control).

Discussion

In this study, where T/S ratio and hTERT-MNS16A-VNTR gene variants were examined in obesity, metabolic syndrome, and hepatosteatosis groups in the adolescent age group, T/S ratio was longer in NAFLD(+) obese adolescents compared to NAFLD(−) obese adolescents. In addition, HOMA-IR values were higher, and T/S ratio was lower in LL genotypic variant patients among obese patients. Moreover, T/S ratio was lower in LL variants across all groups (obese and control). There were no significant difference between healthy and obese adolescents in terms of T/S ratio, but T/S ratio was lower in the obese NAFLD(−) group compared to obese NAFLD(+) and control groups. In addition, T/S ratio was correlated with diastolic blood pressure in obese adolescents. Our study is the first study examining the hTERT gene MNS16A-VNTR variant regarding obesity and hepatosteatosis.

Although studies show that obesity and oxidative stress lead to telomere abrasion, decreased TL, and accelerated biological aging, the findings on this subject have a weak to moderate level of evidence[27]. Studies report that BMI was inversely correlated with TL in adults, and increased telomere abrasion and decreased TL with age were associated with increased morbidity and mortality in various diseases[27,28]. While increasing BMI resulted in higher blood volume, increased blood cells, and telomere shortening, weight loss was positively associated with telomere lengthening[13]. A recent meta-analysis examining the relationship between obesity and TL in children and adolescents has similarly shown that obesity is associated with TL shortening[29]. Tang et al.[30] examined considerable population data and revealed that long TL is associated with lower NAFLD incidence.

	T/S Ratio				hTERT-MNS16A-VNTR			
	Obese		Lean		Obese		Lean	
	Pearson	BF_{10}	Pearson	BF_{10}	Kendall	BF_{10}	Kendall	BF_{10}
Age	0.198	0.663 [AI]	0.053	0.166[MI]	0.167	1.703 [A]	093	0.293[MI]
BMI SDS	0.196	0.642 [AI]	0.132	0.264 [MI]	0.025	0.152 [MI]	0.119	0.409 [AI]
WC SDS	0.148	0.32 [MI]	0.174	0.389 [AI]	0.138	0.719 [AI]	0.062	0.212 [MI]
SBP SDS	0.122	0.248 [MI]	0.076	0.188 [MI]	0.070	0.222 [MI]	0.071	0.23 [MI]
DBP SDS	0.402	**90.2[VS]**	0.064	0.179 [MI]	0.066	0.213 [MI]	0.020	0.171 [MI]
HOMA-IR	0.037	0.147 [MI]	-0.101	0.219[MI]	0.166	1.526 [A]	-0.052	0.207 [MI]
AST(U/L)	0.059	0.159 [MI]	-0.142	0.291 [MI]	0.155	1.135 [A]	0.065	0.215 [MI]
ALT(U/L)	0.043	0.149 [MI]	-0.029	0.156 [MI]	0.234	**16.1[S]**	0.113	0.388 [AI]
GGT(U/L)	0.09	0.192 [MI]	0.022	0.169 [MI]	0.182	2.081 [A]	0.316	**51.9[VS]**
T. Bil.(mg/dL)	-0.078	0.185 [MI]	0.323	2.158 [A]	0.009	0.16 [MI]	0.049	0.21 [MI]
Chol.(mg/dL)	0.09	0.189 [MI]	0.020	0.157 [MI]	0.015	0.146 [MI]	0.041	0.182 [MI]
Trig.(mg/dL)	0.102	0.208 [MI]	-0.019	0.157 [MI]	0.028	0.153 [MI]	-0.064	0.213 [MI]
LDL-C(mg/dL)	0.184	0.532 [AI]	-0.028	0.159 [MI]	0.007	0.144 [MI]	0.034	0.176 [MI]
HDL-C(mg/dL)	-0.104	0.21 [MI]	-0.047	0.166 [MI]	0.106	0.387 [AI]	-0.151	0.757 [AI]
Crea.(mg/dL)	-0.023	0.141 [MI]	0.186	0.466 [AI]	0.056	0.189 [MI]	0.162	1.011 [A]
BUN(mg/dL)	0.107	0.216 [MI]	0.056	0.168 [MI]	0.059	0.194 [MI]	0.088	0.273 [MI]
Albumin(g/dL)	0.01	0.142 [MI]	0.246	1 [A]	0.058	0.195 [MI]	0.094	0.291 [MI]
fT4(ng/dL)	-0.052	0.156 [MI]	0.045	0.167 [MI]	0.165	1.385 [A]	0.121	0.428 [AI]
TSH(mIU/L)	0.012	0.141 [MI]	-0.029	0.161 [MI]	0.151	0.99 [AI]	0.068	0.223 [MI]
Cortisol(ng/mL)	0.103	0.258 [MI]	0.375	0.618 [AI]	0.122	0.363 [AI]	-0.178	0.524 [AI]
ALT/AST	0.012	0.14 [MI]	0.049	0.165 [MI]	0.233	**15.7[S]**	0.129	0.506 [AI]
TyG	0.097	0.2 [MI]	-0.047	0.165 [MI]	0.064	0.206 [MI]	-0.078	0.244 [MI]

Table 2. The descriptive status and statistical results of the study group. *BMI SDS* body mass index standard deviation score, *WC* waist circumference. **Comparison between NAFLD + and NAFLD – obese patients. ** Comparison between NAFLD- obese patients and the control. All calculations were performed with students t test after transforming data to normal in case of non-normal distribution*. Significant values are in bold.

In the meta-analysis conducted by Khosravaniardakani et al.[31], it is mentioned that telomere length decreased with weight gain in adults. However, this meta-analysis also suggested that age distribution might be related to telomere size. In our study, which examined the adolescent group with a limited age range, there was no significant difference between obese and control patients regarding T/S ratio.

Focusing on NAFLD diagnosis among pediatric age group, there are limited studies examining the relationship between TL and NAFLD in obese children. Ooi et al.[32] diagnosed NAFLD with liver enzyme levels and suggested that there was no relationship between TL and NAFLD in obese children. We evaluated NAFLD with ultrasonography and found that T/S ratio was lower in the obese group compared to healthy adolescents, but TL was higher in the NAFLD(+) group compared to the NAFLD(-) group within the obese group. In the study by Wojcicki et al.[33], which included histological liver findings and 96-week follow-up of TL course in NAFLD(+) children, prolonged TL was associated with increased lobular inflammation in the liver after liver biopsies. They suggested that this situation might lead to complications related to NAFLD in later ages[33]. Similar to this study, we found shorter T/S ratio in NAFLD(-) obese adolescents compared to NAFLD(+) obese adolescents in our study. In addition to Wojcicki's study, our study population consists of obese NAFLD patients. We predict that the increased T/S ratio in the NAFLD (+) group in our data might be because we studied with a more specific population group. When we focus on the adult period, a study conducted in the adult age group reported that TL was shorter in NAFLD (+) individuals with increased fibrosis, and TL was shortening with aging[34]. Since our study included adolescents, we could not perform diagnostic biopsy and assessment of fibrosis. In light of the literature and our data, we can say that TL was longer in obese patients compared with the control group and in NAFLD (+) obese adolescents compared with NAFLD (−) obese. But this situation reverses with the progression of NAFLD to fibrosis in adulthood.

There was no statistically significant difference between the NAFLD (−) and (+) groups by LL allele. However, the longer TL in the NAFLD (+) group compared with the NAFLD(−) group might be due to multiple factors, such as genetic/epigenetic factors, compensatory mechanisms in the early period of NAFLD presence, or metabolic/inflammatory features and abnormal fat distribution (in more critical regions) in NAFLD (−) group. However, we need extended studies to answer this question.

Regarding the studies examining the relationship between TL and blood pressure, Martens et al.[35] reported a negative correlation between telomere length and diastolic blood pressure in newborn children. In the same study, the authors did not find a relationship between TL and systolic blood pressure in newborns. Al-Attas et al.[36] also reported no relationship between blood pressure values and TL in the young child age group. A meta-analysis based on adult studies concluded that telomere length was shorter in hypertensive adults[37].

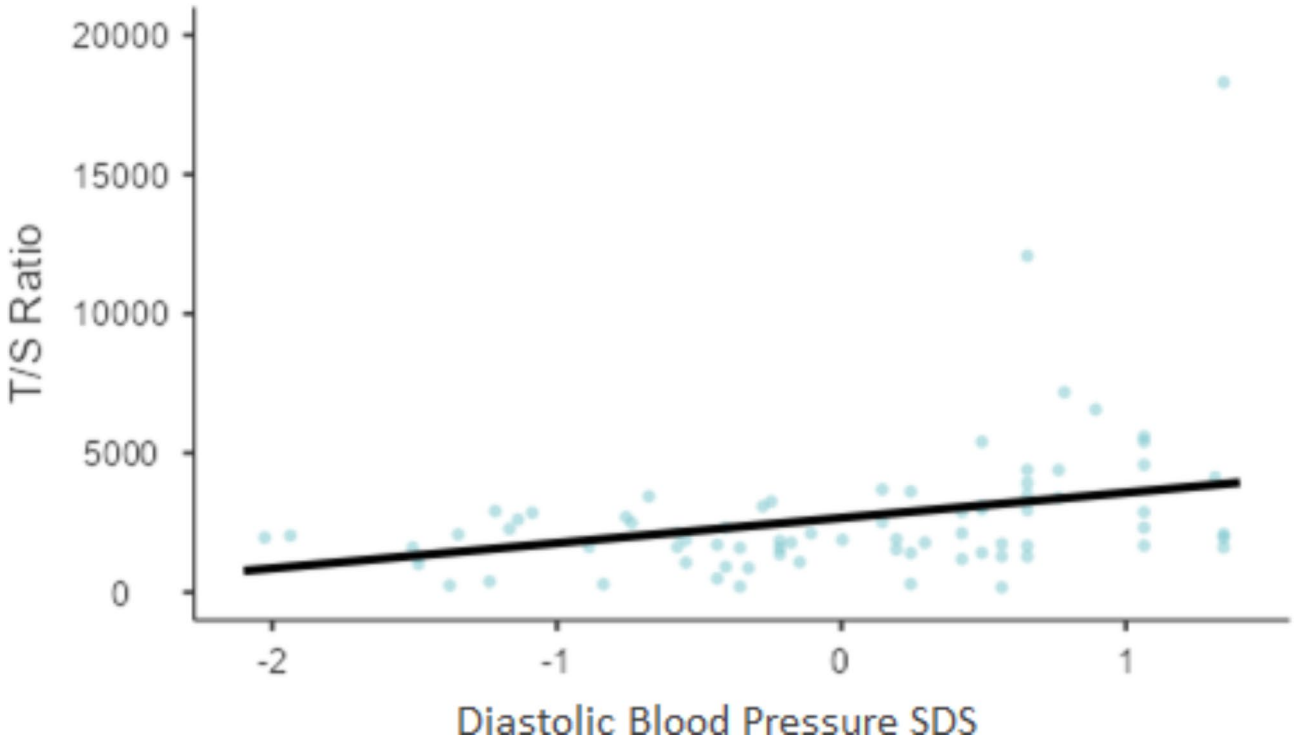

Fig. 1. The correlation graph regarding diastolic blood pressure SDS and T/S ratio among obese group (R^2:0.195, $p < 0.01$).

Diastolic blood pressure SDS	Mean T/S ratio
−2	861 (838–884)
−1	1762 (1748–1775)
0	2671 (2659–2682)
1	3579 (3560–3598)
2	4480 (4450–4510)

Table 3. The estimated marginal means table regarding diastolic blood pressure and T/S ratio.

However, another study conducted on adult patients reported a non-linear relationship between TL and diastolic hypertension: A positive correlation between diastolic blood pressure with TL at the early ages, a mild decrease, and then a constant continuation in diastolic blood pressure was observed in telomere lengths at later ages[38]. Our study results, in which we found a strong positive correlation between diastolic blood pressure and T/S in the obese adolescent group, fit this literature's findings. We predict that T/S can be considered a risk factor in the development of hypertension. This positive correlation between diastolic blood pressure and TL might be because of compensatory mechanisms in early hypertension, genetic influences, or lifestyle. We emphasize that similar findings suggesting compensatory mechanism were presented by Huang et al.[38]. However, we need multicenter and multinational prospective longitudinal studies for the development of diastolic hypertension in obese patients and the relationships between T/S, weight change, and diastolic blood pressure SDSs.

There is limited literature data on the hTERT-MNS16A-VNTR L/L genotype, but it is associated with a short life span in some studies[39]. The studies on the hTERT gene MNS16A-VNTR variant were conducted primarily with malignant patient groups[18,40]; hence, to our knowledge, this study is the first study examining the hTERT gene MNS16A-VNTR variant in an obese adolescent group. In our results, the LL variant was associated with high ALT, ALT/AST rate, HOMA-IR, and GGT in obese adolescents, and these results significantly demonstrated the relationship between the presence of NAFLD and the hTERT gene MNS16A-VNTR variant, especially for the LL allele. Huda et al.[41] investigated the effect of the hTERT gene MNS16A-VNTR variant in adult diabetic patients and reported that SS alleles had an association with type 2 diabetes. However, another study reported that the hTERT gene MNS16A-VNTR LL allele shortens human life[42]. In other studies in the literature, the MNS16A VNTR-243 short allele is seen more frequently in low-stage patients diagnosed with chronic lymphocytic leukemia, and longer TL was detected in these patients[43]. In our study, we found that the T/S rate in the LL variant was lower both in the obese patient group and in the whole study group (obese + control). However, the short allele does not always have a good prognosis, as the short allele was associated with progression and poor

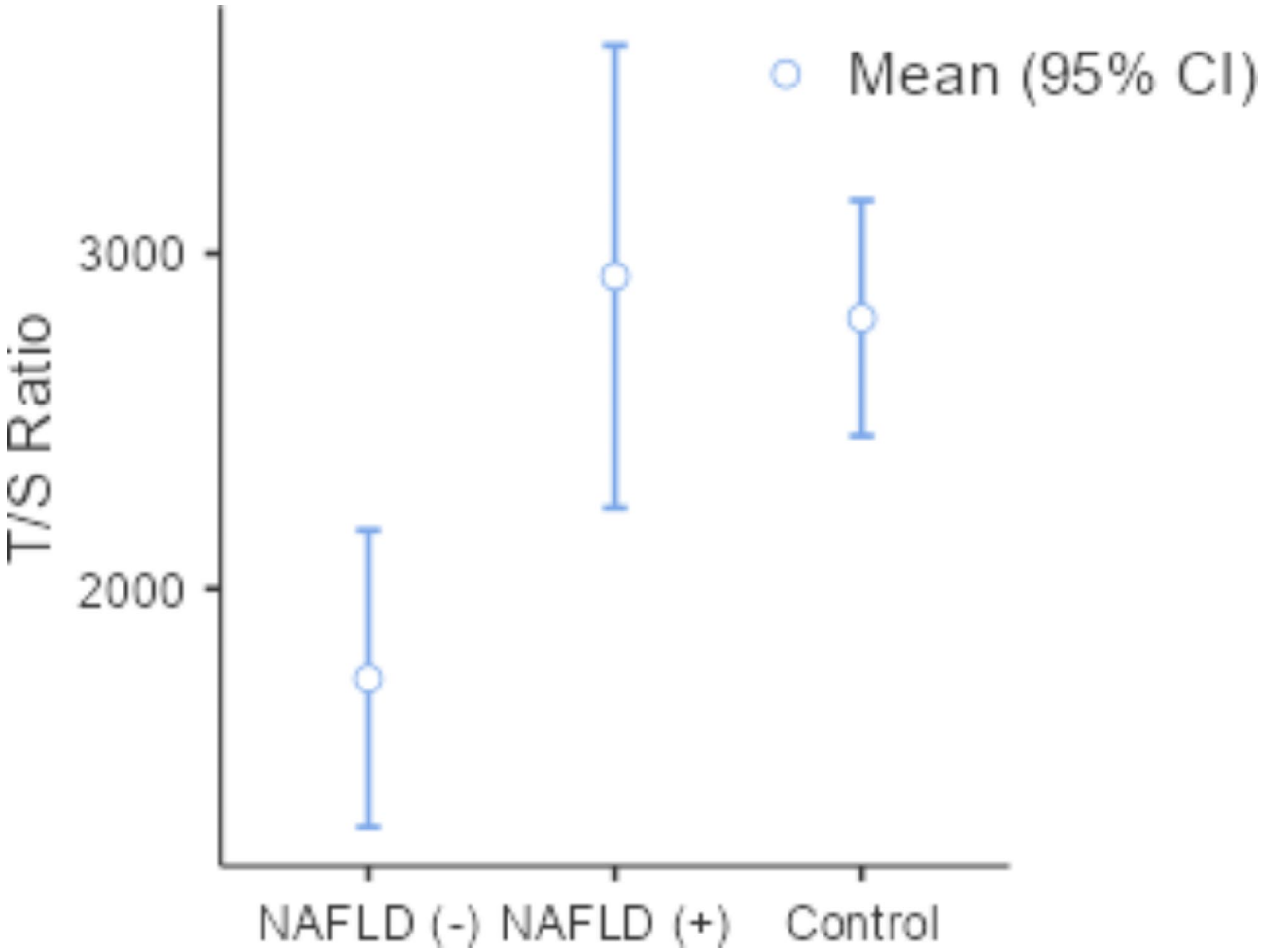

Fig. 2. Comparison of the three groups regarding T/S ratio. ($p = 0.009$).The table is formed with the exact values.

response to treatment in patients diagnosed with non-Hodgkin lymphoma and in patients diagnosed with non-Hodgkin B-lymphoma[44]. According to the literature data, we need more detailed studies on the MNS16A-VNTR long and short alleles.

Lastly, North American society for pediatric gastroenterology, hepatology and nutrition (NASPGHAN) suggests using ALT levels for screening NAFLD[45], hence European society for pediatric gastroenterology hepatology and nutrition (ESPGHAN) advices using ultrasonographic imaging with ALT levels[46], not to miss cases. However, we established the diagnosis with ultrasonographic imagings which were performed by a highly specialized radiologist.

The most important limitation of our study is that we established the diagnosis of steatosis with ultrasonography-there was no tissue diagnosis. In addition, our study was cross-sectional -we did not investigate the T/S alteration with treatment. Other limitations were that we did not include tanner stage, we did not diagnose NAFLD with magnetic rezonans imaging, and we did not assess metabolic reasons of NAFLD. The positive aspect of our study was that it evaluated a specific population, the obese adolescent age group. We think these are contributory analyses for this age group and reveal the relationship between T/S and NAFLD in young people. In addition, this is the first study to examine the hTERT gene MNS16A-VNTR variant in the pediatric age group.

Conclusion

As a result, T/S was higher in obese patients compared to the control group. T/S was higher in the NAFLD (+) compared to the NAFLD (-) among the obese group. Although T/S was higher in patients with metabolic syndrome, it did not reach statistical significance. T/S was lower, and ALT and ALT/AST ratios were higher in the LL allele of the telomerase MNS16A VNTR variant in obese adolescents. There was a strong positive correlation between T/S and diastolic blood pressure in obese adolescents. The relationship between T/S and NAFLD and metabolic syndrome in obese adolescents should be clarified with further studies. In addition, longitudinal studies on telomerase gene variants should be performed and evaluated with patient follow-up.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

Authors' contributions: Conceptualization: AYS, SK, FB, SPMethodology: IK, AYS, SK, FB, SPFormal analysis and investigation: IK, MSFigures: IK, MSWriting - review and editing: IK, AYS, YO, SK, MS, MTA, MP, FB, SPResources: IK, AYS, YO, SK, MS, MTA, MP, FB, SPSupervision: AYS, MP, FB, SP.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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