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Clinical Research Article

Elevated Liver Enzymes in Turner Syndrome: The Role of Low-grade Inflammation and Hormonal Imbalances

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Abstract

Context: Turner syndrome (TS) is a chromosomal disorder in females characterized by the partial or complete absence of 1 X chromosome. Women with TS face a higher risk of liver disease, elevated enzymes and fibrosis, potentially linked to inflammation, and hormonal imbalances, though the cause remains unclear.

Objective: This paper investigates the associations between liver parameters, inflammatory markers, and hormonal factors in women with TS compared with age-matched female controls.

Methods: We included 82 women with TS and 59 female controls. Participants underwent clinical examinations, anthropometric measurements, and fasting biochemical assessments of liver enzymes (γ-glutamyl transferase [GGT], aspartate aminotransferase [AST], alanine aminotransferase [ALT], FIB-4), inflammatory markers (C-reactive protein [CRP], soluble CD163 [sCD163]), sex hormones, and 11-oxygenated C19 steroids. We also assessed myeloperoxidase (MPO) and neutrophil elastase gene expression levels and performed FibroScan and dual-energy X-ray absorptiometry.

Results: Women with TS had higher levels of liver enzymes (GGT, AST, ALT) and FIB-4 than controls ($P < .001$, all). The inflammatory markers CRP and sCD163 were both correlated with elevated liver parameters in women with TS. Hormonal variables such as 11β-hydroxytestosterone levels, were also associated with elevated liver enzymes in women with TS. The neutrophil activation marker MPO was elevated in TS and correlated with liver parameters and sCD163.

Conclusion: Women with TS have elevated liver enzymes associated with low-grade chronic inflammation and hormonal imbalances. These findings highlight the importance of regular monitoring of liver function, inflammatory markers, and hormonal levels in women with TS to enable early intervention and potentially improve clinical outcomes.

Key Words: Turner syndrome, inflammation, liver dysfunction

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; CRP, C-reactive protein; DEXA, dual-energy X-ray absorptiometry; E2, estradiol; ELANE, neutrophil elastase; ERT, estrogen replacement therapy; FSH, follicle-stimulating hormone; GGT, γ-glutamyl transferase; HRT, hormone replacement therapy; KA4, 11-ketoandrostenedione; KT, 11-ketotestosterone; LH, luteinizing hormone; MPO, myeloperoxidase; OHA, 11β-hydroxyandrostenedione; OHT, 11β-hydroxytestosterone; OLS, ordinary least squares; PCA, principal component analysis; SHBG, sex hormone–binding globulin; TS, Turner syndrome; VIF, variance inflation factor; WBC, white blood cell count; WHR, waist–hip ratio.

Turner syndrome (TS) is a chromosomal disorder affecting approximately 1 in 2000 female live births, characterized by the partial or complete absence of 1 X chromosome [1]. Clinically, TS manifests with short stature, gonadal dysgenesis, estrogen deficiency, cardiovascular anomalies, and metabolic disturbances. Recent studies have highlighted that women with TS are at an increased risk of hepatic complications, including elevated liver enzymes and liver fibrosis, contributing to higher morbidity and mortality in this population [2, 3].

While the exact mechanisms underlying liver dysfunction in TS remain unclear, evidence suggests that chronic low-grade inflammation and hormonal imbalances may play pivotal roles [3-5]. Inflammatory markers associated with hepatic abnormalities indicate a state of heightened immune activation [4]. Also, hormonal factors, including estrogen deficiency and alterations in androgen metabolism, may exacerbate liver dysfunction, positively modulated by estrogen replacement therapy (ERT) [5-7]. Architectural changes, including nodular regenerative hyperplasia, multiple focal nodular hyperplasia,

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and cirrhosis have been observed in women with TS. These architectural changes are associated with congenitally abnormal vessels and are related to the risk of severe liver-related complications, such a portal hypertension, uncontrollable bleeding, and liver transplantation [8].

Despite these insights, there is a lack of studies that simultaneously examine the interplay between liver function, inflammation, and hormonal profiles in women with TS compared with healthy female controls. This gap limits our understanding of the pathophysiological processes that lead to hepatic complications in women with TS. Understanding this relationship is crucial for developing effective monitoring strategies and therapeutic interventions to mitigate liver disease risk in women with TS.

The present study aimed to investigate the associations between liver parameters, inflammatory markers, and hormonal factors in a cohort of women with TS and age-matched female controls. Here, we included all women with TS regardless of whether they received hormone replacement therapy (HRT) or not, and, as such, the study was not designed to investigate the impact of HRT on liver parameters. We hypothesized that women with TS exhibit elevated liver enzymes due to increased inflammation and hormonal imbalances and wish to elaborate the interplay between these biochemical markers. This study was designed as an exploratory study.

Materials and Methods

Study Design and Participants

This was a single-center, cross-sectional study, conducted in Aarhus, Denmark. We recruited through the Outpatient Clinic at the Department of Endocrinology, specializing in patients with sex chromosome disorders. For this study, age-matched female controls were recruited through websites (forsøgsperson.dk) and newspaper advertisement. Twenty participants from both the TS group and the control group were part of the EMKI project (NCT05425953), which included a dual-energy X-ray absorptiometry (DEXA) scan, and a FibroScan.

Inclusion and Exclusion Criteria

The inclusion criteria for the TS group were a confirmed diagnosis of TS and an age range of 18-60 years. For the control group, inclusion criteria required age-matched females. Specifically, we included all women with TS regardless of whether they received HRT or not.

Exclusion criteria for both groups included active cancer, ongoing infection, or disease at the time of enrollment. For the control group, individuals with autoimmune diseases were also excluded.

Demographics, Anthropometrics

Demographic data and body measurements were collected during clinical examination. Body measurements included height, body weight, and hip and waist measurements. Body weight was rounded off to the nearest 0.1 kg, and height, hip, and waist measurements were rounded off to the nearest millimeter.

Medical History

Medical history was retrospectively extracted from electronic patient records. The primary focus was on comorbidities potentially associated with increased liver parameters, including congenital malformations, metabolic conditions, and autoimmune diseases. The administration route of HRT was also recorded.

Biochemical Indices

All biochemical indices were analyzed in 1 batch to avoid analytical bias. We chose biomarkers based on existing literature and their practical applications. Fasting routine measurements of serum γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), high sensitivity C-reactive protein (hsCRP), follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone–binding globulin (SHBG), estradiol (E2), and testosterone were performed. Fibrosis was evaluated using the FIB-4 index with the following cutoff values: less than 1.3 for low risk, greater than 1.3 for moderate risk, and greater than 2.67 for high risk [9, 10]. Levels of soluble CD163 (sCD163) were measured using an in-house enzyme-linked immunosorbent assay, and values exceeding 3.86 mg/L were considered increased [11]. 11-Oxygenated androgens—including 11β-hydroxyandrostenedione (OHA), 11-ketoandrostenedione (KA4), 11β-hydroxytestosterone (OHT), and 11-ketotestosterone (KT)—were measured using an in-house liquid chromatography-tandem mass spectrometry assays (Sciex, Framingham, USA). The assay was calibrated using a 3-point calibration curve prepared from charcoal-stripped fetal bovine serum (Sigma-Aldrich, Darmstadt, Germany) spiked with accurately weighed pure compounds (Steraloids) and using stable isotope–labelled OHA as internal standard.

The gene expression level of neutrophil activation markers, neutrophil elastase (ELANE) and myeloperoxidase (MPO), was assessed in whole-blood samples in a subgroup of participants. Total RNA sequencing data was obtained and analyzed as described in a previously published study [12] (European Genome-Phenome Archive, EGAS00001006996). Normalized counts of ELANE and MPO were used for downstream analyses.

FibroScan and DEXA Scan

Transient elastography was performed by FibroScan (Ecchosense, France) at the Department of Hepatology & Gastroenterology, Aarhus University Hospital by a single operator (L.O.R.). FibroScan elasticity values above 8 kPa indicate increased fibrosis. Steatosis was assessed using controlled attenuation parameter (CAP) values, categorized into grades S1, S2, and S3. Specifically, CAP scores of 238 to 260 dB/m correspond to S1 steatosis (11-33% of the liver affected by fatty change), 260 to 290 dB/m to S2 steatosis (34-66%), and 290 to 400 dB/m to S3 steatosis (more than 67%), and here both categorical and continuous data are included. Body composition, including fat and muscle distribution, was assessed by DEXA using a Hologic QDR2000/w osteodensitometer (Hologic, Inc., Waltham, MA, USA), which were done at the Department of Endocrinology by an experienced biotechnician.

Statistical Analysis

Correlation analyses

To explore the relationships between liver parameters and explanatory variables, we conducted Pearson correlation analyses. Prior to analysis, skewed variables—including liver

enzymes and hormonal markers—were logarithmically transformed using natural logarithm to achieve normality and homoscedasticity, satisfying the assumptions required for Pearson correlation. Given the exploratory nature of our study and our aim to minimize Type II errors, we did not apply Bonferroni correction for multiple comparisons. While this approach increased the risk of Type I errors, it allows for the identification of potential correlations that may warrant further investigation. The numerous associations calculated, provide an overview for the reader and do not influence other statistical assessments in this paper, as more robust methods, such as Elastic Net regression, were employed to identify relevant predictors.

Elastic Net Regression and Multiple Linear Regression

To investigate the complex relationships among inflammatory markers, metabolic parameters, sex hormones, and 11-oxygenated C19 steroids with liver function, we employed an Elastic Net regression approach followed by multiple linear regression using ordinary least squares (OLS). Elastic Net regression was chosen for its ability to handle multicollinearity and prevent overfitting by combining the penalties of both Lasso (least absolute shrinkage and selection operator) and Ridge regression methods. We included 16 independent variables encompassing sex hormones (LH, FSH, SHBG, E2, and testosterone), inflammatory markers (white blood cell count [WBC], sCD163, and CRP), metabolic parameters (Hba1c), age, 11-oxygenated C19 steroids (OHT, KT, OHA4, and KA4) and anthropometric measures (body mass index [BMI], waist–hip ratio [WHR]). The Elastic Net model was applied using 10-fold cross-validation to select the optimal regularization parameters, alpha (α) and lambda (λ). The α parameter balances the Lasso and Ridge penalties, where an α of 1 corresponds to a pure Lasso model and an α of 0 corresponds to a pure Ridge model, with LASSO removing variables completely from the analysis. Cross-validation plots were inspected to confirm the selection of the optimal lambda, ensuring that the model was neither overfitted nor underfitted.

Variables identified as significant by the Elastic Net model were included in subsequent multiple linear regression models using OLS. To facilitate direct comparison of variable impacts, all variables in the multiple linear regression models were standardized by subtracting their mean values and dividing by their standard deviations. Due to uncontrollable confounding factors related to age and treatment status, we did not include ERT status in the multivariate analyses.

After fitting the OLS models, we assessed multicollinearity among the predictors using the variance inflation factor (VIF). If the Elastic Net model preferred an α of 1 (indicating a pure Lasso model), we calculated the VIF for each variable. Variables with a VIF greater than 2.5 were considered to exhibit significant multicollinearity and were omitted from the final model to improve model stability and interpretability [13].

We report both the adjusted R-squared from the final multiple linear regression models and the out of sample R-squared from the Elastic Net models (see Table S1 [14]) to evaluate model performance. The contributions of individual variables were interpreted based on their standardized OLS coefficients. This analytical approach was applied to the following liver parameters: ALT, GGT, AST, and FIB-4 score. Analyses were conducted separately for female controls and individuals with TS to assess differences in associations between explanatory and dependent variables across the 2 groups.

To determine whether the effects of independent variables on liver parameters differed between individuals with TS and female controls, we included interaction terms between the group variable (TS vs control) and each independent variable in the regression models. The inclusion of interaction terms allows for the examination of differences in variable effects across groups. Significant interaction terms indicate that the relationship between a predictor and a liver parameter differs between individuals with TS and controls, highlighting potential group-specific mechanisms.

We performed a principal component analysis (PCA) to visually explore the complex interplay between liver parameters and explanatory variables. Only biochemical variables were included in the PCA to focus on metabolic, hormonal, and inflammatory factors influencing liver function. This unsupervised dimensionality reduction technique helped in identifying patterns and clustering within the data, providing a visual guide to understand the underlying relationships.

Statistical analyses and graphs were performed using Stata 17.0. PCA was conducted using R with the packages "FactoMineR," "missMDA," "FactoInvestigate," and "factoextra." A 2-tailed *P* value of less than .05 was considered statistically significant for all tests.

Results

Participant Characteristics

A total of 82 participants with TS and 59 female controls were included in the primary analysis, with 58 (71%) of TS participants receiving ERT. The treated TS participants were, on average, 8 years younger than the untreated group (42.6 [40-45] vs 50.5 [45-56]). Of the 82 TS participants, 33 were mosaics, of which 3 had Y-chromosome material. The remaining 49 participants had a 45, X karyotype. Measurements of body composition and anthropometric data, such as height, weight, BMI, waist circumference, and WHR, varied significantly between female controls and participants with TS (Table 1). Height, weight, and WHR were the most dominant differences between TS and controls. Treatment, cardiac comorbidities, metabolic conditions and autoimmune diseases (Table 2) did not influence liver parameters when adjusted for age. Specifically, we found no differences in liver parameters when comparing oral and transdermal routes of HRT administration, and we found no difference between those receiving HRT and those not receiving HRT when we adjusted for age.

Investigating clinical and biochemical parameters, multiple differences were observed between the TS and female control group (Table 1). Liver function parameters, including FIB-4, GGT, AST, and ALT, were markedly higher in the TS group (Fig. 1). E2 levels were similar, and testosterone levels were lower in participants with TS than in controls, whereas LH, FSH, and SHBG levels were similar between the 2 groups. Inflammatory markers, such as CRP and WBC, but not sCD163, were elevated in participants with TS. Levels of 11-oxygenated C19 steroids, KA4, KT, OHA4, and OHT, did not differ between the groups. Comparing the treated and untreated TS, no statistically significant differences were found in liver parameters or inflammatory markers, except for sCD163, which was significantly higher in the untreated group (Table S2 [15]). Levels of sCD163 were generally with normal

Table 1. Comparison of anthropometrics and biochemical indices between females and TS

Variable	TS (n = 82)	Controls (n = 59)	*P* value
Anthropometrics			
Age (years)	46.2 (37.1, 55.0)	45.0 (29.1, 54.6)	.3
Height (cm)	149.4 (147.6, 151.2)	169.7 (168.0, 171.4)	<.001
Weight (kg)	61.3 (53.1, 70.4)	68.0 (62.2, 76.2)	<.001
BMI (kg/m^2)	26.9 (23.6, 31.5)	23.2 (21.9, 28.0)	<.001
Hip circumference (cm)	97.82 [95.4, 100.3]	100.3 [98.0, 102.7]	.1
Waist circumference (cm)	83.0 (72.0, 96.5)	76.0 (70.0, 85.1)	.011
Waist–hip ratio	0.86 (0.81, 0.91)	0.79 (0.75, 0.85)	<.001
Estrogen replace therapy % (yes/no)	70.1% (58/82)	0%	
Liver parameters			
GGT (U/L)	80.9 (23.7, 148.0)	18.0 (14.1, 23.5)	<.001
AST (U/L)	19.2 (12.5, 28.6)	10.9 (7.9, 15.5)	<.001
ALT (U/L)	32.0 (24.0, 49.2)	19.7 (15.2, 25.1)	<.001
FIB-4 index	0.61 (0.41, 0.85)	0.42 (0.30, 0.60)	<.001
Hormones			
Estradiol (pg/mL)	177 (35, 466)	95 (23, 324)	.1
Testosterone (ng/mL)	0.56 (0.39, 0.74)	0.86 (0.69, 1.09)	<.001
SHBG (nmol/L)	57.3 (40.8, 98.8)	61.2 (46.4, 87.3)	.6
LH (mIU/mL)	10.9 (5.6, 25.7)	11.7 (5.2, 36.3)	.5
FSH (mIU/mL)	16.1 (8.2, 43.4)	8.10(4.6, 68.3)	.3
Inflammatory markers			
sCD163 (ng/mL)	1.40 (1.05, 2.02)	1.60 (1.21, 2.11)	.08
CRP (mg/L)	2.77 (0.99, 5.51)	0.70 (0.36, 2.01)	<.001
WBC	5.9 (5.2, 6.8)	5.2 (4.2, 6.4)	.003
Platelet count (10^9/L)	248 (236, 261)	239 (226, 253)	.3
11-C19 steroids			
KA4	0.69 (0.55, 0.92)	0.64 (0.50, 0.80)	.3
KT	1.00 (0.63, 1.39)	1.03 (0.78, 1.46)	.5
OHA4	4.38 (3.06, 6.10)	4.08 (3.22, 4.51)	.3
OHT	0.43 (0.29, 0.64)	0.50 (0.40, 0.63)	.06
Substudy	TS n = 20	Female control, n = 20	
Age (substudy, years)	37.9 (31.7, 44.1)	34.5 (28.1, 40.9)	.4
FibroScan			
CAP value (dB/m)	237 (208, 261)	227 (207, 247)	.6
Liver stiffness	4.25 (3.57, 5.30)	3.90 (3.50, 4.95)	.4
DEXA scan			
Total fat mass (%)	39.4 (36.4, 42.4)	37.1 (34.4, 39.9)	.3
Total lean mass (%)	57.4 (0.55, 60.1)	59.5 (56.9,62.1)	.2
Abdominal fat (%)	37.0 (33.1, 41.0)	32.8 (29.3, 36.3)	.2

This table shows the differences in median (25th, 75th IQR) and mean (95% CI) values of anthropometrics, liver parameters, inflammatory markers, C19-steroids, and hormones. Additionally, a substudy is presented with participants who had FibroScan and DEXA scan.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CRP, C-reactive protein; DEXA, dual-energy X-ray absorptiometry; FIB-4 Index, Fibrosis-4 Index; FSH, follicle-stimulating hormone; GGT, γ-glutamyl transferase; KA4, 11-ketoandrostenedione; KT, 11-ketotestosterone; LH, luteinizing hormone; OHA4, 11β-hydroxyandrostenedione; OHT, 11β-hydroxytestosterone; sCD163, soluble CD163; SHBG, sex hormone–binding globulin.

ranges in both the control and TS groups, and only 1 with TS had scores above the reference limit of 3.86 mg/L.

In the substudy involving 20 TS participants and 20 female controls who underwent FibroScan and DEXA scan assessments, no statistically significant differences were found in CAP values, median liver stiffness, total fat mass, total lean mass, or abdominal fat percentage between the groups (Table 1).

Regarding the FIB-4 index, 5 individuals in the TS group were at moderate fibrosis risk and 1 at high fibrosis risk. In the control group, 1 individual exhibited a moderate fibrosis risk according to the FIB-4 index. Regarding FibroScan elasticity measurements, only 1 individual with TS had a value exceeding 8 kPa, indicating that most participants had minimal or no fibrosis (F0-F1) (Table 1). For steatosis grading based on CAP values, the control group had 1 individual at

S1, 6 at S2, and 1 at S3. In the TS group, 3 individuals were graded as S1, 3 as S2, and 5 as S3. Leukocyte levels had high unadjusted correlation with CAP values but only in women with TS (β-coef. 18, $P < .01$).

Table 2. Cardiac, metabolic, autoimmune conditions, and HRT use among the 82 TS patients in the study

	TS = 82	%
Cardiac abnormalities		
Aortic ectasia	19	23.2%
Aortic insufficiency	5	6.1%
Aortic stenosis	3	3.7%
Bicuspid aortic valve	15	18.3%
Metabolic complications		
Hypertension	44	53.7%
Type 2 diabetes	14	17.1%
Autoimmune conditions		
Hypothyroidism	29	35.4%
Type 1 diabetes	2	2.4%
Ulcerative colitis/Crohn disease	2	2.4%
Coeliac disease	4	4.9%
Hormone replacement therapy		
No HRT	24	29.2%
Transdermal HRT	9	11.0%
Oral HRT	49	59.8%

Abbreviations: HRT, hormone replacement therapy; TS, Turner syndrome.

Correlation Between Liver Parameters and Biochemical Indices

In the TS group, multiple 11-oxygenated C19 steroids—specifically KA4, OHA4, and OHT—showed significant positive correlations with GGT and ALT (Table 3). sCD163 was identified as the strongest predictor for liver parameters in both TS and control groups, independent of HRT. Age was positively correlated with GGT in TS. Overall, GGT and the FIB-4 index exhibited the highest number of significant correlations with other independent variables. In contrast, variables such as SHBG, LH, and BMI did not show significant correlations with liver parameters in either group.

Within the substudy including participants who underwent FibroScan and DEXA scan assessments, liver stiffness was significantly correlated with GGT in both women with TS and controls, while FIB-4 only was correlated with liver stiffness among TS, but not in controls (Table 3).

For the dependent variable the FIB-4 index, the elastic net model explained 23% of the variance in FIB-4 scores in participants with TS (Table 4). Significant predictors included CRP, BMI, WBC, OHT, sCD163, and SHBG. Notably, BMI and WBC were negatively correlated with the FIB-4 index. In controls the model accounted for 40% of the variance in FIB-4 scores. Significant predictors were SHBG, FSH, and testosterone.

For ALT, the model explained 13.9% of the variance in ALT levels among TS. Significant predictors were sCD163 and OHT. In controls the model accounted for 17.9% of the variance in ALT levels. Significant predictors were E2 and sCD163.

Regarding GGT the model explained 36.5% of the variance among TS. Significant predictors were CRP, CD163,

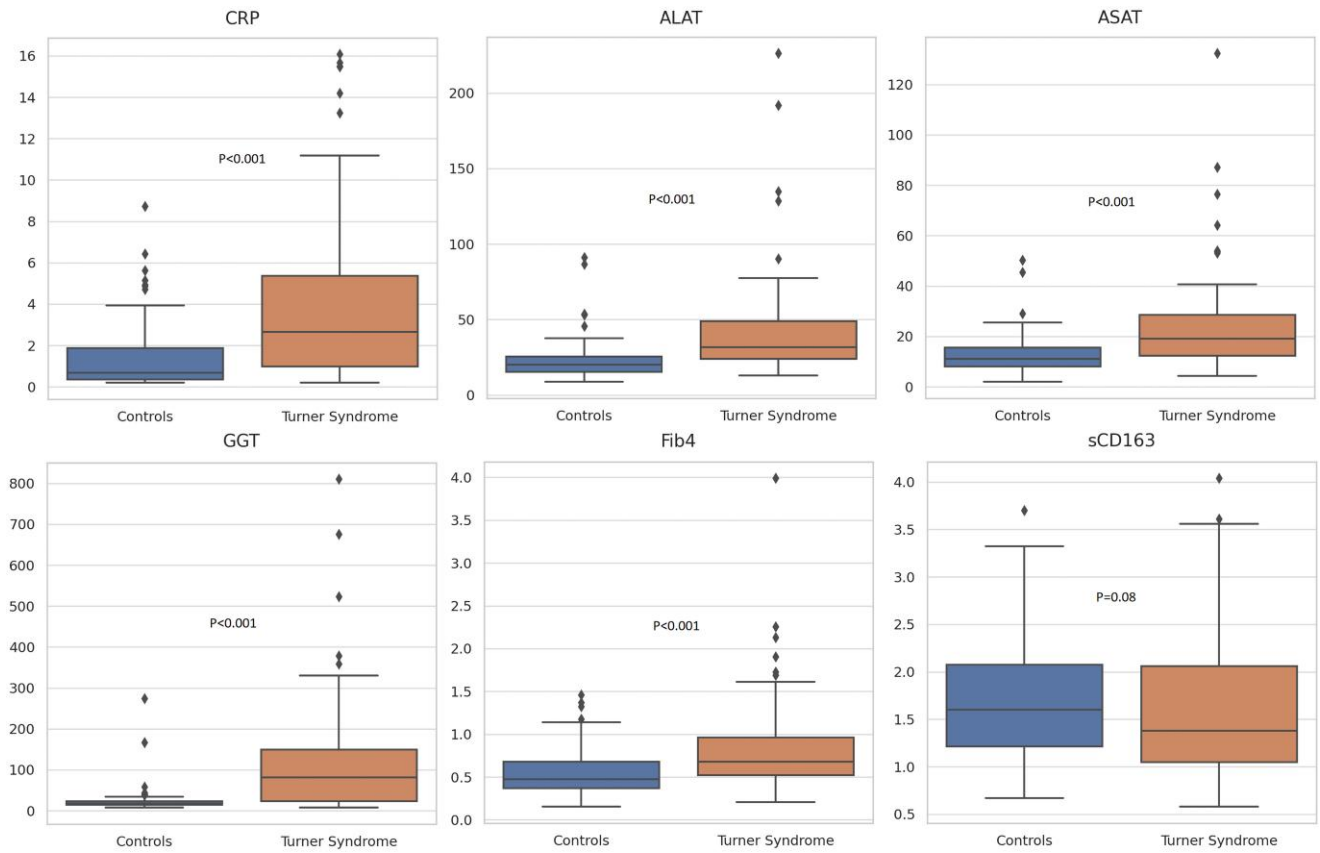

Figure 1. Data are presented for liver parameters, sCD163, and CRP. *P* values are calculated using Mann–Whitney rank sum test.

Table 3. Unadjusted correlations between liver parameters and explanatory variables

Independent variables	GGT (TS n = 76)	GGT (Controls n = 58)	AST (TS)	AST (Controls)	ALT (TS)	ALT (Controls)	FIB-4 (TS)	FIB-4 (Controls
KA4	0.27*	0.13	0.17	0.18	0.29*	0.17	0.17	0.03
KT	0.13	0.03	0.09	0.14	0.16	0.17	0.06	−0.13
OHA	0.25*	0.04	0.14	0.1	0.30*	0	0.17	0.01
OHT	0.34**	0.19	0.15	0.16	0.32*	0.18	0.21	0.07
sCD163	0.40**	0.22	0.33**	0.31*	0.32*	0.25	0.21	0.16
CRP	0.32*	0.35*	0.18	0.02	0.12	0.16	0.14	−0.04
WBC	0.09	0.2	0.04	0.01	0.09	−0.04	−0.13	−0.29*
LH	−0.12	0.21	0.03	0.02	0.03	0.04	0.21	0.18
FSH	−0.09	0.31*	0.07	0.16	0.07	0.23	0.22*	0.40**
E2	−0.09	−0.31*	−0.02	−0.24	−0.05	−0.38*	−0.15	−0.33**
T	0.11	−0.13	0.16	−0.07	0.15	−0.22	0.12	−0.27*
BMI	0.18	0.25	−0.07	0.03	0.1	0.1	−0.11	0.17
WHR	0.30**	0.29*	0.05	0.17	0.12	0.22	0.11	0.30*
Age	0.33**	0.22	0.2	0.01	0.19	0.1	NA	NA
SHBG	−0.10	−0.03	0.06	0.10	−0.17	0.06	0.16	0.18
Hba1c	0.13	0.24	−0.09	0.14	0.13	0.24	0.01	0.33*
Independent variables	GGT (TS n = 20)	GGT (Controls n = 19)	AST (TS)	AST (Controls)	ALT (TS)	ALT (Controls)	FIB-4 (TS)	FIB-4 (Controls)
Truncal adipose tissue	0.36	0.31	0.09	−0.22	0.30	0.03	0.06	−0.06
Cap_value	0.26	0.00	0.21	−0.09	0.37	−0.01	0.03	0.22
Liver stiffness	0.46*	0.56*	0.43	0.17	0.22	0.22	0.55*	−0.19

Presents the correlations between liver parameters and explanatory variable levels separately for female controls and women with TS. Variables such as GGT, AST, ALT, FIB-4 score, KA4, KT, OHA, OHT, CRP, LH, FSH, E2, and T were log-transformed to normalize their distributions. Significant correlations are denoted with 1 asterisk (*) for *P* values less than .05 and 2 asterisks (**) for *P* values less than .001. *P* values were not adjusted for multiple comparisons.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; E2, estradiol; FSH, follicle-stimulating hormone; GGT, γ-glutamyl transferase; KA4, 11-ketoandrostenedione; KT, 11-ketotestosterone; LH, luteinizing hormone; SHBG, sex hormone–binding globulin; OHA, 11β-hydroxyandrostenedione; OHT, 11β-hydroxytestosterone; T, testosterone; WBC, white blood cell count; WHR, waist–hip ratio.

Table 4. Multiple linear regression models

Dependent variable	Group	Adjusted R^2	*P* value (Model)
FIB-4	TS	23%	.0009
Explanatory variables	Coefficient	*P* value	Coef. 95% CI Int.
CRP	0.22	.009	.057 to .38
BMI	−0.19	.018	−.35 to −.033
WBC	−0.15	.043	−.30 to −.0052
OHT	0.15	.012	.034 to .27
sCD163	0.14	.03	.014 to .27
SHBG	0.14	.04	.0064 to .28
WHR	*0.15*	*.054*	*−.003 to .31*
Testosterone	*−0.0026*	*.9*	*−.13 to .12*
Dependent variable	**Group**	**Adjusted R^2**	***P* value (Model)**
FIB-4	Controls	40%	.0001
Explanatory variables	Coefficient	*P* value	Coef. 95% CI
SHBG	0.29	<.001	.14 to .43
FSH	0.16	.005	.050 to .27
Testosterone	*−0.20*	*.050*	*−.39 to −.000072*
BMI	*0.17*	*.088*	*−.026 to .36*
Hba1c	*0.16*	*.2*	*−.087 to .41*
CRP	*−0.16*	*.074*	*−.33 to .016*
sCD163	0.11	.1	−.025 to .24
WBC	*−0.06*	*.3*	*−.20 to .078*
KA4	*0.057*	*.4*	*−.082 to .20*
Dependent variable	**Group**	**Adjusted R^2**	***P* value (Model)**
GGT	**TS**	36.5%	<.0001
Explanatory variables	Coefficient	*P*-value	Coef. 95% CI
CRP	0.43	.001	.17 to .69
Age	0.30	.020	.048 to .55
LH	−0.27	.026	−.50 to −.033
sCD163	0.26	.012	.060 to .47
OHT	0.25	.010	.064 to .44
WHR	*0.23*	*.082*	*−.030 to .49*
BMI	*−0.23*	*.085*	*−.48 to .032*
WBC	*−0.14*	*.2*	*−.38 to .097*
Testosterone	*0.09*	*.3*	*−.11 to .30*
SHBG	*0.07*	*.4*	*−.14 to .29*
Dependent variable	**Group**	**Adjusted R^2**	***P* value (Model)**
ALT	TS	13.9%	.002
Explanatory variables	Coefficient	*P* value	Coef. 95% CI
sCD163	0.14	.018	.025 to .26
OHT	0.12	.023	.017 to .23
Testosterone	*0.04*	*.516*	*−.082 to .16*
Dependent variable	**Group**	**Adjusted R^2**	***P* value (Model)**
ALT	Controls	17.9%	.005
Explanatory variables	Coefficient	*P* value	Coef. 95% CI
E2	−0.17	.014	−.31 to −.037
sCD163	0.15	.020	.024 to .27
Testosterone	*−0.04*	*.6*	*−.21 to .12*
FSH	*0.01*	*.8*	*−.098 to .11*
Dependent variable	**Group**	**Adjusted R^2**	***P* value (Model)**
AST	TS	9.5%	.003
Explanatory variables	Coefficient	*P* value	Coef. 95% CI
sCD163	0.19	.003	.068 to .32

All variables are log-transformed and standardized. The Coefficients therefore represent changes in SDs and are comparable. Explanatory variables are sorted by coefficients and *P* value. No models were applicable for GGT and AST in controls. Nonsignificant explanatory variables are in italics.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Coef., coefficient; CRP, C-reactive protein; Dep., dependent; E2, estradiol; Exp., explanatory; FSH, follicle-stimulating hormone; K4A, 11-ketoandrostenedione; OHT, 11β-hydroxyandrostenedione; SHBG, sex hormone–binding globulin; WBC, white blood cell count; WHR, waist–hip ratio.

age, OHT, and LH, with LH being the only negatively correlated variable. No applicable models were identified for GGT in controls.

Regarding AST in individuals with TS, the model explained 9.5% of the variance in AST levels, with sCD163 as the sole significant predictor. No applicable models were identified for AST in female controls.

Across multiple models, sCD163 consistently emerged as a significant predictor of liver parameters in both individuals with TS and controls. Additionally, CRP and age were significant predictors in several models for women with TS, while SHBG and E2 were significant in models for controls. Some variables exhibited unexpected correlations with liver parameters (eg, negative associations of BMI and WBC with FIB-4 in individuals with TS).

Interaction Effects and Group Comparisons

CRP demonstrated a significantly stronger positive correlation with FIB-4 in women with TS than in healthy controls (Table 5). Similarly, LH exhibited a significantly stronger negative correlation with GGT in women with TS than in controls. These interactions were adjusted for sCD163, WBC, OHT, FSH, WHR, BMI, and age, indicating that the relationships between these variables and liver parameters may be causally modified by TS status.

Myeloperoxidase From Activated Neutrophils

For a subgroup of the cohort (38 individuals with TS and 20 female controls), we assessed the expression level of MPO, a biomarker for neutrophil activation and inflammation, and ELANE, a protease produced in neutrophils. MPO correlated significantly with GGT, AST, FIB-4, and sCD163, whereas ELANE correlated significantly with CD163 (Fig. S1 [16]), suggesting that increased neutrophil activation and neutrophil-mediated inflammation may be linked to liver dysfunction in women with TS.

Principal Component Analysis

To visually depict the complex interplay between the variables, a principal component analysis was conducted using

ALT, GGT, AST, CRP, sCD163, SHBG, E2, FSH, LH, testosterone, KT, OHA4, KA4, and OHT as inputs (excluding FIB-4 since it incorporates ALT and AST). A clear separation between women with TS and controls was observed along the second principal component (Fig. 2). The primary contributors to this separation were liver parameters and inflammatory markers, explaining dimension 2, highlighting their significant roles in distinguishing the biology of women with TS from controls. The 11-oxygenated C19 steroids explained most of the first principal component and the overall variability in the data, but did not significantly contribute to the separation between the groups, suggesting a lesser role in group differentiation.

Table 5. Interaction effects between predictors and group on liver parameters

Dependent variable	Explanatory variable	Coefficient	SE	*P* value
FIB-4	CRP	0.30	0.12	.013
GGT	*CRP*	*0.2743*	0.139	*.051*
	LH	−0.41	0.16	.015

Interaction effects between predictor variables and group (TS vs Control) on liver parameters (FIB-4, GGT). Models were adjusted for CD163, white blood cell count, 11β-hydroxytestosterone, follicle-stimulating hormone, LH, waist–hip ratio, body mass index, and age. Significant interactions are indicated by *P* values <.05. Italics indicate explanatory variables that are not statistically significant.
Abbreviations: CRP, C-reactive protein; FIB-4, Fibrosis-4 Index; GGT, γ-glutamyl transferase; LH, luteinizing hormone.

Discussion

This study reveals important associations between inflammatory markers, hormonal factors, and liver parameters in women with TS compared to female controls. We found that individuals with TS exhibit elevated liver enzymes (ALT, AST, GGT) and higher FIB-4 indices, indicating an increased risk of liver fibrosis and cirrhosis. Our analyses suggest that

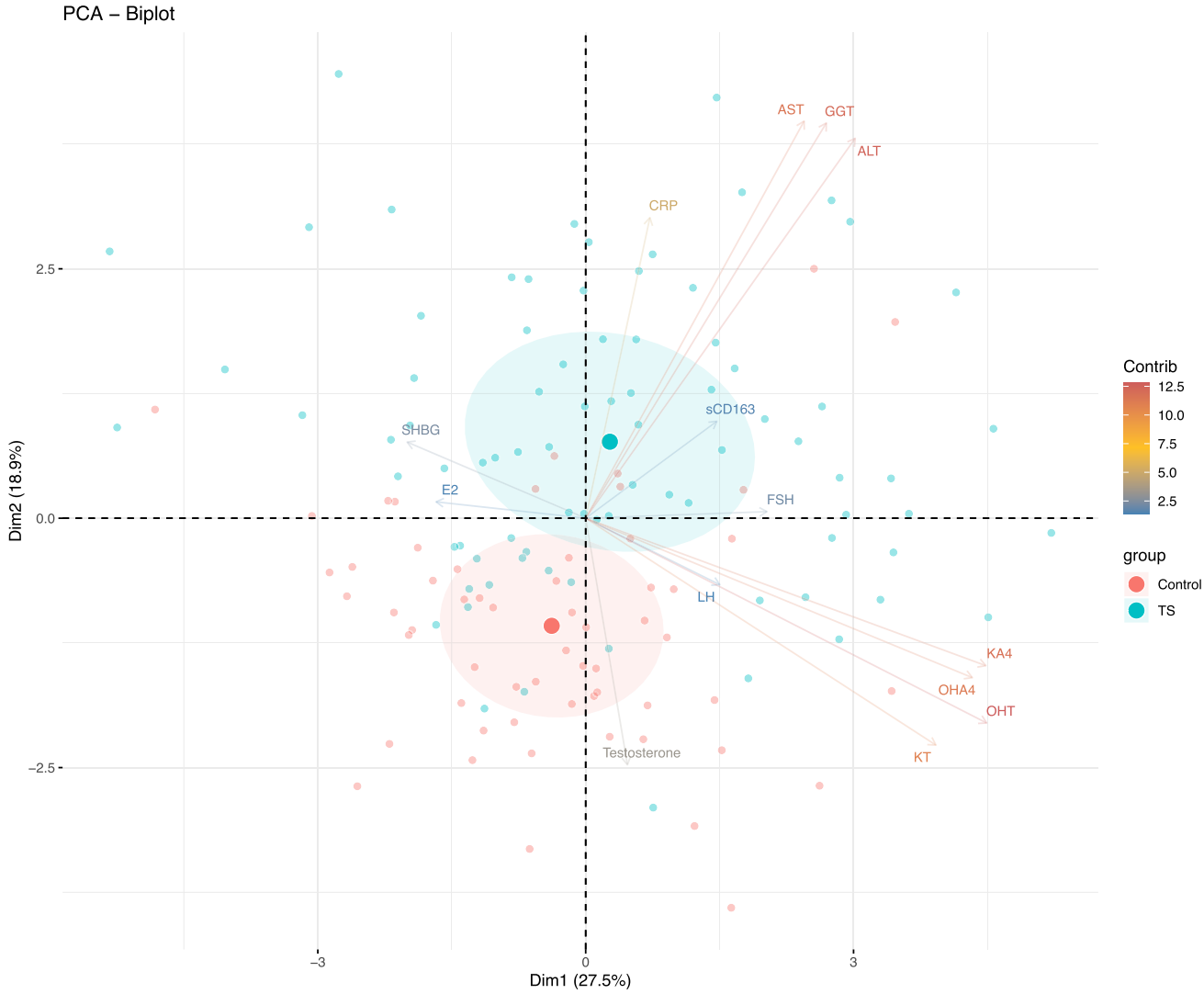

Figure 2. PCA plot incorporating inflammatory markers, hormones, 11-oxygenated C19 steroids, and liver parameters. Controls are shown in red and TS in green. Dimension 1 explains 27.5% of the variance, and Dimension 2 explains 18.9% of the variance. Variables are colored according to their contribution.

Abbreviations: OHA4, 11β-hydroxyandrostenedione; KA4, 11-ketoandrostenedione; KT, 11-ketotestosterone; OHT, 11β-hydroxytestosterone; SHBG, sex hormone–binding globulin; GGT, γ-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; sCD163, soluble CD163; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol.

low-grade chronic inflammation and hormonal imbalances may contribute to these elevations.

A key finding is the strong association between inflammatory markers, specifically CRP and sCD163, and liver parameters in women with TS. Both CRP and sCD163 were independent predictors of elevated liver enzymes and FIB-4 scores, as demonstrated in the PCA plot shown in Fig. 2. The interaction analyses further demonstrated that CRP was more strongly correlated with liver parameters in individuals with TS than in controls. Zaegel et al [4] have demonstrated an increased neutrophil to lymphocyte ratio in TS patients with liver dysfunction, supporting the role of neutrophils in liver disease development. The elevated MPO levels further suggest that neutrophil activation might be increased in TS, reinforcing this connection. sCD163 consistently emerged as a significant predictor of liver parameters in both individuals with TS and controls, even though the levels of sCD163 were within normal ranges. We have previously shown that sCD163 is lowered by both HRT and oral contraceptive therapy in TS [17], and this was confirmed in the present study, with 48% lower sCD163 levels in participants with TS receiving ERT than in participants with TS not receiving ERT. Participants not receiving ERT had sCD163 similar to controls. sCD163 has been associated with liver disease severity (eg, inflammation and fibrosis) in a number of chronic inflammatory liver diseases [18].

These findings support the notion by Zaegel et al [4] that chronic inflammation is central to the hepatic dysfunction observed in TS. Together, these results suggest that HRT may partially modulate inflammation positively, though it does not normalize the inflammatory state completely. Additionally, no differences in liver parameters were observed when comparing oral and transdermal routes of HRT administration. However, as only 9 patients received transdermal HRT, further studies are needed to draw definitive conclusions.

Hormonal factors also emerged as significant predictors of liver parameters in women with TS. Notably, OHT was positively associated with ALT, FIB-4, and GGT levels after adjustments. OHT has previously been linked to metabolic disturbances in polycystic ovary syndrome [19]. This parallel suggests that altered androgen metabolism may also contribute to liver enzyme elevations in TS, although the exact mechanisms remain unclear and warrant further research. Potential pathways could involve OHT's activation of androgen receptors in hepatic tissue, influencing liver metabolism and promoting inflammation [20]. Additionally, we observed a negative association between LH and GGT levels in women with TS, indicating that higher LH levels are associated with lower GGT levels. A possible explanation for this finding is a nonlinear relationship between LH and liver parameters. This highlights the importance of carefully monitoring and adjusting HRT to optimize liver health in individuals with TS. Previously, we and others have shown that optimized ERT can positively influence liver function in TS [21-24].

The substudy involving FibroScan assessments revealed that GGT and FIB-4 are positively correlated with liver stiffness in both individuals with TS and controls. However, the correlation between FIB-4 and liver stiffness was stronger in individuals with TS. This indicates that the FIB-4 index may be a valuable non-invasive marker for assessing liver fibrosis risk in TS, potentially guiding clinical decisions regarding further imaging or interventions, other studies have shown similar conclusions. Although our findings suggest that the FIB-4 index may help assess liver fibrosis risk in adults with TS, it is important to note that its utility in pediatric populations is limited. While FIB-4 has shown value in adults and older adolescents, further research is needed to determine its accuracy and applicability in younger patients with TS [2, 3, 24]. While individuals with TS have increased mortality due to liver diseases, elevated liver enzymes are not highly specific indicators of liver pathology. Markers like GGT and FIB-4 are sensitive but lack specificity. To enhance diagnostic accuracy, incorporating FibroScan assessments may provide greater specificity by directly measuring liver fibrosis [3, 6].

Some variables exhibited unexpected correlations with liver parameters. Notably, BMI and WBC were negatively associated with the FIB-4 index in individuals with TS after adjusting for WHR and CRP, respectively. The negative association between BMI and FIB-4, when controlling for WHR, may indicate that higher BMI reflects increased lean body mass or a healthier fat distribution, which could be protective against liver fibrosis. In any case, these data underscore the importance of assessing WHR and not only BMI, as this measure may not fully explain the specific body composition of a given individual.

The clinical implications of the current study for monitoring and intervention are important. Regular monitoring of liver function tests is already recommended [25]. However, similar to the suggestion by Zaegel et al, we propose that regular monitoring of inflammatory markers in individuals with TS may also may hold clinical relevance [4]. Early identification of elevated liver parameters allows for timely interventions to prevent progression to liver fibrosis or cirrhosis, although this needs to be confirmed in longitudinal studies. Among liver function tests, FIB-4 and GGT have been identified as sensitive markers [4]. Additionally, FibroScan is a valuable noninvasive tool for assessing liver stiffness and detecting fibrosis.

We believe that the current study shows a clear and strong correlation between inflammation and increased liver parameters. Therefore, addressing chronic inflammation through lifestyle modifications or pharmacological interventions could be a strategy to mitigate liver dysfunction in TS. Careful management of hormonal replacement therapy, ensuring appropriate estrogen and androgen levels, may have beneficial effects on liver function. Monitoring LH levels, in addition to FSH, could help in assessing the adequacy of hormonal therapy. Evaluating body composition beyond BMI, such as assessing WHR and lean body mass, may provide better insights into liver disease risk in individuals with TS. While hormonal imbalance and inflammation appear central, other potential mechanisms, including vasculopathies and congenital vascular anomalies, may also contribute to elevated liver enzymes and hepatic dysfunction in TS and warrant further exploration.

Several limitations should be considered. First, the cross-sectional nature of the study limits the ability to infer causality between the identified associations. Second, the relatively small sample size, particularly in subgroup analyses and the FibroScan substudy, may affect the generalizability of our findings. Third, the mRNA measurements of MPO did not enter the strict statistical assessment involving post-Lasso OLS, potentially introducing confounding in the association observed. Additionally, the control group did not adequately control for the higher prevalence of obesity in individuals with TS, which could potentially influence observed

differences in liver parameters. Another important limitation is the absence of concurrent thyroid function testing, as undiagnosed or untreated hypothyroidism may impact liver enzyme and inflammatory marker assessments. Furthermore, information regarding the duration and cumulative estrogen exposure from ERT was not included but could provide insight into the potential protective effects of estrogen on liver function. Lastly, the absence of data on liver enzyme abnormalities during adolescence limits our ability to assess how early liver dysfunction might correlate with abnormalities identified later in life.

Future research should focus on longitudinal studies to establish causal relationships between inflammation, hormonal factors, and liver dysfunction in TS. Investigating the underlying mechanisms of immune dysregulation and hormonal metabolism in TS could provide insights for targeted therapies. Additionally, larger studies incorporating genetic analyses may help identify specific genetic contributors to liver disease risk in individuals with TS. Targeting inflammation in future protocols could well be a sensible course.

Conclusion

Our study demonstrates that women with TS exhibit significantly elevated liver enzymes and risk of hepatic dysfunction compared to female controls, possible influenced by chronic low-grade inflammation. Inflammatory markers like CRP and sCD163 are strongly correlated with liver parameters in individuals with TS. Hormonal imbalances, particularly increased OHT, predict higher liver enzyme levels, suggesting altered androgen metabolism may contribute to hepatic dysfunction. These findings highlight the importance of regular monitoring of liver function, inflammatory markers, and hormonal levels in individuals with TS to enable early intervention and improve clinical outcomes.

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Disclosures

Authors and co-authors declare no conflicts of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author, LOR. The data are not publicly available due to restrictions, eg, their containing information that could compromise the privacy of research participants.

Ethics Statement

Written consent was obtained on all participants. The Aarhus Regional Ethical Scientific Committee approved the trial protocols M-20010248, 1-10-72-131-15, and 1-10-72-186-21. All clinical investigations were conducted in accordance with the principles expressed in the Declaration of Helsinki.

Clinical Trial Information

These studies were preregistered at ClinicalTrials.gov (NCT 05425953 [registered June 13, 2022] and NCT00624949 [registered February 28, 2008]).

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