Eur J Pediatr (2000) 159: 143–148

ORIGINAL PAPER

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Serum liver enzymes in Turner syndrome

Received: 1 November 1998 / Accepted: 20 May 1999

Abstract Increased serum concentrations of liver enzymes are sometimes observed, in the absence of clinical symptoms of liver disease, in patients with Turner syndrome. The purpose of this study was to evaluate, in our Turner patients, serum liver enzyme levels and to find a cause for their increase. In 70 Turner patients, serum AST, ALT, GGT levels were evaluated every 6 months during a period of 0.8–21.9 years. In patients in whom increased values of liver enzymes were found, serological markers for infectious hepatitis, serum hepatitis C virus RNA and virus genotype, IgG and IgA antibodies to gliadin and endomysium, coeruloplasmin, copper, α_1-antitrypsin, total proteins and electrophoresis, IgG, IgA, IgM, fibrinogen, prothrombin, alkaline phosphatase, creatine kinase and total and direct bilirubin were also determined. Antinuclear, anti-smooth muscle and anti-liver-kidney microsome antibodies together with antithyroglobulin and anti-thyroid peroxidase antibodies were determined in all patients and in 166 age-matched female controls. In 22 patients, increased liver enzymes were observed, not related to karyotype. Follow-up showed that the hepatic disorder did not worsen with the time. Serological markers of hepatitis C virus were positive in three patients. When the serum liver enzyme increase was first observed in the other 19 patients with high enzyme levels (group A), 14 patients had never been submitted to hormonal treatment, 4 were on oestrogen/gestagen treatment and 1 was being treated with both growth hormone and oestrogen. Coeliac disease, α_1-antitrypsin deficiency and Wilson disease were ruled out by appropriate investigations. In 8/19 group A patients, antinuclear and/or anti-smooth muscle antibodies were present versus 6/48 of patients with normal liver enzymes (group B). Thyroid antibodies were found in 8/19 patients in group A and in 13/48 in group B. Weight excess SDS was significantly higher in Turner girls with liver enzyme increase. Ultrasonography, performed in 17 patients of group A, showed mild hepatomegaly in 4 and increased echogenicity with fatty infiltration in 6.

Conclusion Hepatic abnormalities in Turner syndrome are not progressive. Oestrogen should not be considered the main cause of increased liver enzymes in Turner syndrome since most of our patients with this finding had not been previously treated with oestrogens. An auto-immune pathogenesis might be considered in some cases, whereas the association with weight excess seems the most frequent cause of liver disorder in Turner syndrome.

Key words Turner syndrome · Liver enzymes · Oestrogen therapy · Weight excess

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Abbreviations *AGA* antigliadin antibodies · *ALP* alkaline phosphatase · *ANA* antinuclear antibodies · *EMA* anti-endomysium antibodies · *HCV* hepatitis C virus · *LKM* anti-liver-kidney microsome antibodies · *SMA* anti-smooth muscle antibodies · *TgA* thyroglobulin antibodies · *TPO* thyroid peroxidase antibodies · *TS* Turner syndrome

Introduction

Increased serum concentrations of liver enzymes are sometimes observed, in the absence of clinical symptoms of liver disease, in patients with Turner syndrome (TS). The frequency of this finding is not well established and the cause is not clear.

In a study of medical status and social life in 49 middle-aged women with TS, high levels of one or more liver enzymes were reported in most of them and the authors stated that the increase was not related to alcohol abuse, without suggesting a possible cause [21]. In a recent paper [24], the increase in serum liver enzymes observed in 8/35 girls with TS was ascribed to oestrogen/gestagen treatment.

In this study we have retrospectively evaluated serum liver enzyme levels in our patients with TS. The subjects in whom an increase was observed were submitted to a series of investigations with the aim of finding a possible pathogenesis of the hepatic disorder.

Patients and methods

Patients

A total of 70 patients with TS, attending regular follow-up at the Endocrine Clinic of the Department of Paediatrics of the University of Pavia, were considered in this study in whom 43 had a 45,X karyotype and 27 had mosaicisms and/or structural abnormalities (isochromosome of the long arm, ring, deletions) of the X chromosome. Mean age was 9.7 ± 4.4 years (range 0.3–20.8 years) at the first evaluation and 18.7 ± 6.9 years (range 3.4–33.7 years) at the last visit; the mean duration of follow-up was 9.0 ± 5.6 years (range 0.8–21.9 years). Weight excess SDS (Weight SDS – Height SDS according to Tanner standards [22]) at the last visit was evaluated in all patients.

Methods

Serum AST, ALT and GGT levels had been monitored every 6 months in all patients as part of routine blood analyses which also included cell blood count, glucose, lipids, creatinine and urea determinations. Turner girls were considered to have an increase in liver enzyme levels when their values were above the normal range at least twice during the follow-up. Given the long duration of follow-up and the variations in reference values for liver enzymes through the years, we have expressed their levels as increase rate versus upper normal limit (enzyme level/upper normal limit). Antinuclear antibodies (ANA), anti-smooth muscle antibodies (SMA), anti-liver-kidney microsome antibodies (LKM), thyroglobulin antibodies (TgA) and thyroid peroxidase antibodies (TPO) were determined in all patients and in 166 age-matched female controls.

In patients with increased liver enzymes, serological markers for hepatitis A virus, hepatitis B virus, hepatitis C virus (HCV), serum HCV RNA and virus genotype, antigliadin antibodies (AGA) (IgG and IgA), anti-endomysium antibodies (EMA), coeruloplasmin, copper, α_1-antitrypsin, total proteins and electrophoresis, IgG, IgA, IgM, fibrinogen, prothrombin, alkaline phosphatase (ALP), CK, and total and direct bilirubin were also determined. Since reference values for Ig vary according to chronological age, we expressed serum Ig levels in our patients as SDS of the age-matched normal mean.

AST, ALT, GGT, ALP, CK and bilirubin were determined by kinetic colorimetric tests. AGA (IgG and IgA) were tested by fluorescence enzyme immunossay. ANA, SMA, LKM, and EMA antibodies were determined by indirect immunofluorescence, TPO and TgA by radioimmunoassay. Coeruloplasmin, α_1-antitrypsin, IgG, IgA and IgM were determined by nephelometry. Serological markers of hepatitis A, B and C were evaluated by enzyme linked immunosorbent assay and/or recombinant immunoblot assay. Serum HCV-RNA and viral genotype were determined by polymerase chain reaction amplification. Serum copper was determined by atomic absorption spectrophotometry. Total serum proteins were determined by the biouret method. Fibrinogen and prothrombin evaluation was performed by colorimetry during coagulation process.

Liver morphology, echostructure and volume were investigated in the group of patients with abnormal liver enzymes by ultrasound using a real-time Toshiba 270 instrument with a 3.5 MHz Convex transducer. We also evaluated the presence or absence of lymph nodes at the level of the hilum and the presence of stones in the gallbladder and biliary tree. The normal liver echostructure is usually homogeneous and less echogenic than the renal parenchyma.

Statistics

All data are presented as mean ± SD. Differences of the means between groups were compared using Student's *t*-test for unpaired data. The Chi-squared (χ^2) test was used to assess differences in the prevalence of auto-antibodies in the groups. $P < 0.05$ was defined as significant.

Results

We found 22 patients who, during the follow-up, had one or more liver enzymes above the normal range, while 48 subjects had normal values at all evaluations throughout the years. Serological markers of infectious hepatitis were positive for HCV in 3 of the 22 patients; in two of whom (both of them had received blood transfusions) HCV RNA (Ib genotype) was detected in the serum. One girl (without a history of parenteral exposure to blood or derivatives) was HCV RNA negative.

The remaining 67 patients were divided in two groups: A ($n = 19$), with increased liver enzymes levels not related to viral hepatitis and B ($n = 48$) with normal liver enzymes. In group A Turner girls, clinical signs of liver dysfunction, such as jaundice, pruritus, anorexia, abdominal pain or distension, bleeding were not present. Mean age at the first visit in group A was 9.7 ± 3.8

years (range 2–14 years), in group B 9.8 ± 4.5 years (range 0.8–20.8 years); the duration of follow-up was 8.7 ± 5.3 years (range 0.8–19.5 years) and 9.3 ± 7.2 years (range 0.8–21.9 years) respectively.

Weight excess SDS was 2.390 ± 0.994 in group A Turner girls and 1.587 ± 1.047 in patients with normal liver enzymes ($P = 0.0055$).

No difference in the frequency of X monosomy was observed between the two groups; 45,X karyotype was present in 11/19 patients of group A (57.9%) and in 29/48 in group B (60.4%). In patients with karyotypes other than 45,X, a higher frequency of X isochromosome was present in group A (4/19, 21.05% vs 6/48, 12.5%; P = N.S.).

AST, ALT and GGT were all elevated in ten cases, AST and ALT in seven, only AST in one and only ALT in one. When the increase in serum liver enzymes was first observed, the mean age of patients was 13.3 ± 5.6 years (range 1.9–26.8 years). Fourteen patients had never been submitted to hormonal treatment, four were on oestrogen/gestagen treatment and one was being treated with both growth hormone and oestrogens. Coeliac disease, α_1-antitrypsin deficiency and Wilson disease were ruled out in patients of group A, since AGA and EMA, coeruloplasmin, copper, and α_1-antitrypsin levels were normal and patients did not present any symptoms or signs of these diseases. Albumin, coagulation factors and bilirubin were normal. No pathological increase of immunoglobulin levels was observed in these Turner girls. Most of the patients had IgG levels between 0 and −2 SDS and 6/48 group B patients had IgG values below −2 SDS, while no group A patients nor HCV-positive girls had this characteristic.

The study of auto-antibodies showed that 8/19 patients in group A were positive for ANA (three cases), SMA (three cases), and ANA + SMA (two cases). In group B we found 6/48 positive Turner girls, four for ANA and two for LKM, while in controls 16 subjects were positive for ANA and three for SMA (42.1%, 12.5% and 11.4% respectively; $\chi^2 = 13.3$; $P = 0.001$). Table 1 depicts the frequencies of ANA, SMA and LKM in groups A and B Turner patients and in normal controls. Antithyroid antibodies were present in 42.1% of group A, in 27.1% of group B Turner subjects and in 2.4% of controls ($\chi^2 = 44.9$; $P < 0.00001$). Of the three patients positive for HCV serological markers, one was auto-antibody-negative, one was positive for TgA and TPO and the girl negative for HCV RNA was positive for both SMA and TgA. Mean age at the first evidence of increase in liver enzyme levels was 11.7 ± 5.8 years in the eight subjects with ANA and/or SMA antibodies and 13.7 ± 6.3 years in the others. No difference related to karyotype was found between patients positive or negative for auto-antibodies. When we compared the pattern of AST, ALT and GGT in Turner girls of group A with ($n = 8$) and without ($n = 11$) ANA and/or SMA antibodies (Fig. 1), we found that positive girls had higher levels of AST and GGT. Furthermore, we did not observe any worsening during the follow-up.

Finally, to study the possible influence of oestrogen therapy, we evaluated AST, ALT and GGT pattern before and during treatment with oestrogens in five patients with TS of group A and found that liver enzymes did not increase during oestrogen therapy (Table 2).

Ultrasonography, performed in 17/19 patients of group A, showed increased echogenicity with fatty infiltration in six, associated with mild hepatomegaly in four. No relation was found between liver structural abnormalities seen on ultrasound and frequency of auto-antibodies (36% of steatosis in patients with auto-antibodies and 25% in those without); a non significant higher value of weight excess SDS was found in Turner girls with liver steatosis (2.845 ± 0.626 vs 2.321 ± 0.870; $P = 0.21$ N.S.). Liver ultrasound in the three Turner girls positive for HCV serological markers showed hyperechogenicity and volume at the upper limit of normal in the two RNA HCV positive cases and normal sonographic appearance in the RNA HCV negative girl.

Discussion

On the basis of our data, liver enzyme increase is fairly frequent in Turner patients even from childhood. Excluding the three patients positive for HCV serological markers, we found that 28% of Turner patients had transient or persistent elevation of liver enzymes of unknown cause. Our results agree with those of Wemme [24], who observed high enzyme values in 23% of patients with the same age range as our case series. In a study on 49 middle-aged Turner women [21], this characteristic was found in 80% of cases, suggesting that in TS the elevation of liver enzyme levels becomes more frequent with aging.

Table 1 Prevalence of ANA, SMA, LKM auto-antibodies in patients with TS with increased liver enzyme levels (group A), normal liver enzyme levels (group B) and healthy controls

Auto-antibodies	Group A Turner patients (n = 19)		Group B Turner patients (n = 48)		Controls (n = 166)		Group A vs Group B	Group A vs controls
	Positive (n)	%	Positive (n)	%	Positive (n)	%		
ANA	5	26.3	4	8.3	16	9.6	P = 0.051	P = 0.0299
SMA	5	26.3	0	0.0	3	2.2	P = 0.00022	P = 0.000079
LKM	0	0.0	2	4.2	0	0.0		

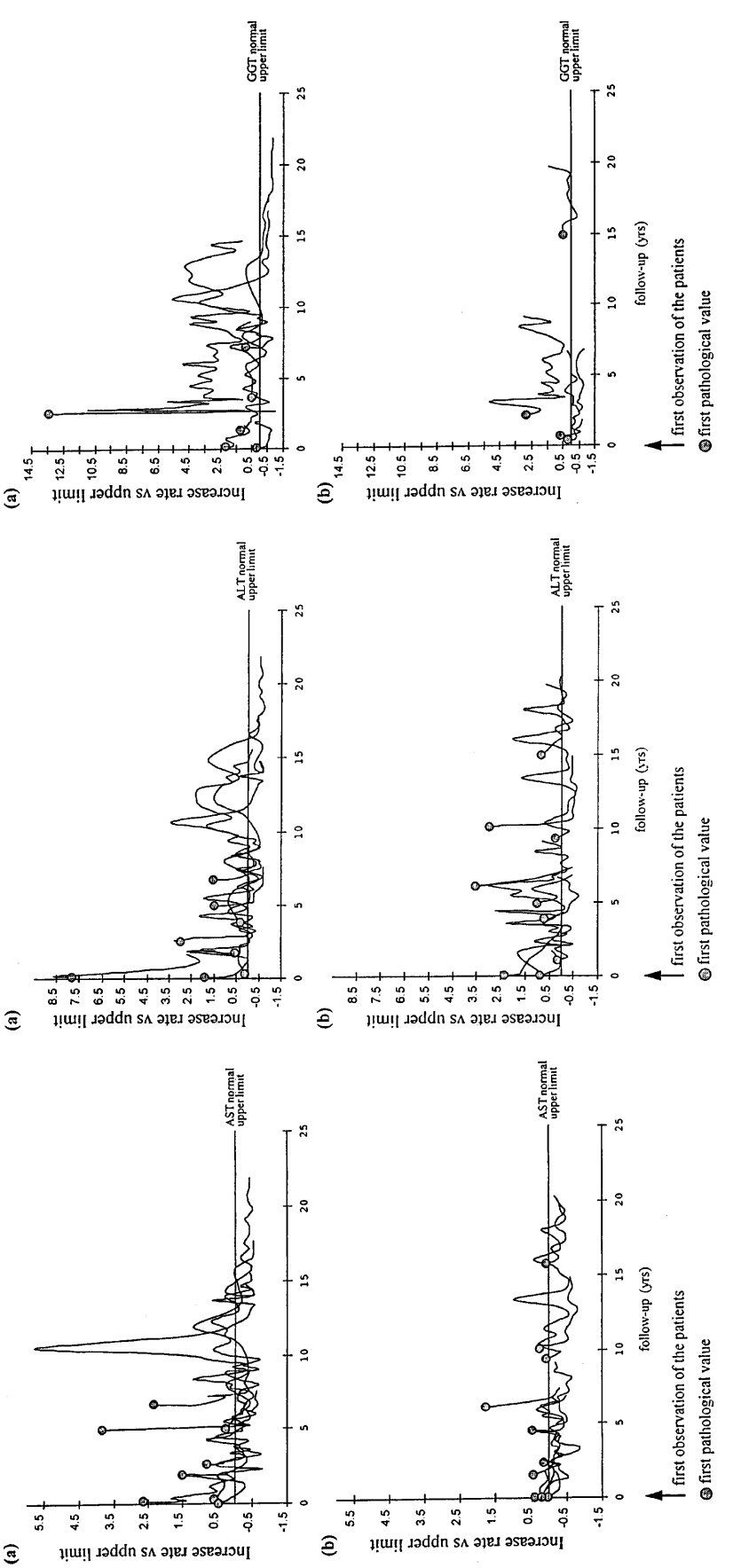

Fig. 1 AST, ALT, GGT pattern, expressed as increase rate versus upper normal limit, during the follow-up in group A Turner patients. (*a*) Patients positive for ANA and/or SMA; (*b*) patients negative for these auto-antibodies. Only patients in whom enzyme level was found increased are reported

Table 2 AST, ALT and GGT mean levels (±SD), expressed as increased rate versus upper normal limit in five Turner patients of group A before and during oestro-progestin treatment (*NS* not significant)

Patient number		Before treatment	During treatment	*P*
1	AST	−0.19 (±0.21)	−0.51 (±0.08)	0.002
	ALT	0.27 (±0.49)	−0.44 (±0.18)	0.003
	GGT	−0.48 (±0.22)	−0.66 (±0.10)	NS
2	AST	−0.19 (±0.16)	−0.32 (±0.16)	NS
	ALT	0.07 (±0.49)	−0.32 (±0.31)	NS
	GGT	−0.11 (±0.28)	0.06 (±0.12)	NS
3	AST	−0.19 (±0.35)	−0.33 (±0.21)	NS
	ALT	0.80 (±1.09)	0.33 (±0.56)	NS
	GGT	1.82 (±1.36)	2.26 (±0.92)	NS
4	AST	−0.12 (±0.43)	−0.31 (±0.07)	NS
	ALT	0.24 (±0.62)	0.59 (±0.83)	NS
	GGT	0.51 (±0.53)	3.6 (±1.76)	0.020
5	AST	1.58 (±0.84)	0.81 (±1.61)	NS
	ALT	6.47 (±2.42)	2.37 (±2.84)	0.050
	GGT	1.94 (±0.25)	0.38 (±0.83)	0.014

Given the fact that oestrogens are known to influence the metabolic activity of the liver [4], Wemme et al. [24] attributed the increase in liver enzymes in TS to therapy with oestrogens, since their eight patients developed conspicuous serum liver enzyme levels during oestrogen therapy and in three of them discontinuation of therapy was followed by a decrease in enzyme levels. Since in their study no such increase of liver enzymes was observed in 41 tall girls treated with oestrogens, the authors suggested that Turner patients might have a greater vulnerability to the hepatic effect of oestrogens than eukaryotic individuals [24]. However, the results could have been biased by the fact that in that study all Turner girls had received oxandrolone prior to oestrogen replacement and oxandrolone might induce or worsen hepatic damage. Our data suggest that oestrogens should not be considered an important cause of the high levels of liver enzymes in TS because most of our patients with this finding had not yet been treated with oestrogens and did not show a further increase of enzyme levels during oestrogen therapy. Furthermore, at the last observation, 29/48 patients of group B, in whom liver enzymes were normal at all times during the follow-up, were on oestrogen/gestagen replacement therapy.

Given the presence of auto-antibodies associated with auto-immune liver disease (ANA, SMA) [11, 16–18] in some of our Turner patients with elevated enzymes, together with the well-known higher prevalence of auto-antibodies and auto-immune diseases in Turner subjects than in normal women [3, 13], an auto-immune disorder might be the cause of liver enzyme increase in TS, at least in some cases. ANA, SMA and LKM auto-antibodies are considered important markers of auto-immune hepatitis, particularly in children, where they are very rare [18]; however, they may also be found in other diseases such as malignancies or other auto-immune disorders [19, 25] and recently the incidence of ANA has been reported to be higher in paediatric patients with auto-immune thyroid disease [10]. The higher prevalence of antithyroid antibodies in our group A patients might also be considered a further indication of an auto-immune pathogenesis of the elevation of liver enzymes since auto-immune thyroiditis is one of several conditions that have been reported to be associated with auto-immune hepatitis [11, 16–18]. However, in our Turner patients the low titres of ANA and SMA, the finding of low-normal serum IgG levels and the lack of progression of the hepatopathy without any immunosuppressive therapy weakens this hypothesis.

To our knowledge, one girl with TS affected by primary sclerosing cholangitis [12] and five Turner patients with chronic asymptomatic intrahepatic cholestasis [1, 14] have been described, but none with auto-immune hepatitis has been reported in the literature. In a study on morbidity in TS, the relative risk for cirrhosis of the liver was reported to be slightly higher than expected, while the relative risk of diseases of the liver, biliary system and pancreas was not increased [8].

The finding of a significant association between increase of liver enzymes and weight excess SDS might suggest that obesity, which is a characteristic feature of girls with TS [2], is related to the liver abnormality in most cases. Obesity has been identified as a risk factor for liver disease [7, 15]. Hepatic involvement, characterized by ultrasonographic image of liver steatosis and elevated transaminase levels both responsive to loss of weight, has been reported in obese children [5].

Ultrasound showed fatty infiltration of the liver in six of our patients, associated with hepatomegaly in four. Steatosis is usually an aspecific abnormality of liver structure [23]; in our patients it might be related to both obesity and auto-immune disorders, in which, however, no specific echographic findings have been described [9, 23].

We observed that in a high number of Turner patients hepatobiliary enzyme levels are increased and that the origin of their elevation is unclear, unless a viral infection is found. On the basis of our data, hepatic abnormalities are not induced by oestrogen therapy. An auto-immune pathogenesis might be suggested in some cases, however the significant association of liver enzyme increase with weight excess SDS could indicate that overweight can be responsible for hepatic disorders in many Turner girls. Both the long follow-up of our patients and the data reported in the literature on middle-aged Turner women [6, 20, 21] indicate that hepatic disorders in Turner patients do not worsen with time and that liver disease is not a relevant cause of morbidity or death in these patients.

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