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Original Article

The outcomes of transient elevation of maternal liver enzymes preceding laser treatment for twin-twin transfusion syndrome

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ABSTRACT

Background: A proportion of twin-twin transfusion syndrome (TTTS) patients may have elevated liver enzymes (ELEzs) before fetoscopic laser therapy, but the incidence of ELEzs before laser therapy and the association with the perinatal outcomes after laser therapy remain unclear.

Methods: From October 2008 to April 2015, 93 patients with TTTS who received fetoscopic laser therapy at our hospital were included in this study, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured within 24 h before therapy. If ELEzs (AST > 34 U/L or ALT > 36 U/L) were observed before therapy, the AST and ALT levels were evaluated within 24 h after therapy. The pre-operative characteristics and post-therapy outcomes were compared between patients with and without ELEzs.

Results: Among 93 TTTS patients before laser operation, 18 patients (were found with ELEzs (19.4%) before laser therapy. In 17 (94.4%) of the 18 cases, their liver enzymes values dropped after laser surgery. Maternal body mass index, age, gestational age of laser therapy, hemoglobin level before laser therapy and survival rates after laser therapy were not significantly different between TTTS with and without ELEzs. The maternal hemoglobin dropped significantly from 10.8 [1.6] g/dL before surgery to 9.6 [1.5] g/dL after laser therapy in TTTS with ELEzs ($p < 0.001$).

Conclusion: An elevated liver enzyme was not associated with poor perinatal outcomes in patients with TTTS after laser therapy. The authors suspected that the reduced liver enzymes values after laser therapy could partly arise from the hemo-dilution effect.

Twin-twin transfusion syndrome (TTTS) occurs in approximately 9% of monochorionic, diamniotic twin pregnancies [1]. Fetoscopic laser therapy is recognized as the first-line treatment for stage II to IV TTTS diagnosed before 26 weeks of gestation [2]; however, whether it is the preferred treatment for stage I TTTS remains debatable [3,4].

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At a glance of commentary

Scientific background on the subject

Fetoscopic guide laser therapy is the first-line treatment for all stage twin-twin transfusion syndrome (TTTS) diagnosed before 26 weeks of gestation. Elevated maternal liver enzymes in TTTS before laser therapy had not been reported; the etiology of its occurrence is unclear.

What this study adds to the field

Elevated maternal liver enzymes was observed in 19.8% of the patients with TTTS, and in most cases, liver enzyme levels normalized after therapy largely due to hemodilution . Elevated maternal liver enzymes in TTTS do not influence post-laser outcomes for TTTS.

Maternal reactions to twin pregnancies and to laser operations because of TTTS are well known. Twin pregnancy is characterized by an even more hyper-dynamic circulation than is singleton pregnancy [5,6] and amnioreduction for severe polyhydramnios in TTTS induces maternal hemodynamic changes after treatment [7]. In 2008, a case of TTTS with elevated liver enzymes (ELEzs), including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), before laser therapy caught our attention and prompted us to routinely examine AST and ALT levels before laser operation.

The etiologies of elevated AST and ALT in pregnancy include hyperemesis [8–10], cholestasis [11,12], severe preeclampsia [8], a combined hemolysis, elevated liver enzymes and low platelets syndrome (HELLP) [13,14], fatty liver [15], gallbladder stones [16,17] and hepatitis [18]. There is yet no report about elevated maternal AST and ALT in TTTS patients to our knowledge.

Therefore, the aims of this study are to evaluate the incidence of maternal liver enzyme abnormalities before laser therapy, and examine whether ELEzs before laser therapy is in relation to neonatal outcomes in patients with TTTS.

Materials and methods

The present study featured a retrospective design and was performed at Chang Gung Memorial Hospital, Taoyuan, Taiwan, a tertiary referral center with 3668 beds available and also the largest laser center for TTTS treatment in Taiwan; from October 2008 to April 2015, patients with TTTS who received fetoscopic laser therapy were included in this study. Surgery was performed in the operating room under regional or local anesthesia with methods of selective laser photocoagulation of communicating vessels (SLPCV) [19] with or without Solomon technique [20].

Inclusion criteria for this study included patients diagnosed as TTTS detected before 26 weeks of gestation, receiving laser therapy at our hospital, and having AST and ALT checked 24 h before laser therapy. AST and ALT were measured at our central biochemistry laboratory by enzymatic method (FORMOSA BIOMEDICAL TECHNOLOGY CORP. Taipei, Taiwan) with a matched instrument application (Hitachi LST008, Tokyo, Japan). Exclusion criteria were as follows: TTTS detected after 26 weeks of gestation, receiving laser therapy at our hospital, but undergoing termination before 24 gestational weeks, lacking AST or ALT data 24 h before laser therapy, without AST or ALT data available within 24 h after laser therapy when ELEzs was found before laser therapy, preeclampsia, chronic hepatitis, and fetal chromosome or major fetal structural anomaly.

The patients were divided into ELEzs and non-ELEzs groups (with and without ELEzs within 24 h before therapy, respectively). ELEzs is defined as AST >34 U/L or ALT >36 U/L within 24 h before laser therapy. The primary outcomes are to measure the basic characteristic between TTTS patients with and without ELEzs and AST and ALT levels within 24 h after laser therapy for TTTS in ELEzs group. The secondary outcomes are to evaluate the neonatal survival and gestational age at delivery between the two groups of TTTS. This study was approved by the Chang Gung Medical Foundation Institutional Review Board, and patient consent was not required because of the retrospective study design. Maternal body weight and height, serum levels of complete blood count (CBC) and differential blood count (DC), blood urine nitrogen (BUN), creatinine (Cr), C-Reactive Protein (CRP), PT (Prothrombin time), APTT (activated partial thromboplastin time) other than AST and ALT were determined before operation in all patients. In the ELEzs group, CBC, DC, AST and ALT levels were reevaluated within 24 h after laser therapy. But only CBC and DC were checked in cases of TTTS without ELEzs. If the post-laser-therapy AST or ALT level was higher than the pre-laser-therapy level, AST and ALT levels were assessed again after 24 h. On detection of a more than 10-fold increase in the AST or ALT level, the patients were referred to the gastrointestinal department for further management. The diagnosis of TTTS was based on the Quintero staging system [21]. Stages III and IV were classified as high Quintero stages, and stages I and II were categorized as low stages. After the patients received the diagnosis of TTTS (stages I to IV) before 26 weeks of gestation, treatment options, including serial amnioreduction, laser therapy, or expectant management, were offered. Fetoscopic laser therapy was performed in an operating room under regional (either epidural or spinal) or local anesthesia as described previously [22]. After completing photocoagulation, the amniotic fluid was drained to reduce the patients' maximum vertical pocket (MVP) to a value less than 8 cm.

The preoperative (pre-OP) characteristics, including Quintero staging, gestational age at operation, BMI (Body Mass Index) of mother and MVP values of both fetuses, were assessed and documented in the ELEzs and non-ELEzs groups. The outcomes of fetoscopic laser therapy for TTTS, including fetal survival (defined as living more than 30 days after delivery), gestational age at delivery, and interval between laser therapy and delivery, were compared between the two groups.

Statistical analysis was conducted using SPSS (version 11.0 for Windows; SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation, median [inter-quartile range] and frequency (%) as when appropriate. Qualitative data were

compared by means of X2 test or Fisher exact test (when cells have expected count less than 5). Continuous variables were tested for normality. Two-sample Student t test or Mann–Whitney U test was used to compare between groups for the continuous variable. Paired samples test or Wilcoxon signed ranks test was used to compare the pre-operation and post-operation data such as maternal serum AST, ALT, hemoglobin (Hb) and hematocrit (Hct) values. A probability value of less than 0.05 was considered statistically significant.

Results

During the study period (October 2008 to April 2015), 98 patients with TTTS received fetoscopic laser therapy at our hospital. However, two cases (2.0%) had hemolytic blood samples and therefore, their serum AST and ALT data were unavailable. Two patients with two viable fetuses after laser therapy underwent early pregnancy termination; in the first case, trisomy 21 was confirmed through karyotyping, and the pregnancy was terminated at the gestational age of 20 weeks. In the second case, the patient opted for termination of pregnancy at the gestational age of 18 weeks for personal reasons. Another case with single twin survival after laser therapy, was terminated at 19 weeks of gestation on the patient's request. After excluding the five cases (5.1%) described above and the prior two cases (2.0%) without analyzable serum samples, a total of 93 (94.9%) cases were incorporated into this study. Pre-operation ELEzs was observed in 18 patients (19.4%), and preeclampsia or chronic hepatitis was not diagnosed in any study patients before laser therapy.

The mean gestational age at operation is 20.9 ± 2.6 weeks. Of the patients, 19 are at Quintero stage I (20.4%), 29 are at stage II (31.2%), 29 are at stage III (31.2%), and 16 are at stage IV (17.2%). The mean gestational age at delivery is 31.9 ± 5.5 weeks, total fetal survival rate is 74.2% (138/186), single survival rate is 86.0% (80/93), and dual survival rate is 62.4% (58/93). The Hb and Hct levels dropped significantly after laser therapy: the median pre-operation Hb at 10.8 [1.6] g/dL versus post-operation Hb at 9.6 [1.5] g/dL (Wilcoxon Signed Ranks Test, $p < 0.001$)), and pre-operative Hct of 32 [4.2] % compared with post-operative Hct of 28.5 [13.8] % (Wilcoxon Signed Ranks Test, $p < 0.001$).

[Table 1] lists the characteristics and neonatal outcomes of the ELEzs and non-ELEzs groups. The two groups do not differ significantly in the maternal age and BMI at operation, gestational age at operation, ratio of high Quintero staging, and recipient and donor MVP values. Likewise, all the 93 TTTS display no significant discrepancy in their pre-operation and post-operation Hb and Hct data s between ELEzs and non-ELEzs groups. The fetal outcomes of the 93 patients with TTTS, including the single survival rate, dual survival rate, gestational age at delivery, and interval between operation and delivery are not significantly different between the two groups [Table 1].

All the patients with brief ELEzs did not eventually develop preeclampsia or placental abruption during the rest of the pregnancy period and no apparent adverse postnatal consequences were observed in the ELEzs group. Of the 18 cases with ELEz, 17 (94.4%) exhibit increased AST and ALT levels and 1 (5.6%) case has only elevated AST level. The postoperative (post-OP) AST and ALT levels of all 18 cases with ELEzs were reassessed within 24 h. The average levels of both enzymes decrease significantly after laser therapy; maternal Hb and Hct also drop significantly after operation [Table 2]. Only one patient (5.5%) exhibited a higher post-op AST value than pre-op AST value (pre-op AST vs post-op AST: 116 vs 128 U/L). In addition, one patient (5.5%) exhibited a more than 10-fold elevation in enzyme levels (AST, 509 U/L; ALT, 485 U/L). The two aforementioned patients (11.1%) were referred to the gastrointestinal department for further investigation. Levels of hepatitis markers, including those for hepatitis B and C, and liver ultrasounds were normal, and AST and ALT levels in both patients returned to normal values prior to delivery.

In the ELEzs group, one case (5.5%) had more than 10-fold and one case (5.5%) had more than 5-fold elevations in AST levels, and three cases (16.7%) had more than 2-fold elevations in AST levels as compared to the normal limits. With regard to

Table 1 Characteristics and neonatal outcomes of twin-twin transfusion syndrome (TTTS) patients after fetoscopic laser therapy between elevated liver enzymes (ELEzs) and non-ELEzs groups.

	TTTS without ELEzs (n = 75)	TTTS with ELEzs (n = 18)	statistical test	*p* value
Maternal age (years)	31.2 ± 3.4	32.3 ± 3.9	Student t test	0.29
Gestational age of operation (weeks)	20.8 ± 2.6	21.02 ± 2.7	Student t test	0.61
High Quintero stage (stage III or IV)	38/75 (50.6%)	7/18 (36.8%)	Chi-square test	0.37
Body mass index (BMI) at laser therapy (Kg/M^2)	23.9 ± 3.3	24.1 ± 2.6	Student t test	0.85
maximum vertical pocket (MVP) of recipient (cm)	10.2 [3.5]	10.6 [4.1]	Mann–Whitney U test	0.43
MVP of donor (cm)	0 [1.1]	0.2 [1.1]	Mann–Whitney U test	0.93
Hemoglobin (g/dL) before operation	10.7 ± 1.1	10.6 ± 1.2	Student t test	0.87
Hemoglobin (g/dL) after operation	9.6 [1.5]	9.5 [1.3]	Mann–Whitney U test	0.56
hematocrit (%) before operation	32.0 [4.0]	32.1 [4.9]	Mann–Whitney U test	0.85
hematocrit (%) after operation	28.2 ± 2.8	28.7 ± 2.9	Student t test	0.55
Interval between operation and delivery (days)	74.6 ± 44.0	88.5 ± 40.6	Student t test	0.22
Gestational age at delivery (weeks)	32.4 [9.4]	35.6 [19.4]	Mann–Whitney U test	0.066
At least one survival rate	63/75 (84%)	16/18 (88.9%)	Fisher's exact test	1.0
Two survival rate	47/75 (62.7%)	11/18 (61.1%)	Chi-square test	0.90
Total survival	110/150 (73.3%)	27/36 (75.0%)	Chi-square test	0.83

Data are expressed as mean ± standard deviation, median [inter-quartile range] and frequency (%) as when appropriate.

Table 2 The maternal serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin (Hb) and hematocrit (Hct) values in twin-twin transfusion syndrome (TTTS) with elevated liver enzymes (ELEzs) (n = 18) before and after laser therapy.

	Before laser therapy	After laser therapy	*p* value
AST (U/L)	107 [146]	32 [19]	0.001
ALT (U/L)	163 [238]	100 [154]	<0.001
Hb (g/dL)	10.8 [1.6]	9.6 [1.5]	<0.001
Hct (%)	32.0 [4.2]	28.5 [4.0]	<0.001

p values were generated by Wilcoxon Signed Ranks Test.
Data are expressed as median [inter-quartile range].

ALT level, one case (5.5%) showed more than 10-fold, one case (5.5%) had more than 5-fold, and seven cases (38.9%) displayed more than 2-fold elevations in relation to the normal limits. Over all, there were 10 cases (55.5%) with either AST or ALT of more than 2-fold elevation before laser therapy.

Discussion

In this study, the authors found that 19.4% (18/93) of the patients with TTTS (diagnosed before 26 weeks of gestation) had ELEzs. Both the AST and ALT levels decreased after laser therapy in 17 (94.1%) of the 18 TTTS with ELEzs.The post-laser-therapy neonatal outcomes and gestational age of delivery are not associated with maternal ELEzs before operation.

Among the liver injury markers, ALT and AST are probably the most commonly used in both clinical diagnosis and research involving liver damage [23]. ALT is localized exclusively in the cytosol and essentially restricted to the hepatocytes and renal tubular epithelium [10]. By contrast; AST is widely distributed throughout the body, with the highest activity in the heart, liver, skeletal muscles, brain, gastric mucosa, kidney, pancreas, spleen, and lungs [24]. AST is typically elevated during hepatocellular injury caused by several factors, such as drugs, viral hepatitis, alcoholic hepatitis, autoimmune hepatitis, and ischemic hepatopathy. Throughout the course of pregnancy, ELEzs can occur in various conditions as mentioned above. However, our study cases did not exhibit the aforementioned conditions. Drug-induced liver dysfunction was also unlikely because the study patients were not administered with any drugs before assessing their blood liver enzyme levels.

A study ever reported that the serum AST activity and total bile acid concentrations did not differ between pregnant and non-pregnant women [25], and that liver disease during pregnancy could complicate up to 3% of all pregnancies [26]. Therefore, the fact that there was a relatively high incidence (19.4%) of ELEzs and that even setting the cut-off point at two-fold elevation of AST or ALT, there were still 10.7% (10/93) abnormal liver enzymes elevations observed in this study certainly warrants additional investigation.

Polyhydramnios occurring in TTTS is due to increased urinary output by the recipient twin, which markedly enlarges the uterus. Authors suspect a rapidly enlarged uterus may compress the liver in a very fast and intense way unlike in a normal multiple pregnancy where the pregnant woman can accommodate the gradually enlarged uterus. So the rapid process sequence caused liver congestion, resulting in elevation of liver enzymes in patients with TTTS. Laser therapy involves coagulating the intertwin anastomosis vessels, thus eliminating the pathological cause of TTTS. Furthermore, amnioreduction at the end of the procedure could relieve polyhydramnios. Thus, we hypothesize that maternal ELEzs that decreased after laser therapy as observed by this study is due to elimination of pathological TTTS following laser therapy and amnioreduction, which together relieved the liver congestion and subsequently improved the liver enzymes levels. Even though the mean MVP values in the ELEzs group were not significantly higher than those in the non-ELEzs group, it could mean that the value of MVP might not reflect the degree of liver congestion or the small case number in this study failed to deliver a significant difference. Amnioreduction after laser therapy with the removal of fluid more than 1000 cc had been reported to be associated with changes in Hb, Hct and protein plasma levels and significant hemodilution had been suggested as the cause [7]. The median Hb and Hct levels in the 19 TTTS cases with ELEzs drop significantly after laser therapy [Table 2], suggesting the hemodilution effect should also play a certain role in reducing maternal AST and ALT after laser therapy for TTTS.

This study had limitations: first, because of its retrospective study design, each TTTS patient with ELEzs did not receive a complete liver work-up and therefore other causes of ELEzs could not be ruled out; second, authors did not reassess the AST and ALT levels after therapy in the non-ELEzs group and thus how many patients exhibiting ELEzs after therapy is not known; third, the sample size was small and might not have an adequate statistical power to make a strong conclusion in terms of maternal characteristics like BMI or age, or even the difference of MVP of recipient twin between TTTS patients with or without ELEzs. Fourthly, authors do not check the post operation liver enzymes in TTTS without ELEzs, so the effect of hemo-dilution on the liver enzyme values in this group of patient had not been evaluated.

Conclusions

ELEzs is observed before laser therapy in 19.4% of the patients with TTTS diagnosed before 26 weeks in our series. A majority of 94.1% of patients had their liver enzymes levels decreased after laser therapy in this study. ELEzs before laser therapy do not significantly relate to the post-laser-therapy perinatal outcomes of the TTTS patients.

Conflicts of interest

All authors declare they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2019.05.006.

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