A Single Centre Prospective Study of Liver Function Tests in Post Liver Transplant Patients

Pradeep Naik · Venkataraman Sritharan ·
Premsagar Bandi · Mallikarjuna Madhavarapu

Received: 16 January 2012 / Accepted: 20 July 2012 / Published online: 17 August 2012
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Abstract Liver transplantation means surgical replacement of a diseased liver with a healthy liver. The survival rate used to be 30 % after 1 year and LTx was considered to be the last procedure when all medical or surgical intervention failed. Advances in donor organ preservation, surgical techniques, patient selection, immunosuppressive regimens and treatments for opportunistic infections all have contributed to substantially improve the survival rates. Despite substantial technological, medical and surgical advances, liver transplantation remains a complex procedure that is accompanied by significant morbidity and mortality. The post-operative outcome of each patient varies greatly depending on the patient's pre-operative state, quality of the donated organ and the complexity of the surgery. Complications occur both immediately post transplant and in the long term. Most of the problems can be satisfactorily assessed with a panel of routine LFTs results of which are generated quickly, cheaply on the analyzer which operates 24 h. Liver Function Test identifies the presence of problem but not problem itself. Abnormal results can be meaningful only when used with clinical data, radiological findings. The study includes 75 post LTx patients in three groups adults (non ACR), Pediatrics and ACR. All recipients were on immunosuppressive therapy (tacrolimus, mycophenolate and methylprednisolone), antiviral (ganciclovir), antipprotozoal, antibacterial and antifungal (fluconazole). 5 mL of blood was drawn in plain vacutainer from the post LTx patients every

day for 15 days and LFT and GGT was done. Routinely performed liver function tests correlates well with clinical complications involving liver in the transplant patients. Instead of daily testing, may be alternate day analysis of LFT should be sufficient for effective monitoring of patients. The total protein and albumin and the transaminases offer little help in monitoring LFT post LTx. The elevated levels of serum GGT and ALP may be related to chronic immune damage to the transplanted liver. Serum GGT and ALP can be used as early markers for diagnosing biliary complications and can be used to assess adequacy of endoscopic treatment in the group of patients presenting early. Thus, most of the problems can be satisfactorily assessed with a panel of routine LFTs generated quickly, cheaply on analyzer which operates 24 h each day. However, it must be emphasized that LFTs may identify the presence of problems but not the problem itself and the abnormal results are meaningful only when correlated with other clinical information.

Keywords Liver transplant · AST · ALT · GGT · ALP · Acute cellular rejection

Introduction

Liver transplantation (LTx) means surgical replacement of a diseased liver with a healthy liver. The survival rate used to be 30 % after 1 year and LTx was considered to be the last procedure when all medical or surgical intervention failed. The first LTx was done in 1963 by Dr. Thomas Starzl and coworkers in Denver [1]. However, since 1980s advances in donor organ preservation, surgical techniques, patient selection, immunosuppressive regimens and treatments for opportunistic infections all have contributed to substantially improve the survival rates [2, 3]. Currently

P. Naik (✉) · P. Bandi · M. Madhavarapu
Department of Clinical Biochemistry, Global Hospitals,
Lakdikapool, Hyderabad 500004, India
e-mail: pradnaik408@yahoo.com

V. Sritharan
Department of Molecular Diagnostics and Biomarkers,
Global Hospitals, Hyderabad, India

Table 1 Liver function tests

Function assessed	Analytes	Other data
Synthetic	Albumin (ALB)	Clinical symptoms, coagulation
	Ammonia	
	Prothrombin time	
Multi compartmental	Bilirubin (total) (BIL T)	Clinical symptoms, transfusion
Biliary	Alkaline phosphatase (ALP)	Clinical symptoms, ultrasound biopsy
	Gamma glutamyl transferase (GGT)	
Hepatocyte integrity	Aspartate transaminase (AST)	Clinical symptoms
	Alanine transaminase (ALT)	

survival rates of over 90–95 and 70 % respectively at 1 and 5 years post-LTx are common [4]. LTx is now an established therapy for end stage liver disease [5]. While cirrhosis is the reason for more than 80 % of transplantations performed in adults, biliary atresia and metabolic liver disease [6] are the major indications in children.

Despite substantial technological, medical and surgical advances, liver transplantation remains a complex procedure that is accompanied by significant morbidity and mortality.

The post-operative outcome of each patient varies greatly depending on the patient's pre-operative state, quality of the donated organ and the complexity of the surgery. Complications occur both immediately post transplant and in the long term.

The main complications in the immediate post transplant period are related to the functioning of the graft (dysfunction or rejection), surgical technique, infections (bacterial, fungal and viral) and other systemic problems (pulmonary, renal or neurological). In the long-term, typically as a consequence of the prolonged immuno suppressive therapy, diabetes mellitus, systemic arterial hypertension, de novo neoplasia and organ toxicities, particularly nephrotoxicity [7] and recurrence of the original disease may threaten survival and graft loss.

After transplant a variety of clinical problems may require laboratory services for accurate, timely diagnosis, monitoring and intervention. Rapid turnaround time (2 h or less) is a critical factor which will support clinical decision making and provide real diagnostic value and save cost.

Certain clinical problems, whose early presentation must be anticipated, include graft ischemia, primary non-function and arterial thrombosis. Acute cellular rejection (ACR) is common at 5–10 days post LTx, the principal target being biliary tree. A successful LTx program can be adequately supported with a battery of simple automated tests that are cheap, fast and available at all times.

Most of the problems can be satisfactorily assessed with a panel of routine LFTs [8] results of which are generated quickly, cheaply on the analyzer which operates 24 h. Liver function test (Table 1) identifies the presence of

problem but not problem itself. Abnormal results can be meaningful only when used with other data.

The studies of LFT Liver functions have been investigated and reported from other countries [8, 9] but to the best of our knowledge this study embodies results of first prospective investigation of liver functions in post LTx from India.

Materials and Methods

The study was conducted at Global Hospitals Hyderabad India, for 7 years (Feb 2003–Dec 2009) and it includes 75 post LTx patients. All patients were categorized into three groups (A) adults ($n = 63$) non-ACR, (B) pediatrics ($n = 6$), (C) adults ($n = 6$) ACR. This included 50 cadaveric donors and 25 live donors. All the patients had an-hepatic phase between 60 and 80 min. Post-liver transplant, all recipients were on immunosuppressive therapy (tacrolimus, mycophenolate and methylprednisolone), antiviral (ganciclovir), antiprotozoal, antibacterial and antifungal (fluconazole). 5 mL of blood was drawn in plain vacutainer from the post LTx patients every day for 15 days. Serum was separated from blood by centrifugation after clot formation and analyzed for AST, ALT, total protein (TP), ALB, ALP, GGT, BIL (T & D) in a chemistry autoanalyser (Olympus AU 400). Methods used are shown in Table 2. Bio-Rad Quality controls were used to check accuracy and precision.

Results

Mean AST activity in adults was 12 times of the URL (Upper Reference Limit) on day one and reached normal limits by 9th day (Fig. 1a). Where as in pediatric recipients the mean AST activity was 5 times the URL on day one and it peaked on day 3 to 10–11 times of URL and stabilized by 10th day, though the mean activity remained one time higher than the URL.

In the adults the peak ALT activity (Fig. 1b) was 10 times of URL after transplant surgery which gradually

Table 2 Following were the methods used for analytes on auto analyzer AU 400

Analytes	Method	References
AST	IFCC	[10]
ALT	IFCC	[11]
ALP	IFCC (pNPP)	[12]
GGT	IFCC	[13]
BIL (T)	DPD	[14]
BIL (D)	DPD	[15]
TP	Biuret	[16]
ALB	BCG	[17]

decreased and reached normal levels by 7th day. In pediatric group it peaked on day 3 then declined gradually to normal limits on day 9.

Serum alkaline phosphatase levels (Fig. 2c) were unchanged immediately post LTx in adults and subsequently started increasing. In pediatric group ALP activity was twice as much higher than adults may be due to age and stabilized on day 7th. In ACR group though the activity of ALP was normal immediately after surgery its

levels gradually increased and it remained higher than normal thereafter.

In the adults and pediatric group the GGT activity (Fig. 2b) was normal after surgery and subsequently it started increasing. The increase in GGT activity was much more exaggerated in adults than in children.

The levels of TP (Fig. 1c) and albumin (Fig. 1d) were low after surgery in both adults and pediatric recipients which increased gradually indicating revival of synthesis function of liver.

Though there was marginal elevation of serum total bilirubin (Fig. 2a) immediately after surgery, it gradually decreased and reached normal levels on day 15 in both adult and pediatric LTx patients.

ACR in adult group, the activity of AST (Fig. 3d), ALT (Fig. 3c) was 20 times above the URL on first day which gradually decreased and reached normal range on 9th day while in the uncomplicated group of adults the activity of AST, ALT were 10–12 times above the URL on first day which gradually decreased and reached normal range on 9th day.

ALP levels in ACR in adult group (Fig. 4b) was normal till the day 9th after which it gradually increased and peaked on day 14th and then reached to normal range on day 15th.

Fig. 1 Comparisons parameters in adults versus pediatric

Fig. 2 Comparisons parameters in adults versus pediatrics

In uncomplicated group of adults, the activity of ALP was normal till the 8th day and then gradually decreased till day 15th.

The GGT (Fig. 4c) activity reached four times above the URL in adult ACR group and after treatment the activity decreased and peaked again on day 14th and when it reached to 3 times above the URL.

In uncomplicated group of adults initially the GGT activity was normal and continued to increase from day 3 and remained elevated.

The levels of TP (Fig. 3a) and albumin (Fig. 3b) were low after surgery in both ACR and uncomplicated recipients of adults who increased gradually indicating revival of synthesis function of liver.

In ACR group of adults the concentration of total bilirubin (Fig. 4a) peaked on 8–11 day and declined thereafter reaching normal levels on day 15th.

In uncomplicated group of adults there was marginal elevation of serum total Bilirubin immediately after surgery, it gradually decreased and reached normal levels on day 15.

Discussion

Despite improvements in immunosuppressive therapy, hepatic allograft rejection remains an important cause of morbidity and late graft loss in patients undergoing liver transplantation [18–23]. In this study all liver panel tests, LFTs, were done every day for 15 days in post-LTx patients. The LFTs were done to assess the status of the liver in post transplant patients and to monitor the recovery of liver functions and to predict risk of rejection if possible.

Early acute cellular rejection occurs within 5–10 days of the liver transplant and it will be useful if this could be determined and reported to the hepatologists. Elevation of the liver enzyme levels is the probably the earliest biochemical marker of liver dysfunction. Abnormal results reflect clinical complications in these patients after the transplant. The present study observed a marked increase in all the enzymes immediately after the surgical procedure which correlated with studies done by Goldberg et.al. [24].

There are a number of studies reporting the role of endoscopy in diagnosis and management of biliary complications. Endoscopic retrograde cholangiography with sensitivity and specificity of 53 and 98 % respectively is best in predicting overall biliary complications. Not many studies in India reveal the role of liver function tests and their significance in this regard.

TP and total albumin, though estimated in all patients for 15 days did not show appreciable sensitivity to be used as markers for revival of synthetic function of liver.

Bilirubin being a parameter for excretory function of liver is very important analyte. An increase in the level of bilirubin is observed immediately after the procedure in all these groups, but gradually returns to normal levels within 15 days. Wang et al. [32] reported rapid increase of serum total bilirubin from 4.8 mg/dL and reached peak levels of 63.8 mg/dL. Whereas, our results showed similar trend, the peak level was 22 mg/dL on day 8th in non-ACR group.

Fig. 3 Comparisons parameters in ACR versus non ACR in adults

Even in the cases of ACR though the levels of bilirubin rose very high between 8 and 10 days, they drastically came down to normal levels by day 15. The high levels of bilirubin immediately after the procedure could have resulted from pre-transplant liver disease, tissue injury during surgery and blood transfusion intra operatively, make it likely that bilirubin concentration will be increased without any diagnostic importance being attached [9]. Decrease in levels of bilirubin is indicative of return of conjugation and detoxification process in liver and hence good graft functioning.

All the transplant patients were treated with tacrolimus and steroids for immuno suppression [25]. Steroids are known inducers of liver enzyme activities, although effect of tacrolimus on serum enzymes is unknown.

The present study observed an increase in the AST and ALT to multiples of normal levels initially in all three groups, but they reached normal level within a weeks' time in uncomplicated cases. Transaminases reflect hepatocytes integrity and health. Therefore, their elevated levels should not be considered as indicative of rejection immediately post-LTx. Further, majority of transplant cases, the initial target in rejection is the biliary system and therefore transaminases activity which reflects hepatocyte damage should not rise until rejection is well established. Therefore an increase in transaminase activity should not be used as principle index of liver rejection. It has been reported that

an early rise in transaminase activity is associated more with severe disease although this assertion is disputed [26] the early peaking of transaminases represents the preservation injury to donor's organ acquired during its removal from donor and subsequently transplantation. Hickman reported as the transaminases are 4–5 times above the URL in uncomplicated groups on day 1. Our results showed that the transaminases activity was 10–12 times above the URL reflecting more e preservation related injury to donor organ compared that reported by Hickman [30].

As already discussed the initial attack in the rejection episode is specifically directed against the biliary system, it may be anticipated that biliary markers like ALP and GGT would be the most informative [28] and usually will be confirmed by liver biopsy [29].

The study observed the initial levels of ALP in pediatric were several folds higher than the URL due to the age factor. In the adults initial levels were high and enhanced two fold by the end of 15 days. In the ACR group it started as normal but increased by fivefold by day 15 indicating a progressive decline in liver function raising the possibility that this could be a good marker for risk profiling and prognosis of transplant associated complications or rejection.

GGT as a marker of rejection is gaining value [27] and is considered a better marker than ALP as the latter increased in bone disease whereas GGT does not. Serum GGT levels were elevated several folds in both adult and

Fig. 4 Comparisons parameters in ACR versus non ACR in adults

pediatric LTx patients and the ACR category of patients also showed a mean GGT levels progressively higher than normal. The increase in GGT may reflect poor health and integrity of hepatocytes secondary to a viral infection of the liver. Immunosuppressant drugs predispose patients to various viral infections that can involve the liver to lead to increase in GGT level. GGT may be increased as a result of immune-mediated liver damage also.

Studies showed [30, 31] the relative utility of GGT and ALP in diagnosis of rejection and found GGT to be very

much more useful with a sensitivity for clinically significant rejection of 91 % compared with ALP's diagnostic sensitivity of 68.7 %. The positive predictive value for GGT was 70.9 % and 67.6 % for ALP.

Hickman et al. [30] reported peak GGT activity 2–3 times of normal on day 3 and 4 and it rose steadily thereafter and reached 6 times normal levels on 11th day. We obtained similar results in our study also; the levels peaked early during day 3–4 and subsequently continued to increase with fluctuations indicating a deteriorating health of the transplanted liver in ACR cases.

Hickman did not observe any significant elevation in the levels of ALP till day 9 after which it started increasing continuously. In our study also, ALP activity did not change much till day 11 after which it started increasing apparently indicating a deteriorating liver function in ACR cases.

Total protein, albumin, ALT did not correlate with the failing liver function in these ACR cases during 15 days following transplant.

Our study and the report published by Hickman et al. showed that the total bilirubin was elevated (about 5 mg/dL) immediately after the transplant and it continued to increase post-transplant in ACR cases and reached a peak of about 20 mg/dL on day 10 and decreased thereafter due to treatment.

To summarize, Hickman's reports and our findings show that impending/risk of acute rejections post liver transplant could be evaluated by monitoring the levels of GGT, ALP and total bilirubin.

GGT and ALP are quite sensitive in predicting biliary complications, on the other hand transaminases AST and ALT and bilirubin were found to be of little clinical significance in diagnosing these complications. GGT and ALP levels are sensitive indicators of rejection related biliary duct injury and should correlate well with sensitive imaging results. If there is no evidence of biliary obstruction, then these elevated activities are likely due to drug induced injury.

To sum up, the above results of LFT in adults and pediatrics groups show that the quality of donor organs was good and an-hepatic phase of 60–80 min was also good. The results of ACR group show that the immunosuppressive and steroid therapies were effectively monitored through these LFTs. The early peaking of transaminases apparently represents the injury to donor's organ acquired during its removal from the donor and subsequently during preservation and transportation prior to transport. Total proteins and albumin in adults and pediatrics recipients though low immediately post-LTx, returned to normal limits indicating the revival of synthetic function of liver. The rise in GGT and ALP are significant and can be used as early, non-invasive and inexpensive markers for diagnosing biliary

complications in post liver transplant and for risk profiling of patients for rejection [31, 32].

Conclusion

Routinely performed liver function tests correlates well with clinical complications involving liver in the transplant patients. Instead of daily testing, may be alternate day analysis of LFT should be sufficient for effective monitoring of patients. The total protein and albumin and the transaminases offer little help in monitoring LFT post LTx. The elevated levels of serum GGT and ALP may be related to chronic immune damage to the transplanted liver. Serum GGT and ALP can be used as early markers for diagnosing biliary complications and can be used to asses adequacy of endoscopic treatment in the group of patients presenting early. Thus, most of the problems can be satisfactorily assed with a panel of routine LFTs generated quickly, cheaply on analyzer which operates 24 h each day. However, it must be emphasized that LFTs may identify the presence of problems but not the problem itself and the abnormal results are meaningful only when correlated with other clinical information.

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