

Transition of Serum Alkaline Phosphatase Isoenzymes during Liver Regeneration in  
Humans

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#### Abbreviations

ALP alkaline phosphatase

POD postoperative days

LRR liver regeneration rate

CT computed tomography

HG high regeneration group

LG low regeneration group

## ABSTRACT

**Background/ Aims:** Serum alkaline phosphatase (ALP) levels tend to increase after hepatectomy, however, no previous examinations have yet focused on the relationship between liver regeneration and the individual ALP isoenzymes levels.

**Methodology:** Forty living liver transplantation donors who underwent hemi-hepatectomy were herein investigated. We evaluated the serum ALP levels and ALP isoenzyme levels preoperatively and postoperatively. The liver regeneration rate (LRR) was calculated using volumetry. According to the LRR, we divided the donors into two groups, consisting of a high regeneration group (HG) and a low regeneration group (LG).

**Results:** The total serum ALP levels increased gradually after hepatectomy and peaked on postoperative days (POD) 14. ALP-1 was not detected in any donor preoperatively; however it was detected after hepatectomy, peaking on POD 7. The serum ALP-2 level increased after hepatectomy, reaching a peak level on POD 14. The ALP-2 levels gradually increased after hepatectomy and reached peak levels on POD 14 in both groups. However, the ALP-2 level on POD 14 was significantly higher in HG than LG.

**Conclusions:** The serum ALP- 2 levels after POD 14 might therefore be a useful indicator of favorable liver regeneration following hepatectomy, especially in patients who have a normal liver function.

## INTRODUCTION

The liver is one of the few organs that has the ability to regenerate in the human body.

Several indices are used as markers of liver regeneration, including alpha-fetoprotein

and interleukin-6 (1,2). Alkaline phosphatase (ALP), which is a nonspecific

phosphomonoesterase usually linked to the external cell surface via glycosyl

phosphatidylinositol, may also be applicable as a marker of liver regeneration. Partial

hepatectomy is known to cause an increase in the activity of ALP (3). Several reports

have discussed the relationship between the total serum ALP levels and liver

regeneration (4). In 1950, Burke et al. (3) reported that ALP is present in the cellular

membrane and is associated with liver regeneration. Osada et al. (5) described the

monitoring of the postoperative levels of ALP, and suggested that ALP may be a

convenient indicator to predict liver failure after hepatectomy. However, the total

serum levels of ALP during liver regeneration are not specific, and using the total ALP

level as a marker of liver regeneration is controversial. We therefore focused on the

levels of individual ALP isoenzymes in this study.

ALP determinations were first applied to the investigation of bone disease on a

theoretical basis by Robinson (6). ALP consists of several isoenzymes in humans.

ALP-1 originates in the liver (biliary system and hepatocyte), ALP-2 the liver (intrahepatic bile duct), ALP-3 in the bone, ALP-4 in the placenta, ALP-5 in the small intestine and ALP-6 in the IgG-bound ALP (7). Therefore, measuring the fraction of each isoenzyme in patients with elevated ALP is considered to be a useful diagnostic tool (8). For example, an elevation of the ALP-1 or 2 levels can indicate the existence of an obstruction or malignant tumor in the biliary system (9). The serum ALP-3 level also increases under certain normal conditions, especially during the growth period (10).

To our knowledge, there have not been any studies which examined the relationship between liver regeneration and serum ALP isoenzymes. To clarify the transition of serum ALP isoenzymes during liver regeneration, we analyzed the ALP isoenzymes expression levels of donors who underwent a hemi-hepatectomy for living donor liver transplantation. We herein report the results of our examination of living liver transplantation donors who had normal liver function; a normal range for both the serum transaminase levels and total bilirubin levels, a normal coagulation profile and a negative hepatitis B and C status. We believe that our results may reflect the physiological phenomenon of liver regeneration in humans.

## **PATIENTS AND METHODS**

Forty donors who underwent hemi-hepatectomy for living liver transplantation, including 17 right lobe grafts and 23 left lobe grafts, at our institution between April 2004 and December 2009 were included in this study. Right or left lateral section graft donors were excluded. To clearly determine the relationship between liver regeneration and isoenzyme serum levels of ALP, we evaluated the serum ALP levels and ALP isoenzyme (ALP-1, ALP-2, ALP-3, ALP-4, ALP-5) levels preoperatively, and again on postoperative days (POD) 3, 7, 14, and 28. The liver regeneration rate (LRR) (LRR = regenerated liver volume of POD 28/remnant liver volume of POD 0) was calculated using volumetry based on computed tomography (CT). We divided the donors into two groups according to their LRR, a high regeneration group (HG; LRR  $\geq$  1.5) and a low regeneration group (LG; LRR < 1.5). We analyzed each isoenzyme level of ALP at each time point, and also compared the changes of each isoenzyme between the HG and LG groups postoperatively.

## **STATISTICAL ANALYSIS**

The Mann-Whitney *u*-test was used for statistical analysis. *P* values < 0.05 were

regarded as statistically significant.

## RESULTS

Twenty-five donors were male and 15 were female, with a median age of 32 years-old (range: 20-63y). Seventeen donated right lobe grafts and 23 donated left lobe grafts.

The postoperative total serum ALP levels of all patients are shown in **Figure 1**. The total serum ALP levels increased gradually after the hepatectomy, and reached peak levels on POD 14. Concerning the individual isoenzymes of ALP (**Figure 2**), ALP-1 was not detected in any donor preoperatively; however it was present after the hepatectomy, peaking on POD 7 (10.5% of total ALP) and gradually decreasing thereafter. ALP-2 levels also elevated after the hepatectomy and reached peak levels on POD 14 (68.7% of total ALP).

We also compared the serum ALP levels and ALP isoenzyme levels between the high regeneration group (HG) and the low regeneration group (LG). Sixteen patients were included in the HG and twenty-four patients were included in the LG. There were no significant differences in the donors' ages between the two groups. In the HG, the resected liver volume was significantly higher than in the LG (62.0% vs



35.1%). The percentage of resected patients in the HG group was significantly higher than that in the LG group (**Table 1**). The total serum ALP levels in the HG tended to be higher than the LG (**Figure 3**). The ALP-2 levels gradually increased after hepatectomies and reached peak levels on POD 14 in both groups. However, the level of ALP-2 on POD 14 was significantly higher in the HG than the LG (**Figure 4**). The other ALP isoenzyme levels were not significantly different between the patients in the HG and the LG.

## **DISCUSSION**

The regenerative capacity of the liver has been known for hundreds of years, but the mechanisms underlying liver regeneration are still unclear. We have shown that the levels of each ALP isoenzyme change during liver regeneration. One of the interesting points presented in this study is that we examined donors involved in living liver transplantation. This enabled us to elucidate the mechanisms of liver regeneration, because it presents an ideal method for monitoring liver regeneration *in vivo*. Previous reports about liver regeneration in humans have focused on dysfunctional livers, including cases of hepatocellular carcinoma (11).

Mori et al. (12) described that ALP activity increased in rat hepatocyte culture after the cell density reaches confluency. They also showed that the induced ALP activity is mainly localized at the apical surface membrane of the cells. These results suggest that during the regeneration period, the hepatocyte produces ALP on its membrane. In our study, we showed that serum ALP-2 levels increased after hemi-hepatectomy in living liver transplant donors. Moreover, the levels of ALP-2 in the HG were higher than those in the LG. We hypothesize that ALP-2, which is synthesized in the liver, increases during the regeneration period in tandem with the increase in liver growth.

On the other hand, Kaplan et al. (4) hypothesized that serum ALP levels increase after hepatectomy because the increased portal or hepatic venous pressure might cause swelling around the intrahepatic vascular, which in turn compresses the intraparenchymal biliary tract mimicking an obstructive process. However, we also observed that even if serum ALP-1 was not detected preoperatively, it could be detected after the hepatectomy. There is a possibility that pseudo-biliary tract obstruction after the hepatectomy causes the increase in ALP-1. More investigations are needed to clarify the mechanisms underlying the increases in both of the isoenzymes and their role in liver regeneration.

Concerning the LRR, the serum ALP-2 levels on POD 14 were significantly higher in the HG than in the LG, although there was a peak at POD 14 in both groups, and a gradual decrease thereafter in both groups. The serum ALP-2 levels may therefore reflect the speed of liver regeneration or the amount of regenerated liver.

The isoenzyme levels of ALP, with the exception of ALP-1 and ALP-2, were not affected by liver resection, possibly because these isoenzymes do not originate from the hepato-biliary tract. This phenomenon also supports our hypothesis that serum levels of ALP-1 or ALP-2 may be a marker for liver regeneration.

In conclusion, the ratio of each fraction of ALP isoenzymes changes in a time-dependent manner, after the hemi-hepatectomy in living liver transplant donors, who have normal remnant livers. It might therefore be possible to use the ratio of ALP-2 levels preoperatively and at various times postoperatively as an indicator of favorable liver regeneration following hepatectomy, especially in patients who have a normal liver function.

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***Figure Legends***

Figure 1. Total ALP levels after hepatectomy in donors of living donor liver transplantation. Abbreviations : ALP, Alkaline phosphatase; POD, Postoperative day.

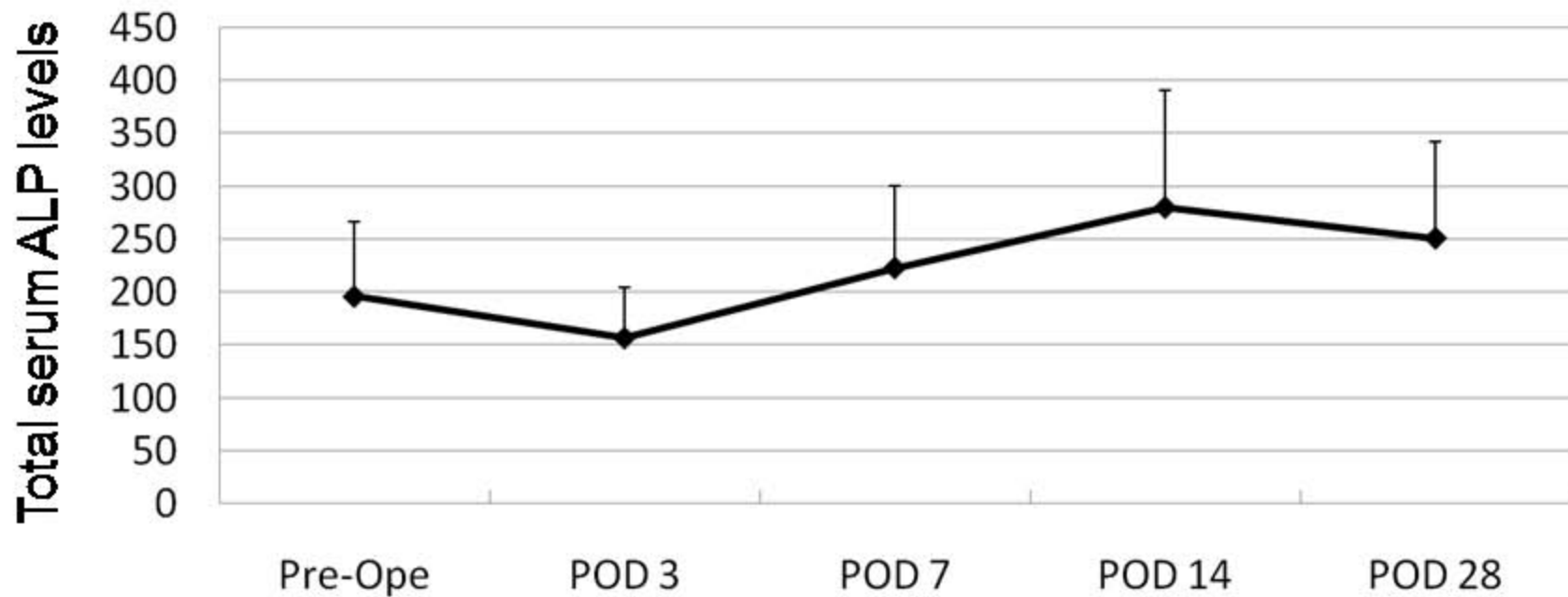
Figure 2. Postoperative transition of the ratio of ALP isoenzymes in donors of living donor liver transplantation. Abbreviations : ALP, Alkaline phosphatase; POD, Postoperative day.

Figure 3. Postoperative total ALP levels in each group according to liver regeneration rates. Abbreviations : ALP, Alkaline phosphatase; H group, High regeneration group; L group, Low regeneration group.

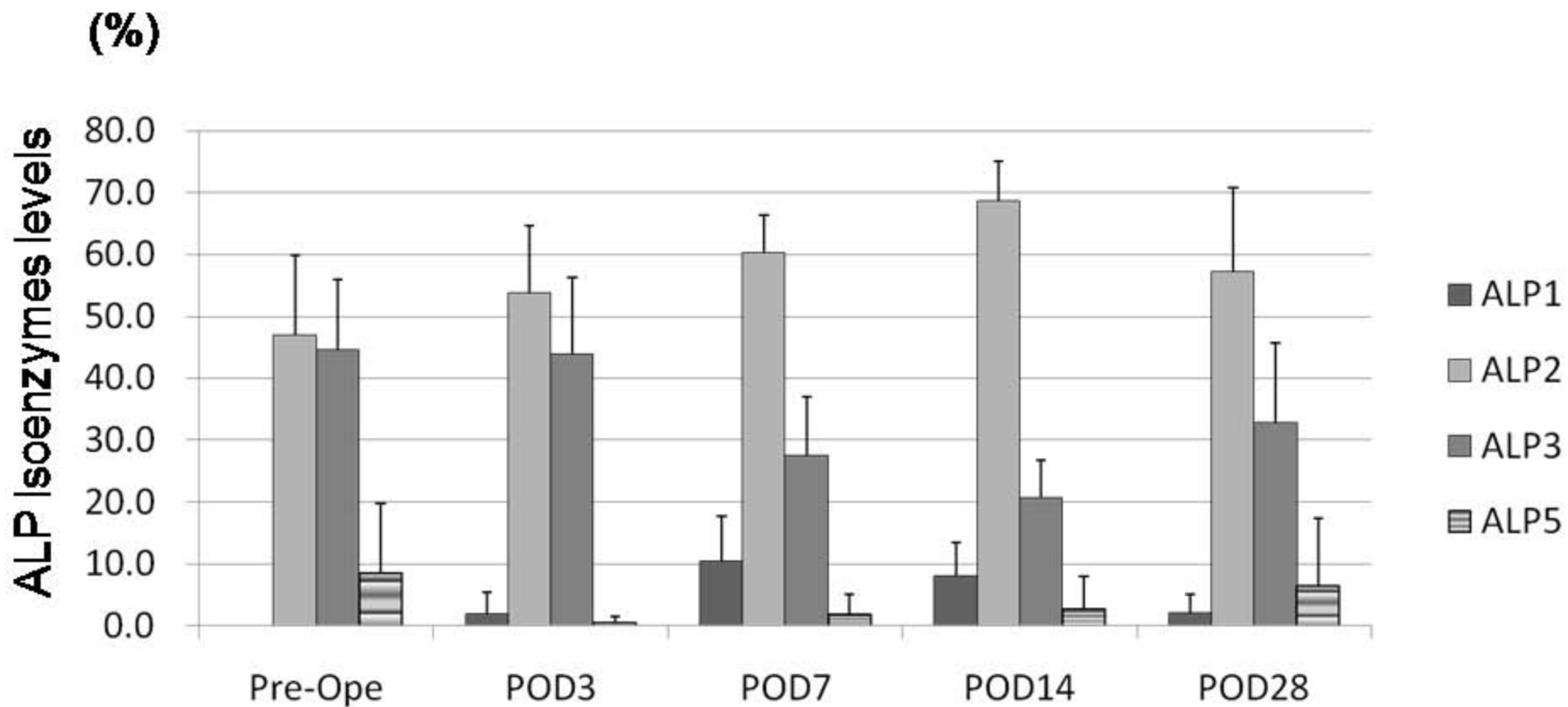
Figure 4. Expression of the ALP isoenzyme levels according to liver regeneration rates. Abbreviations : ALP, Alkaline phosphatase; H group, High regeneration group; L group, Low regeneration group.

# Figure 1

(IU/l)

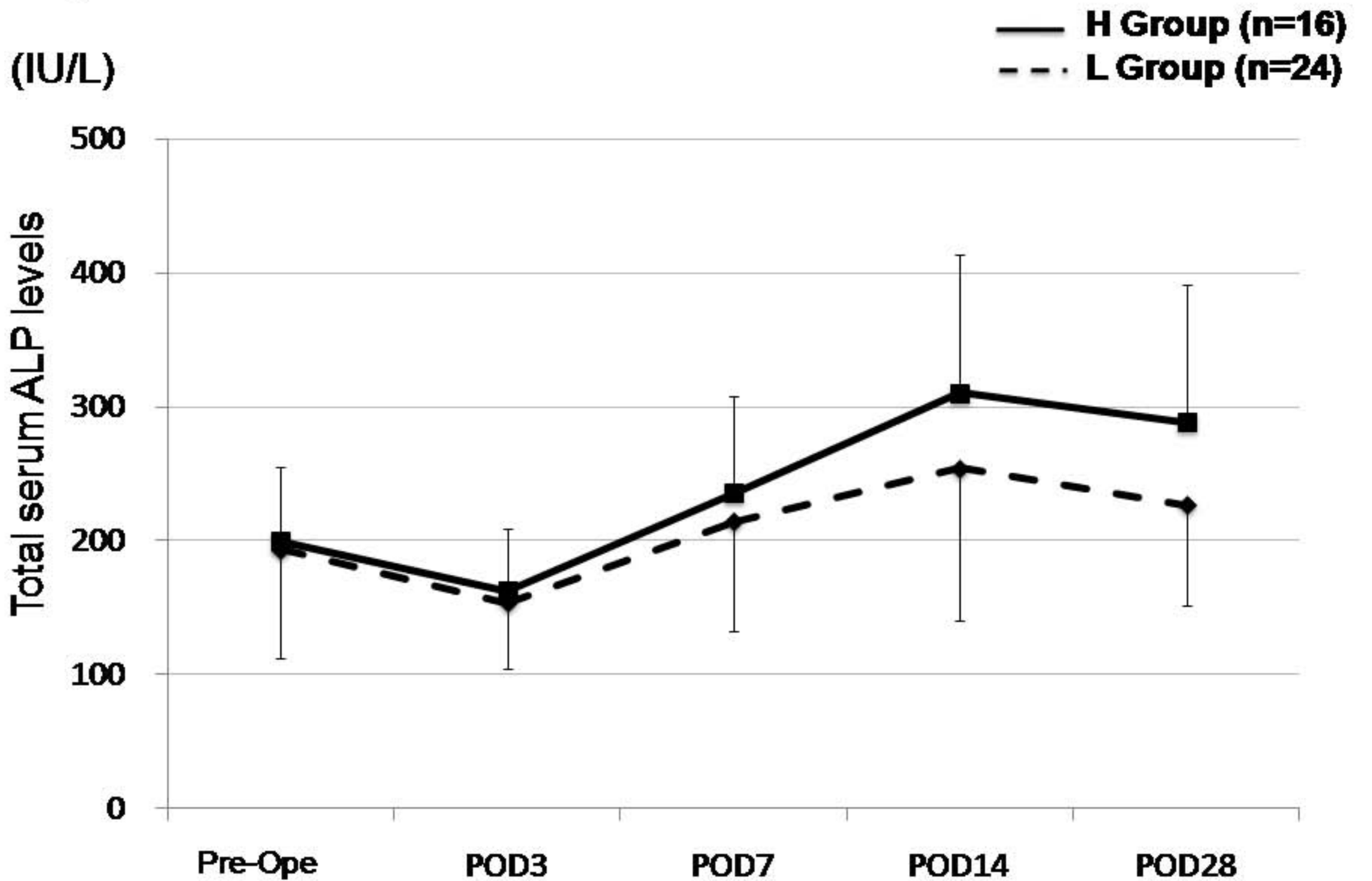


# Figure 2





# Figure 3

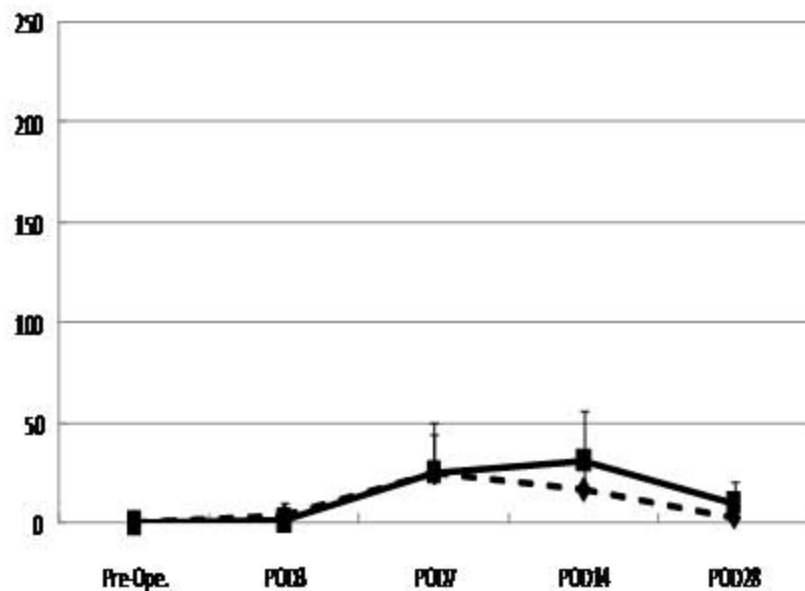


# Figure 4

— H Group (n=16)  
- - L Group (n=24)

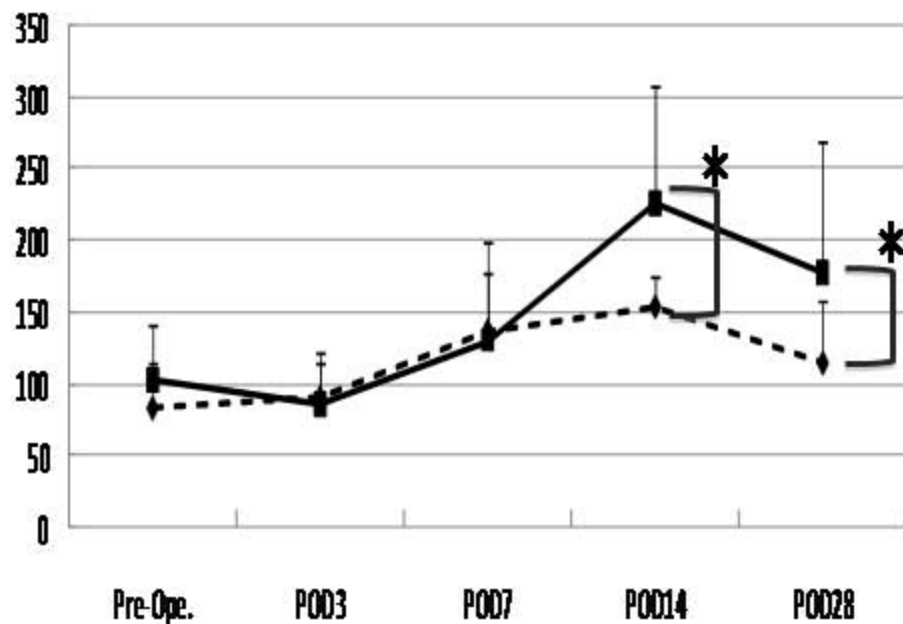
## ALP1

(IU/l)



## ALP2

(IU/l)



\* P < 0.05

**TABLE 1**

Characteristics of LDLT donors in each group according to liver regeneration rates.

	H group (n=16)	L group (n=24)	p-value
Age	37 (21 - 63)	32 (20 - 55)	N.S.
Resected liver volume (%)	62.0 (37.4 - 70.8)	35.1 (27.8 - 66)	<0.001
Rt lobe vs. Lt. lobe	13 : 3	4 : 20	<0.001

H group: high regeneration rate group

L group: low regeneration rate group