Association Between Serum 25-hydroxy-vitamin D Level, Liver Function and Liver Fibrosis in Biliary Atresia Infants One Year After Kasai Procedure

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Abstract

Objective: To investigate the relationship between serum 25 (OH) D level, liver function, and liver fibrosis in infants with in biliary atresia (BA) infants one year after Kasai procedure.

Methods: This retrospective study was conducted using the clinical data of children with BA confirmed by cholangiography and/or surgery admitted to Children Hospital of Shanxi Province between October 2018 and October 2020, as well as the follow-up data at 1, 3, 6, and 12 months after Kasai Portoenterotomy so as to analyze the relationship between serum 25(OH) D level, liver function, and liver fibrosis.

Results: Preoperative data of 57 infants with BA were collected. All infants had vitamin D insufficiency or deficiency status before surgery. Three months after surgery, data were collected from 55 infants, 15 of whom did not take vitamin D supplements and 40 of whom received regular vitamin D supplementation. It was found that 15.38%, 47.22%, 75%, and 92.31% of infants living with regular vitamin D supplementation and native liver had sufficient vitamin D levels at 3, 6, and 12 months after Kasai surgery, respectively. The serum 25(OH) D level was significantly lower in patients without vitamin D supplements at 3 months after surgery (t=-2.974，P＜0.05), and the aspartate aminotransferase to platelet ratio index (APRI) was higher in patients with vitamin D supplementation. There was no significant correlation between vitamin D level, liver function, and liver fibrosis preoperatively (P＞0.05). The level of serum 25(OH) D was negatively correlated with liver fibrosis and liver function in infants with the native liver at 12 months after surgery ($P < 0.05$).

Conclusion: Vitamin D deficiency was commonly found in children with BA before operation. Nearly 70% of infants living with native liver reached sufficient vitamin D levels within 6 months after surgery with routine vitamin D supplementation. Serum 25 (OH)D level can reflect the degree of liver fibrosis and liver function in biliary atresia infants living with the native liver at 12 months after the Kasai procedure.

Introduction

Biliary atresia (BA) is characterized by inflammation and fibrosis of the intra- and extra-hepatic biliary tree. Without timely treatment, it may lead to death within two years of age. Kasai portoenterotomy, which is the first choice of treatment for BA[1], can facilitate the bile flow, thus prolonging the survival time of the native liver in some children with extra-hepatic biliary obstruction. Nonetheless, about 40–50% of children still need liver transplantation within two years after surgery [2, 3].

Vitamin D is an essential fat-soluble vitamin whose absorption, transport, and utilization are closely related to liver function. The current studies have shown that the level of serum 25(OH) VD is closely related to liver fibrosis and related complications caused by chronic liver disease in adults [4,5]. In children and adolescents with liver disease, serum vitamin D is low [6]. Similarly, the children with BA generally suffer from vitamin D insufficiency or deficiency [7, 8]; however, few studies have reported on the serum vitamin D levels and the relationship between liver fibrosis and liver function within one year after the Kasai procedure. The purpose of this study was to analyze preoperative and postoperative 25(OH) D levels from 3 to 12 months after the Kasai procedure and their relationship with liver function and liver fibrosis.

Materials And Methods

Subjects

This retrospective study was conducted using the clinical data of children with BA confirmed by cholangiography and/or surgery admitted to Children Hospital of Shanxi Province between October 2018 and October 2020, as well as the follow-up data at 1, 3, 6, and 12 months after Kasai portoenterotomy.

Observation indicators

Sex, age, serum 25-hydroxyvitamin D (25(OH) VD) level, liver function, and liver fibrosis indicators were collected. Biochemical parameters of liver function included: aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (γGT), alkaline phosphatase (ALP), and total bile acid (TBA). Indicators of liver fibrosis include aspartate aminotransferase to platelet ratio index (APRI).

Definitions

Vitamin D levels were defined according to serum 25(OH)VD level recommended by global consensus recommendations in 2016 [10]: vitamin D sufficiency: serum 25(OH)VD: 50-250nmol/L (20-100ng/mL); insufficiency: serum 25(OH)VD: 30-50nmol/L (12-20ng/mL); deficiency: serum 25(OH)VD: <30nmol/L (<12ng/mL). The supplement plan included: vitamin D 400-800 IU/ D taken orally once per day. Radiographs of bone epiphysis were taken for children suspected of rickets. Vitamin D supplements were immediately stopped for those who suffered from overdose poisoning.

Statistical analysis

All data were analyzed by using SPSS 22.0. All the measurement data were tested for normality, and those with normal distribution were expressed as the mean ± standard deviation (SD), while those with non-normal distribution were expressed as median (Q25-Q75). Independent t-test or Mann–Whitney test was used for the analysis of two sample data according to the distribution. Multiple measurement data were compared with analysis of variance. The Student-Newman-Keula (SNK) test was used to compare the differences between groups. Chi-square or Fisher’s exact test was used to examine enumeration data. Correlations between variables were analyzed by using Spearman's correlation analysis or linear and logistic regression models. A P-value < 0.05 was considered statically significant.

Results

Clinical data

A total of 65 children diagnosed with BA were enrolled in this study, including 29 males and 36 females, with a median operation age of 60 days (50.75-70d). Among these children, 8 did not undergo the Kasai procedure. Serum samples were collected from 57 patients before surgery. Fifty-five samples (96.49%) were collected three months after the Kasai procedure until November 1, 2021; 15 postoperatively from infants without vitamin D supplementation (non-Vitamin D supplementation Group, non-VDS) and 40 from infants

with regular vitamin D supplementation (Vitamin D supplementation Group, VDS). At 6 and 12 months after the Kasai procedure, data were collected from 28 and 26 children with native liver and regular vitamin D supplementation, respectively. During the follow-up period, no children suffered from rickets or vitamin D poisoning, and the survival time of native liver was < 1 year in 9 cases.

Serum Vitamin D level in different periods before and after Kasai procedure

All the children were with vitamin D deficiency or insufficiency, where 31.58% were with vitamin D insufficiency, and 68.42% were vitamin D deficient (Table 1). Although there was no significant difference in the preoperative level of 25(OH) VD between the 15 children in the non-VDS group and the 40 children in the VDS group 3 months after surgery (t=-0.327, P=0.745, Table 2), the level of 25(OH) VD in the non-VDS group was significantly lower than that in the VDS group at 3 months after surgery (t=-2.974, P=0.004, Table 2). Among the 40 children who lived with the native liver at 3, 6, and 12 months after the Kasai procedure with regular oral vitamin D supplementation, 38.18%, 75%, and 92.31% were in vitamin D sufficiency status, respectively (Table 1). The level of 25(OH)VD at different periods before and after Kasai procedure significantly differed (F=50.802, P=0.000, Figure 1).

Table 1. Vitamin D status in children with regular vitamin D supplementation before and after Kasai procedure

	cases	Gender		Serum vitamin D level			
		male	female	$\bar{x}$±SD (nmol/L)	Sufficiency (%)	Insufficiency (%)	deficiency (%)
preoperative	57	27	30	27.01±9.14	0	18 (31.58)	39 (68.42)
3 months after surgery	40	21	19	55.73±27.64*	19 (38.18)	13 (36.36)	8 (25.45)
6 months after surgery	28	15	13	98.86±53.29	21 (75.00)	4 (14.29)	3 (10.71)
12 months after surgery	26	13	13	115.06±50.92	24 (92.31)	1 (3.85)	1 (3.85)

Table 2. Vitamin D status and liver fibrosis degree of patients with vitamin D supplementation 3 months after Kasai procedure

	Cases	Gender		25(OH)VD level		APRI	
		male	female	Before surgery	3 months after surgery	Before surgery	3 months after surgery
non-VDS	15	6	9	26.52±7.94	32.92±17.37	1.57±0.81	1.24±0.55
VDS	40	21	19	27.44±9.70	55.73±27.64*	1.60±1.25	0.93±0.59**

Note: * indicates that the serum 25(OH)VD level in the VDS group was significantly higher than the non-VDS group (t=-2.974, P=0.004). ** indicates that the degree of liver fibrosis in the VDS group was less than the non-VDS group, but the difference was not statistically significant (t=1.787, P=0.08).

There was no significant correlation between serum 25(OH)VD level and ALT, AST, γGT, ALP, TB, DB, TBA preoperatively (P□0.05, Table 3). The serum 25(OH)VD level was not significantly correlated with APRI value (r=-0.183□P=0.173).

Table 3. Association between 25(OH) VD level, liver function, and liver fibrosis indicators before and after Kasai procedure

	Preoperative			**Postoperative**		
	M□Q25-Q75□	*R*	*P*	*M□Q25-Q75□*	*R*	*P*
ALT(U/L)	163(107.58~225.23)	-0.261	0.050	77.25(48.73~142.23)	-0.234	0.023
AST(U/L)	246.50(159.50~323.18)	-0.191	0.155	106.80(61.00~191.98)	-0.406	0.000
γGT(U/L)	419.00(181.75~810.00)	0.166	0.216	497.00(248.25~738.25)	-0.425	0.000
ALP(U/L)	559.00(436.00~765.00)	-0.251	0.059	268.25(141.75~268.25)	-0.465	0.000
TBA	121.90(94.13~121.90)	0.144	0.290	39.90(13.20~101.98)	-0.619	0.000
TB(μmol/L)	190.80(157.65~262.73)	-0.216	0.106	12.45(5.00~38.18)	-0.697	0.000
DB(μmol/L)	116.85(94.93~147.35)	-0.133	0.324	22.95(4.88~61.43)	-0.258	0.012

Association of vitamin D level with liver function and liver fibrosis after Kasai procedure

In infants with native liver and regular vitamin D supplementation after surgery, the level of 25(OH)VD was negatively correlated with ALT, AST, γGT, TB, DB, and TBA (Table 3), and was negatively correlated with APRI (R^2=0.072, P=0.009, Figure 2). There were statistically significant differences in the degree of liver fibrosis at different periods before and after the Kasai procedure (F=5.033, P=0.002). In infants living with native liver, APRI at 3 and 6 months after surgery was lower than that before surgery. The APRI level 12 months after surgery was lower than before, but the difference was not statistically significant (Figure 3). The degree of preoperative liver fibrosis in the non-VDS group was not significantly different from that in the VDS group

(t=-0.069, P=0.945), and APRI was slightly higher compared to the VDS group at 3 months after surgery; however, the difference was not statistically significant (T =1.787, P=0.08, Table 2).

Discussion

Vitamin D deficiency is a global health problem. VD deficiency in infancy can lead to rickets and pathological fracture. Therefore, understanding the changes in vitamin D levels in children with biliary atresia and different liver cirrhosis degrees after the Kasai procedure is of great importance for evaluating the liver function and liver fibrosis degree after the Kasai procedure and making postoperative supplementary plans.

Vitamin D levels before and after Kasai procedure in biliary atresia children

Our results revealed that preoperative vitamin D deficiency was common in children with biliary atresia, which was consistent with previous studies [7, 8, 11]. Currently, there is no universal consensus on vitamin D supplementation after the Kasai procedure. Davenport *et al.* [7] reported that the vitamin D levels in children with BA after the Kasai procedure were very low even though bile drainage was unobstructed and fat-soluble vitamin was orally supplemented. Yet, in the present study, we used the recommended dose of vitamin D to prevent rickets after the Kasai procedure (oral vitamin D 400-800 U per day), and 75% of the infants living with native liver reached sufficient vitamin D levels at 6 months after surgery. In our study, serum 25(OH) VD level was higher in 40 patients with regular vitamin D supplementation than in 15 patients who did not receive supplementation 3 months after the Kasai procedure. Also, the APRI value showed that the degree of liver fibrosis was reduced in the VDS group compared with the non-VDS group. We hypothesized that routine vitamin D supplementation could increase the level of 25(OH) VD and delay the progression of liver fibrosis. Although the sample size was small, the data we used were real data from a province in China, which can provide a reference for postoperative vitamin D supplementation for the Kasai procedure.

The association between vitamin D level and liver function and fibrosis

Previously, it was believed that the low vitamin D level in children with BA was mainly related to the obstruction of hepatoenteric circulation, the reduction or loss of intestinal bile acid concentrations causing the absorption barrier of fat-soluble vitamin, and the impaired liver cells leading to the transformation of the active component of vitamin D [8]. However, many clinical studies have found that vitamin D deficiency is also prevalent in adults and children with non-obstructive chronic liver disease [12-14]. Laboratory studies have also shown that vitamin D can delay the progression of liver fibrosis. The combination of Vitamin D and Vitamin D receptor (VDR) inhibits the activation of hepatic stellate cells and alleviates cholestatic liver injury and liver fibrosis by regulating TGF-β/Smad and Hh signaling pathways [15-17]. Our results revealed that the degree of liver fibrosis in children 1-year-old living with native liver could be improved by taking vitamin D supplementation.

We suspected that liver fibrosis alleviation might be related to enterohepatic circulation recovery, reduction of toxic effects of bile acids on hepatocytes, and vitamin D supplementation, which can delay the process

of liver fibrosis.

Zheng *et al*. [18] and Peng *et al*. [19] confirmed the correlation between serum vitamin D levels and the degree of liver fibrosis in children with BA but were not consistent with our study. Zheng *et al*. [20] mainly studied children with BA in the period of operation and found that preoperative vitamin D levels had no significant correlation with liver function level and were negatively correlated with liver fibrosis. In the present study, we found no correlation between preoperative vitamin D levels and liver fibrosis grade, which may be related to the inconsistency in sample size, geographical latitude, and criteria for defining vitamin D levels. On the other hand, liver fibrosis was evaluated by the Batts-Ludwig grading system and serum markers of type III procollagen, type IV collagen, laminin, and hyaluronic acid in their research. In the current study, Ohkumas and APRI [20, 21] were used to evaluate the degree of liver fibrosis. As these two evaluation methods were inconsistent, the relationship between preoperative serum 25(OH) D levels and the degree of liver fibrosis needs to be further studied. Peng *et al*. [19] found that the level of serum 25(OH) D was negatively correlated with the degree of liver fibrosis after the Kasai procedure, which was consistent with the results of our study. Nevertheless, the main subjects in this previously mentioned study underwent the Kasai procedure more than 1 year ago, while the main subjects in our study were those who underwent the Kasai procedure within 1 year and were living with native livers. Although we found postoperative serum 25 (OH) D levels were negatively correlated with the degree of liver fibrosis, we still could not clarify the causal relationship between vitamin D levels and liver fibrosis. Accordingly, future large-scale, multicenter studies are needed to reveal the causal relationship between vitamin D levels and liver fibrosis. It is expected that improving vitamin D levels could delay liver fibrosis progression and prolong the autologous liver's survival time.

This study suggested that children with BA generally suffer from preoperative vitamin D deficiency; however, no obvious correlation was found with the degree of liver function and liver fibrosis. Our results revealed that oral vitamin D supplements in infants with the native liver could provide enough vitamin D. This can provide a theoretical basis for a postoperative vitamin D supplement plan in children with BA.

However, there are some limitations in the present research: (1) this study is a cross-sectional study, so the relationship between vitamin D levels and liver fibrosis cannot be clarified. (2) This research did not exclude other confounding factors, which may affect vitamin D levels such as: environmental factors, i.e., the geographical latitude, season, sunshine time; personal factors such as diet, outdoor activity time, protecting the skin from light during the outdoor activities, genes, as well as the classification of BA, age at surgery and whether the children were taking corticosteroids postoperatively. In addition, there may be some bias in the results. (3) Only intraoperative histopathological examination results of liver fibrosis were available in this study, and liver biopsy results at different postoperative periods were lacking. Whether vitamin D supplementation can prevent the occurrence of liver fibrosis remains to be further observed. Further studies are needed to confirm the causal relationship between serum 25(OH) D levels and liver fibrosis.

Abbreviations

BA: Biliary atresia; 25(OH) D: 25-hydroxy-vitamin D; AST: aspartate transaminase; ALT: alanine transaminase; γ GT: gamma-glutamyl transferase; ALP: alkaline phosphatase; TBA: total bile acid; APRI:

aspartate aminotransferase to platelet ratio index; VD: Vitamin D.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Xiaoxia Wu analyzed the data and drafted the manuscript. Hongxia Ren critically revised important intellectual content in the manuscript. Hui Zhang, Baohong Zhao, Yuanyuan Jin, Wenyue Liu, Liang Zhao, Xue Sun, Xin Guo, and Wei Li collected the data. All authors read and approved the final manuscript.

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Availability of data and materials

Our raw data can be shared dy a public repository.

https://pan.baidu.com/s/1L7NLPi8nH2a-7YhpcWC4kA?pwd=yvla

Declarations Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the ethics committee of Children's Hospital of Shanxi Province, PRC(IRB-KY-2019-001). Because of the retrospective nature of the study, patient consent for inclusion was waived by the ethics committee of Children's Hospital of Shanxi Province, PRC.

Consent for publication

Not applicable.

Competing interests

The authors have no conficts of interest to disclose.

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Figures

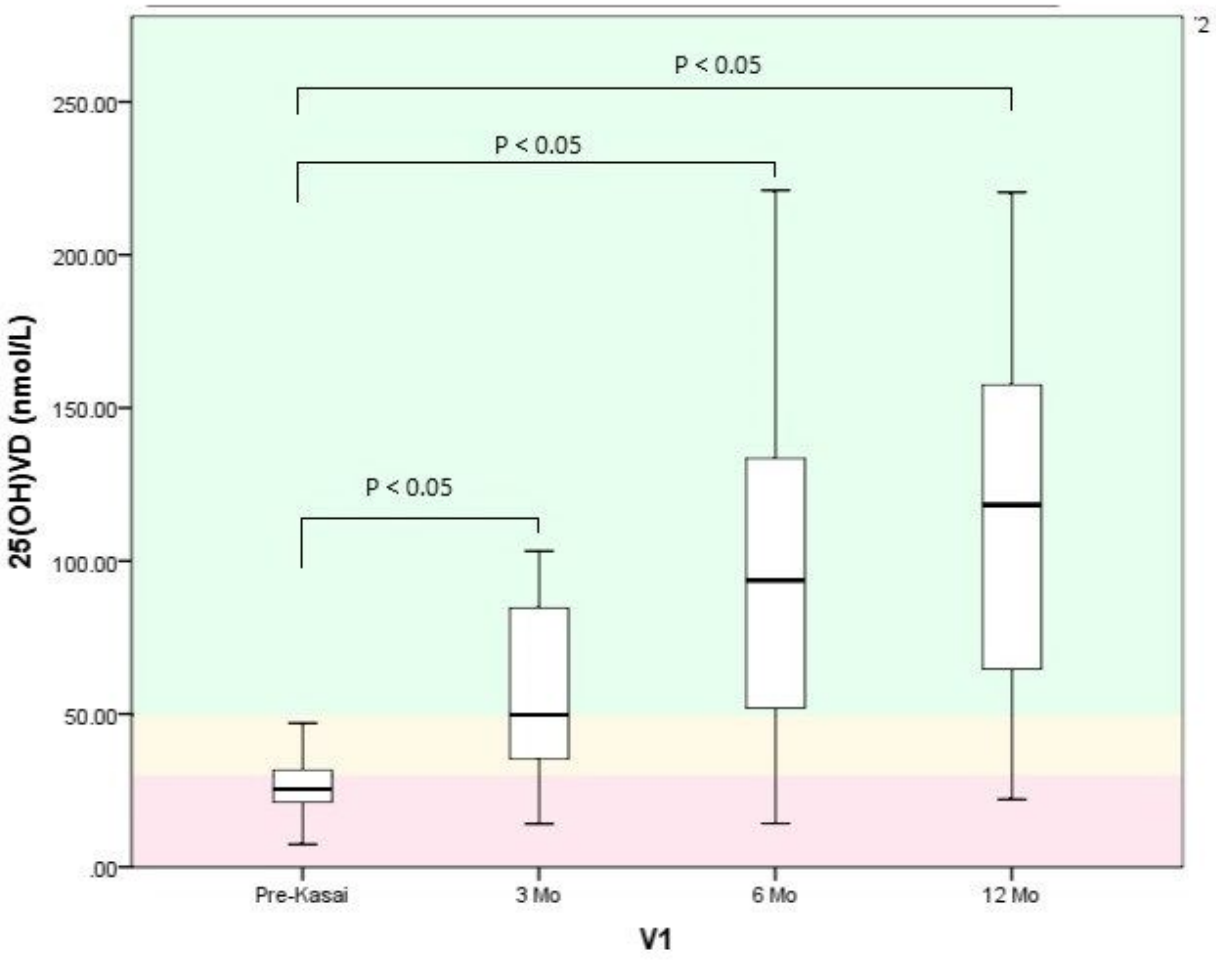

Figure 1

Vitamin D levels before and after the Kasai procedure. Vitamin D was generally deficient preoperatively, and it became significantly higher at different time points postoperatively.

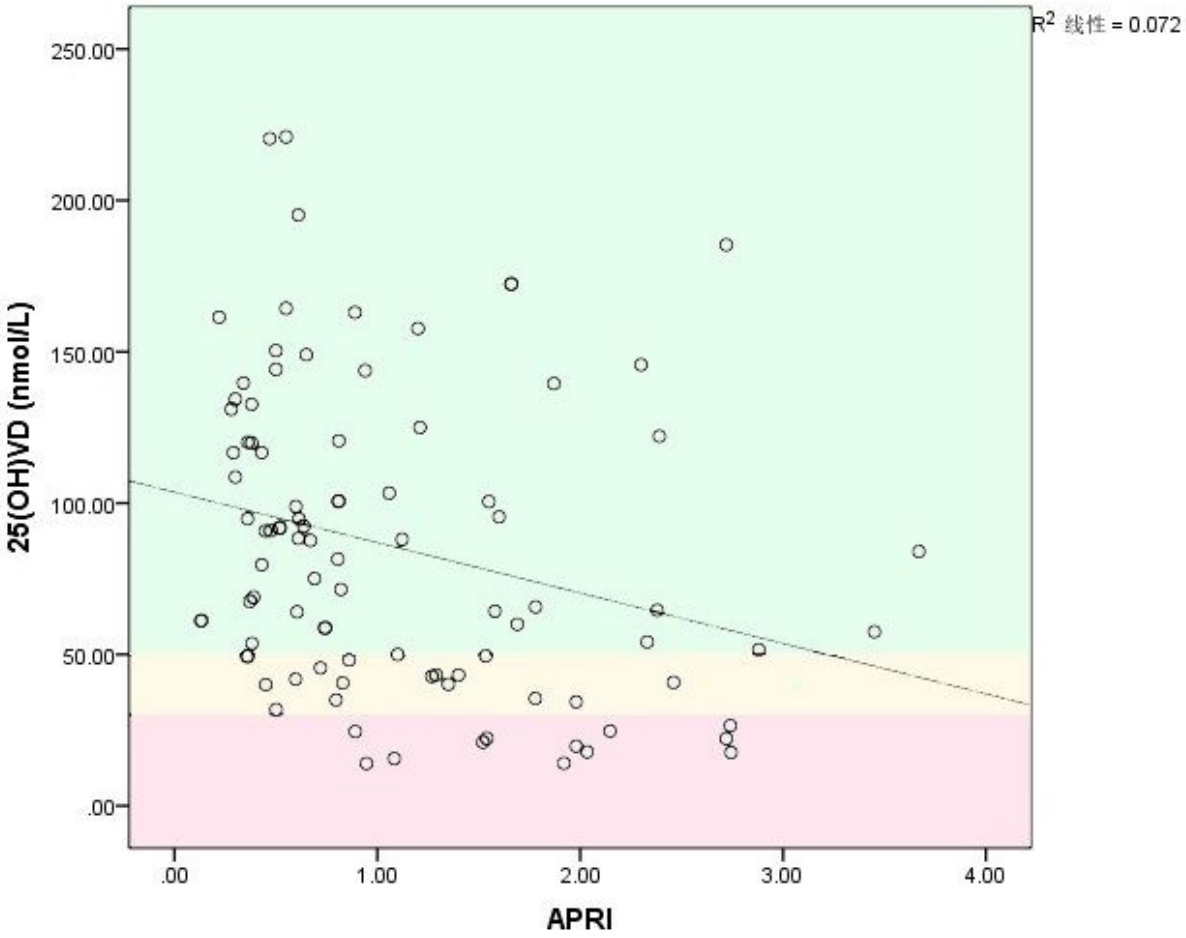

Figure 2

25(OH)VD level after Kasai procedure was negatively correlated with APRI.

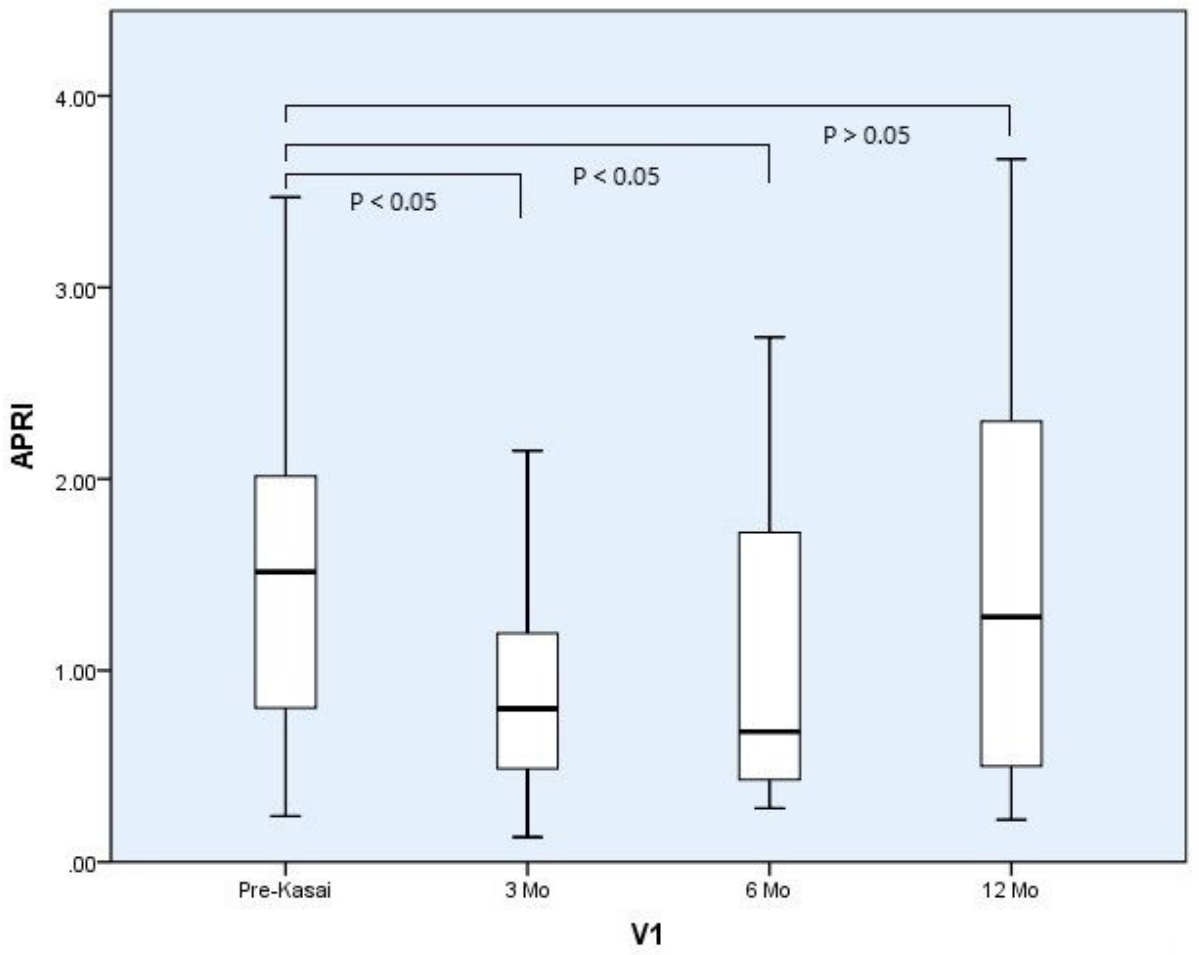

Figure 3

Degree of liver fibrosis at a different time before and after Kasai procedure.