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의학석사 학위 논문

MRI로 측정한 생체 기증자 간의
지방 분포 및 조직 검사와의 상관
관계

2012년 7월

서울대학교 대학원

의학과 외과학 전공

최 영록
The Distribution of Fat in the Liver and Correlation between Estimated Fat Fraction Using MRI and Liver Biopsy

by

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A Thesis Submitted to the Department of Surgery in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medicine (Surgery) at the Seoul National University College of Medicine

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Abstract

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(Background) This study was aimed to evaluate the correlation between the real fatty change on liver biopsy and the estimated fat fraction using MR triple-echo chemical shifting gradient echo imaging (MRC) as well as the difference in fat fraction for each segment, section, hemi-liver under the estimated fat fraction on MRC. (Patients and Methods) Between June 2011 and February 2012, 81 liver donors who had both the pathologic report for the liver biopsy and MRC images were selected in living donor liver transplantation. Fat fraction was estimated in the deep portion of eight segments, in four sections and two hemi-livers. Fatty change of the peripheral portion of segment 4, 5, 6, 7 and 8 were measured separately on the MRC. Then correlations among each fat faction were analyzed and the uneven distribution of fat in the liver was investigated statistically. (Results) The estimated fat fraction on peripheral part of S5 from MRC had a strong correlation with pathologic macro-vesicular fatty change of the liver wedge biopsy(r=.816, p=.000). And this peripheral S5 fat fraction had positive correlations with all peripheral and deep portion of each segment (p=0.000). But, there were differences of fat fraction between peripheral and deep part of S4, S6, S7 and S8 (p=000, .004, .000 and .006). And there were significant differences in fat fraction among deep parts of eight segments (F(4.003, 58.032)=8.684, p=.000)
as compared with no difference among peripheral parts of S4, S5, S6, S7 and S8 \((F(2.9, 5.3) = 1.3, p = .272)\). Although there were no difference of fat fraction among section and hemi-liver, the range of the fat fraction had an increasing tendency according to the fat fraction on MRC \((p=0.063)\)

**Conclusion** Although the fat fraction of peripheral S5 reflects the pathologic fatty change for the liver biopsy as well as the other parts of liver, the distribution of fat in the liver is uneven statistically. Therefore, when fatty change getting severe, we should be cautious for predicting fat fraction for the whole liver under liver biopsy or estimated fat fraction using MRI under the specific area in the liver.

Key Words) fatty liver, liver transplantation, MRI chemical shifting gradient

Student Number) 2007-21983
CONTENTS

Abstract ................................................................................................................................. i

Contents ............................................................................................................................... iii

List of tables ........................................................................................................................ iv

List of figures ......................................................................................................................... v

List of abbreviations ........................................................................................................... vi

Introduction ........................................................................................................................... 1

Patients and Methods ......................................................................................................... 1

Results .................................................................................................................................. 3

Discussion ............................................................................................................................. 4

References ............................................................................................................................. 17

Abstract in Korean .............................................................................................................. 19
LIST OF TABLES

Table 1  Average fat fraction in each segment relative to their true fat change on liver biopsy ................................................................. 8

Table 2  Pearson correlation between the fat fraction on biopsy and the fat fraction on MRI in each segment of liver ............................................. 9

Table 3  Correlations between the fat fraction in the PP of segment 5 and each section, between the fatty change on liver biopsy and each section .................................................................................................... 10

Table 4  Paired T-test for the fat fraction between Peripheral and deep portion in segment 4,5,6,7 and 8 .............................................................. 11

Table 5  Paired samples test between fat fraction in the deep portion of each segment ....................................................................................................... 12
LIST OF FIGURES

Figure 1 the method to estimate the fat fraction in each segment and in each section on MRC ................................................................. 13

Figure 2 Linear regression between peripheral fat fraction in segment 5 on MRI and fat fraction on liver biopsy ........................................ 14

Figure 3 Linear regression between peripheral fat fraction in segment 5 on MRI and fat fraction on liver biopsy ........................................ 15

Figure 4 Uneven distribution of the fat in the liver ............................... 16
LIST OF ABBREVIATIONS

DP : deep portion
LT : liver transplantation
MRC : MR triple-echo chemical shifting gradient echo imaging
MRS : MR spectroscope
PP : peripheral portion
ROI : region of index
Introduction

Nonalcoholic fatty liver disease may affect 10-30% of adults in the general population\(^1\). Cirrhosis develops in 20%-25% of patients with nonalcoholic steatohepatitis\(^2\). And macrovesicular steatosis of more than 30% is generally considered a risk factor for both recipient and donor in liver transplantation (LT)\(^3\). Therefore, we need to know the exact quantification of graft liver fat before LT.

There are several indirect and direct methods to estimate it. Roughly estimating methods are visual grading\(^4\), LS ratio and a liver attenuation index (the difference between liver and spleen) in non-enhanced liver CT\(^5\). Recently MR triple-echo chemical shifting gradient echo imaging (MRC) or MR spectroscope (MRS) are more accurate to measure fat content of the donor liver\(^6\). Of course, the most exact method in quantifying liver steatosis is liver biopsy. But, it could not evaluate whole liver and its information is limited within biopsy sites. And sampling error of liver biopsy can result in substantial misdiagnosis\(^7\). In addition, it’s invasive procedure that can cause infection, bleeding and bile leakage for living donors.

Sometimes, liver has focal fat deposition, diffuse deposition with focal sparing, multifocal deposition, perivascular deposition, and subcapsular deposition\(^8\). From these, we supposed that the distribution of fat in the liver is uneven according to the location in the liver. This imply the possibility of the occurrence of errors to analysis the liver fat fraction when we take the value of the fatty change from using MRI or biopsy in the limited area in the whole liver.

Although liver biopsy is usually required to exclude other diseases, MRC and MRS can inform us of the close real fatty percentage in donor liver and MRC could quantify the fatty change in the liver at different sites several times before biopsy and LT. Therefore, this study was aimed to evaluate the correlation between intraoperative liver biopsy and estimated fat change using MRC as well as interrelationship between estimated fat fractions in a specific area and in another part of the liver under the MRI.

Patients and Methods

Patients

88 consecutive living donor liver transplantations underwent between June 2011 and February 2012. The intraoperative liver biopsy for the evaluation of graft
steatosis, periportal inflammation and fibrosis was done in all cases. 3 donors who underwent left liver graft with biopsy on it and another 4 cases had no MRC images were excluded.

This study was conducted with 81 patients who had the results of liver biopsy and MRC images. MRI was performed within 32 days before the LT except 3 cases (77, 42 and 45 days).

MR imaging & three-point DIXON

MR imaging was performed by using 3.0T MR imaging system (Siemens Medical Solutions, Erlangen, Germany). An axial, triple-echo, Dixon water-fat separation image with T2* correction was also acquired using a 3D gradient echo prototype sequence provided by the manufacturer (Siemens Healthcare, Erlangen, Germany). The triple echo data consisted of in-phase (360 degrees)/opposed-phase (540 degrees)/in-phase (1080 degrees) images and the imaging parameters were: relaxation time (TR) 9.9 ms; echo times of triple echos, TE1/TE2/TE3 2.5 / 3.7 / 7.3 ms; flip angle 11 degrees; matrix 256x167; slice thickness 3.5 mm (56 slices); and FOV 380x327mm. A single, continuous ROI was defined in each of the source images by including a maximum amount of parenchyma tissue of the liver avoiding major blood vessels. From the calculated water-only and fat-only images, hepatic fat fraction images were obtained.6,9,12

Measuring fat fraction on MRC

All images were interpreted on PACS (Marosis m-view 5.4, Marotech, Seoul, Korea) that allowed the extraction of fat fraction and T2 maps fitted on a pixel-by-pixel basis. The estimated fat fraction was the one-tenth mean value in the region of interest (ROI) on PACS.

Peripheral portion was defined as the area out of 3/4 from main portal vein bifurcation. Deep portion was the area within 3/4. To estimate fat fraction in peripheral portion (PP) and deep portion (DP) of the liver, an region of index (ROI) of which size was about 1-2cm² was drawn in each segment. ROI in the PP of the segment 4, 5, 6, 7, 8 was a long rectangle shape along the liver edge. And a circle type of ROI, devoid of hepatic vein and portal vein was used in the DP at the same segments. But, peripheral fat fraction could not be measured in segment 1, 2 and 3, because its parenchyma was too small to divide into deep and peripheral part. For measuring fat fraction of each section, free type of ROI devoid of major vessels was drawn fully along the segment boundary based on Brisbane 2000 system. The value
for fat change was the average of 3 values which were estimated at different slice of MRI in the same segment and section (Figure 1).

Liver biopsy

Wedge resection of the liver was performed before liver mobilization in donor surgery. Biopsy site was in the edge of the segment 5. Biopsy specimen was about $0.5 \times 0.5 \times 0.5 \text{ cm}^3$ sized. The degree of macrovesicular and microvesicular steatosis was reported on a percentage scale under hematoxylin-eosin stained slides.

Group

Donors were divided into 3 groups based on the fat fraction in the PP of segment 5 on MRC($<5\%, \geq 5\%$ and $<15\%, \geq 15\%)$.

Statistical analysis

Correlations between the estimated fat percentage using MRC and the pathologic macrovesicular fatty change of the biopsy and interrelation among estimated fat fractions in PP, DPs, each section and hemi-livers were assessed with Pearson correlation, paired T-test, linear regression and repeated measure ANOVA. After analysis with repeated measure ANOVA, Bonferroni adjustment was applied to control for Type I error. All statistical analysis were performed by using commercial software SPSS for windows (version 19.0 : SPSS, Chicago, Ill). A \textit{P} value of 0.05 was considered to show significant difference.

Results

Characteristics of donors

Average age of donors was $31.9 \pm 11.4$ (average $\pm$ SD) years old and BMI was $23.7 \pm 2.7$ (average $\pm$ SD) Kg/m$^2$. Male was dominant and mean macro-vesicular fat change was $3.24 \pm 4.6$ % on liver biopsy. Table I shows estimated fat fraction in peripheral portion and in deep portion using MRC in the each segment and true
fatty change on biopsy.

The estimated fat fraction in the PP of the segment 5, where wedge biopsy was usually performed, correlated well with the real macrovesicular fatty change under the intraoperative liver wedge biopsy (Table 2, r=.816, p=.000) and they had linear relation (Figure 2, R² =0.667, p=0.000). Moreover, the pathologic result of liver biopsy looked like the represent of fat distribution in the other segments as well as the fat fraction in segment 5 using MRC, because the correlation coefficients were from .736 to 868.

And the fat fraction on liver biopsy and fat fraction in the PP of segment 5 using MRC had good correlations with right anterior, posterior and left lateral section (all p=0.000) (Table 3). From these results, we guessed that liver biopsy and focal fat fraction in the liver would represent the fatty change of the whole liver.

However, there was significant difference of fat fraction between PP and DP of the segment 4, 6, 7 and 8 statistically (p=.000, .004, .000 and .006) (Table 4). Besides, analysis of variance showed a statistically difference at the p < .000 level in fat fraction for the DP of eight segments : F(4.0, 58.032) = 8.7, p=.000. It stood for significant within-subjects main effect. Table 5 shows the paired sample test for pairwise comparisons among the fat fraction in the DP of each segment. Caudate lobe and segment 4 had distinct differences from the other segment in fat fraction in paired test among the deep portion. But, there was no difference in fat fraction for the PP of five segments : F (2.9, 5.3) = 1.3, p = .272.

Consequentially, the distribution of fat in the deep portion of the liver was uneven (Figure 3). That was different from even distributed PP in the liver. Furthermore, the range of the fat fraction (the difference between the highest value and the lowest value among the fat fractions in eight segments per case) had an increasing tendency with the more severe fat fraction. Patients with more severe fatty liver had a wide range of fatty distribution with more high the lowest value of fatty change than groups with less fatty liver. Also the highest difference in each group became wide as the fat change (Figure 4). The difference of fat distribution in the liver is bigger, as the more it is fatty.

Discussion

Severe fatty liver disease is the common cause of graft dysfunction resulting in primary non-function and it can delay the recovery of donor after liver surgery.
some institutions evaluate the steatosis of the liver with routine liver biopsy. But, it is invasive and has a risk of bleeding for healthy donors. Therefore, several non-invasive methods have been developed to estimate the liver status.

Liver spleen attenuation index (LS index) under unenhanced liver CT is highly reliable in the diagnosis of 30% or higher macrovesicular steatosis in living liver donor candidates. But, it can’t show the exact fat change of the liver and its accuracy is so low in the diagnosis of less than 30% steatosis. With the development in MRI, we could see the fat change in the liver parenchyma indirectly without any invasive procedure. MRS has high accuracy to estimate fat fraction in the liver using a signal change. But it has the limitation that it could not estimate the fat fraction of the whole liver at the same time. And the region it can estimate is usually fixed to the limited small area at one time. MR chemical shift images using the Dixon method or modified Dixon method for quantification of fat in the liver provided us benefits such as easy manipulation, whole liver coverage, minimal vulnerability to confounding factors and absence of radiation.

Limitations to the use of MR imaging in liver fat quantification have the differences of result according to MR imaging system, scanning parameters and methods of analysis. And it’s expensive and too sensitive to show the exact fat content in iron deposition. In spite of that, high degree of correlations between fat fraction using MRC, MRS and the pathologic result of liver biopsy were well known to date. The result of this study also showed that there was linear relationship between fat fraction in the PP of the S5 and fat change on liver biopsy, fat fraction in the each segment has a strong relation with liver biopsy. Judging from these things, if the distribution of fat is even in the liver, we could analyze the whole liver fat fraction out of the fat fraction on segment 5 using MRI.

However, the distribution of the fat was uneven in the liver. Although its pathogenesis has been controversial, that could be caused by relative ischemia due to decreased portal venous flow or decreased delivery of unknown substance via the portal vein, and a part of this distribution has been attributed to variant venous circulation. Therefore, the results from liver biopsy or intraoperative wedge resection could not represent the exact fatty change in the whole liver or the other regions of the liver that biopsy was not done.

This study showed that the distribution of fat in liver is uneven statistically. First, there are significant differences between fat fraction in the PP and in the DP of the same segments. Second, under repeated measure ANOVA, analysis indicated that the fat fraction in the some segments was different from the other ones. Because the
fat fraction in the PP of each segment was not different among them, liver had uneven distribution of fat in DP of the liver which was away from peripheral region in this study. Left medial and left lateral section of the liver were different from right hemiliver in the distribution of fat.

As fatty change getting worse, the range of fat fraction has a tendency to increase. And the result showed that the difference in fat deposition in each group became more wide as the fatty change more severe. It imply that the estimated value of fat change in limited region might be different from fat fraction in the other region of the liver to the highest range. And it contain the information about fatty change in a segment using MRC or on liver biopsy, it could not represent the fat fraction in another region, especially in a severe fatty liver.

For the safety of both donor and recipient, we need to know status of the liver graft as well as the remnant liver before LT. In spite of the importance of the accuracy for the pathologic fatty change, the report for frozen biopsy is inaccurate in operation and the permanent pathology is reported postoperatively. Although liver biopsy is still needed to exclude graft with steatohepatitis and another liver disease, we can get the accurate information about fatty change with non-invasive MRI and avoidance of unnecessary biopsy in donors preoperatively.

Because the distribution of fat in liver is uneven, we should be cautious for predicting fat fraction for the whole liver with the small part of liver biopsy or the estimated fat fraction in the specific region using MRC. In case of severe fatty liver, we need to estimate fat fraction on both hemiliver or all sections separately.

This study has limitations. First, the numbers of patient with fatty liver (only 13 cases were over 5% of macrovesicular fatty change) was small in our study. Second, although MRC showed the whole fatty change of hepatocytes which included macrovesicular and microvesicular fatty change, only macro-vesicular fatty changes on liver biopsy were investigated in our study. Finally, we used liver biopsy as the reference, but the pathologic reports for fatty change in liver biopsy could be vary considerably depending on the methods used by pathologist. The results might be different a little.

In spite of that, this paper give us important messages clinically. First, it was proven that the distribution of fat is uneven by statistical methods. Second, it revealed the possibility of misleading of fat contents in the liver by biopsy or MRI in a specific small region the liver. Third, the more fatty change is severe in the liver, the more fat distribution of the liver deviates. Therefore, author suggests that
multifocal fat estimation is needed in the different segment or hemiliver, or that the estimation of fatty change on graft liver as well as the other remnant liver should be considered.
<table>
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<th>Deep portion</th>
<th>Biopsy</th>
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<tr>
<td></td>
<td>S4</td>
<td>S5</td>
<td>S6</td>
</tr>
<tr>
<td>Fat fraction</td>
<td>3.8 ± 2.7</td>
<td>3.5 ± 3.0</td>
<td>3.6 ± 3.7</td>
</tr>
<tr>
<td>(%) mean ± SD</td>
<td>2.7</td>
<td>3.0</td>
<td>3.7</td>
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Table 2 Pearson correlation between the fat fraction on liver biopsy and the fat fraction on MRC in each segment of liver

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<td>S5</td>
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<td>.816</td>
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<td><em>p</em> value</td>
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Table 3 Correlations between the fat fraction in the PP of segment 5 and each section, between the fatty change on liver biopsy and each section

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<th>Right posterior section</th>
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<td>Fatty change on biopsy</td>
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<td>.872</td>
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Table 4 Paired T-test for the fat fraction between Peripheral and deep portion in segment 4, 5, 6, 7 and 8.

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<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
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<tr>
<td>Fat fraction in PP (%; mean ±SD)</td>
<td>3.8 ± 2.7</td>
<td>3.5 ± 3.0</td>
<td>3.6 ± 3.7</td>
<td>3.9 ± 2.8</td>
<td>3.7 ± 3.4</td>
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<td>Fat fraction in DP (%; mean ±SD)</td>
<td>3.1 ± 2.8</td>
<td>3.4 ± 3.1</td>
<td>3.4 ± 3.6</td>
<td>3.3 ± 3.4</td>
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<tr>
<td>t</td>
<td>5.3</td>
<td>0.4</td>
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<td>p value</td>
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Table 6 Paired samples test between fat fraction in the deep portion of each segment

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<th>Segment (A)</th>
<th>Segment (B)</th>
<th>Difference (A-B)</th>
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<th>p value</th>
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† Bonferroni adjustment
Figure 1 the method to estimate the fat fraction in each segment and in each section on MRC

A : The fat fraction in PP was estimated under rectangular ROI about 1-2cm², 3/4 away from main portal vein bifurcation to liver margin. And it in DP was estimated under circular ROI about 1-2cm², 3/4 inner area. The fat fraction in PP : 17.5%, DP : 21.7%

B : The fat fraction in each section, its boundary based on Brisbane 2000 system, was calculated under free style ROI devoid of major vessels and bile duct. The fat fraction in the section : 21.7%
Figure 2 Linear regression between peripheral fat fraction in segment 5 on MRI and fat fraction on liver biopsy ($Y=1.225(p=.000)X-1.129(p=0.016)$)
Figure 3 Uneven distribution of fat in the liver. The mean value of ROI, that express the fatty change on MRC, was different between segment 7 and segment 8: fat change, 11.5% in segment 7, 5% in segment 8.
Figure 4 The increasing range of the fat fraction according to the group based on fat fraction in Segment 5 using MRC
Reference


국문 초록

서론 : 본 연구는 간 조직 검사에서 보고된 지방 변성과 MRI 화학적 변위영상(MRC)을 이용하여 측정한 간의 지방 비율의 상관 관계 및 각의 분절 및 절편의 지방 비율 사이의 상호 연관성을 조사하고자 하였다.

방법 : 2011년 6월에서 2012년 2월까지의 생체 간 기증자 중에서 조직검사와 MRC 영상이 있는 81명을 대상으로 하였다. MRC을 이용하여 간의 각 8분절의 심부와 각 4절편의 지방 비율을 측정하였다. 분절 4,5,6,7,8에서는 말초 부위의 지방 비율을 따로 측정했다. 이들 각각의 지방분율의 상관 관계 및 조직검사와의 연관성, 간의 불균등한 지방 분포를 통계적 방법을 사용하여 조사하였다.

결과 : MRC를 이용하여 측정한 간 5번 분절의 말초부위 지방 분율은 실제 간 조직의 거대수포성 지방 변화 비율과 연관성이 있었다(r=.816, p=.000). 또한, 이 부위의 지방 비율은 간의 다른 부위의 지방 비율과도 유의한 연관성이 있었다(p=.000). 한편, 간 분절 4번, 6번, 7번, 8번에서는 말초 부위와 심부 부위의 지방비율 간에 유의한 차이가 있었고 (p=.000, .004, .000 and .006), 심부의 지방 비율은 각 8분절 간에 유의한 차이가 있었다 (F(4,003, 58.032)=8.684, p=.000). 하지만, 간 분절 4번, 5번, 6번, 7번, 8번의 말초 부위의 지방 비율 간에는 유의한 차이가 없었다(F(2,9, 5,3) = 1.3, p = .272).

그리고, 환자별 간 8분절의 최대 지방 비율 값과 최소 지방 비율 값의 차이인, 지방의 편차는 간의 지방 변화가 심할수록 커지는 경향을 가졌다.
결론: 지방 간이 심혈수록 간의 일부의 조직 검사 결과나 특정 부위에서 MRC을 사용한 측정한 지방 비율은 전체 간의 지방 비율을 나타내지 못하므로 간의 여러 부위나 분절 및 전체 간에서의 지방 비율의 측정이 필요하다.

주요어: 간이식, 기증간, 지방분율, MRI 화학적 변위영상

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