Point Shear Wave Acoustic Radiation Force Impulse Elastography (pSW-ARFI) is a Non-invasive Tool to Diagnose Acute and Chronic Hepatic Graft-Versus-Host Disease in Adult Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Background: The gold standard method of diagnosis of hepatic graft versus host disease (GVHD) is liver biopsy. However, liver biopsy is an invasive procedure with risk of complications especially bleeding.

Objective: Therefore, in the current study, we used acoustic radiation force impulse (ARFI) imaging that is a non-invasive, sensitive, and accurate tool for the assessment of liver fibrosis during routine conventional ultrasonography (US) evaluations.

Methods: The study was conducted prospectively on 30 adult patients undergoing allogeneic Hematopoietic stem cell transplantation (HSCT). Point shear wave Acoustic radiation force impulse (pSW-ARFI) elastography (stiffness and score) was performed for all patients: pre-transplant, within 3- and 6-months post-transplant.

Results: Overall, ARFI score and stiffness were higher in patients who developed acute hepatic GVHD in comparison to those who did not develop acute GVHD (P=0.028 and P=0.03, respectively). Furthermore, the mean ARFI stiffness during the first 3 months post-transplant was directly correlated to the grade of acute hepatic GVHD (P=0.012). Moreover; ARFI score and stiffness within 6 months post-transplant were higher in patients who developed chronic hepatic GVHD in comparison to those who did not develop (P=0.020 and P=0.018, respectively).

Conclusion: pSW-ARFI is a reliable non-invasive tool for the early diagnosis of acute and chronic hepatic GVHD, especially if a liver biopsy is not feasible. It can also be used in the grading of acute hepatic GVHD.

KEYWORDS: Acoustic radiation force impulse; Graft versus host disease; Liver stiffness; Transplant, Stem cells; Hematopoietic stem cell transplantation

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the curative treatment option for many diseases including hematological and non-hematological diseases. HSCT is performed via the intravenous infusion of stem cells collected from the patient himself (autologous HSCT) or a donor (allogeneic HSCT). Donor stem cells may be collected from bone marrow, peripheral blood, or the umbilical cord blood [1].

Despite advances in this field, HSCT is not a safe procedure. Many complications may occur during or after transplant with significant morbidity and mortality. These complications

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include but not limited to Veno-occlusive disease (VOD), mucositis, infections, septic shock, disseminated intravascular coagulation (DIC), and graft versus host disease (GVHD) [1].

Liver injury is a common complication of HSCT. Hepatic complications may occur early (due to drug toxicity, veno-occlusive disease, acute GVHD, infection, and cholestasis) or late post-transplant (due to liver cirrhosis, secondary malignancy, and chronic GVHD) [2]. Acute hepatic GVHD may occur in up to 35 % of patients undergoing allogeneic HSCT, while chronic hepatic GVHD may occur in up to 30 % of them [3].

Acoustic radiation force impulse (ARFI) imaging is an imaging method that can be done by the conventional B-mode ultrasonography [4]. It targets a certain anatomic region and tests its elastic properties. As stiffness increases, the shear-wave speed increases as it travels through this region. Being non-invasive and easily done during conventional US evaluations, without requiring additional transducers or equipment, ARFI may serve as a simple clinical tool for the assessment of liver injury. It has been used with promising success to differentiate between cirrhotic and non-cirrhotic liver [5].

To our knowledge, few previous studies investigated the utility of point shear wave elastography ARFI (pSW-ARFI) in the diagnosis of hepatic GVHD in adult patients subjected to allogeneic HSCT. This study aims to explore if pSW-ARFI can help the diagnosis or grading of hepatic GVHD in those patients.

MATERIALS AND METHODS

Patients

The study was conducted prospectively on 30 adult patients with benign and malignant hematological diseases, who underwent peripheral blood allogeneic HSCT from a fully matched related donor in first or second complete remission (CR) according to disease state and risk stratification. All Patients were followed over 12 months with a minimum follow-up of 6 months.

All procedures performed in the study were following the ethical standards of the institutional and national research committee and in accordance with the 1964 Helsinki declaration and its later amendments. Written informed consent has been obtained from all patients.

Pre-transplant Work-up

All patients were subjected to the following pre-transplant work-up which included CBC, ABO blood group test, kidney function tests, liver function tests and prothrombin activity, HBV DNA by PCR, HCV RNA by PCR, CMV antibodies (IgM & IgG), toxoplasma antibodies (IgM & IgG), CMV and EBV PCR, HIV serology, and bone marrow aspiration.

Patients also underwent radiological investigations in the form of chest x-ray, pulmonary function tests, echocardiography, and abdominal ultrasonography. Point shear wave elastography acoustic radiation force impulse was done pre-transplant for all patients and the hepatic comorbidity index (HCI) score was calculated according to the study reported by Sorror *et al.* [6].

Conditioning Regimen and Transplant Procedure

Patients were isolated in high efficiency particulate air (HEPA) filter cube and received conditioning regimens according to each disease: 60% (n=19) of the patients received busulfan/ fludarabine , 13.3% (n=5) received fludarabine /cyclophosphamide , 3.3% (n=2) underwent total body irradiation (TBI) and 6.7% (n=4) of patients received anti-thymocyte globulin (ATG) according to each disease.

All patients received GVHD prophylaxis in the form of cyclosporine for a minimum of 120 days (initially intravenous cyclosporine was given for a total daily dose of 3 mg/kg in two divided doses over 2-4 hours, then oral route cyclosporine was given for a total daily dose of 2.5 mg/kg/12h) and methotrexate 10 mg/m^2 on days 2, 4, and 6.

Table 1: Descriptive data of studied group at pre-transplant.

Variables	Sub-groups	Statistics (n= 30)
Age (yrs)	16-25 years	10 (33.3%)
	25-35 years	12 (40.0%)
	35-45 years	7 (23.3%)
	45-55 years	1 (3.3%)
Sex	Male	17 (56.7%)
	Female	13 (43.3%)
Diagnosis	Acute myeloid leukemia	18 (60.0%)
	Acute lymphoblastic leukemia	9 (30.0%)
	Aplasia	3 (10.0%)
Pre-transplant hepatic comorbidity index score	0	19 (63.3%)
	1	8 (26.7%)
	3	3 (10.0%)
HBV serology	Negative	27 (90.0%)
	Positive	3 (10.0%)
PCR HBV	Negative	29 (96.7%)
	Mild viremia	1 (3.3%)
HCV Ab	Negative	23 (76.7%)
	Positive	7 (23.3%)
PCR HCV	Negative	28 (93.3%)
	Mild viremia	2 (6.7%)
HIV Ab	Negative	30 (100.0%)
	Positive	0 (0.0%)
PCR CMV	Negative	30 (100.0%)
	Positive	0 (0.0%)
PCR EBV	Negative	30 (100.0%)
	Positive	0 (0.0%)
Pre-transplant ARFI score	F0	27 (90.0%)
	F1	3 (10.0%)
ARFI stiffness pre-transplant	Mean ± SD	0.78 ± 0.18
	Range	0.53 - 1.16
Abdominal ultrasound before HSCT	Normal	18 (60.0%)
	Fatty liver	12 (40%)
Conditioning regimen	Busulphan\fludarabine	19 (60.0%)
	Fludarabine\cytoxan	5 (13.3%)
	TBI based	2(3.3%)
	ATG based	4 (6.7%)
Stem cell dose (CD34) x (10)6/kg of recipient weight	Mean ± SD	6.63 ± 1.47
	Range	4.9 - 10.9
CMV PCR reactivation during transplant	Negative	28 (93.3%)
	Positive	2 (6.7%)

Data present as Mean ± SD or number (%).
ARFI: Acoustic Radiation Force Impulse Elastography; HSCT: Hematopoietic Stem Cell Transplantation

Table 2: Descriptive data of studied group at pre-transplant.

Variables	Sub-groups	Statistics (n= 30)
Post-transplant ARFI in first 3 months	F0	20 (66.7%)
	F1	5 (16.7%)
	F2	5 (16.7%)
post-transplant ARFI stiffness in first 3 months	Mean ± SD	0.97 ± 0.28
	Range	0.56 – 1.43
Acute hepatic GVHD	Negative	19 (63.3%)
	Positive	11 (36.7%)
Grade of acute hepatic GVHD	1	2 (20%)
	2	5 (45%)
	3	4 (35%)
Post-transplant ARFI stiffness at 6 months	Mean ± SD	1.23 ± 0.51
	Range	0.5– 2.1
Post-transplant ARFI at 6 months	F0-F1	13 (43.3%)
	F2-F4	17 (56.6%)
Chronic hepatic GVHD	Negative	17 (56.7%)
	Positive	13 (43.3%)
Grade of chronic hepatic GVHD	1-2	6 (46.0%)
	3	3 (24.0%)
	4	4 (30%)

Data present as Mean ± SD or number (%).
ARFI: Acoustic Radiation Force Impulse Elastography; HSCT: Hematopoietic Stem Cell Transplantation; GVHD: Graft Versus Host Disease

Post-transplant Diagnosis of Hepatic Chronic GVHD

Liver GVHD was diagnosed via clinical and laboratory data and in accordance with the National Institutes of Health (NIH) consensus criteria [7].

Patients were followed over a period of 12 months with a minimum follow-up of 6 months. ARFI score and stiffness were measured among patients three times: 1) pre-transplant; 2) 3 months post-transplant: ARFI was done once the patient developed AHGVHD or within 100 days in comparison to patients who did not develop aHGVHD; 3) 6-months post-transplant: ARFI was done once the patient developed CHGVHD or within 6 months in comparison to patients who did not develop cHGVHD.

Point Shear Wave Acoustic Radiation Force Impulse Methodology

We used a Siemens Acousan S3000 virtual touch ultrasound system (Siemens AG, Erlangen, Germany) with 6 CI transducer to perform pSW-ARFI on the right liver lobe for all patients.

ARFI is a type of push pulse that can be done during the conventional ultrasound scan using the ultrasound probe. The probe is directed to the side of a region of interest (ROI), which has a predefined size, provided by the system (10 mm long and 5 mm wide). The acoustic "push" pulse generates shear waves that propagate into the tissue, perpendicular to the "push" axis. Then the speed of the shear wave is measured and expressed in m/s unit.

After fasting for at least 6 hours, patients were put in the supine position with the right arm in maximum abduction and were asked to hold their breath. Minimal scanning pressure was applied by a clinically blind operator to the right lobe of the liver. We used the intercostal approach, 1–2 cm under the liver capsule. The mean of 8-10 valid measurements were calcu-

Table 3: Comparison between acute hepatic GVHD with pre-transplant ultrasound, pre-transplant and post-transplant hepatic comorbidity index, post-transplant ARFI score and its stiffness during first 3 months.

Variables	Sub-group	Negative acute hepatic GVHD	Acute hepatic GVHD	Test value	P-value
ARFI score in first 3 months	F0-F1	18 (94.7%)	7 (63.6%)	4.852	0.028
	F2-F4	1 (5.3%)	4 (36.4%)		
ARFI stiffness post- transplant in first 3 months	Mean± SD	0.86± 0.25	1.16 ± 0.22	3.315	0.003
	Range	0.56 – 1.43	0.89 – 1.42		
Pre-transplant hepatic comorbidity index score	0	12 (63.2%)	7 (63.6%)	7.194	0.027
	1	7 (36.8%)	1 (9.1%)		
	3	0 (0.0%)	3 (27.3%)		
Post-transplant hepatic co-morbidity index in first 3 months	0	9 (47.4%)	0 (0.0%)	13.612	0.001
	1	7 (36.8%)	2 (18.2%)		
	3	3 (15.8%)	9 (81.8%)		
Abdominal ultrasound before HSCT	Normal	12 (63.2%)	6 (54.5%)	0.395	0.821
	Fatty liver	2 (10.5%)	2 (18.2%)		
	Hepatomegaly	5 (26.3%)	3 (27.3%)		

Data present as Mean ± SD or number (%).

ARFI: Acoustic Radiation Force Impulse Elastography; HSCT: Hematopoietic Stem Cell Transplantation; GVHD: Graft Versus Host Disease

lated.

pSW-ARFI results were scored from F0 to F4 using the following references range: F0 less than 1.1 m/s; F1 from 1.1 - 1.29 m/s; F1-2 from 1.29 - 1.37 m/s; F2 from 1.37 - 1.45 m/s; F3 from 1.45 - 1.72 m/s; F4 more than 1.72 m/s.

Statistical Analysis

Statistical analysis was done using the SPSS software, version 20 for windows. The quantitative data are presented as mean, standard deviations and qualitative variables are presented as number and percentages. The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test. The comparison between two independent groups with quantitative data and parametric distribution was done using the independent t-test. The comparison between more than two independent groups with quantitative data and parametric distribution was done using the one-way ANOVA test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the pre-transplant characteristics of the patients included in this study.

Our patients included 17 males (56.7%) and 13 females (43.3%). Their age ranged from 16-55 years old. Overall, 90% (n=27) of patients' initial diagnosis was acute leukemia, while 10% of included patients (n=3) were suffering from aplastic anemia. Among acute leukemia patients, 60% (n=18) were diagnosed as acute myeloid leukemia (AML), while 30% (n=9) were diagnosed as acute lymphoblastic leukemia (ALL).

All included patients were negative for EBV and CMV by PCR, HIV by serology. During transplant, only 2 patients (6.7%) became CMV positive by PCR. Three patients (10%) were HBV positive by serology (HBsAg, HBcAb, and HBsAb). Only one patient (3.3%) had mild viremia by HBV PCR. HCV (Ab) was positive in seven patients (23.3%) while only two patients (6.7%) had mild viremia by HCV PCR.

Table 4: Comparison between ARFI stiffness in 1st 3 months post-transplant and grade of acute hepatic GVHD.

Variables	Post-transplant ARFI stiffness in first 3 months		Test value	P-value
	Mean ± SD	Range		
1	0.94 ± 0.0	0.94 – 0.94		
2	1.28 ± 0.2	0.95 – 1.42	4.512	0.012
3	1.13 ± 0.24	0.89 – 1.38		

ARFI: Acoustic Radiation Force Impulse Elastography; GVHD: Graft Versus Host Disease

HCI Score and Abdominal Ultrasound Pre-transplant

Pre-transplant HCI score was 0 in 19 patients (63%), 1 in eight patients (26,7%), and 3 in three patients (10%). Normal abdominal ultrasound before HSCT was detected in 70% (n=21) of patients while the rest had fatty liver by ultrasound (30%).

Results of Pre-transplant ARFI Score

Regarding the results of pre-transplant ARFI score, 27 patients had F0 (90%) and 3 patients had F1 (10%) with mean stiffness of 0.78 ± 0.18 m/s (range from 0.53 to 1.16 m/s). Pre-transplant ARFI stiffness showed no significant differences between patients who later developed acute hepatic GVHD and those who did not develop acute hepatic GVHD (Range 0.59–1.13 vs 0.53 – 1.16 with P= 0.067).

Also; pre-transplant ARFI score showed no significant difference between patients who later developed acute hepatic GVHD or not (F0= 9 (81.8%) vs 18 (94.7%); F1= 2 (18.2%) vs 1 (5.3%) with P= 0.256).

During the First 3 Months Post-transplant

As regard results of ARFI score done within 3 months post-transplant; twenty patients (66.7%) had F0 score, five (16.7%) had F1 score and another five patients (16.7%) had F2 score (their mean stiffness was 0.97 ± 0.28 m/s; range from 0.56–1.43 m/s) (see Table 2).

Acute hepatic GVHD developed in eleven patients (36.7%). Two patients (20%) had grade 1 hepatic GVHD; 5 patients (45%) had grade 2 and 4 patients (35%) had grade 3 hepatic GVHD (see Table 2).

Results of ARFI score in 1st 3 months post-transplant, were significantly higher in patients who developed acute hepatic GVHD in comparison to those who did not develop acute hepatic GVHD (P= 0.028). Among patients who developed acute hepatic GVHD; seven patients (63.6%) had F0-F1 score and 4 patients (36.4%) had F2-F4 score. In comparison, among patients who didn't develop acute hepatic GVHD, 18 patients (94.7%) had F0-F1 and one patient (5.3%) had F2-F4 (Table 3).

The mean ARFI stiffness in the first 3 months post-transplant in patients who developed acute hepatic GVHD was higher than those patients who didn't develop acute hepatic GVHD with a highly statistically significant difference (1.16 ± 0.22 m/s vs. 0.86 ± 0.25 m/s, P=0.03) (Table 3).

No statistically significant difference was detected between the development of acute hepatic GVHD and pre-transplant hepatic abnormalities in ultrasound (P= 0.821) (Table 3).

The mean ARFI stiffness in the first 3 months post-transplant was higher in patients who had high grade GVHD (grade 2, 3 acute hepatic GVHD) than those patients who developed low grade acute hepatic GVHD (grade 1 acute hepaticm GVHD) (grade 3: 1.28 ± 0.2 m/s, grade 2: 1.13 ± 0.24 m/s; grade 1: 0.94 ± 0.0 m/s; P=0.012) (Table 4).

HCI Results

Pre-transplant HCI score in patients who later developed acute hepatic GVHD was 0 in 7 patients (63.6%), 1 in one patient (9.1%), 2 in 3 patients (27.3%). In comparison, among patients who did not develop acute hepatic GVHD, score 0 was documented in 12 patients (63.2%), score 1 in 7 patients (36.8%) and score 2 in 0 patients (0.0%). A statistically significant dif-

Table 5: Comparison between chronic hepatic GVHD and ARFI stiffness after 6 months post-transplant.

Variables	Sub-group	Negative chronic hepatic GVHD	Chronic hepatic GVHD	Test value	P-value
ARFI score 6 months post-transplant	F0-F1	11 (66.7%)	2 (16.7%)	5.452	0.020
	F2-F4	6 (33.3%)	11 (83.3%)		
ARFI stiffness 6 months post-transplant	Mean± SD	0.95 ± 0.58	1.45 ± 0.32	2.577	0.018
	Range	0 – 2.1	0.72 – 1.92		

Data present as Mean ± SD or number (%).
ARFI: Acoustic Radiation Force Impulse Elastography; GVHD: Graft Versus Host Disease

ference was detected between the development of acute hepatic GVHD and increased pre-transplant HCI (Table 3).

Post-transplant HCI (within first 3 months) in patients who developed acute hepatic GVHD showed a score of 3 in 9 patients (81.8%). A highly statistically significant difference was detected between the development of acute hepatic GVHD and increased post-transplant HCI (P=0.001) (Table 3).

Pre- and post-transplant HCI showed no statistically significant diffrence with ARFI score and stiffness (P=0.310 and P=0.111) respectively.

Six Months Post-transplant

Thirteen patients (43.3%) were diagnosed to have chronic hepatic GVHD by clinical; laboratory data and liver biopsy within six months after transplantation. Grade 1-2 chronic hepatic GVHD was documented in 6 patients (46%), grade 3 in 3 patients (24%) and grade 4 in 4 patients (30%) (Table 2).

Regarding ARFI score 6 months post-transplant, thirteen patients (43.3%) had F0- F1 score and 17 patients (56.6%) had F2-F4 score. The mean ARFI stiffness was 1.23 ± 0.51 m/s (range 0.5 to 2.1 m/s) (Table 2).

ARFI score 6 months post-transplant was higher in patients who developed chronic hepatic GVHD [11 patients (83.3%) had F3-F4 score] in comparison to those who did not develop chronic hepatic GVHD [6 patients (33.3%), P= 0.020].

The mean ARFI stiffness 6 months post-transplant was higher in patients who developed chronic hepatic GVHD (1.45 ± 0.32 m/s) than those who didn't develop chronic hepatic GVHD (0.95 ± 0.58 m/s, P= 0. 018) (Table 5).

All patients who developed grade 4 chronic hepatic GVHD had F2-F4 ARFI score but this was not translated into a statistically significant correlation (P= 0.097) (Table 6).

The mean ARFI stiffness 6 months post-transplant was higher in patients who had grade 4 chronic hepatic GVHD (1.66 ± 0.29m/s) vs. (1.43 ± 0.19m/s) in patients who had grade 1-2 and grade three (1.23 ± 0.44 m/s) without statistical significance (P= 0.115) (Table 6).

DISCUSSION

HSCT showed many advances in recent decades. It is being used by many centers aiming to achieve cure in many hematological and non-hematological diseases [8]. However, HSCT remains to be a dangerous procedure due to peri- and post-transplant complications, of which, hepatic GVHD is one of the most common complications [8].

Hepatic GVHD may occur as an acute event usually within the first 100 days after transplant (acute hepatic GVHD) or as a chronic event within 2-12 months after HSCT (chronic hepatic GVHD). chronic hepatic GVHD is usually associated with other organ affection like skin, eyes, mouth, or other organs [8]. In general, patients with hepatic GVHD show progressive elevation of all liver enzyme tests

Table 6: Correlation between ARFI score and stiffness with grade of chronic hepatic GVHD 6 months post-transplant.

Variables		Grade of chronic GVHD			Test value	P-value
ARFI score 6 months Post-transplant	F0-F1	2 (33.3%)	1 (33.3%)	0 (0.0%)	6.300	0.097
	F2-F4	4 (66.6%)	2 (66.7%)	4 (100.0)		
ARFI stiffness 6 months Post- transplant	Mean± SD	1.43 ± 0.19	1.23 ± 0.44	1.66 ± 0.29	2.287	0.115
	Range	1.19 – 1.71	0.72 – 1.53	1.39 – 1.92		

Data present as Mean ± SD or number (%).
ARFI: Acoustic Radiation Force Impulse Elastography; GVHD: Graft Versus Host Disease

including direct bilirubin and alkaline phosphatase, usually as the earliest findings [3].

Diagnosis of both acute and chronic hepatic GVHD is mainly achieved by liver biopsy. Liver biopsy has been considered the 'gold standard' method for the diagnosis of hepatic GVHD. However, liver biopsy has many disadvantages such as being an invasive procedure with many subsequent complications especially bleeding. Also, sampling error, variability in histopathological interpretation, and the reluctance of patients to do repeated biopsies for follow-up are common problems [9, 10]. Subsequently, a non-invasive method is needed.

ARFI was first reported in 2011 [11]. It was first used in the diagnosis of sinusoidal obstruction syndrome. Shear wave velocities were higher than normal in examined patients and returned to normal level after treatment. Thus, in this early study, ARFI showed to help in diagnosis and in monitoring the treatment response [11, 12].

The increase of shear wave, in parallel to the decrease in elasticity and increase stiffness, was then documented in patients with different hepatic diseases (hepatic congestion, portal hypertension, hepatic fibrosis, inflammation, and cholestasis). Thus, ARFI may have a potential role in the diagnosis, staging and follow -up of different hepatic diseases like chronic hepatitis and liver cirrhosis and fibrosis [12-17].

ARFI can be used in diagnosing liver fibrosis in chronic viral hepatitis B and C [18], non-viral liver diseases [19], after eradication of hepatitis C virus [20], in cases with intrahepatic cholestasis [21], in autoimmune and cholestatic liver diseases [22], hepatic iron overload [23], non-alcoholic fatty liver disease [24, 25] and alcoholic liver disease [26].

Recent studies used ARFI to predict esophageal varies in cirrhotic patients [27-29] and to predict recurrence of hepatocellular carcinoma (HCC) after radiofrequency ablation [30].

The role of ARFI in the detection of increased stiffness encouraged researchers to investigate its possible role in different diseases. Recent studies point to a possible role of ARFI in the diagnosis of stiff pancreas in chronic pancreatitis [31], acute cellular rejection in patients undergoing hepatic transplantation [32], and assessment of graft fibrosis after liver transplant [33, 34].

Also, ARFI may be helpful in patients with ulcerative colitis [35], children with short bowel syndrome [36], and even myocardial stiffness [37]. Furthermore, recent studies point to a possible role of ARFI in the prediction and diagnosis of breast cancer [38, 39] and malignant thyroid nodules in patients with Hashimoto's thyroiditis [40].

The role of ARFI in the diagnosis of hepatic GVHD remains to be clarified. In our study; we recruited 30 patients who underwent peripheral blood allogeneic HSCT. We assessed the development of hepatic GVHD by using (right lobe hepatic point shear wave elastography ARFI: pSW-ARFI) score and stiffness. ARFI was done pre-transplant, within 3 months and 6 months post-transplant for all patients. We correlated our results to clinical data and liver biopsy results.

We found that ARFI score and stiffness done within the first three months of transplant can significantly diagnose the occurrence of acute hepatic GVHD once it occurred. In our cohort of patients; acute hepatic GVHD developed in eleven patients (36.7 %). ARFI score and stiffness were significantly higher in patients who developed acute hepatic GVHD in comparison to those who did not develop acute hepatic GVHD with significant P-value. (P= 0.028 and 0.03 respectively)

Furthermore, the mean ARFI stiffness in 1st three months post-transplant was directly correlated to the grade of acute hepatic GVHD (P= 0.012) indicating ARFI stiffness may be helpful in grading acute hepatic GVHD.

We think that the role of ARFI is not predictive but more diagnostic in hepatic GVHD. In our study, pre-transplant ARFI stiffness or score showed no significant differences between patients who later developed acute hepatic GVHD and those who did not. This is expected as GVHD occurs after transplant. Other studies reported a possible role of ARFI in predicting hepatic complications after HSCT not only GVHD but also sinusoidal obstruction syndrome and drug-induced liver injury. While our study only investigated patients with GVHD as a homogenous cohort of patients [41].

On the contrary, pre-transplant HCI showed a significant difference between patients who later developed acute hepatic GVHD and those who did not. We think that the HCI has a predictive value in predicting occurrence of acute hepatic GVHD, while ARFI has a diagnostic value. Combining both tools together may show double benefits (prediction and diagnosis) and warrant further studies.

In our study, we repeated ARFI within six months post–transplant for all patients. ARFI score and stiffness in 6 months post-transplant were higher in patients who developed chronic hepatic GVHD in comparison to those who did not develop. Furthermore, ARFI score and stiffness in 6 months post-transplant tend to be higher in higher grades of chronic hepatic GVHD, yet this did not show a statistically significant correlation in our study.

Few previous studies investigated the utility of ARFI in diagnosis and grading of GVHD after bone marrow transplant in adults. In the study done by Karlas and his colleagues in 2014, they included 59 patients with different hematological diseases who underwent allogeneic HSCT. The initial diagnosis of those patients included variable hematological diseases like chronic leukemia, lymphoma, myelodysplasia, and acute leukemia. Moreover, they used both reduced intensity and myeloablative transplant technique. In our study; we used a homogenous group of patients (acute leukemia and aplastic anemia only) and all our patients received myeloablative HSCT. The authors in this study performed ARFI pre- and post-transplant for three or 4 measurements [42].

In contrary to our results, Karlas and his colleagues [42], found that ARFI values were significantly elevated in patients that later developed severe complications after HSCT. Thus, they used pre-transplant ARFI in prediction not for diagnosis of post-transplant hepatic complications including hepatic GVHD, drug-induced liver injury, and veno-occlusive disease.

In another pilot study done on six patients with chronic hepatic GVHD by Zhang *et al.*, authors documented higher shear wave velocity in patients with chronic hepatic GVHD in comparison to normal controls. Our study included patients with acute and chronic hepatic GVHD in a bigger sample size. Yet our results are in accordance with this study [24].

No previous studies to our knowledge investigated the role of ARFI in diagnosis and grading of acute hepatic GVHD.

In conclusion, ARFI score and stiffness are significantly increased post-transplant with the occurrence of acute and chronic hepatic Graft-versus-host disease, So ARFI could be a reliable non-invasive tool for the early diagnosis of hepatic GVHD, especially if a liver biopsy is not feasible. Furthermore, higher ARFI

score may correlate to the grade of acute and chronic hepatic GVHD. Further studies are needed to confirm our results.

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