**Name of journal: World Journal of Hepatology**

**ESPS Manuscript NO: 15993**

**Columns: ORIGINAL ARTICLE**

***Basic Study***

**Normal liver stiffness: a study in living donors with normal liver histology**

Alsebaey A *et al.* Liver stiffness in living donors

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**Author contributions:** Alsebaey A performed the research; Allam N wrote the paper; Alswat K contributed research tools and contributed to the writing of the paper; and Waked I supervised the study.

**Ethics approval:** The study was reviewed and approved by the Institutional Review Board of the National Liver Institute.

**Informed consent:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest:** Imam Waked is a speaker for Hoffman La Roche, MSD, BMS, GSK, Bayer, Gilead, and Minapharm, has sat on advisory boards of Janssen, Hoffman La Roche, MSD, and GSK, and has acted as investigator in clinical trials for Hoffman La Roche, BMS, GSK, Bayer, and Minapharm. All authors declare no conflicts of interest related to this work.

**Data sharing:** No additional data are available.

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**Received:** December 20, 2014

**Peer-review started:** December 23, 2014

**First decision:** January 20, 2015

**Revised:** February 10, 2015

**Accepted:** April 1, 2015

**Article in press:**

**Published online:**

**Abstract**

**AIM:** To define the normal range of liver stiffness values using transient elastography in living-related liver transplantation candidate donors with normal liver histology.

**METHODS:** Liver stiffness was measured using Fibroscan in 50 (16 women, 34 men) healthy potential donors (mean age 28.4 ± 5.9 years) who were being evaluated for liver donation for their relatives at the National Liver Institute, Menoufeya University, Egypt. All potential donors had normal liver tests and were negative for hepatitis B or C virus infection. Abdominal ultrasounds showed normal findings. None of the subjects had diabetes, hypertension, renal impairment, heart disease, or body mass index > 30 kg/m2. All subjects had normal liver histology upon liver biopsy. They all donated the right lobe of their liver with successful outcomes.

**RESULTS:** The mean liver stiffness was 4.3 ± 1.2 kPa (range: 1.8–7.1 kPa). The 5th and 95th percentiles of normal liver stiffness were 2.6kPa and 6.8kPa, respectively, with a median of 4kPa; the interquartile range was 0.6 ±0.4. Liver stiffness measurements were not significantly different between men and women (4.4 ± 1.1 kPa *vs* 3.9 ± 1.3 kPa) and did not correlate with age. However, stiffness values were significantly lower in subjects with a body mass index < 26 kg/m2 compared to those with an index ≥ 26 kg/m2 (4.0 ± 1.1 kPa *vs* 4.6 ± 1.2 kPa; *P* <0.05). There were no differences in hospital stay or postoperative bilirubin, albumin,alanine and aspartate transaminases, or creatinine levels (at discharge) between donors with livers stiffness ≤ 4 kPa and those with stiffness > 4 kPa.

**CONCLUSION:** Healthy donors with normal liver histology have a median liver stiffness of 4kPa. Stiffness values are elevated relative to increase in body mass index.

**Key words:** Fibroscan; Liver stiffness; Living donors; Normal liver histology; Transient elastography

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**Core tip:** Although some studies have measured liver stiffness by transient elastography in healthy populations, few reports evaluate these with respect to liver biopsy results. This study adds to the knowledge of liver stiffness values in clinically and histologically normal livers of an Arab population, which may form the basis for future clinical practice. The results of this study suggest a new normal level of liver stiffness for this particular population, which differs from other populations reported in the literature.

Alsebaey A, Allam N, Alswat K, Waked I. Normal liver stiffness: a study in living donors with normal liver histology. *World J Hepatol* 2015; In press

**INTRODUCTION**

Liver stiffness (LS) measurement (LSM) is a noninvasive method for the evaluation of liver fibrosis, and is usedin clinical practice for the diagnosis and follow-up of liverdiseases[1,2]. As liver fibrosis may develop slowly in subjects showing persistently normal liver tests, identifying subjects with normal liver histology without fibrosis or undiagnosed histologic changes is of paramount importance in defining the true normal range of LS values. However, most studies to date have focused on LSM in patients with different chronic liver diseases[3-11]. A few European studies have addressed LSM in apparently healthy subjects, though these did not correlate LS with liver histology[12–14]. Hence, the primary aim of this study was to define the normal range of LS values using transient elastography in individuals with normal liver histology as determined by liver biopsy during evaluation as candidate donors for living-related liver transplantation. Furthermore, LS values are examined with respect to age, gender, and body mass index (BMI).

**MATERIALS AND METHODS**

***Subjects***

This study involved candidate donors for living-related liver transplantation who passed all stages of evaluation for liver donation for their relatives at the National Liver Institute, Menoufeya University during the period from June 2012 to January 2014. They all had normal liver blood tests and blood pictures, were negative for autoimmune markers and hepatitis B and C virus infection, and had normal abdominal imaging studies. None of the subjects had diabetes, hypertension, renal impairment, heart disease, or BMI > 30 kg/m2. Only the LS of the subjects who had normal histology on liver biopsy were included in analyses.

All subjects provided signed informed consent prior to study enrollment. This study was approved by the Institutional Review Board of the National Liver Institute, Menoufeya University (in 2012), Egypt, and conformed to the ethical guidelines of the 1975 declaration of Helsinki.

***LSM***

LS was measured by transient elastographyusing a FibroScan machine device (EchoSens, Paris, France) according to a previously described method[15]. The procedure was performed in the morning before obtaining a liver biopsy. The physician performing the procedure was blinded to clinical and biochemical data.The median value of tensuccessful measurements was recorded as the representative LS value, and is representative of the elastic modulus of the liver[15]. The success rate was calculated as the number of valid measurements divided by the total number of measurements. The interquartile range (IQR) was defined as an index of the intrinsic variability of LSM, corresponding to the interval of LSM results containing 50% of the valid measurements between the 25th and 75th percentiles. The results were considered unreliable if fewer than ten valid readings were obtained, success rate was < 60%, or IQR/LS value was >30%. LSM failure was recorded when no value was obtained after ten measurements.

***Statistical analysis***

Continuous data were compared using the Student’s *t*-test and categorical data were compared using the Fisher’s exact test. The Mann-Whitney *U* test was used to compare non-parametric variables. A Pearson’s test was used for correlational analysis. All two-sided *P* < 0.05 were considered significant. Statistical analyses were performed using SPSS version 17 for Windows (SPSS Inc., Chicago IL, United States). Data are presented as mean ± SD.

**RESULTS**

A total of 128 healthy subjects underwent liver biopsy for evaluation as potential liver donors for their relatives. Subjects excluded from donation due to histologic changes (*n* = 20) or with minimal histologic changes (*n* = 58) that did not prevent donation were not included in this analysis. Fifty individuals between 19 and 42 years of age were finally included in the study. The baseline characteristics of the fifty recruited subjects are shown in Table 1.

LSM was performed with a 100% success rate. IQR was 0.6 ± 0.4. LS values ranged from 1.8kPa to 7.1 kPa (Figure 1), with a mean of 4.3 ± 1.2 kPa. The 5th and 95th percentiles of LS were 2.6 kPa and 6.8kPa, respectively, with a median of 4kPa.There was no significant difference in LS between men and women (4.4 ± 1.1kPa *vs* 3.9 ± 1.3 kPa). Moreover, LS did not correlate with age. Stiffness values were significantly lower in subjects with BMI < 26 kg/m2 than those with BMI ≥ 26 kg/m2 (4 ± 1.07 kPa *vs* 4.6 ± 1.2 kPa; *P* < 0.05) (Figure 2).

The donors donated their right liver lobes. The duration of their hospital stay and postoperative bilirubin, albumin, alanine and aspartate transaminase levels, and creatinine results (on discharge) were recorded. Using the median LS value to divide the donors into two groups, there were no significant differences found in any of these measures (Table 2).

**DISCUSSION**

The possibility of using LSM as a screening tool for liver disease in the general population has been raised[16], but true normal LS values have not been well-identified, especially among various populations. Using the 5th and 95th percentiles from a non-obese population, the present study tentatively estimates a healthy liver stiffness range of 2.6 kPa to 6.8kPa, with a median stiffness of 4 kPa within an Egyptian population. This is lower than that established by Roulot *et al*[13] (3.3–7.8 kPa in women and 3.8–8.0 kPa in men). However, in their study, patients with potential liver disease were excluded based only on clinical and laboratory data, and no imaging studies or biopsies were performed. Furthermore, there may have been a selection bias, as their study recruited participants from a free health check, and subjects may have had symptoms that triggered their participation. In contrast, a wider range (2.3–8.8 kPa) was reported in another study conducted in 144 normal Romanian subjects[17]. However, that study comprised a large proportion (about 60%) of subjects that did not receive any laboratory testing or imaging studies, thus their definition of normal was less stringent.

In the present study, normal subjects were selected based on a healthy liver histology, without evidence of fatty liver or fibrosis. Similarly, Kim *et al*[18] conducted LSM in 12 biopsied healthy donors and reported a lower range of 3.9 kPa to 5.3 kPa. However, their study was in an East-Asian population, with 84.8% of the subjects having a BMI < 25 kg/m2. The present study includes a large proportion (46%) of individuals with a BMI of 27–30 kg/m2, and shows that LS is higher in individuals with a BMI > 26 kg/m2. Importantly, the biopsies did not reveal steatosis, which may influence LSM. Hence, the potential mechanism for the high LS values in healthy subjects with a higher BMI (without histologic changes of steatosis) remains speculative. The increase of LS with BMI was also reported in the study by Roulot *et al*[13] and Wong *et al*[19],with higher LS values in subjects with BMI > 30 kg/m2.

Some studies observed higher LS values in healthy men than in women[12,13]. However, consistent with reports by Kim *et al*[18] and Fung *et al*[20], this study shows no significant sex effect. However, the lack of significance may be due to the small sample size. There are intrinsic differences between men and women in the density of the extracellular matrix of the liver [21-23], and normal ranges need to be established for each sex in larger studies using the same stringent selection utilized in the present study.

In the current study, age had no significant impact on the LS value. However, the age range is narrow (19–42 years), as older persons are seldom accepted as living liver donors. Sirli *et al*[17] also found no difference in LS with age within a wider age group (18–69 years), which is consistent with results from other studies in France, Korea, and India[12,18,24]. On the other hand, a study in a Chinese population demonstrated a decline in median LS in the older age group, from 4.2 kPa in those < 25 years of age to 3.4 kPa for those > 55 years[25].

Although racial differences have not been reported, it is speculated that different cutoff values for normal ranges are needed for various populations[16]. The distribution of body fat varies with race[26-30], and this may affect rates of successful LSM acquisition. This has implications for the normal values used in areas of high ethnic diversity. All previous studies that included biopsies (and reported lower LS values) were performed in the FarEast; Fung*et al*[20] reported a median LS of 4.6 kPa (all < 7.2 kPa), and Kim *et al*[18] reported values all < 5.3 kPa. Consequently, the present study is important because it suggests a new normal level of LS for an Arab population, and provides further evidence that normal LS values should be defined for various populations.

Despite having a small sample size, the present study has considerable strengths. The subjects were living-related liver transplantation donors who were extensively evaluated clinically, chemically, radiologically, and histologically, making this the largest reported cohort of histologically normal livers. The healthy condition of the livers in our subjects was further confirmed intraoperatively during and postdonation. Another important aspect to consider is the large range of LS values obtained in studies that did not rely on histology to define normal liver;studies that include liver histology show a narrower range (< 7.2 kPa)[18,20]. A stiff liver is rarely found in the absence of any pathology. Hence, transient elastography may be used to screen the general population and to identify those that require further evaluation. The LS threshold requires further investigation and should take into account the population demographics as well as the likely prevalence of the condition to be screened for.

**COMMENTS**

***Background***

Liver stiffness (LS) measurement is a noninvasive method for the evaluation of liver fibrosis, and is used in clinical practice for the diagnosis and follow-up of liver diseases. Identification of the true normal LS value is an important prerequisite for widespread application of LS measurement.

***Research frontiers***

Although some studies have investigated LS as measured by transient elastography in healthy populations, few have correlated these values with results of liver biopsy in normal individuals. Therefore, the stiffness of livers with normal histology needs further assessment.

***Innovations and breakthroughs***

This study adds to the knowledge of LS values in a clinically and histologically normal liver population, which may form the basis for future clinical practice.

***Applications***

Transient elastography may be used in screening the general population and subsequent selection of sub-populations that require further evaluation.

***Peer-review***

This article presents LS values from healthy livers that were evaluated for living-related liver transplantation in an Arab population. The results are useful in establishing the normal range of LS values in a specific population, which can be used as a reference for further clinical applications.

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**P-Reviewer:** Arai M, Malnick SDH, Savopoulos cg **S-Editor:** Ma YJ

**L-Editor:** **E-Editor:**

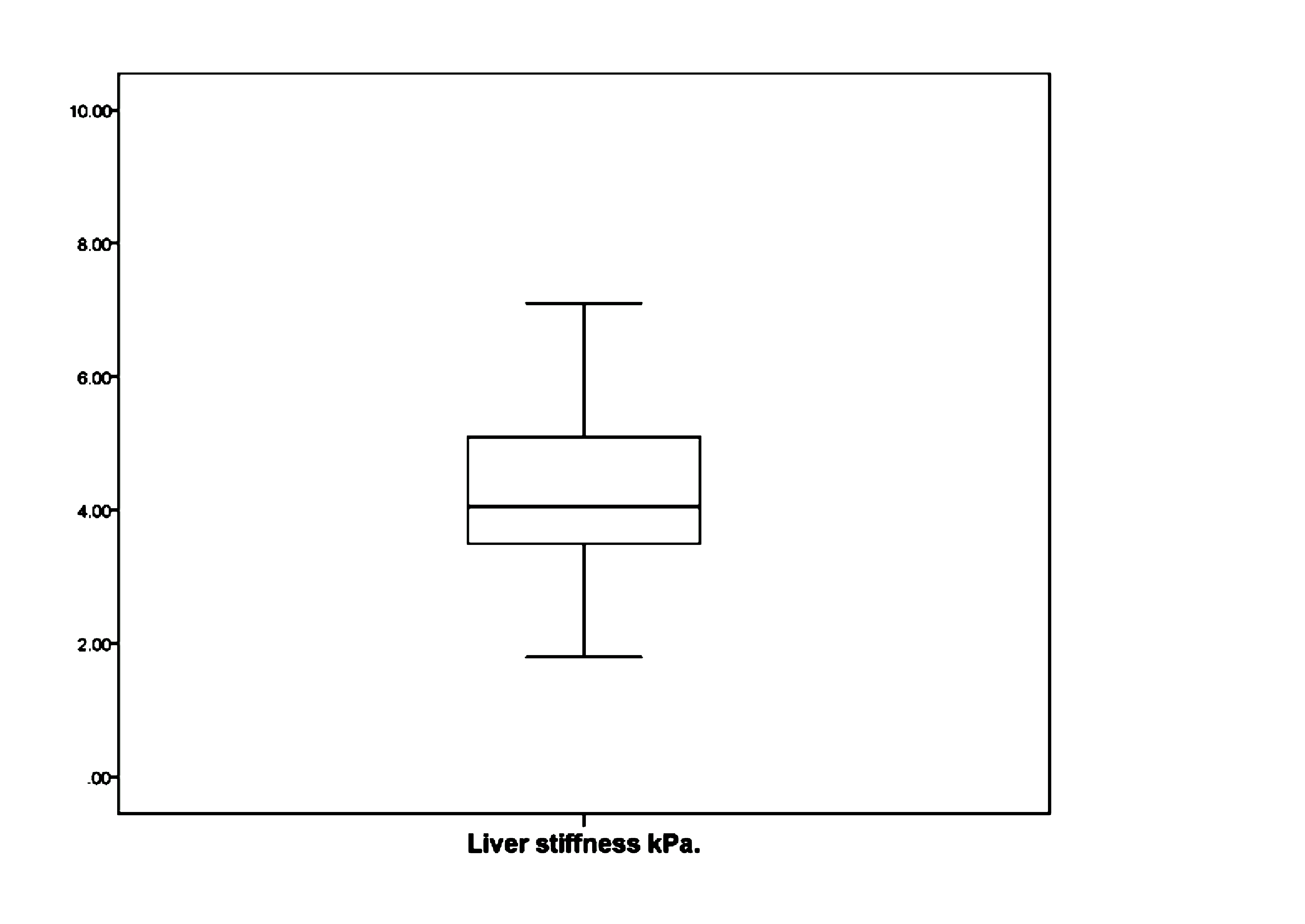


Figure 1 Boxplot diagram of the liver stiffness measures of the potential donors.

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**Figure 2 Stiffness values in donors with body mass index < or ≥ 26 kg/m2.**

**Table 1 Baseline characteristics of the enrolled donors (*n* = 50)**

|  |  |
| --- | --- |
| **Characteristic** | **Value** |
| Age (yr) | 28.4 ± 5.9 (range: 19–42) |
| Sex (male/female) | 34/16 |
| BMI (kg/m2) | 25.9 ± 2.8 (range: 18–30) |
| Total bilirubin (mg/dl) | 0.6 ± 0.3 |
| ALT (U/L) | 16.8 ± 7.2 |
| AST (U/L) | 18.8 ± 3.9 |
| Albumin (g/dL) | 4.7 ± 0.3 |
| Alkaline phosphatase (U/L) | 76.5 ± 17.7 |

Data are presented as mean ± SD unless otherwise indicated. ALT: Alanine transaminase; AST: Aspartate transaminase; BMI: Body mass index.

**Table 2 Donor characteristics according to liver stiffness**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **Stiffness < 4 kPa**  **(*n* = 21)** | **Stiffness ≥ 4 kPa**  **(*n* = 29)** | ***P-*value** |
| Sex (male/female) | 14/7 | 21/8 | 0:58 |
| Age (yr) | 28.70 ± 6.38 | 28.24 ± 5.81 | 0.79 |
| BMI | 25.00 ± 3.34 | 26.42 ± 2.26 | 0.11 |
| Hospital stay (d) | 10.00 ± 2.89 | 10.50 ± 4.40 | 0.80 |
| Bilirubin (mg/dL) | 0.42 ± 0.39 | 0.57 ± 0.46 | 0.60 |
| Albumin (g/dL) | 3.68 ± 0.36 | 3.74 ± 0.22 | 0.75 |
| AST (U/L) | 37.25 ± 27.28 | 39.6 ± 28.99 | 0.90 |
| ALT (U/L) | 57.25 ± 39.43 | 55.6 ± 48.29 | 0.96 |
| Creatinine (mg/dL) | 0.79 ± 0.12 | 0.67 ± 0.09 | 0.05 |
| INR | 1.09 ± 0.14 | 1.04 ± 0.02 | 0.42 |

Data are presented as mean ± SD unless otherwise indicated. ALT: Alanine transaminase; AST: Aspartate transaminase; BMI: Body mass index; INR: International normalized ratio.