Abstract

Background: Renal transplantation is the treatment of choice in end-stage renal disease. Chronic allograft dysfunction is the leading cause of chronic allograft failure. Surveillance biopsy is the only reliable tool to detect early fibrosis in the allograft. There is a need for non-invasive methods for the detection of early development of renal allograft fibrosis. Aims: To analyse the reliability of sonographic elasticity index and resistive index measurements in the evaluation of renal transplant fibrosis using linear and convex transducers according to segmental anatomy. Materials and methods: Elasticity index and resistive index were measured in 28 renal transplants and correlated with clinical prognostic parameters. Donor age above 50 years old, post transplantation time over 60 months and serum creatinine level above 1.5 mg/dl were defined as poor prognostic parameters. Results: Renal transplant recipients with serum creatinine level above 1.5 mg/dl demonstrated higher mean elasticity index (p=0.006) with a convex probe and higher elasticity index in the middle segments both with a convex and a matrix linear probe (p=0.026, p=0.001). Renal transplant recipients with post-transplantation time of 60 months and more demonstrated higher resistive index values in the middle segments (p=0.016). Conclusions: Convex probe was able to detect the changes in mean stiffness better than the matrix linear probe. The measurements from subsegments might suggest that diffuse changes in stiffness can truly be detected in the middle segments or that fibrotic processes start from the middle segments of the renal transplants. Further studies correlated with histopathology are required to validate the findings.

Keywords: renal transplant, sonoelastography, elasticity index

Introduction

Renal transplantation is the treatment of choice in end-stage renal disease [1]. Despite the significant reduction in acute rejection rates over the last decades, chronic allograft dysfunction remains the leading cause of chronic allograft failure among renal transplant recipients [2]. Chronic allograft dysfunction, previously named chronic allograft nephropathy, is a multifactorial process associated with progressive interstitial fibrosis and tubular atrophy. Fibrosis is the final outcome of various types of injury [3].

Factors related to the donor, surgery, and the recipient determine the initial function of a renal transplant. Interstitial fibrosis and arteriolar hyalinosis lead to progressive glomerular sclerosis causing a decline in the glomerular filtration rate. The progressive decline in renal function is detected by a rise in serum creatinine and clinical programs rely on monitoring the change in serum creatinine for identification of patients at risk. But this change occurs late in the course of the disease and has shown to have limited predictive value for consequent graft loss and usually underestimates the decline of renal function [4,5]. There is also accumulating evidence that subclinical rejection may be a key issue in early graft loss [6]. Subclini-
real rejection refers to allografts with normal renal function associated with tubulitis and interstitial infiltrate [7].

If clinical screening solely depends on serum creatinine for the identification of patients at risk for graft dysfunction, then measures to prevent subsequent graft loss will be delayed [8]. In this sense surveillance biopsies remain the only reliable tool to detect early fibrosis in the graft [9,10]. Transplant biopsy is an invasive method which is potentially associated with sampling errors and complications. Biopsy has been reported to be associated with haematuria, haematomas, obstruction of the graft by clots, arteriovenous fistula and intraperitoneal haemorrhage [11,12].

Therefore, there is a need for non-invasive methods for the detection of early development of graft fibrosis. The aim of the study is to evaluate the reliability of cortical elasticity index (E) measurement of renal transplants by using real time sonoelastography (RTS) and resistive index (RI) measurements by pulsed wave Doppler ultrasonography in correlation with clinical prognostic parameters.

Material and methods

After obtaining the approval of the instutional review board and informed patient consent, 28 renal transplant recipients (10 women, 18 men; mean age, 37 years, age range: 23 - 65 years) underwent RTS by Logiq E9 Elasto (General Electric, Milwakuee, WI, USA) with a convex probe of 1 - 5 MHz (C 1-5-D) and a matrix linear probe of 6 - 15 MHz (M L6-15-D). Patients were examined during routine post-transplantation renal ultrasound protocols and all of them had stable allograft function. An experienced radiologist with 20 years of sonography experience who was blinded to the clinical paramaters of the patients performed the examinations.

First gray scale images were obtained for each transplant kidney in longitudinal and transverse planes. Afterwards RI measurements were done from the interlobar artery of the renal cortex in each third of the transplant kidney with the convex probe (fig 1). RI was calculated as (peak systolic velocity - end diastolic velocity)/ peak systolic velocity. The mean RI value of each transplant was also calculated.

In the RTS evaluation mild repetitive pressure was applied. A compression indicator bar with a scale ranging from 1 to 7 on the screen made the examiner aware of the optimal compression level. Images were obtained when the optimal compression bar was in the range of 6–7. The elastogram was displayed over the B-mode image in a color scale. Circular region of interest (ROI) selections of the same size were placed in the renal cortex and E values were obtained by the elastography quantitative analysis. This is a strain elastography method which provides relative information about stiffness compared to other techniques based on Young’s modulus measuring absolute stiffness of the material in kPa. E is defined as a value from 0.0 to 6.0. It is reflected as a color distribution within the measured ROI. Low E value indicates less
stiffness and red color is dominant (fig 2). Higher E value indicates higher stiffness and blue color is dominant (fig 3). Three E values were measured in the cortex of the upper pole, three in the middle third and three in the lower pole resulting in nine values for each transplant kidney. The E measurements were first done by the convex probe and repeated with the matrix linear probe. A mean E value was calculated for both convex and matrix linear probes.

The patients were later divided into prognostic groups according to clinical prognostic parameters similar to the study of Syversveen et al [13]. Donor age above 50 years old, post transplantation time over 60 months and serum creatinine level above 1.5 mg/dl were defined as poor prognostic parameters. The patients gained 1 point for the presence of each one of these parameters or 0 points for their absence for the calculation of the total prognostic score. A total prognostic score of 0 indicated the best clinical prognosis, while score 3 indicated the worst clinical prognosis.

Statistical Analysis

SPSS for Windows 15 programme was used for statistical analysis. Mann Whitney-U, independent sample T-test and Kruskal Wallis methods were used for analysis of the correlation between the sonographic measurements and the clinical prognostic parameters. P values <0.05 were accepted as statistically significant. Area under the curve (AUC) measurements of the Receiver Operating Characteristic (ROC) curve were analysed for the determination of cut-off points of the elasticity measurements.

Results

The mean donor age was 44 ± 12 years. Half of the donors were younger than 50 years old and the other half were 50 years old or older. Transplants from cadavers were received by nine patients (32.1 %) while 19 of the patients (67.9 %) had living donors. The mean post-transplantation time was 66 ± 42 months. In 17 patients (60.7 %) the post-transplantation time was below 60 months, in 11 patients (39.3 %) it was 60 months or more. The mean serum creatinine level was 1.47 ± 0.48 mg/dl (0.80 - 2.50). In 17 patients (60.7 %) serum creatinine was below 1.5 mg/dl, in 11 patients (39.3 %) it was 1.5 mg/dl or higher. Total prognostic score was 0 in 6 patients (21.4 %), 1 in 12 patients (42.9 %), 2 in 6 patients (21.4 %) and 3 in 4 patients (14.3 %).

The mean E value was 3.04 ± 0.90 (1.53 - 5.24) for the convex probe and 3.38 ± 0.74 (1.68 - 5.27) for the matrix linear probe. The mean RI was 0.69 ± 0.16 (0.52 - 1.20).

Table I. Patient characteristics.

<table>
<thead>
<tr>
<th>Females/Males</th>
<th>10 /18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>BMI</td>
<td>25.24 ± 4.22</td>
</tr>
<tr>
<td>Donor age (mean ± SD)</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Living donor/deceased donor</td>
<td>19 / 9</td>
</tr>
<tr>
<td>Post-transplantation time (months)</td>
<td>66 ± 28</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.47 ± 0.48</td>
</tr>
</tbody>
</table>

Table II. Sonographic measurements.

| E average (C) | 3.04 ± 0.90 |
| EU (C) | 3.50 ± 1.47 |
| EM (C) | 3.04 ± 1.42 |
| EL (C) | 2.59 ± 1.14 |
| E average (ML) | 3.38 ± 0.74 |
| EU (ML) | 3.69 ± 1.23 |
| EM (ML) | 3.28 ± 0.95 |
| EL (ML) | 3.20 ± 1.06 |
| RI average | 0.69 ± 0.16 |
| RIU | 0.68 ± 0.18 |
| RIM | 0.68 ± 0.16 |
| RIL | 0.68 ± 0.19 |

Descriptive statistics of the renal transplant recipient characteristics are shown in Table I and descriptive statistics of the sonographic measurements is shown in Table II.

Analysis According To Prognostic Parameters

When the data were analysed according to donor age, which is the first prognostic parameter, renal transplant recipients whose donors were 50 years old and older had higher serum creatinine levels (p=0.016). E and RI measurements did not differ significantly.

When the data were analysed according to post-transplantation time, which is the second prognostic parameter, renal transplant recipients with post-transplantation time of 60 months and more demonstrated higher RI values in the middle part of the kidneys (p=0.016). E measurements did not differ significantly.
When the data were analysed according to serum creatinine level, which is the third prognostic parameter, renal transplant recipients with serum creatinine level of 1.5 mg/dl and higher showed higher E values in the middle third and lower pole of the kidneys by the convex probe according to segmental anatomy, also showed higher mean E values by the convex probe (p=0.026, p=0.007, p=0.006, respectively). They also had higher E values in the middle third of the kidney by the matrix linear probe (p=0.001). RI measurements did not differ significantly.

None of the measured sonographic parameters had a statistically significant correlation with the total prognostic score.

For the differentiation of poor clinical prognosis cut-off E values by matrix linear probe were calculated with 95 % confidence interval (CI). The cut-off point for E was 3.550 in the middle third of the kidney (p=0.002, AUC=0.858 ± 0.069, CI=0.723 - 0.993); 3.015 in the lower pole (p=0.047, AUC=0.709 ± 0.101, CI=0.511-0.906) and 3.635 for the mean E value (p=0.049, AUC=0.663 ± 0.106, CI=0.456 - 0.870) (fig 4, Table III). According to the AUC values the best discriminative graph was obtained from the measurements of the middle third of the kidney. The distribution of the rest of the data obtained did not allow the analysis of the cut-off E values.

**Discussions**

In this research, patients with high serum creatinine level displayed higher mean E value as well as higher E value in the middle segments and lower poles of the renal transplants with convex probe measurements. On the other hand the mean E value with matrix linear probe measurements did not differ significantly. Convex probes can visualize organs located deeper in the abdomen better due to the deeper penetration ability as a result of a low frequency band. Since transplanted kidneys are located superficially in the body, the difference in the penetration ability of both probes should not create a significant difference [14].

Looking at the probe geometry, linear probes have linearly structured crystals which send and receive acoustic signals perpendicular to the investigated area. The matrix linear probe we utilized has over a thousand crystals organized in a matrix structure with three rows and three columns. In this way dipole shift is prevented, acoustic signals are sent and received equally in every depth. On the other hand convex probes have crystals designed in a convex structure. Only the acoustic signals from the middle part of the probe are sent and received perpendicularly to the investigated area. The acoustic signals from the lateral sides are sent and received with an angle to facilitate the visualization of the organs which do not have a straight structure such as the kidney in our case [15]. Elastography image is highly dependent on an

![Fig 4. ROC curves for the calculation of cut-off values of the cortical elasticity index measurements by the matrix linear probe (EM = Cortical elasticity index in the middle segment, EL = Cortical elasticity index in the lower lobe, E mean = Mean cortical elasticity index).](image)

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Standard Error</th>
<th>Asymptotic Significance</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>E^M</td>
<td>.858</td>
<td>.069</td>
<td>.002</td>
<td>.723</td>
</tr>
<tr>
<td>E^L</td>
<td>.709</td>
<td>.101</td>
<td>.047</td>
<td>.511</td>
</tr>
<tr>
<td>E</td>
<td>.663</td>
<td>.106</td>
<td>.049</td>
<td>.456</td>
</tr>
</tbody>
</table>

EM: Cortical elasticity measurement of the middle third segment of the renal transplant by matrix linear probe
EL: Cortical elasticity measurement of the lower pole of the renal transplant by matrix linear probe
E: Average cortical elasticity measurement of the renal transplant by matrix linear probe
of renal transplant fibrosis. In the literature a few articles have been published concerning the utility of quantitative elastographic methods in the prediction of renal transplant dysfunction in the past decade. Grenier et al conducted a study on forty-three renal transplant recipients to assess the role of shear wave elastography imaging for renal allograft stiffness. Renal cortical elasticity measurements were not associated with any clinical parameters, any single Banff score or the level of interstitial fibrosis. Nevertheless a correlation was present between cortical stiffness and the total Banff scores. Authors concluded that cortical stiffness measurement was achievable and reproducible for the evaluation of renal transplants [22].

Arndt et al investigated the feasibility of transient elastography (FibroScan), which calculates shear wave velocity, to evaluate renal allograft fibrosis in 57 patients. There was a significant correlation between renal stiffness and the degree of interstitial fibrosis [23].

Syversveen et al evaluated the role of acoustic radiation force impulse (ARFI) elasticity method, which calculates shear wave velocity, in 30 renal transplant recipients. The measurements were compared to the degree of interstitial fibrosis in the biopsy specimens. Authors did not find a correlation between renal stiffness and the grade of fibrosis. They concluded that the use of ARFI quantification was not capable of assessing low-grade fibrosis in renal transplants. Besides they indicated high intraobserver and interobserver variation as a disadvantage of this technique [13].

In a consequent study Syversveen et al researched the ability of ARFI quantification to detect fibrosis in renal transplants and the affect of variations in the transducer force applied to the skin in this technique. Cortical shear wave velocity measurements were carried out in 31 renal allografts referred for protocol biopsies. The transducer was attached to a mechanical device and applied different predetermined forces. Mean shear wave velocity results were significantly different in repeated measurements. Authors concluded that ARFI results depend on applied transducer force and this technique cannot evaluate renal allograft fibrosis [24].

Stock et al measured the stiffness of 18 renal transplants by ARFI and evaluated the correlation with histological fibrosis score. A positive but moderate correlation was found between mean ARFI measurements and the grade of fibrosis [25]. In another study Stock et al. aimed to prospectively assess changes in ARFI values between clinically stable renal allografts and transplant dysfunction proven with biopsy in eight patients [26]. Renal transplant patients were first examined during stable allograft function and baseline ARFI values were obtained. Later a second allograft examination was performed on subsequent presentation with transplant dysfunction prior to biopsy. An average increase in renal stiffness of more than 15 % was only detected in transplants with histologically proven acute rejection. Mean RI values did not display an increase in none of the pathologies.
The only RTS study utilizing strain ratio measurements was conducted by Ozkan et al. Authors examined 42 renal transplants with sonoelastography to evaluate the interobserver variability of the technique. They calculated the strain ratio of the central echo complex to the renal parenchyma. Authors concluded that there was a correlation between parenchymal stiffness with Doppler parameters. They indicated a wide range of intraobserver agreement and low interobserver agreement in renal transplants [27]. This variability declared by Ozkan et al [30] and Syversveen et al [13] could be attributed to the complex anatomical structure of the kidney which is anisotropic as indicated by diffusion-weighted magnetic resonance imaging [28]. In the assessment of renal allograft fibrosis there is not a focal lesion to examine. The examiner is expected to evaluate the kidney as a whole and express the stiffness value as a single mean measurement which is very difficult with the complex anatomical structure of the kidney composed of different compartments.

Because elastographic examination is generally done by a freehand method, the pressure and speed of compression of the probe depend highly on the operator as indicated by Syversveen et al [24]. Some techniques have been suggested such as a minimum of five elastogram acquisitions per lesion to make the results more accurate and reproducible [29]. The sources of variability can also occur during the selection of an imaging plane and selection of ROI. Yoon et al has recently published significant interobserver variability of the ultrasound elastography technique [30].

In this research we evaluated the elasticity of the renal transplants both by matrix linear and convex probes. In the patients with high serum creatinine values, the middle thirds of the renal transplant cortex were significantly stiffer by both matrix linear and convex probes. With the use of convex probe the lower poles of the renal transplant cortex and the average of the whole kidney were also stiffer in the same group of patients. At this point one can hypothesize that the convex probe can be more sensitive in the evaluation of renal transplant fibrosis which needs be investigated with further studies. Whether the inflammatory and fibrotic processes start from the middle part of the renal cortex is unclear and may be a subject for further research. Compressibility differences of lower and upper poles of the kidney compared to the middle part of the kidney is also another possible explanation of the observed differences in our study and should be supported with further studies.

A noninvasive technique such as RTS is not expected to achieve the diagnostic accuracy of histological examination. It has to be emphasized that RTS cannot replace biopsy to detect renal transplant fibrosis. The most promising usage of RTS could be monitoring a change of allograft parenchyma over time. Another significant contribution of RTS could be guiding allograft biopsies to sample the stiffest area during routine surveillance biopsies and during biopsies performed in the situation of declining allograft function.

**Limitations of the study.** The first limitation of this preliminary study is the lack of correlation with the histopathology. In this study we examined the patients during routine post-transplantation renal ultrasound protocols and biopsy was not necessary since all of them had stable allograft function at the time. The second limitation is that intraobserver reproducibility was not analysed; instead the mean of the three measurements were used for the elastographic evaluation according to segmental anatomy and the mean of the nine measurements were used for the mean elastographic evaluation. The third limitation is that the interobserver agreement could not be studied since a radiologist with 20 years of sonography experience performed the examinations.

**Conclusion**

Clinical experience with RTS is still preliminary for the evaluation of renal transplant fibrosis. In this study a convex probe was able to detect the changes in mean stiffness better than the matrix linear probe in the evaluation of renal transplant fibrosis. The results of the measurements from upper, middle and lower segments might also suggest that diffuse changes in stiffness can truly be detected in the middle segments of the renal transplants both by convex and linear probes or that fibrotic processes start from the middle segments. Further studies with a larger number of patients correlated with histopathology are required. Intraobserver and interobserver variability of the RTS technique remains to be further investigated.

**Conflict of interest:** any

**References**

2. Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? Am J Transplant 2004; 4: 1289-1295.


