ANALYSIS OF THE VOLUME INDICES OF EXTRA- AND INTRA-CELLULAR FLUID OBTAINED FROM THE MULTI-FREQUENCY IMPEDANCE TECHNIQUE IN EXTREMITIES

INTRODUCTION

About 60% of the adult human body is fluid. The most of this fluid is inside the cells and called intracellular fluid (ICF) and one third is in the space out side the cells and called extracellular fluid (ECF) (1). Evaluation of the two components of ECF and ICF is important in various disease conditions. The standard method for this purpose in clinical practice is dilution principle using a tracer of isotopes (2), but that method is rather elaborate and there is a need of noninvasive methods with characteristics of safe, not expensive, requiring little operator skill and subject cooperation. The measuring of electrical impedance is considered as one of the methods to fulfill that need, and the application of bioelectrical impedance analysis for that needs started from the measurement of the volume of ECF using a single

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ABSTRACT

The information on the volume indices of extra-cellular fluid (ECF) and intra-cellular fluid (ICF) is critical in many disease conditions. The multi-frequency technique was primarily produced to estimate ECF and ICF in tissues, and now be used to estimate body composition such as total blood volume, fat free mass etc. in health and disease. However, little is known on adequacy of those indices of ECF and ICF using this method in clinical conditions. This report describes the analysis of the indices of ECF and ICF obtained from the measurements with a multi-frequency impedance meter technique of legs in post-hysterectomy patients for uterine cancer and in hemodialysis on the end-stage chronic renal disease. The results appear to indicate adequacy of these indices not only of ECF but also of ICF in practice.

Keywords: intra-cellular fluid, extra-cellular fluid, impedance

ANALIZA INDICILORDE VOLUM INTRA-ȘI EXTRACELULAR OBȚINUȚI PRIN TEHNICA IMPEDANȚEI VU MULTIFRECVENȚĂ LA NIVELUL EXTREMITĂILOR

REZUMAT:

Informațiile referitoare la indicii de volum intra- și extracelular sunt foarte sumare în special legat de condiții patologice. Tehnica de multifrecvență a fost inițial folosită pentru a evalua ECF și ICF în țesuturi iar acum pentru a estima compoziția corpului uman cum ar fi volumul săngei, masa de țesut adipos etc., la populația sănătoasă și la pacienți. Oricum sunt cunoscute puține informații referitoare la importanța acestor indicii ECF și ICF, folosind această metodă de determinare în condiții patologice. Acest studiu descrie analiza acestor indicii obținuți prin măsurători cu impedanța multi-frecvență la nivelul membrilor inferioare la paciențele posthisterectomie pentru cancer uterin și la pacienții hemodializați în stadiu terminal de insuficiență renală cronică. Rezultatele studiului arată că este utilă determinarea acestor indicii și în condiții patologice folosind această tehnică.
frequency of 1KHz (3). The wide application of this technique such as the measurement of total blood volume, fat free mass was reviewed (4), and progressed to the segmental impedance analysis (5). However, the measurement of body composition using single-frequency impedance technique has been criticized based on the applied hypothesis, that body behaves as a uniform isotopic conductor of electricity, and that the body can be viewed as a single cylinder, and that volume is a function of resistivity ($\rho$)·length$^2$/impedance (6).

The electrical properties of living tissues is known to have three kinds of frequency dispersions, called $\alpha$, $\beta$, and $\gamma$ dispersions, due to the three different mechanisms of relaxation (7). The application of multi-frequency electrical impedance technique was developed using $\beta$ dispersion, which is well known as a structural relaxation and occurs at the radio frequencies between 10KHz and 10 MHz. (Fig. 1) (8). From the measured results using the frequency range of 1-500 kHz, information on ECF and ICF can be obtained: when a low frequency voltage is applied to the tissue, current flows mainly through ECF due to large impedance of cell membrane, and when the applied current having higher frequencies, the current flows through both of ECF and ICF (Fig. 2), and the measurement using wide current frequency range allows to detect the equivalent electrical circuit embedded in tissues (8). Using the apparatus developing multi-frequency technique, many reports have been published on the distribution of body water, the effect of diuretics, the management of haemodialysis on end-stage renal disease, and measurement of lymph-oedema in extremities (9,10,11, 12). However, that method can not escape the above mentioned critics on the single-frequency impedance analysis and segmental application was recommended (6, 11). Furthermore, there are few reports to evaluate adequacy of the indices of ECF and ICF obtained from multi-frequency technique in clinical conditions. This report will concentrate to the analysis of these indices in the oedematous legs of postoperative lymphatic obstruction and that on haemodialysis.

**METHODS**

A multi-frequency impedance apparatus developed by Kanai's laboratory or an apparatus produced from Nihon Koden designed by Kanai’s laboratory was used. Measurements were made using four electrodes. Current electrodes (rectangular or band) were placed around the lower part of the thigh and just above the ankle. One of the two voltage electrodes was placed below the head of the fibula with the other also placed 10 cm more distal. Circumference were measured at the midpoint between the two voltage electrodes. The
apparatus provides the information of an equivalent electrical circuit for a cell or tissue shown in Fig. 3a, or 3b (13). Where, Re is the resistance of ECF, Ri is that of ICF, and Cm is the capacitance of cell membrane. The admittance locus of tissue is represented in Fig. 4 (8, 13).

In order to compare results obtained from different individuals, it was necessary to "normalize" the data. The equivalent resistivity of ECF (RE) and the equivalent resistivity of ICF (RI) were calculated assuming that the volume of a leg between two voltage electrodes was a cylinder with cross-sectional area S and length L, as follows:

\[
RE = Re \cdot S / L \quad \text{(I)}
\]

\[
RI = Ri \cdot S / L \quad \text{(II)}
\]

Alternately, RE and RI can be represented as,

\[
RE = \rho e \cdot (S / Se) \quad \text{(III)}
\]

\[
RI = \rho i \cdot (S / Si) \quad \text{(IV)}
\]

Where, \(\rho e\) and \(\rho i\) are the real resistivities of ECF and ICF, respectively, and Se and Si are the effective areas of ECF and ICF in S, respectively. Since the real resistivities of \(\rho e\) and \(\rho i\) are nearly identical in many individuals, RE and RI are mainly affected by changes in the ratios S/Se and S/Si, respectively.

From formula (III) and (IV),

\[
Se / Si = \rho e / \rho i \cdot RI / RE = K \cdot RI / RE \quad (13,14)
\]

Statistics: A paired t test was used for analysis of the measurements of leg oedema in patients with lymphatic obstruction in the first study. In the second study using CT measurements, initially "statistical analysis of variance for split designs" was used for analysis. The sources of variation were considered to be group, leg, and interaction between group and leg. The interaction between group and leg was significant, except for \(C_m\) (p<0.05). Analysis was performed both on the oedematous legs (OLs) in group 2 and the left legs (LLs) in group 1, and on the contra-lateral legs (CLs) in group 2 and right legs (RLs) in group 1 (Table 3), as LL is said to be more easily oedematous than the RL because of the cross of right common iliac artery over the left common iliac vein. The difference of means was estimated using a t-test. In the case of unequal variance, estimation was done by Welch’s method. The difference of means between legs in both groups was examined using a paired t-test. Correlation coefficients were calculated. In the third study on haemodialysis, Welch’s method was used to examine the difference of means.
The data was expressed by mean±SD. P<0.05 was considered to be significant.

**RESULTS**

**Evaluation of leg oedema with the impedance indices**

Measurements were performed on oedematous legs and contra-lateral legs in 9 patients with unilateral oedema after hysterectomy for cervical uterine cancer (14). The decrease of RE and the increase of RI/RE was observed in oedematous legs. Significant differences were observed in RE, Cm and RI/RE in comparison between the oedematous legs and contra-lateral legs (Table 1). Then, the same indices were compared between both legs in the 3 patients out of 9 in changing leg position: from supine with leg raised with angle of 45 degree to standing position (Table 2). Only the slight decrease of RE was observed in the oedematous legs in contrast to the significant change in the contra-lateral legs.

**Table 1.** Sequential measurements of impedance indices in both legs of 9 edematous patients (ref. 14).

<table>
<thead>
<tr>
<th>Side of legs</th>
<th>Circumference cm</th>
<th>RE (Ω·cm)</th>
<th>RI (Ω·cm)</th>
<th>Cm (nF/cm)</th>
<th>RI/RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>edematous leg</td>
<td>37.2 ± 6.0</td>
<td>212±60</td>
<td>874±225</td>
<td>2.7±1.0</td>
<td>4.6±2.4</td>
</tr>
<tr>
<td>contra-lateral</td>
<td>33.5±4.0</td>
<td>246±45</td>
<td>698±106</td>
<td>3.1±1.0</td>
<td>2.9±0.6</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.01</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;.05</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

The data was expressed by mean±SD. P<0.05 was considered to be significant.

**Table 2.** Comparison of the changes of impedance indices after a change of leg posture in both legs in 3 post-hysterectomy patients (ref. 14).

<table>
<thead>
<tr>
<th>Side of legs</th>
<th>RE (Ω·cm)</th>
<th>RI (Ω·cm)</th>
<th>C_m</th>
<th>RI/RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examined</td>
<td>raised</td>
<td>standing</td>
<td>raised</td>
<td>standing</td>
</tr>
<tr>
<td>edematous</td>
<td>230±40</td>
<td>203±19</td>
<td>915±202</td>
<td>1201±381</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>contra-lateral</td>
<td>288±39</td>
<td>236±28</td>
<td>782±37</td>
<td>1078±270</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.025</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
CT analysis of the edematous legs

In order to demonstrate the relationship between the decrease in RE and the increase of ECF in subcutaneous tissue and muscles, the following study was performed in 9 patients with leg oedema after hysterectomy for uterine cancer and 8 age matched female healthy subjects (15). Seven CT slices were taken at the same level of the calf of both legs in both groups. The CT area and numbers of the three regions of interest (subcutaneous tissue, muscle, and bone) were measured and the means of areas and CT numbers in subcutaneous tissue and muscles were calculated using the data obtained from the seven scans. The volume ratio of the interstitial fluid (IF) in the subcutaneous tissue area (SA) ($IF_s$) to SA and that of IF in muscle area (MA) ($IF_m$) to MA, and that of the sum of $IF_s$ and $IF_m$ to cross-sectional area (CSA) of legs were calculated by Wegener’s method (16) (Fig. 5). The change in $IF_s$/SA and $IF_s+m$/CSA was related to the change in RE and RI/RE (Fig. 6). The results indicate that the decrease in RE and the increase in RI/RE coincided with ECF increase in tissues.

The impedance indices on haemodialysis

The change of fluid distribution in legs was examined following haemodialysis in 19 patients, involving 13 patients of the first haemodialysis and 6 patients of the maintenance haemodialysis, on end-stage renal disease and 13 controls (17). The sodium concentration in

Table 3. Comparison of the data with multi-frequency impedance meter and CT in legs of controls and patients of post-hysterectomy lymphatic obstruction (ref.15)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Circumference</th>
<th>RE $\Omega$·cm</th>
<th>RI $\Omega$·cm</th>
<th>RI/RE</th>
<th>CSAcm$^2$</th>
<th>SA/CSA %</th>
<th>MA/CSA %</th>
<th>$IF_s$/SA %</th>
<th>$IF_m$/MA %</th>
<th>$IF_s+m$/CSA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL m</td>
<td>34.0</td>
<td>328</td>
<td>517</td>
<td>1.6</td>
<td>76.8</td>
<td>28</td>
<td>62</td>
<td>-1</td>
<td>5</td>
<td>-5</td>
</tr>
<tr>
<td>SD</td>
<td>2.4</td>
<td>30</td>
<td>89</td>
<td>0.2</td>
<td>11.4</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>LL m</td>
<td>34.2</td>
<td>328</td>
<td>505</td>
<td>1.4</td>
<td>76.1</td>
<td>28</td>
<td>62</td>
<td>0</td>
<td>-5</td>
<td>2</td>
</tr>
<tr>
<td>SD</td>
<td>2.3</td>
<td>39</td>
<td>75</td>
<td>0.2</td>
<td>12.2</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Group 2</td>
<td>OL m</td>
<td>38.5</td>
<td>212</td>
<td>950</td>
<td>4.9</td>
<td>107.9</td>
<td>42</td>
<td>50</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>7.6</td>
<td>40</td>
<td>526</td>
<td>3.7</td>
<td>44.5</td>
<td>14</td>
<td>12</td>
<td>19</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>CL m</td>
<td>34.1</td>
<td>248</td>
<td>676</td>
<td>2.8</td>
<td>80.8</td>
<td>30</td>
<td>60</td>
<td>15</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>SD</td>
<td>3.8</td>
<td>35</td>
<td>323</td>
<td>1.5</td>
<td>17.4</td>
<td>9</td>
<td>7</td>
<td>16</td>
<td>34</td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations: *P<0.05, †<0.01, ‡<0.001.

Table 4. The change of the impedance indices in patients with end-stage renal disease after haemodialysis (ref. 17).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age</th>
<th>RE $\Omega$·cm</th>
<th>RI $\Omega$·cm</th>
<th>$C_m$ nF/cm</th>
<th>RI/RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>M4, F9</td>
<td>51±9</td>
<td>312±32</td>
<td>475±98</td>
<td>1.1±0.4</td>
<td>1.5±0.0</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>haemodialysis</td>
<td>M13, F6</td>
<td>51±1</td>
<td>before 271±88</td>
<td>616±461</td>
<td>0.3±9.7</td>
<td>3.1±3.5</td>
</tr>
<tr>
<td>(n=19)</td>
<td></td>
<td></td>
<td>after 312±89</td>
<td>538±433</td>
<td>2.0±36.7*</td>
<td>2.2±2.4</td>
</tr>
<tr>
<td>p</td>
<td>0.029</td>
<td>0.143</td>
<td>0.856</td>
<td>0.086</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: *n=18.
Dialysate was 140 mEq/L. The fluid removed at the haemodialysis was 1.1 ± 1.1 L. The change in impedance indices were shown in Table 4. RE significantly increased after haemodialysis. The decrease in RI was not significant, but the increase in RI/RE was nearly significant.

**Fig. 5.** Calculation of ratios of interstitial fluid to subcutaneous tissue and to muscle in mixed tissue using CT number of group 1 as normal control (ref. 15).

Abbreviations be = ratio of interstitial fluid in subcutaneous tissue, ab = the difference of CT number in subcutaneous tissue between each leg and the mean in control group.

**Fig. 6.** Relationship between impedance measurements and CT analysis in four groups (ref. 15).

Abbreviations • = CL and R group, ○ = CL and L group, ▲ = OL and L group, △ = OL and R group.
DISCUSSION

The decrease in RE was observed in oedematous legs in the first study and that decrease in RE did not show significant change after changing body position. This result suggested ECF retention in oedematous legs due to combined fibrous tissue proliferation which inhibits gravitational shift of fluid in legs, and coincided with the character of non-pitting oedema with lymphatic obstruction. The adequacy of this inference was exhibited by the increase in IF in edematous legs in the second study of CT analysis. Furthermore, significant correlation between RE and RI/RE with IF/S or IF/C/S CSA shown in figure 6 will support the interpretation of the increase of RE after haemodialysis as the decrease of ECF in legs which was caused by fluid removal from dialysis (8). Concerning the change in ECF and ICF after haemodialysis, an analysis using isotope was reported: when the dialysate sodium concentration is equal to the serum sodium concentration, essentially all the water removed during dialysis comes from the extra-cellular compartment, however, when the dialysate concentration is below the serum concentration, fluid shift occurs from the extra-cellular compartment to the intra-cellular compartment (18). Using a multi-frequency impedance meter, in a patient with maintenance haemodialysis, RE increased and RI almost unchanged following haemodialysis using high sodium concentration dialysate but RI decreased in contrast to the continuous increase in RE on the haemodialysis using regular dialysate (138 mEq/L) performed alternately, on the repeated tests (13) (Fig. 7). This result indicates the increase of ICF during haemodialysis with regular dialysate. Accordingly, the decrease in RI combined with the nearly significant increase in RI/RE presented after haemodialysis in the current study suggests the increase in ICF, though further studies are necessary to confirm this hypothesis.

CONCLUSION

The measurement of RE, RI, and RI/RE with multi-frequency technique appears to be useful not only to examine the change in ECF but also ICF in biological tissues.

**Fig. 7.** Change of the impedance indices after haemodialysis using high and regular dialysate in a patient of maintenance haemodialysis (ref. 13).

REFERENCES:
REFERENCES (continued):


