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RESEARCH ARTICLE

Evaluating Dielectric Properties for Assessing Water Content at Acupuncture Points: New Methodology

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Importance: Understanding acupuncture point microenvironments is vital for optimizing treatment efficacy. Evaluating changes in water content at these points can provide further insights into the effects of acupuncture on tissues.
Objective: This study aimed to measure tissue dielectric constant (TDC) and assess changes in water content, specifically at stomach 36 (ST36, Zusanli) and spleen 6 (SP6, Sanyinjiao) acupuncture points.
Methods: In a controlled, blinded, randomized trial, 113 healthy volunteers were divided into six groups based on TDC sensor diameters (XS, M, and L): three control groups and three acupuncture groups. They were assessed at three time points: T1, baseline; T2, 20 min post-needle withdrawal; and T3, 40 min post-needle withdrawal. Electrical impedance (EI) was also analyzed. Significance level was set at $p < 0.001$.
Results: TDC at ST36 and SP6 significantly decreased with the XS probe at T2 and T3 compared with that at T1 (F8, 452: 54.61). TDC did not significantly vary between T2 and T3 with M and L probes. EI data indicated that the current passage increased in the SP (F2, 226: 39.32) and ST (F2, 226: 37.32) groups during T2 and T3 compared with that during T1 within their respective groups and controls.
Conclusions and Relevance: This study demonstrated the efficacy of TDC measurements in detecting water content fluctuations at acupuncture points and their responses to needles. TDC measurements, which were validated against EI, provide valuable insights into acupuncture point microenvironments and thus help optimize treatments.

Keywords: Dielectric constant, Electrical impedance, Acupuncture points, Acupuncture meridians, Spleen meridian, Stomach meridian

INTRODUCTION

The efficacy of acupuncture in traditional Chinese medicine (TCM) is precisely stimulating acupuncture points and channels [1]. According to this concept, the accurate needling of acupuncture points can yield local and systemic effects. The main channels connect these points, which run along the length of the body and connect to specific organs in the thorax [2,3].

Distinct electrical properties, including increased conductance, reduced impedance and resistance, enhanced capacitance, and elevated electrical potential compared with those in adjacent non-acupuncture points, commonly define acupuncture points [4]. However, the observed differences in electrical properties may be influenced by variations in anatomical structures, skin moisture levels, and measurement techniques. Moreover, the precise nature and significance of the electrical properties of acupuncture points and their therapeutic effects are still debated [5].

If additional evidence is required to investigate the electrical characteristics of acupuncture points, other research methods or experimental approaches could be used to support and strengthen the findings. In this case, one current theory proposes measuring hydraulic resistance to determine if the resistance along the acupuncture channels is low [6]. If the channels operate with low hydraulic resistance, interstitial fluid flow velocity increased; therefore, assessing the water content at acupuncture points could be an alternative to analyzing electrical impedance (EI).

A method to assess the quantity of local tissue water involves measuring the tissue dielectric constant (TDC) at a frequency of 300 MHz by using a coaxial reflection line

[7]. Through this methodology, changes in local tissue water content under various health conditions can be effectively assessed [8-10]. Considering the theory that acupuncture channels function as hydraulic channels, dielectric constant assessment at acupuncture points provides an alternative perspective to EI analysis [11]. These approaches can complement each other in elucidating the dynamic characteristics of acupuncture points and channels. They contribute to advancing our knowledge, including potential alterations after point stimulation, without the complexities associated with EI assessment [12].

This study aimed to validate a novel methodology for establishing concepts related to acupuncture points by investigating changes in local water after needling, thereby supporting the theory of low hydraulic resistance channels. This hypothesis stated that distinct probes of a TDC measurement device could elucidate changes in water content in the stomach 36 (ST36, Zusanli) and spleen 6 (SP6, Sanyinjiao) acupuncture points at intervals of 20 and 40 min after needle insertion. EI assessment results corroborated the data acquired using the TDC measurement device.

MATERIALS AND METHODS

1. Ethical aspects

This study was approved by the Research Ethics Committee of the Hospital das Clínicas, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Registration number CAAE: 45339020.6.0000.5440; Clinical Trial NCT04919746). After signing a consent form, all participants provided their informed consent.

2. Characterization of the study

Healthy subjects were included in this study implemented as a controlled, blind, and randomized clinical trial. Three researchers were assigned distinct roles: first researcher, participant recruitment and data collection; second researcher, randomization and interventions; and third researcher, data processing and analysis.

3. Subjects and groups

A total of 113 healthy male and female volunteers were included, and 452 acupuncture points were evaluated. Their age ranged from 18 years to 38 years. The eligibility criteria were as follows: availability throughout the required study duration, absence of any known medical conditions that could interfere with study outcomes, and willingness to provide informed consent. The exclusion criteria were as follows: women with a menstrual delay exceeding 28 days, pregnant or breastfeeding women, and individuals taking medications that could potentially interfere with the autonomic nervous system.

4. Groups and randomization

The volunteers were randomly divided into six groups: three control groups (control XS, CXS; control M, CM; and control L, CL) and three acupuncture groups (acupuncture XS -AXS; acupuncture M, AM; and acupuncture L, AL). In the control groups, the volunteers not stimulated by any form were subjected to TDC evaluation at acupuncture points ST36 (Zusanli) and SP6 (Sanyinjiao) with an XS, M, or L probe. Similarly, the volunteers in the acupuncture groups were placed under TDC evaluation at the same acupuncture points by using the XS, M, or L probe.

The subjects were randomized using six evenly distributed pieces of paper to divide the participants into six groups. Before the experiment, each participant drew a piece of paper to determine their assigned group (Fig. 1).

5. Point localization

The finger-cun (F-cun) measurement method, a proportional technique based on the size of a person's fingers and commonly used in acupuncture, was used to locate acupuncture points. It is mainly used by acupuncturists to accurately locate acupuncture points. The proportional bone (skeletal) measurement method (B-cun) was also utilized to determine the location of the acupuncture point. As suggested by the World Health Organization, this dual approach ensures the accurate placement for each examined subject [13,14].

The acupuncture points ST36 and SP6 are on the lower limbs. ST36 is on the tibialis anterior muscle, which is the anterior aspect of the leg and the line connecting ST35 to ST41 and 3 B-cun inferior to ST35. On the tibial aspect of the leg, which is posterior to the medial border of the tibia, 3 B-cun is superior to the prominence of the medial malleolus [14]. ST36 and SP6 are widely used in clinical and scientific practice and

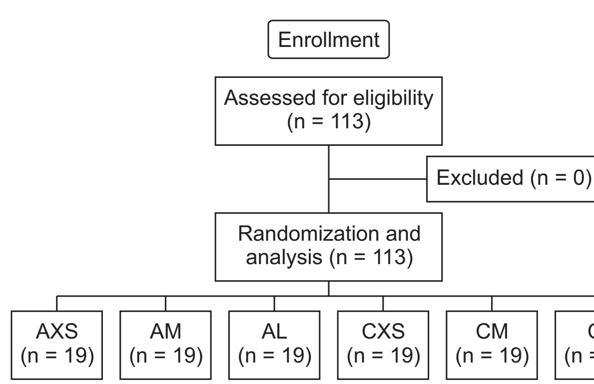

Fig. 1. Flowchart of the study. CXS, CM, and CL = Control groups with XS, M, and L probes; AXS, AM, and AL = Acupuncture groups with XS, M, and L probes.

can be quickly found [1,15]. The experimental protocol was implemented by placing the healthy volunteers in a dorsal decubitus position, with their lower limbs extended and arms relaxed, thus fully supporting the body.

6. Subject evaluation

The volunteers were instructed to avoid hot baths, apply any cosmetic or topical medication in the areas of points ST36 and SP6, engage in vigorous physical exercise, and consume any stimulating substances 2 h before the evaluation. If a volunteer had excess hair in the acupuncture point, the hair was removed using a disposable razor blade before the evaluation.

Evaluations were performed by a trained researcher familiar with the measuring equipment at three time points: evaluation time 1 (T1), 0 min; evaluation time 2 (T2), 20 min; and evaluation time 3 (T3), 40 min. In the acupuncture groups, the following acupuncture points were involved: T1 preceded ST36 and SP6 point stimulation; T2 occurred immediately after needle removal; and T3 occurred 20 min after the needle was removed from the ST36 and SP6 points (Fig. 2A). The control groups were not stimulated during T1, T2, and T3 (Fig. 2B).

7. Acquisition of dielectric constant data

TDC was evaluated using MoistureMeterD (Delfin Technologies Ltd., Finland). Three manual probes equipped with sensors of different diameters were used (Fig. 2): probe XS, 10 mm; probe M, 23 mm; and probe L, 55 mm. Noninvasive measurement was conducted by gently touching the skin surface at ST36 and SP6 points for approximately 10 s [9].

TDC was measured by reframing the electromagnetic field produced by the probe that interacted with the water molecules closest to the contact area (Fig. 3). The transmitted signal with a frequency of 300 MHz was propagated through an open coaxial transmission line. A portion of the signal, mainly water, was absorbed by the tissue, while the other portion was reflected. Through this reflection, the complex reflection coefficient, which determines TDC, can be calculated. Free and bound water molecules jointly contribute to the TDC [16].

8. Acquisition of EI data

EI was measured using Acuspointer (Medichina, São Paulo, Brazil), which consists of a pair of electrodes. A passive electrode was positioned on the patient's hand and aligned with the point under assessment, while the active electrode was placed on the skin surface of the acupuncture point. The device was operated at a continuous current of 12 V and manually calibrated at 200 μA by touching the electrode ends to ensure precise measurements. The current at the acupoints

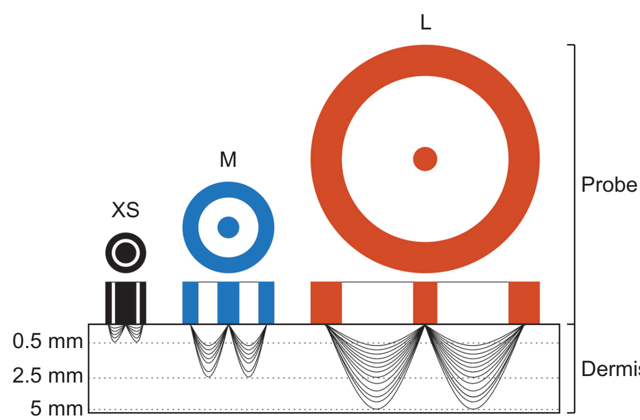

Fig. 3. Dielectric measurement and electric field in tissues. XS = Probe with a 0.5 mm effective measuring depth and 10 mm sensor contact diameter; M = probe with a 2.5 mm effective measuring depth and 23 mm sensor contact diameter; and L = probe with a 5 mm effective measuring depth and 55 mm sensor contact diameter.

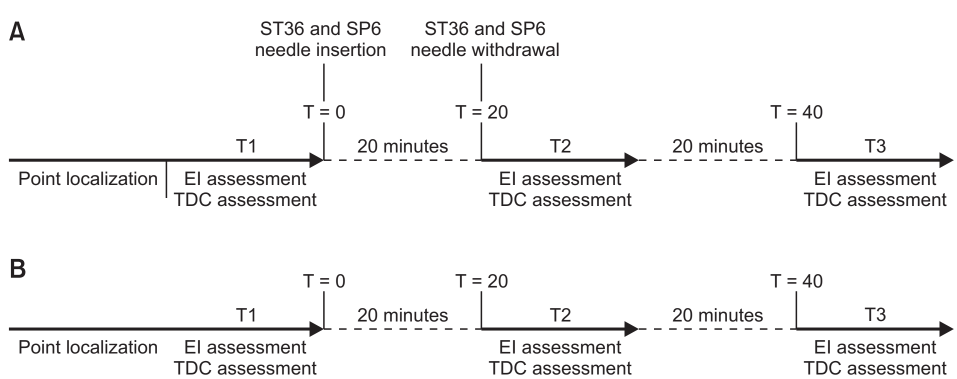

Fig. 2. Experimental design. (A) Acupuncture groups: Tissue dielectric constant (TDC) and electric impedance (EI) assessments were performed at three time points: before acupuncture stimulation (T1), immediately after needle removal (T2), and 20 min after the needle was removed from ST36 and SP6 (T3). (B) Control groups: TDC and EI evaluations were conducted at T1, T2, and T3 without acupuncture stimulation.

may vary between 0 and 200 μA; high current indicates low skin resistance and high current passage from one electrode to another [17]. The obtained value was equivalent to the average of three EI assessments at the pre-marked acupuncture point at T1, T2, and T3. EI was evaluated before TDC (Fig. 2A and 2B). The study subjects were divided into two groups: acupuncture and control.

9. Point stimulation

Acupuncture stimulation was performed by a researcher with previous training and experience in the field.

Sterile and disposable filiform acupuncture needles measuring 0.25 mm × 30 mm (Dong Bang Acupuncture) were used to stimulate the ST36 and SP6 points. They were inserted at a depth of 10 mm and left in place for 20 min. Afterward, the volunteers were asked about the sensation perceived at the needle insertion site to identify the presence of de qi. This sensation, a crucial indicator of the therapeutic effect of acupuncture, activates specific physiological responses in the body [18].

The ST36 and SP6 points of only the subjects in the acupuncture group were stimulated by the needles.

10. Statistical analysis

The TDC and EI data collected with different probes were analyzed via multivariate analysis of variance in the Sigma plot statistical software. Differences between the groups were further examined via Tukey's post-hoc test. Statistical significance was set at $p < 0.05$. The demographic characteristics of the groups were evaluated through one-way ANOVA. Sex was analyzed using a chi-square (χ^2) test.

RESULTS

The demographic characteristics, including height, weight, age, and sex, are presented in Table 1. ANOVA revealed no statistically significant differences in height ($F5, 108 = 0.77$, $p = 0.56$), weight ($F5, 108 = 1.57$, $p = 0.17$), and age ($F5, 108 = 1.09$, $p = 0.36$) among the groups. χ^2 test indicated no sex-related differences between the groups (male, $p = 1.0$; female, $p = 1.0$; Table 1).

The analysis of the results at points ST36 and SP6 revealed that TDC captured by the XS probe significantly decreased during T2 and T3 compared with that during T1 ($F8,452:54.61$; $p < 0.001$). Conversely, the values obtained with M and L probes did not significantly differ between the T2 and T3 assessments (Fig. 4).

The EI data showed that the current passage in the SP and ST groups increased during T2 and T3 compared with that during T1 in their respective groups and in the corresponding points in the control groups ($F2, 226 = 39.32$, $p < 0.001$ and $F2, 226 = 37.32$, $p < 0.001$, respectively; Fig. 5).

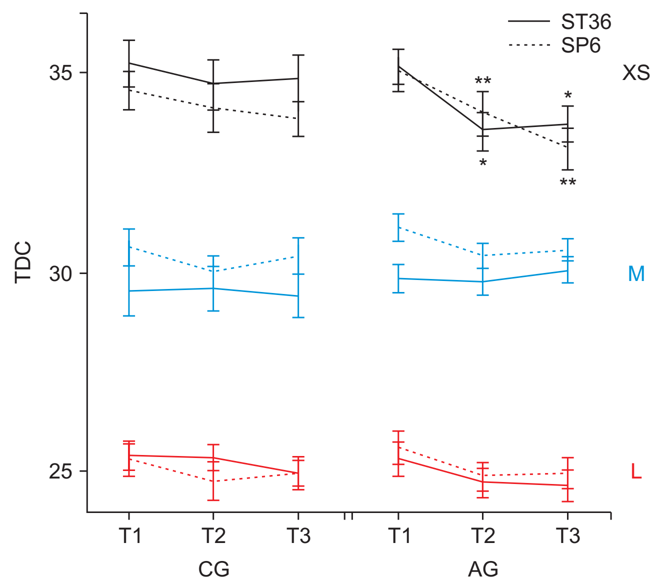

Fig. 4. Measurement of tissue dielectric constant (TDC) in acupuncture and control groups. The lines indicate the means of the TDC at the ST36 and SP6 acupoints obtained at different evaluations (T1, T2, and T3) with XS, M, and L probes. Vertical bars represent the standard error of the mean. *Significance relative to T1, $p < 0.001$; multivariate analysis of variance followed by Tukey's post-hoc test. AG = Acupuncture group; CG = Control groups. All groups had 19 subjects except for CG probe L, which had 18 subjects.

Table 1. Baseline demographic characteristics of study groups

Groups	Age (years)	Weight (kg)	Height (cm)	Female	Male
AXS (n = 19)	22.6 ± 0.6	68.1 ± 3.4	168.0 ± 2.2	13	6
AM (n = 19)	22.1 ± 0.5	61.4 ± 2.9	164.2 ± 7.1	14	5
AL (n = 19)	21.9 ± 0.4	57.6 ± 2.2	164.9 ± 1.8	14	5
CXS (n = 19)	20.9 ± 0.6	62.7 ± 3.2	167.3 ± 2.2	13	6
CM (n = 19)	22.1 ± 0.6	64.8 ± 2.5	168.3 ± 2.0	14	5
CL (n = 19)	21.3 ± 0.6	61.9 ± 2.0	167.4 ± 1.7	13	5
p-value	0.36	0.17	0.56	1.00	1.00

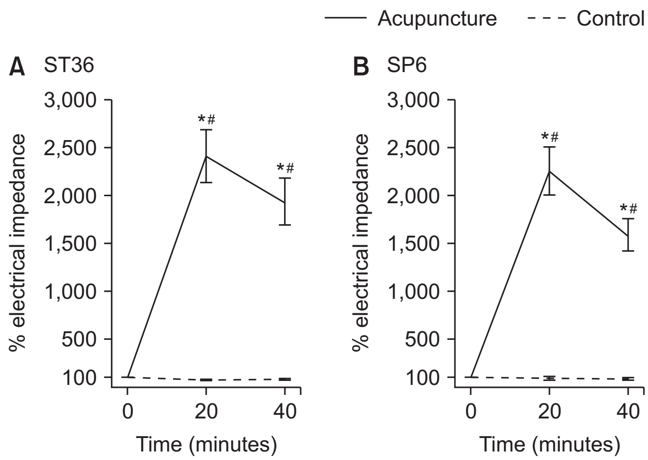

Fig. 5. Measurement of electric impedance in acupuncture and control groups. The lines indicate the electric impedance (EI) percentage at the ST36 (A) and SP6 (B) acupoints obtained at different evaluations (T1, T2, and T3). Vertical bars represent the standard error of the mean. *Significance relative to T1 in the same group. #Significance relative to the control group. Multivariate analysis of variance followed by Tukey's post-hoc test ($p < 0.001$). AG = Acupuncture group; CG = Control groups. The acupuncture group had 57 subjects, and CG had 56 subjects.

DISCUSSION

Our study highlighted the importance of the dielectric constant as a novel and reliable measure for quantifying the water content at ST36 and SP6. Immediately after needle removal and after 20 min, the dielectric constant could be considered an effective physical parameter for assessing the changes in water content at these specific points. This study introduced a new methodology and validated its robustness by comparing it with EI measurements.

Interestingly, our initial hypothesis could not be confirmed because only the 10 mm sensor exhibited sensitivity to water content reduction. The 23 and 55 mm sensors showed no significant changes. As such, the factors contributing to the differential responses observed among the sensor sizes should be further investigated.

Acupuncture points, specific locations on the skin with increased responsiveness to physical stimulation, often coincide with muscle motor points identified by distinct electrical properties [19]. This evidence supports the neural theory of acupuncture points and channels; therefore, skin electrical resistance can be considered a valuable tool for detecting these points [20,21]. Our study demonstrated that EI increased at both points possibly because of neural modulation triggered by acupuncture stimulation [22]. Acupuncture needle insertion may elicit responses from nerve endings and alter the conductivity of surrounding tissues [23,24].

Studies on interstitial fluid movement within acupuncture channels may reveal the potential for a novel approach to evaluate the response of acupuncture points to needle stimulation [11]. Consistent with this concept, our results showed that the water content at ST36 and SP6 acupuncture points with a 10 mm sensor decreased after acupuncture needle stimulation.

MoistureMeterD, a device used to measure the volume of water under the epidermis, is equipped with sensors reaching depths of 0.5, 2.5, and 5 mm below the dermis (XS, M, and L, respectively) by emitting an ultra-high-frequency electromagnetic wave of 300 MHz through a coaxial line [9]. A previous study investigated the dielectric property of the PC4 acupuncture point compared with that of the adjacent tissue unrelated to any acupuncture point in the 50-75 GHz range; its results indicated a particular dielectric property at the acupuncture point [25]. Our study was the first to demonstrate that the dielectric constant changed after an acupuncture point was stimulated.

The assessment of the water content at acupuncture points based on dielectric properties has several advantages. It is a noninvasive and painless technique that provides real-time measurements, thus allowing for dynamic monitoring [7]. Dielectric measurements can contribute to personalized acupuncture treatments by guiding needle insertion and optimizing treatment protocols based on individual water-distribution patterns [10,26].

A decrease in TDC immediately after acupuncture needle insertion may be associated with changes in fluid dynamics within acupuncture points [27]. It is attributed to the influence of acupuncture on local microcirculation and tissue perfusion; needle stimulation may temporarily alter tissue permeability or redistribute interstitial fluids, causing a transient decrease in TDC [28]. Our study was consistent with a previous research on the hydraulic characteristics of acupuncture channels; the analysis of tissue water content based on TDC helps elucidate this concept [11]. According to the TCM theory, acupuncture can promote the free flow of qi and blood [18]. Despite millennia of contemplation by Chinese philosophers, a conclusive scientific understanding of qi still needs to be achieved. Notably, the Chinese character of qi incorporates the elements of vapor and fire; vapor can suggest a link to the interstitial fluid. However, further research should be performed to elucidate the precise nature of this connection [29]. Moreover, ongoing research in this area helps advance our understanding of qi and its role in TCM.

Contrary to our initial hypothesis, our results demonstrated that only the 10 mm sensor detected a change in TDC after acupuncture needle insertion. This finding could be attributed to the specificity of acupuncture points.

Acupuncture points specifically react to inserted acupuncture needles [30]. In their microanatomical aspects, these points have numerous nerve endings and vessels embedded within complex networks of collagen membranes in the hypodermis and muscles [23]. Compared with the neighboring tissue, these distinct tissue characteristics at the acupuncture point change only when the needle correctly reaches the acupuncture point [31].

In our study, methodological rigor ensured that all needles were correctly inserted into the acupuncture points. We hypothesized that the 10 mm sensor, which was the smallest, could more likely capture readings precisely from the acupuncture point area because of its reduced spatial coverage. This hypothesis was consistent with the traditional understanding that acupuncture points occupy relatively small and precise locations on the body's surface. Conversely, the 23 and 55 mm sensors, with larger surface areas, more likely encompass the neighboring tissue and the acupuncture point itself. This inclusion of the surrounding tissue can complicate the measurement of TDC because it may introduce signals from adjacent areas with different physiological properties [16].

Although our study provided valuable insights into the use of the dielectric constant to measure the water content in acupuncture points, it had some limitations. For instance, the imbalance in gender representation was a significant limitation of our study. The number of females was more than that of males, and bias toward younger demographics existed. This imbalance might affect the generalizability of our findings to diverse populations. Future studies should involve a more balanced and representative sample, which includes a broader range of age and gender proportions, to enhance the external validity and applicability of our outcomes. Additionally, our study focused on two specific acupuncture points, ST36 and SP6, which were selected on the basis of their relevance and previous research highlighting their distinct properties [32,33]. However, the characteristics and responses of acupuncture points may vary. Future studies should explore a broader range of acupuncture points to enhance the understanding of the generalizability of our findings.

In conclusion, our study remarkably advances the knowledge of acupuncture mechanisms by introducing a novel method through which the dielectric constant is used to assess the water content at acupuncture points. The unexpected variations in sensor sensitivity highlight the complexities of acupuncture point responsiveness; as such, underlying factors should be further explored. Overall, this study provides the basis for objective assessments of acupuncture therapy and enhancement of related research and clinical applications.

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AUTHORS' CONTRIBUTIONS

Conceptualization, Funding acquisition, Writing - review & editing: JEA; Investigation, Methodology, Writing - original draft: MGP, KZ and GHMR; Formal analysis: ECOG and MLS; Methodology: FBR.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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