



Wound healing effect of acupuncture for treating phonotraumatic vocal pathologies: Cytokine study

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 Manuscripts

Vocal pathology wound healing effect

Wound healing effect of acupuncture for treating phonotraumatic vocal pathologies: Cytokine study

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Conflict of Interest: None

Running title: Vocal pathology wound healing effect

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Wound healing effect of acupuncture for treating phonotraumatic vocal pathologies: Cytokine study

Objective: Acupuncture is a less invasive procedure when compared with surgical treatment for benign vocal pathologies caused by vocal overuse. This study aimed to determine the wound healing effect of acupuncture in treating phonotraumatic vocal fold lesions.

Study Design and Methods: The study used a two-way mixed-model between- and within-subjects, prospective randomized, placebo-controlled, blinded, group design. Seventeen dysphonic individuals with vocal nodules were recruited from a university clinic in Hong Kong. Each participant was randomly assigned to receive one session of either genuine or sham acupuncture. The genuine acupuncture group (N=9) received needles puncturing nine voice-related acupoints for 30 minutes; while the sham acupuncture group (N=8) received blunted needles stimulating the skin surface of the nine acupoints for the same frequency and duration. Laryngeal secretions were suctioned from the surface of the vocal folds immediately before, immediately after, and 24 hours after the acupuncture. The protein concentration levels of wound-healing related cytokines (interleukin IL-1 and IL-10) in these secretion samples were measured.

Results: Following acupuncture, a significant increase in the anti-inflammatory cytokine IL-10 was found in the genuine acupuncture group (N=9) but not in the sham acupuncture group (N=8).

Conclusions: The findings showed that acupuncture of voice-related acupoints facilitated an anti-inflammatory process in phonotraumatic vocal pathologies. This could be considered as supporting evidence to consider acupuncture as a less invasive alternative option, when compared to surgery, for treating phonotraumatic vocal pathologies.

Keywords: voice problems, laryngology, voice therapy, anti-inflammatory effect of acupuncture, alternative treatment, cytokine study

Level of Evidence: 1b

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INTRODUCTION

Benign vocal fold pathologies, such as vocal nodules, polyps or chronic (or non-infectious) laryngitis, are common causes of voice problems that resulted from trauma to vocal fold tissue during phonation (phonotrauma)^{1,2}. Current treatment options generally include conservative behavioral voice therapy that allows the vocal pathologies to resolve through a wound healing process^{3,4} or occasionally requiring surgery in some chronic conditions⁵⁻⁷, supplemented by vocal hygiene education and behavioral voice therapy^{8,9}.

In recent years, more people seek for less invasive alternative treatment besides surgery¹⁰. Acupuncture has been shown to be effective in treating voice problems due to phonotrauma¹¹. Subjects who received acupuncture at voice-related acupoints for 10 30-minute sessions of acupuncture over a period of one month¹¹ demonstrated significantly greater improvements in the vocal function (maximum fundamental frequency produced)¹¹, voice quality severity (breathiness and roughness)¹¹ and voice-related quality of life¹¹ in terms of voice-related activity and participation according to the International Classification of Function¹².

The mechanism of how acupuncture brings about physiological improvement in treating a variety of ailments is not fully understood. Different hypotheses have been put forward. For example, neurological blocking¹³ and neurological activation leading to corresponding healing activities in the wound^{14,15} have been proposed in pain control studies. It has been shown that acupuncture-like stimulation can promote the healing of wounds that are unresponsive to conventional treatment¹⁶. There are also a number of studies using animal models that showed wound healing are facilitated with the use of acupuncture¹⁷⁻²⁰. Findings from these studies might well be used to hypothesize the wound healing effect of acupuncture on the benign vocal fold lesions.

The events of wound healing are highly organized and are governed by numerous biochemical markers including cytokines, growth factor, prostaglandins and metalloproteinases^{21,22}. In a study investigating the effects of acupuncture in patients with asthma²³, acupuncture stimulation was found to affect the immune system by

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3 normalizing the concentrations of plasma cytokines. Based on this, we hypothesized
4 that the acupuncture protocol reported in the study by Yiu et al¹¹ was capable of
5 promoting wound healing in benign laryngeal injuries commonly associated with
6 phonotrauma. One would expect the concentrations of biochemical markers in
7 damaged vocal folds to be altered by treatment. We therefore investigated whether
8 biological changes (using cytokines as surrogate healing markers) would be observed
9 in the vocal folds as a result of needling at the designated acupoints. The research
10 hypothesis was that acupuncture, but not sham treatment, would improve the
11 inflammatory profile of the vocal folds, which was estimated from the cytokine levels
12 in the laryngeal secretions.
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20 21 **Objective**

22 The objective of the study was to determine the level of anti-inflammatory cytokine
23 (interleukin (IL)-10) and pro-inflammatory cytokine (IL-1 β) level in the laryngeal
24 secretion of subjects who were given acupuncture or sham acupuncture at the voice-
25 related acupoints.
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31 **METHODS**

32 The experimental protocol was approved by the Institutional Review Board of The
33 University of Hong Kong/ Hospital Authority Hong Kong West Cluster/ (IRB# UW
34 07/004).
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39 **Participants**

40 Seventeen dysphonic individuals with vocal nodules, recruited in February 2011 at
41 the University of Hong Kong Voice Clinic, were assigned to either the genuine (N=9)
42 or sham (N= 8) acupuncture groups. Participants had no knowledge of how
43 acupuncture would be conducted. They were also free from any cardiovascular,
44 neurological or respiratory disorders and did not receive any medication at the time of
45 the study.
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51 **Procedures**

52 Suctions of secretions from the larynx were carried out before the acupuncture
53 procedure. Local anesthetics (5% lidocaine + 0.5% phenylephrine) were dropped
54 down to the laryngeal inlet via the channel of the flexible laryngoscope under direct
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vision to provide local anesthesia for the suction to be carried out. A flexible laryngoscope (Olympus ENF-P4, Japan) with a disposable endoscope sheath with channel (Slide-On™ EndoSheath®, Medtronic, Jacksonville, FL) were used by an otolaryngologist (RT, co-author) to collect the secretion on the vocal fold surface²⁴. A new endoscope sheath was used for each suction procedure. The secretions from both vocal folds were suctioned with the vocal folds adducted during phonation. The amount of secretion sample collected was approximately 1 cc. The channel was flushed with 0.5mL saline to flush out any remaining secretion in the channel. Each sample was transferred into an airtight eppendorf tube and immediately frozen to -80°C and stored in a freezer for subsequent biochemical analysis.

A 30-minute session of acupuncture (genuine or sham) was then given to each participant. The experimental group received needling that punctured nine acupoints, while the sham acupuncture group received stimulation on the skin surface of the acupoints using blunted needles. These acupoints are Hegu (Large Intestine 4) and Lieque (Lung 7) at the wrists, Lianquan (Conception vessel 23) and Renying (Stomach 9) around the neck and Zhaohai (Kidney 6) points on the ankles (Figure 1)¹¹. With the group that received genuine acupuncture, each of the disposable stainless steel needles (0.25 mm diameter, 25 mm long with a 25 mm extended handle) was inserted through a 25 mm long guide tube (Hua Tuo Brand, Suzhou Medical Appliance, Suzhou, China) with one end attached to an opening in the center of a foam board (1 cm x 1 cm) taped to the skin surface of each acupoints (see Figure 1 showing the needle in a guide tube).

Put Figure 1 here.

The needling depth is typically about 13 mm for Zhaohai, Hegu and Lianquan and 5-8 mm for Lieque and Renyin acupoints²⁵. For the group that received the sham acupuncture, blunted acupuncture needles were used²⁶. The blunt needle end was used to exert pressure on the skin surface without penetrating the skin. Each blunt needle was held in place by using a stopper inserted into the opening of the guide tube (see also Figure 1).

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Each needle was twirled in the acupoint (acupoint twirling for genuine acupuncture) or on the skin surface of the acupoint (skin surface twirling for sham acupuncture) every five minutes for 30 minutes. Since all the subjects had no prior experience of acupuncture, it is reasonable to expect that the participant could not tell whether they had genuine acupuncture (acupoint twirling) or the sham acupuncture (skin surface twirling). Vocal fold secretions were collected again immediately after the acupuncture procedure and then again 24 hours after the acupuncture.

Sample analysis

All laryngeal secretion sample analyses were carried out at the Oral BioSciences Laboratory, The University of Hong Kong using enzyme-linked immunosorbent assay (ELISA) kit (by RayBiotech, Inc., Norcross, GA). The concentrations of biochemical markers (in pg / mg of protein) pro-inflammatory cytokine IL-1 β and anti-inflammatory cytokine IL10 were determined.

Data analysis

This study employed a prospective, randomized, 2-way mixed-model, repeated-measures placebo-control group design. The between-subjects independent variable was group assignment (i.e. genuine acupuncture versus sham acupuncture), and the within-subjects independent variable was the time point (a pre-needling and 2 post-needling time points). Dependent variables were concentrations of two major biochemical markers, namely pro-inflammatory (IL-1 β) and anti-inflammatory cytokine IL10, as these markers have been shown to predominate in acute and chronic phonotraumatic injury²⁴. Statistical tests were carried out using SPSS version 20.

RESULTS

Results showed that the pro-inflammatory IL-1 β level increased significantly over time (Friedman's 2-way ANOVA=7.6, df=2, p=0.02; see Figure 2) in the sham acupuncture group while the changes in the genuine acupuncture group was not significant (Friedman test: chi square =1, df=2, p=0.61). While for the anti-inflammatory cytokine IL10, the genuine acupuncture group demonstrated a marginally significant increase after the acupuncture (Friedman's 2-way ANOVA=6,

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df=2, p=0.05; see Figure 3), while the sham acupuncture group showed no significant changes over time (Friedman test: chi square =2.57, df=2, p=0.28). The anti-inflammatory effect was, however, only short living and the increased IL10 level could not be maintained at the 24-hour follow-up time point.

Put Figures 2 & 3 here

DISCUSSION

The pro-inflammatory IL-1 β level continued to increase significantly over time in the group with sham-acupuncture. There was, however, no significant change with the IL-1 β level in the group with genuine acupuncture. These results suggested that the inflammatory condition in the sham acupuncture was continuing, while that of the genuine acupuncture group was stabilized. On the other hand, the anti-inflammatory IL-10 level increased (p=0.05) following genuine acupuncture but decreased with the sham acupuncture. Admittedly, the sample size was small (N=17). Nevertheless, this effect suggested that acupuncture indeed facilitated an anti-inflammatory process at the laryngeal level of the body. It should be noted that the anti-inflammatory effect was only short living and the increased IL-10 level could no longer be maintained after 24 hours (see Figure 3).

CONCLUSION

Contemporary treatment options for benign vocal fold pathologies related to phonotrauma involved conservative voice exercise therapy and possibly surgery^{6,9}. Given the invasiveness and risk associated with phono-surgery for phonotraumatic vocal lesions, it is not difficult to understand that many patients would look for alternative treatment options. Many anecdotal reports and more recently randomized trials¹¹ have shown the acupuncture can effectively treat this problem. Being a less invasive procedure compared to surgical treatment, we have proposed that acupuncture can be considered as an alternative management option for chronic phonotraumatic vocal fold lesions. The underlying mechanism of the effectiveness of acupuncture in treating phonotraumatic lesions could probably be attributed to its facilitation of wound healing.

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3 The increase of anti-inflammatory IL-10 level in the laryngeal secretion following
4 acupuncture but not in the sham-acupuncture group suggested that the acupuncture
5 itself facilitated such change. The anti-inflammatory effect of acupuncture has also be
6 found in other disease models²⁷⁻²⁹. It remains, however, uncertain how and why
7 acupuncture could facilitate such wound healing effect. We hypothesize that the use
8 of needling at the Renyin acupoints near the larynx could have facilitated the blood
9 circulation and nerve endings stimulation around the larynx, which subsequently
10 enhanced wound healing. If this were the case, then would the needling at the other
11 acupoints distal from the larynx be necessary? According to the traditional Chinese
12 medicine principle, needling at the other acupoints are essential, if not supplementary,
13 in order to achieve a balanced outcome. In evidence-based practice medicine, this
14 remains to be answered by setting up trials to compare the effects of acupuncture with
15 just the acupoints next to the larynx with those when a complete set of traditional
16 acupoints are to be used.
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Figure legends

Figure 1. Needling position and needle design.

Figure 2. Mean level of pro-inflammatory IL-1 beta over time.

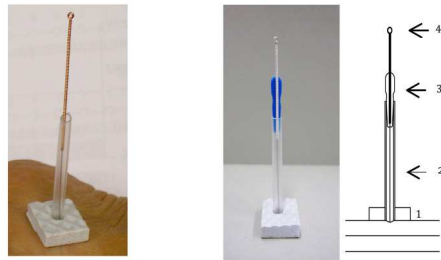
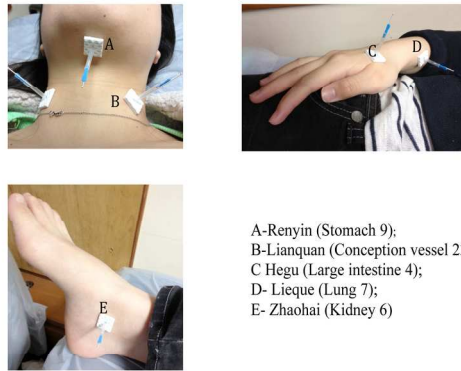
Figure 3. Mean level of anti-inflammatory IL10 over time.

For Peer Review

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Figure 1. Needling position and needle design.



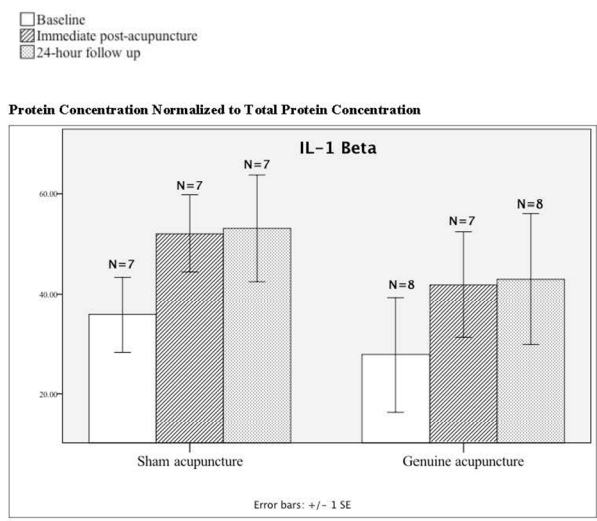
Genuine acupuncture with a stand and shortened guide tube.

Sham acupuncture showing a stand (1), guide tube (2), needle-holding tag (3) and a blunt needle (4).

Needling position and needle design.
76x118mm (600 x 600 DPI)

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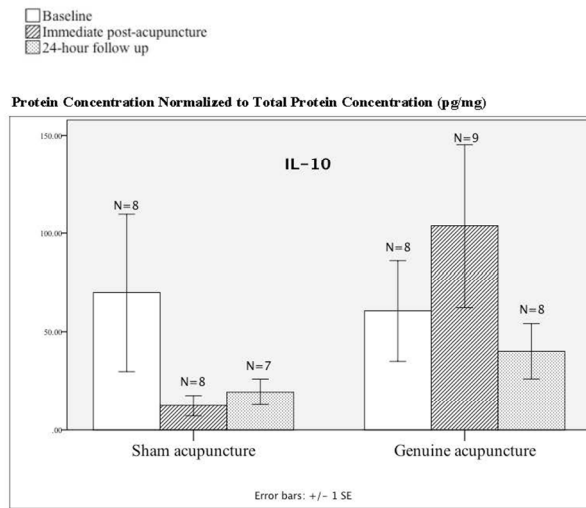
Figure 2. Mean level of pro-inflammatory IL-1 beta over time



Mean level of pro-inflammatory IL-1 beta over time.
210x297mm (134 x 131 DPI)

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Figure 3. Mean level of anti-inflammatory IL10 over time



Mean level of anti-inflammatory IL10 over time.
210x297mm (137 x 131 DPI)