Journal of Advanced Zoology

ISSN: 0253-7214

Volume 44 Issue S-3 Year 2023 Page 112:116

Vagus Nerve Acupucture-Like Transcutanious Electrical Nerve Stimulation on Immunity After Liver Resection

Mohamed Abd El -Azem Nour[1], Hesham Galal Mahran[1], Islam Ismail Ayoub[2], Reham Salah El Zaiat[3], Samah Mohammed Awad[4], Marwa Mahdy Abd EL-Hameed[1]

[1]Department of Physical Therapy for Surgery, Faculty of Physical Therapy, Cairo University, Giza, Egypt.

[2]Department of Hepato-pancreato-biliary surgery, National Liver Institute, Menofiea University, Egypt.

[3]Department of Medical Clinical pathology, Faculty of Medicine, Menofiea University, Egypt.

[4]Department of Medical Microbiology and Immunology, National Liver Institute, Menofiea University, Egypt.

**Corresponding author's E-mail: ptrservices2022@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 September 2023
Accepted: 21 September 2023

Abstract

Objective: *To find out the therapeutic impact of acupuncture like-transcutaneous electrical nerve stimulation of vagus nerve on immunity after liver resection.* ***Methods*** *This was a single-blind randomized controlled trial. A total of sixty individuals who had undergone liver resection at the National Liver Institute Hospital at Menofiea University were randomly divided into two groups: study group A (n=30) and control group B (n=30). The study group had vagus nerve stimulation with acupuncture like-TENS parameters include low-frequency (2–10Hz), pulse width (100–400 μs) from (15 - 30 min) in addition to medical treatment the control group had medical treatment only. Immune response calculated by measuring CD3/CD4 ratio and CD3/CD8 ratio by flow cytometry, measured at the start of treatment, after 4 weeks, and again after 8 weeks.* ***Results****: There was a statistically substantial difference in the CD3/CD4 ratio and CD3/CD8 ratio in favor of the study group post I and post II (p = 0.001). The mean, standard deviation for CD3/CD4 ratio 29.73±6.88, 38.6±5.15 in the study group and 22.6 ±5.07, 27.1 ±4.76 in the control group. The mean, standard deviation for CD3/CD8 ratio 23.60 ±5.69, 30.83±4.94 in the study group and 19.90 ±4.30, 24.36 ±4.21 in the control group.* ***Conclusion:*** *Incorporating acupuncture, specifically transcutaneous electrical nerve stimulation of the vagus nerve, into standard physical therapy protocols may yield enhanced efficacy in boosting immune function following liver resection surgery, as supported by a notable elevation in CD4 and CD8 cell levels.*

Keywords: *Acupuncture like-TENS, Vagus nerve, CD4, CD8, Liver resection*

1. Introduction

Liver resection is the standard treatment for any liver lesion. Liver resection is surgery to remove a piece of the liver **(1).** Pathophysiological effects of liver resection include hepatocellular damage and decreased liver mass. The stress of the operation is also acknowledged after liver resection. This ultimately affects immune system and liver metabolism, which has an effect on postoperative recovery **(2).** The immune system (including innate and adaptive immune responses) is impacted by surgical stress. Significant surgical trauma encourages immunologic dysfunction, which puts the patient at risk for serious morbidity **(3)**.

The occurrence of immunosuppression following surgery is attributed to the physiological response to surgical stress. Patients diagnosed with Stage I gastrointestinal cancer who undergone surgical resection experienced a decline in both the count and function of their peripheral blood lymphocytes for a duration of two weeks following the surgical procedure. In particular, the postoperative acute period saw a decline in the CD4:CD8 T cell ratio **(4)**.

The vagus nerve is crucial for establishing a connection between the immunological and neurological systems **(5).** The celiac vagus nerve is stimulated, which in turn triggers an immunological response in the spleen. In order to generate the required immune activities under clinical situations, bioelectronic stimulation regimens may be able to slow the progression of disease. **(6).**

The application of transcutaneous electrical nerve stimulation techniques, including acupuncture, has been found to effectively induce immunomodulatory effects by targeting the vagus nerve. Acupuncture has been observed to elicit a notable improvement in vagus nerve activity. Hence, it may be postulated that acupuncture has the potential to stimulate the cholinergic anti-inflammatory pathway as well as regulate the immunological response **(7)**.

2. Materials and Methods

Study designs

This was a parallel-group, single-blind, randomized controlled trial carried out from March 2022 to May 2023.

Participants

Sixty patients from National Liver Institute Hospital at Menoufia University. The study (No. P.T.REC/012/003046) was accepted by the Ethical Committee of the Department of Physical Therapy. The eligibility of participants was assessed by a screening process prior to their enrollment in the study. Subsequently, participants were required to provide their informed permission by signing a document prior to their registration.

The inclusion criteria for this study consisted of participants who had undergone hepatectomy and were between the ages of 20 and 60 years. A detailed participant history was obtained, including information on pre- and post-operative symptoms. Participants were excluded from the study if they presented with any of the following conditions: heart disease, psychological problems, neurological diseases, as well as abuse of alcohol.

Randomization

The participants were provided with information regarding the objective as well as nature of the study, as well as their entitlement to withdraw or refuse to participate at any time. Encrypt all data while maintaining anonymity. The participants who had hepatectomy were assigned randomly to two groups (A and B) by the utilization of computer-generated randomization blocks. After allocation, no patients dropped out of the study.

Sample size calculation

Prior to participant enrollment, the sample size was determined using the statistical program G*POWER. (ANOVA: analysis of variance [ANOVA]:(repeated measurements between pre-as well as post I, II interaction measurements) according to the results of measuring the CD3/CD4 ratio and CD3/CD8 ratio with an effect level of 0.74 and a power of 0.8 in addition to the significance level is 0.05. The sample size calculator indicated that 60 participants would be sufficient for the research.

Intervention

The participants were randomly assigned to either Group A (the study group) or Group B (the control group). There were 30 participants in each group, ranging in age from 20 to 60, who were all undergoing hepatectomy. Participants assigned to Group B (the control group) were given medical therapy (Cefalosporin, IV, crystalloids and colloids, and human albumin). Participants assigned to Group A (the study group) had vagus nerve stimulation with TENS-like acupuncture in addition to medical treatment. Alcohol use and vigorous activity should be avoided by all participants for at least 12 hours prior to the start of the study. Participants were also instructed to urinate before to the start of the trial and to abstain from coffee as well as nicotine the morning of the investigation. The investigation started between 8 and 10 in the morning in a specialized research room maintained at

21± 2°C. The participant is requested to lie in a semi-reclined position on a chair while their heart rate, blood pressure, as well as respiration are constantly checked. Percutaneous vagus nerve stimulation was conducted by utilizing a transcutaneous electrical nerve stimulation (TENS) device designed in an acupuncture-style, specifically modified with surface electrodes. Electrodes are positioned on the inside as well as outside of the ear. TENS was used constantly for 15–30 min, with a pulse width of 200 μs along with a pulse frequency of 10 Hz. The amount of amplitude was changed to match the sensory threshold (10–50 mA).

Electrodes of TENS are placed on the tragus, and the amplitude is increased until the participants report feeling a sensation. The participants were thereafter notified that the amplitude would be moderately decreased in order to mitigate any potential discomfort, occurring on a regular basis for a duration of five days per week over a period of four weeks.

Outcome measures

The outcome measures included cluster of differentiation 4(CD4) as well as cluster of differentiation 8 (CD8) by flow-cytometry. Assessment was conducted pre- and post-treatment. Initial measure: Before treatment procedure, peripheral blood samples were taken after 3 days of surgery at 7 am to measure (CD4) as well as (CD8) by flow-cytometry.

Post treatment measure: Peripheral blood samples were taken after 4 and 8 weeks of treatment procedure in EDTA contaminating tubes, at 7 am to measure Cd4 / cd8 by flow-cytometry. Following a 15-minute incubation period of blood samples with a combination of fluorescently labeled monoclonal antibodies targeting CD3 as well as CD8, red blood cell lysis was carried out using a lysis solution known as 10Test 3 (Immun-tech, Beckman Coulter, Marseille, France). Analysis was performed by utilizing an EPICS XL flow cytometer (Coulter Electronic, Fl, USA). In all flow - cytometry investigations, each sample underwent analysis using a suitable isotype control (mouse IgG, Dako-cytoformation, Denmark) in order to discern cells that exhibited negative staining. The following antibodies were used: anti-CD3 labeled with fluorescein isothiocyanate (FITC), anti-CD4 labeled with R-phycoerythrin-cyanine 5 (RPE-CY5), anti-CD8 labeled with phycoerythrin (PE). (immunotech, Beckman Coulter, Marseille, France) was where we got our antibodies **(8).**

Data Analysis

All statistical analyzes were carried out by utilizing the Statistical Package for Social Research (SPSS) version 25 for Windows (IBM SPSS, Chicago, IL, USA). An unpaired t-test was carried out to assess the differences in age between the groups. The chi-square test was employed to assess the differences in gender distribution across different groupings. The Shapiro-Wilk test was employed to assess the normality of the data distribution. The Levene test was used to assess the homogeneity of variance across different groups. A mixed MANOVA was conducted to contrast within- as well as among-group effects on CD3/CD4 as well as CD3/CD8 ratios. Post hoc tests were conducted to make further multiple comparisons, utilizing the Bonferroni correction. The predetermined threshold for statistical significance in all conducted tests was established at a p-value of less than 0.05.

3. Results and Discussion

A total of sixty participants were included in this study following having liver surgery. These participants were then randomly assigned to either group A, referred to as the study group, or group B, referred to as the control group.

Subject characteristics

Table (1) presents the demographic characteristics of both study as well as control groups. There were no statistically significant differences seen between the groups in terms of age and sex distribution (p > 0.05).

Table 1. Basic characteristics of participants.

	Study group	Control group		p-value
Age, mean ± (SD), years	43.5 ± 8.35	45.9 ± 9.6	t = -1.03	0.31
Sex, n (%)				
Females	15 (50%)	13 (43%)	χ^2 = 0.26	0.61
Males	15 (50%)	17 (57%)		

SD, standard deviation; χ^2, Chi squared value; p-value, level of significance.

Effect of treatment on CD3/CD4 and CD3/CD8 ratio

Mixed MANOVA showed that there was a substantial interaction of treatment as well as time ($F = 19.93$, $p = 0.001$). There was a substantial main effect of time ($F = 164.09$, $p = 0.001$). There was a substantial main effect of treatment ($F = 17.64$, $p = 0.001$).

There was a substantial improvement CD3/CD4 as well as CD3/CD8 ratio at post I when contrasted with pretreatment also, there is a substantial improvement at post II when contrasted with that pretreatment and post I in study as well as control groups ($p < 0.001$) (Table 2).

There was no substantial difference among groups pretreatment ($p > 0.05$). There was a substantial improvement in CD3/CD4 as well as CD3/CD8 ratio of study group A at post I as well as post II when contrasted with that of control group ($p < 0.01$) (Table 2).

Table 2. *Mean CD3/CD4 and CD3/CD8 ratio pretreatment, post I and Post II of study and control groups:*

	Pretreatment	**Post I**	**Post II**	**Within group comparison MD (p-value)**		
	mean ± SD	**mean ± SD**	**mean ± SD**	**Pre vs Post I**	**Pre vs Post II**	**Post I vs Post II**
CD3/CD4 ratio						
Study group	20.20 ± 4.05	29.73 ± 6.88	38.6 ± 5.15	-9.53 (0.001)	-18.4 (0.001)	-8.87 (0.001)
Control group	19.03 ± 3.10	22.6 ± 5.07	27.1 ± 4.76	-3.57 (0.001)	-8.07 (0.001)	-4.5 (0.001)
MD	1.17	7.13	11.5			
	p = 0.22	*p = 0.001*	*p = 0.001*			
CD3/CD8 ratio						
Study group	**14.93** ± 4.79	23.60 ± 5.69	**30.83** ± 4.94	-8.67 (0.001)	-15.9 (0.001)	-7.23 (0.001)
Control group	13.50 ± 3.92	19.90 ± 4.30	24.36 ± 4.21	-6.4 (0.001)	-10.86 (0.001)	-4.46 (0.001)
MD	*1.43*	*3.7*	*6.47*			
	p = 0.21	*p = 0.006*	*p = 0.001*			

SD, Standard deviation; MD, Mean difference; p-value, Level of significance

The current study showed a substantial improvement in CD3/CD4 ratio as well as CD3/CD8 ratio post treatment when contrasted with pretreatment in both the study as well as control groups. The outcomes of this study are in line with the findings of preceding studies. Preceding systematic reviews **(9)** have demonstrated that the utilization of VNS within the context of bioelectrical medicine presents novel therapeutic possibilities for addressing chronic inflammatory conditions like IBD. Also, it has direct effects on neurotransmitters of leukocytes.

The application of transcutaneous electrical nerve stimulation techniques, resembling acupuncture, can be employed to induce immunomodulatory effects by stimulating the vagus nerve. Acupuncture has been found to have a notable impact on the stimulation of the vagus nerve, leading to the activation of the cholinergic anti-inflammatory pathway followed by subsequent regulation of the immune response **(7).** Additionally, to measure immune cells, different assessment tools are used to calculate CD3/CD4 and CD3/CD8 ratios using flow cytometry **(10).**

In this study, we provided vagus nerve acupuncture-like transcutaneous electrical nerve stimulation 15-30 min to patients with liver resection_Vagus nerve acupuncture-like transcutaneous electrical nerve stimulation as a form of physical therapy modality more effective in regulation of immune response of patients who suffered from defect in immunological response after liver resection due to VNS works by activating the cholinergic anti-inflammatory pathway, a pathway involved in regulating inflammation. The vagus nerve releases acetylcholine, which then acts on specialized receptors on immune cells, leading to decrease inflammation. VNS can lead to an increase in both CD3\CD4 ratio and CD3\CD8, which explained as increase in CD4 cells and a decrease in CD8 cells.

A similar study conducted by Bonaz et al. **(9)** reported that the use of VNS in the era of bioelectric medicine opens new therapeutic avenues for the treatment of chronic inflammatory disorders such as IBD. Moreover, anti-inflammatory status should be evaluated using the best possible measures. VNS is also used to treat psoriasis and a, two other TNF-mediated disorders.

Regarding the enhancement of the CD3/CD4 ratio, a study conducted by Karimi et al. **(11)** indicated that VNS exhibited a potentially advantageous impact, as it resulted in an increase in both the quantity and activity level of CD3 as well as CD4 cells, both in septic and non-septic circumstances. Concerning the T-helper cells, it was revealed that they transform within 48 h into regulatory T-cells (CD3? CD4? CD25?) as well as cytokine-releasing (CD3? CD4? CD25- cells). Cytokine-releasing T cells have been observed to elicit a more robust inflammatory response 48 hours following in vitro activation, while cholinergic therapy has been found to diminish inflammatory responses.

The findings of the study conducted by Huston et al. **(12)** demonstrated that the application of electrical stimulation to the vagus nerve has the ability to suppress the synthesis of inflammatory cytokines as well as prevent the occurrence of shock in cases of life-threatening systemic inflammation. This effect is achieved through the activation of a specific receptor called alpha7 nicotinic acetylcholine receptor (alpha7nAChR) as well as it is mediated by a pathway known as the cholinergic anti-inflammatory pathway, which operates in the spleen. Pharmacological agonists targeting the (alpha7nAChR) have been found to effectively suppress the synthesis of High Mobility Group Box 1 (HMGB1), a critical inflammatory mediator, thereby demonstrating their potential in rescuing mice from polymicrobial sepsis, a life-threatening condition. Therefore, transcutaneous mechanical stimulation of the vagus nerve can protect mice against fatal sepsis.

4. Conclusion

In conclusion based on the findings of this study acupuncture like-transcutaneous electrical nerve stimulation of vagus nerve has demonstrated an effective role in increasing in cd4 and cd8 levels in patients with liver resection.

Acknowledgments

We thank all patients who participated in the study. And great thanks to **Assistant Prof. Dr. Samah Mohammed Awad**, (Assistant Professor of Medical Microbiology and Immunology, National Liver Institute, Menofiea University) to her support encouragement in assistance throughout collection my data of this work.

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