

Effect of selected osteopathic lymphatic techniques on immune system in healthy subjects: A randomized control trial

Immune system

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Abstract

Aim: This study was designed to investigate and compare the efficacy of selected osteopathic lymphatic techniques on the absolute CD4+ count in healthy subjects. **Material and Method:** Forty-five subjects (33 males and 12 female) with age ranged from 20 to 50 years old were included in the study. They were allocated to three groups; each group had 15 subjects. The first group received sternal pump and sternal recoil techniques for 12 sessions, three sessions per week. The second one received thoracic lymphatic pump and splenic pump techniques for 12 sessions, three sessions per week. The third one (control group) didn't receive OMT. Absolute count of CD4 was used to evaluate participants before and after application of the osteopathic techniques. **Results:** The analysis showed a significant increase in the CD4 count after treatment in the second group also there was no significance in the first and third groups. **P-value** was ≤ 0.05 . **Discussion:** Thoracic lymphatic and splenic pump manipulative techniques are effective methods of enhancing the immune system in healthy subjects (TLPT & SPT).

Keywords

Osteopathy; Lymphatic Techniques; Immune System

DOI: 10.4328/JCAM.6045 Received: 09.10.2018 Accepted: 16.11.2018 Published Online: 18.11.2018 Printed: 01.09.2019 J Clin Anal Med 2019;10(5): 576-80
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Introduction

Osteopathic Manipulation Techniques are therapeutic manual applications used to improve the physiological system as well as restore the fluid base of the body that can be changed by structural problems [1]. It was suggested that fighting disease is naturally stimulating the body's immune system [2]. The immune system is the body's defense against infectious organisms and other invaders [3]. The immune system is divided into two parts, called the innate immune system and acquired immune system (adaptive immune), while each of these plays a role in defending the body [4]. Spleen, lymphatic system, gums, bone marrow, white blood cells, antibody, hormones are the basic components of the immune system [5]. T- and B-cells are types of lymphocytes which represent the adaptive immune system [6]. T-helper lymphocytes regulate both the innate and adaptive immune responses [7]. Thoracic lymphatic pump technique (TLPT) is a method of manipulation used to enhance the rate of lymphatic flow to overcome infection; these methods increase the flow of lymph through the thoracic duct [8]. Splenic pump technique (SPT) is a technique involving manipulation of the spleen that has an important role in lymphatic and macrophage systems by eliminating the damaged RBCs and in producing antibodies [9]. Sternum pump and recoil techniques are working on vegetative system which in turn enhances the lymphatic flow and drainage [10]. This study is unique as regarding to this literature; there is a gap in knowledge addressing these techniques especially those in the first group (sternal techniques), also there is a relationship between these techniques and lymphatic cells, researches need to be done to make it more evidence-based. Thus, this study was designed to investigate and compare the efficacy of selected osteopathic lymphatic techniques on the immune system in healthy subjects.

Material and Method

Forty-five healthy subjects (33 male and 12 female) aged 20 to 50 years participated in this study. Subjects were randomly assigned into 3 equal groups using the permuted blocks randomization in selection. The first group received sternal pump and sternal recoil techniques, the second group received thoracic lymphatic pump and splenic pump techniques and the third was control group and didn't receive any manipulative techniques. This study was conducted at the outpatient clinic of the Faculty of Physical Therapy, Cairo University, Alexandria Main University Hospital "Clinical Pathology Lab" and Outpatient Clinic in Alexandria. The signed consent form for participation in the study was obtained from the subjects. The eligibility criteria for the study were healthy subjects; all subjects who had thoracic trauma, inflammatory or systemic diseases, malignancy, osteoporosis, and autoimmune diseases were excluded. All subjects had signed the consent form. This study was approved by the ethical committee of Cairo University No: P.T.REC/012/00945. All subjects in this study were subjected to the following:

1- Peripheral blood sampling:

Peripheral blood samples (4 ml) were collected under complete aseptic conditions; these were divided into:

a- 2 ml on K2 EDTA for complete blood count (CBC) morphological studies.

b- 2ml on K2 EDTA immune-phenotype for CD4 detection

2- CBC and morphological studies:

CBCs were performed on ADVIA 2120 automated cell analyzer (Siemens healthcare diagnostics, Forchheim, Germany) films were stained with Leishman stain, and were used for morphological identification of the differential count and calculation of the absolute CD4 count [11].

3-Detection of CD4:

In this study, the direct immunofluorescence technique was employed using labeled Ab according to the manufacturer instructions for CD4 detection. Immunofluorescence on the viable peripheral blood cells in suspension was analyzed using Becton Dickinson, FACS caliber flow cytometer equipped with Cell Quest Software (USA) [12].

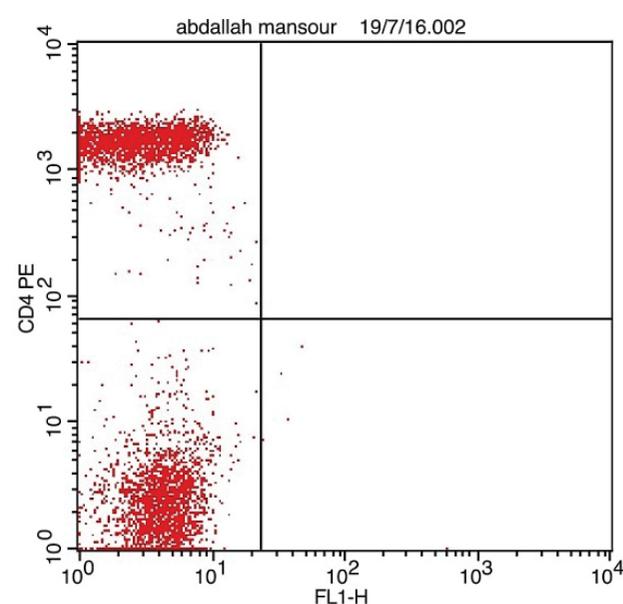


Figure 1. CD4 detection

Treatment techniques:

Group A: Sternal pump and recoil techniques:

- 1- The subject was in the supine position. The practitioner stood at the head of the table.
- 2- Both hands were on top of each other on the subject's sternum, with the thenar eminence on the sternal angle.
- 3- The subject was asked to inhale deeply. During the deep exhalation, pressure was applied with both hands caudally and posteriorly. The pressure was released on the next inhalation.
- 4- This process was repeated five to six times. The Practitioner made recoil technique at the beginning of next inhalation, then left his hands rapidly so this made sternum to recoil [10].

Group B: Thoracic lymphatic pump technique:

- 1-The subject was in supine position with the head turned to one side (to avoid breathing or coughing into the face of the practitioner) and with the hips and knees flexed and the feet rested on the table.
- 2 The practitioner stood at the head of the table with one foot in front of the other.
- 3-The practitioner placed his thenar eminences inferior to the



Figure 2. Sternal pump technique



Figure 4. Thoracic lymphatic pump technique



Figure 3. Sternal recoil technique

subject's clavicles with the fingers spreading out over the upper rib cage. For female subjects, the practitioner placed the hands more midline over the sternum.

4-The subject was asked to take deep inhalation firstly then during exhalation while the practitioner increased the pressure posteriorly and caudally on the anterior rib cage, exaggerating the exhalation motion.

5-At the final exhalation, the practitioner induced a vibratory motion to the rib cage at two compressions per second.

6-The subject was asked to breathe during pressure which was decreased slightly gradually [13].

Splenic pump technique:

1- The subject was in the supine position, legs bent. The practitioner stood on the subject's right side.

2- The fingers of practitioner's right hand with the side of the little finger were placed below the left costal arch.
 3- The left hand was placed on top of the right hand. With both hands, oscillation was in the direction of the spleen, i.e. pressing lightly and intermittently in a cranial-lateral direction with a frequency of 150-180/min and continuing these oscillations for about 2 min [10].



Figure 5. Splenic pump technique

Statistical Analysis

All statistical measures were performed using the Statistical Package for Social science (SPSS) program version 23 for windows. The current test involved two independent variables. The first one was the (tested group) between-subjects factor which had three levels (Group A, Group B, and Group C). The second one was the (measuring periods) within-subjects factor which had two levels (pretreatment and post-treatment). In addition, this test involved one tested dependent variable (CD4). Preliminary assumption checking revealed that data was normally

distributed, as assessed by Normal Q-Q Plot. There were no outliers, as assessed by examination of studentized residuals for values greater than ± 3 . There was the homogeneity of variances ($p > 0.05$) and covariances ($p > 0.05$), as assessed by Levene's test of homogeneity of variances and Box's M test, respectively. All these findings allowed the researchers to conduct a parametric analysis. So, 3×2 mixed design ANOVA was used to compare the tested variables of interest at different tested groups and measuring periods. The alpha level was set at 0.05.

Results

As indicated by the one-way ANOVA, there were no statistically significant differences ($P > 0.05$) between subjects in three groups concerning age, body mass, height and BMI (Table 1). Statistical analysis using mixed design ANOVA analyzed 45 patients assigned into three equal groups. It revealed that there were significant within-subject effect ($F = 14.53$, $p = 0.0001$) and between-subject effect ($F = 11.921$, $p = 0.0001^*$). Additionally, there was significant treatment-time effect ($F = 23.534$, $p = 0.0001$). Table 2 present descriptive statistics (mean \pm SD) and multiple pairwise comparison tests (Post hoc tests) of the detective variable. In the same context regarding within-subject effect, the multiple pairwise comparison tests revealed that there was a significant increase ($p < 0.05$) in the CD4 post-treatment compared with pretreatment in Group B only. Regarding between-subject effects, multiple pairwise comparisons revealed that there was a significant increase in favor to Group B compared to Group A and C at post-treatment ($p < 0.05$).

Table 1. Descriptive statistics and One Way Analysis of Variance (ANOVA) for the mean age, weight, height, and BMI values for the three tested groups.

	Group A (N=20)	Group B (N=20)	Group C (N=20)	F-value	P-value
Age (years)	22.93 \pm 8.12	23.13 \pm 7.64	26.06 \pm 2.15	1.072	0.351
Body mass (kg)	76.6 \pm 1.13	76.9 \pm 1.59	77.97 \pm 2.75	2.044	0.142
Height (cm)	177.68 \pm 0.18	177.72 \pm 0.55	177.68 \pm 0.45	0.037	0.963
BMI (kg/m ²)	24.81 \pm 0.31	24.78 \pm 0.26	24.81 \pm 0.12	0.074	0.929

*Significant at alpha level < 0.05

Discussion

Osteopathic lymphatic manipulative techniques are used in practice to enhance the immune and defense of the body to fight the organisms. This study was designed to investigate and compare the efficacy of selected osteopathic lymphatic techniques on the absolute CD4+ count in healthy subjects. The multiple pairwise comparison tests revealed that there was a significant increase in the CD4 post-treatment compared with pretreatment in Group B only. Regarding between-subject effects, multiple pairwise comparisons revealed that there was a significant increase in favor to Group B compared to Group A and C at post-treatment. Regarding the effects of the first group (sternal pump and recoil techniques) on immune system, according to the analysis in the current study, the results of the Group A (sternal pump and recoil techniques) revealed that

Table 2. Descriptive statistics and 3×2 mixed design ANOVA for CD3 at different measuring periods at three groups.

CD4	Pre-treatment	Post-treatment	Mean difference	95% CI (lower-upper limit)
Group A	0.91 \pm 0.28	0.83 \pm 0.19	0.08	(-0.058, 0.229)
Group B	0.93 \pm 0.21	1.5 \pm 0.50	-0.57	(-0.73, -0.421)
Group C	0.77 \pm 0.21	0.76 \pm 0.22	0.01	(-0.134, 0.153)
Multiple pairwise comparisons between pre- and post-treatment values for CD4 at both groups				
Pre- Vs. post-	Group A	Group B	Group C	
p-value	0.237	0.0001*	0.896	
Multiple pairwise comparison tests (Post hoc tests) for the CD4 among three groups at different measuring periods				
	Group A Vs. group B	Group A Vs. group C	Group B Vs. group C	
Pre-treatment	0.999	0.342	0.267	
Post-treatment	0.0001*	0.999	0.0001*	

*Significant at alpha level < 0.05 , SD: standard deviation, 95% CI: 95% confidence interval.

there was a non-significant effect within and between groups in the values of absolute CD4 pre-treatment Mean/SD values and post-treatment, this was due to the application of these techniques on sternum so they can affect the mobility of manubrium, thorax and or lungs, however the neuro-vegetative effect of these techniques and the pressure changes in thorax after applying these techniques, but they didn't make any change to blood counting. Firsova [14] studied the efficacy of recoil technique on the cardiovascular and the respiratory systems. The result was significant on systolic blood pressure; the study concluded that there was a change in blood pressure after application of recoil technique. There are no studies related to blood counting or lymphocytes according to sternal techniques however they have a vegetative effect on the circulatory system. Regarding the effects of the second group (Thoracic lymphatic pump technique and splenic pump technique) on immune system, according to analysis in the current study, the results of the group B (TLPT&SPT) revealed that there was a significant improvement within and between groups in the values of absolute CD4 pre-treatment Mean/SD values and increased significantly post-treatment, this was due to the forces exerted on the lymph vessels, increase force would also compress lymph vessels and increase lymph flow and manual techniques on spleen so it released the WBCs into bloodstream. Breithaupt et al [15] examined the impact of LPT on the immune reaction incorporates few randomized controlled examinations researching the impact of LPT on immune reaction to vaccination, and the study concluded that application of LPT with flu immunization enhanced general immunity. Mesina [16] studied an application of LPT on seven normal subjects and the study showed an increase in the Basophils, WBCs counts. In a study by Hodge et al. [17], TLPT has shown an increase in blood leukocyte numbers of macrophages, neutrophils and CD4⁺ T-cells in both thoracic and mesenteric duct lymph. Leukocytes released from mesenteric lymph nodes by the effect of TLPT into thoracic duct lymph and stimulate flux of leukocytes in mesenteric duct lymph and thoracic duct lymph. Measel and Kafity [18] studied the pump techniques and showed an increase of WBCs count, B-cells and T-cells, this explains the important role of the spleen in the im-

mune system. Castlio and Ferris-Swift [19] studied the efficacy of different compressions to spleen for different times ranging from 1.5 to 5 minutes for 21 compressions /min and the study showed an increase in the leukocytes count in almost all cases. McMillan et al. [20] studied TLPT and showed that TLPT enhanced thoracic duct lymph and leukocyte concentrations in dogs and rats. In the study by Rockson [21], TLPT and intestinal motility exerted during SPT increase lymphatic transmural pressure due to compressing lymph vessels so the lymph flow increased consequently. Noll et al. [22] examined the importance of osteopathic treatment protocol including the thoracic lymphatic pump on elderly resident patients in hospitals who suffered from pneumonia, the results showed a significant difference in the rate of change in WBCs counts between days 1 and 3, also he dedicated it to the spleen that acts as a reservoir of antibodies and leukocytes that expelled into circulation by contraction of the spleen. This augments importance of the application of SPT to enhance the adaptive immunity in infectious diseases. This study was limited by the lack of studies regarding the use of lymphatic techniques. According to the results of this study, the following further researches are highly recommended: 1- Evaluation of T-helper lymphocyte cells number of normal subjects on a larger sample size. 2- The other study could be done on a wide range of young age group (infant) or different age groups. 3- Other studies could be done to determine the effect of osteopathic manual therapy program on other immune system components (innate and adaptive). 4- Studying the effect of osteopathic manual therapy program in the T-helper lymphocyte cells percentage and cytotoxicity. 5- Studying the effect of thymus gland removal (thymectomy) in myasthenia gravis patients and its effect on muscle efficiency. 6- Studying the effect of osteopathic manual programs on the level of Vit D.

Conclusion

From the finding of the current study, it was concluded that the osteopathic techniques (TLPT & SPT) had a significant effect on the absolute CD4 count than the other techniques (sternal pump & recoil techniques) on enhancing the immune system in healthy subjects.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:

Abdelfattah ASI, Abdel Raouf NAL, Nasef SAS, Ali RR, Swailm RS. Effect of selected osteopathic lymphatic techniques on immune system in healthy subjects: A randomized control trial. J Clin Anal Med 2019;10(5): 576-80.