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Effectiveness of osteopathic manipulative applications on hypothalamic–pituitary–adrenal (HPA) axis in youth with major depressive disorder: a randomized double-blind, placebo-controlled trial

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Abstract

Context: Osteopathic treatments regulate the neurovegetative system through joint mobilizations and manipulations, and myofascial and craniosacral techniques. Despite the growing body of research, the precise impact of osteopathic medicine on the autonomic nervous system (ANS) is not yet fully elucidated. As to Kuchera's techniques, the stimulation of the sympathetic trunk and prevertebral ganglia contributed to harmonization of the sympathetic activity. However, potential relationships between the harmonization of the sympathetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis largely remain uncertain and warrant further exploration.

Objectives: This study was designed to evaluate the effectiveness of the osteopathic sympathetic harmonization

(OSH) on the SNS and the HPA axis in youth with major depressive disorder (MDD).

Methods: The study included 39 youths aged 15–21 years and diagnosed with MDD. The participants were randomly assigned into either the OSH or the placebo group. Stimulation was performed on the sympathetic truncus and prevertebral ganglia in the OSH group. The stimulation of the placebo group was performed with a lighter touch and a shorter duration in similar areas. Each participant completed the Beck Depression Inventory (BDI) and the State and Trait Anxiety Inventory (SAI and TAI) before the application. Blood pressure (BP) and pulse measurements were made, and saliva samples were taken before, immediately after, and 20 min after application.

Results: The baseline BDI ($p=0.617$) and TAI ($p=0.322$) scores were similar in both groups. Although the SAI scores decreased in both groups postintervention, no statistically significant difference was found between the two groups. Subjects who received OSH had a decrease in α -amylase level ($p=0.028$) and an increase in cortisol level ($p=0.009$) 20 min after the procedure.

Conclusions: Following OSH application in depressed youth, SNS activity may decrease, whereas HPA axis activity may increase. Future studies may examine the therapeutic efficacy of repeated OSH applications in depressed individuals.

Keywords: cortisol; depression; osteopathic treatment; sympathetic nervous system

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Depression is a major mental health problem defined as a set of specific symptoms and associated disorders [1]. The clinical and diagnostic features of depression are similar in adolescents and adults [2]. Depression affects the social competence and academic achievements of adolescents and increases the rate of obesity, smoking, and substance addiction [3]. The incidence of major depression in adolescents is between 3 and

8 % [4, 5]. Moreover, it is predicted that 40 % of major depression recurs within 2 years and that 70 % of major depression recurs within 5 years [6] and that chronic depression is resistant to current pharmacological treatments [7]. Evidence from prospective community studies suggests that youths are more likely to have deficiencies in the diagnosis and treatment of depression than adults [8].

The individual under stress may create an adaptive response to the stressor, and this physiological response has two components: neural and endocrine [9]. The first and faster one is the “fight or flight” response provided by the sympathetic adrenal axis (SAA) and the sympathetic neural axis (SNA), whereas the second and slower one is the cortisol response, which is provided by the hypothalamic–pituitary–adrenal (HPA) axis [10]. These processes have broad metabolic and neural effects enabling the body to deal with stress. Thus, these systems can also be considered the body’s center for solving problems and overcoming challenges [11]. In case of any dysfunction in these systems, the individual cannot show the ability to overcome the challenge and thus experiences a chronic process. This dysregulation causes these systems to be overactive or unable to produce an adequate response, depending on whether the condition is acute or chronic, and this condition clinically presents as depression or psychosomatic pain [12, 13].

There are several methods to assess the activity of the sympathetic system, such as heart rate (HR) variability [14], plasma norepinephrine levels [15], and skin sympathetic nerve activity [16]. However, in recent years, salivary α -amylase was identified as a marker for sympathetic nervous system (SNS) activity [17]. The activity of α -amylase reflects the activation of the SNS, specifically the sympathetic–adrenal medullary system [18]. Rohleder et al. [17] reported that “stress-induced increases” in α -amylase are independent of saliva flow rate, further supporting its role as a marker for SNS activity.

Cortisol is a glucocorticoid hormone produced by the adrenal cortex, and its secretion is regulated by the hypothalamus and the pituitary gland. Cortisol levels can be influenced by various factors, including stress, time of day, and metabolic state [19]. Measuring cortisol levels can provide valuable information about HPA axis activity. Various methods can be utilized to assess cortisol levels, including blood, urine, saliva, and hair samples [20]. One commonly utilized method is the assessment of salivary cortisol levels. Saliva sampling is noninvasive and can be easily collected multiple times throughout the day [21]. Salivary cortisol levels are considered to be a reliable indicator of free, biologically active cortisol in the bloodstream [22]. Salivary cortisol levels can reflect acute changes in cortisol secretion and are commonly utilized in research studies [20, 23]. The pulsatile release of cortisol

demonstrates an average half-life of approximately 15–20 min, signifying the swift and ephemeral character of cortisol secretion [24, 25].

The treatments for depression focus on the principle of reducing the activity of these axes [26]. Both the SNA axis and the HPA axis interact with each other on a positive feedback [27]. Wang et al. [26] showed that stellate ganglion block has an antidepressant effect on depression-like behaviors. The potential mechanism appears to involve the HPA axis and the sympathetic–adrenomedullary system [26]. Osteopathic manipulative treatment (OMT) can regulate the neurovegetative system through joint mobilizations and manipulations and through myofascial and craniosacral techniques [28]. By providing SNA and HPA axis regulation in depression and psychosomatic pain, the body’s self-healing – in other words, the body’s problem-solving mechanism – will be activated.

Osteopathy is a holistic manual therapy method developed by Andrew Taylor Still. According to Still, the body has the potential to heal itself. The body utilizes its inherent power to return to homeostatic balance and organization. Applied treatments should be aimed at providing homeostasis by revealing this potential [29].

There is no study examining the effects of osteopathic treatment on the SNA and HPA axes in individuals with major depressive disorder (MDD). This study was planned to evaluate the effect of the osteopathic approach on SNA and HPA axes in youths with depression.

Methods

Participants

This study was conducted at Gazi University Hospital between November 2017 and March 2018. The ethics permission for this clinical study was obtained from the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (Date: 16.11.2016 and number E-16-1106). The study was registered at www.clinicaltrials.gov (NCT04840043). Written informed consent was obtained from all participants (or from their parents if they were under the age of 18 years) after they were informed about the study. Patients aged 15–21 years who were diagnosed by a psychiatrist with MDD according to the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders) criteria were included in the study. Participants were excluded if they met any of the following exclusion criteria: having a fever over 38.5 °C, having an acute injury or infection, rib fracture, cardiac arrhythmia, and intestinal obstruction (vomiting, diarrhea, etc.), utilizing antidepressant, antipsychotics, and mood stabilizer drugs or drugs that affect the cortisol level (birth control pill, hormonal drugs, etc.), being pregnant, and being in the premenstrual or menstrual period.

Power analysis conducted before the study indicated that 34 individuals should be included for 80 % power with a 5 % margin of error. The study included 39 youths with MDD, who were randomly divided into two parallel groups as the osteopathic sympathetic harmonization (OSH) group (n=19) and the placebo group (n=20). Simple randomization was utilized as the randomization method, and the first case for each sex

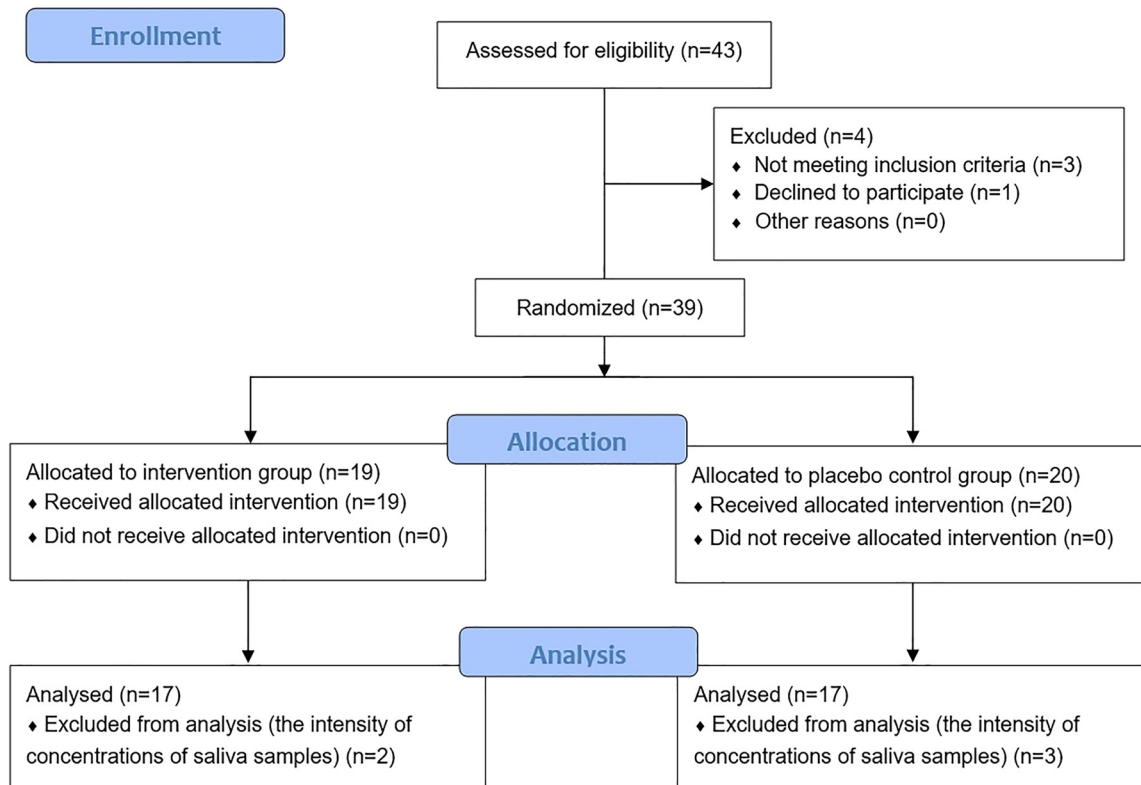


Figure 1: A Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the clinical trial.

was determined by a coin flip. Subsequent participants were divided into groups according to order of recruitment and gender. For example, if the first female participant was placed in the OSH group with a coin flip, the second female participant was placed in the placebo group and the third female participant was placed in the OSH group according to the order of recruitment. The same process was followed for male participants as well. Thus, it was ensured that there were similar numbers of male and female participants in both groups. Randomization and osteopathic interventions (including placebo) were performed by the same investigator. In this double-blind study, participants were blinded to whether the intervention was placebo or actual. The co-researcher who performed the biochemical analysis (the primary outcome measurement) was blinded regarding the group to which the saliva samples belonged (Figure 1).

Data collection tools and steps of the study

Individuals who were clinically diagnosed and met the inclusion criteria were given a new appointment between 10:00 am and 11:00 am the next day and were asked strictly to come on an empty stomach. Detailed sociodemographic information of all participants was obtained. The Beck Depression Inventory (BDI) and the State and Trait Anxiety Inventory (SAI and TAI) were administered to each participant before the application. The SAI was repeated immediately after and 20 min after the application. In addition, to evaluate autonomic responses, saliva samples were taken from the participants before, immediately after, and 20 min after administration, and blood pressure (BP) and HR measurements were performed.

Outcome measures

Beck Depression Inventory: The BDI is a 21-item scale utilized to measure the symptoms of depression. The Turkish adaptation of the scale was made in 1988 by Hisli Şahin [30]. There are four different options for each question in the BDI, with the lowest possible score being 0 and the highest score being 63. The cutoff score of 17 is utilized to indicate clinical depression [31].

State and Trait Anxiety Inventory: The State and Trait Anxiety Inventory (SAI and TAI) consists of two separate scales consisting of 20 items in each scale. These 20-item scales are Form-1 (SAI) and Form-2 (TAI) on two separate pages. Whereas the SAI consists of items that require the person to answer by taking into account their feelings about the moment, the TAI consists of items that evaluate how the person generally feels. The total score obtained from both scales is at least 20 and at most 80. A high score indicates a high level of anxiety, and a low score indicates a low level of anxiety. A Turkish validity and reliability study of the SAI and TAI was carried out by Öner and le Compte [32] in 1998.

Protocols for saliva collection and analysis: Salivette® (Sarstedt, Germany) tubes were utilized in the collection of samples. These tubes provide results with high analytical performance from small volumes of samples. A special cotton swab in the tube was chewed for 60 s for saliva stimulation. After chewing, the saliva-absorbed swab was placed in tubes and centrifuged for 2 min at 1,000 rpm. The centrifuged saliva samples were taken in an Eppendorf Tube to prevent contamination and refrigerated at -80°C [33]. When the target sample size was obtained,

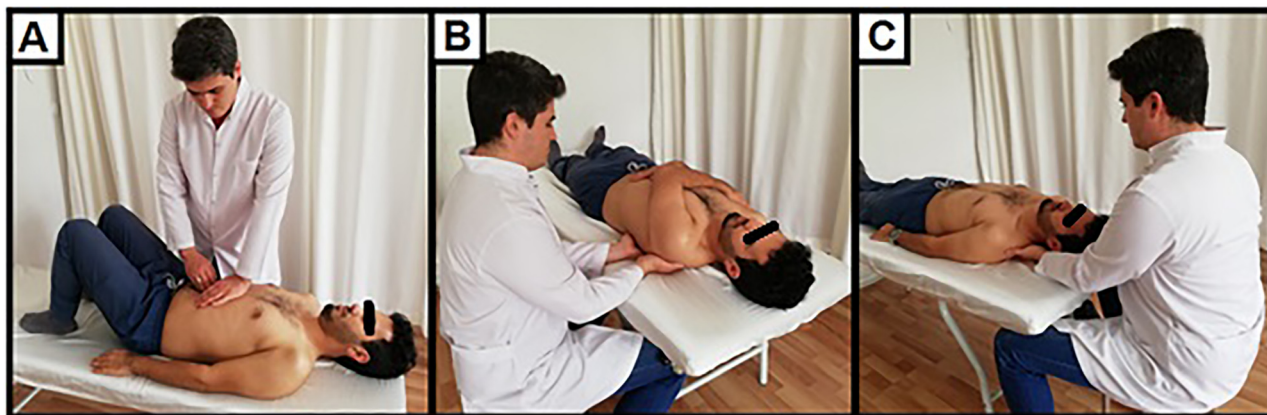


Figure 2: Osteopathic sympathetic harmonization (OSH) application. (A) Stimulation of the prevertebral ganglia. (B) Stimulation of the T5–T12 paravertebral ganglia. (C) Stimulation of the T1–T4 paravertebral ganglia.

samples were analyzed utilizing the Enzyme-Linked Immunosorbent Assay (ELISA) with the Human Cortisol Saliva Elisa Kit (Diametra-Immunodiagnostic Systems Ltd., Perugia, Italy) Human Alpha-Amylase Saliva Elisa Kit (Shanghai YL Biotech Co., Ltd., Shanghai, China).

Blood pressure and heart rate measurement: We utilized the Microlife BP 3BTO-A device, which is a clinically validated automatic BP monitor for both BP and HR measurement. BP was measured on the brachial artery when individuals were sitting with their feet touching the ground and arm supported at the heart level [34]. The device can automatically measure systolic and diastolic BP and HR. Measurements were performed before, immediately after, and 20 min after the application, and the values on the screen of the device were recorded. The timing between measurements was based on the minutes of saliva collection due to the cortisol value. A saliva sample was taken immediately after the application, followed by BP and HR measurements. Thus, the effect of being in the supine position on BP during the application was minimized. This process was repeated after 20 min.

Interventions

Osteopathic sympathetic harmonization (OSH): These applications, intending to harmonize the sympathetic system, according to Kuchera's technique, are such that they follow the course of sympathetic nerves and plexuses. They contain the stimulation of the paravertebral and prevertebral ganglia. The paravertebral ganglia generally are located in front of the heads of the ribs and are stimulated by the rib-raising technique. The prevertebral ganglia are stimulated through the abdominal wall between the umbilicus and the xiphoid process [35]. Both the OSH and placebo applications were performed by a physiotherapist who received 1350 h of special training on the subject, passed the qualifying examination, and received the title of osteopath.

Stimulation of the prevertebral ganglia: The patient is in the supine position. The osteopath stands on the side of the patient, places the fingertips between the xiphoid process and the umbilicus, and declines into the depth of the abdomen until reaching the plexus (Figure 2A). Pressure is maintained until obtaining the fascial release [35].

Stimulation of the paravertebral ganglia: The patient is in the supine position, with the arms at the sides of the body. The practitioner stands on the side of the patient and places the fingertips of both hands

in contact with the costal angle (for levels T5–T12) (Figure 2B). The same application is repeated for the left side. The practitioner sits on the side of the patient's head and places the fingertips of both hands in contact with the costal angle (for levels T1–T4) (Figure 2C). This position is maintained until fascial release takes place. Then the practitioner pushes the patient's thorax rhythmically above the positioned fingers 8 to 10 times for sympathetic stimulation [35].

Placebo application: The patient is in the same position as in the OSH group. The practitioner places the fingertips to the costal angle more laterally. Unlike the OSH group, more light touches and shorter pushes are made anteriorly from the costal margin [36].

Statistical analysis

Statistical analyses were performed utilizing the Statistical Package for Social Sciences (SPSS) software version 22. The variables were investigated utilizing visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilks's test) to determine whether or not they were normally distributed. Descriptive analyses are presented utilizing means and standard deviations for the normally distributed variables and medians and interquartile ranges for the nonnormally distributed variables. Differentiations between groups and delta values were performed utilizing the independent t test for the normally distributed variables and the Mann–Whitney U test for the nonnormally distributed variables. Analyses of changes within groups at three separate times were performed utilizing repeated-measure analysis of variance (ANOVA) with Bonferroni corrected t tests for the normally distributed variables and the Friedman test with Bonferroni correction for Wilcoxon test for the nonnormally distributed variables. An overall 5 % type-I error level was utilized to infer statistical significance.

Results

The mean age of the OSH group was 16.58 ± 1.53 years, and 5 (26.3 %) individuals in the group were male and 14 (73.7 %) were female.

were female. The mean age of the placebo group was 17.30 ± 1.41 years, and 6 (30 %) individuals in the group were male and 14 (70 %) were female. The groups were similar in terms of age ($p=0.121$), duration of education ($p=0.312$), duration of sleep ($p=0.777$), and body mass index ($p=0.522$) (Table 1).

Trait anxiety ($p=0.322$) and depression levels ($p=0.617$) were similar before the application in both groups (Table 2). It was observed that the state anxiety levels of both groups (OSH group: $p<0.001$; placebo group: $p=0.001$) were significantly reduced after the application. This decrease in state anxiety levels was similar in both groups immediately after ($p=0.185$) and 20 min after ($p=0.221$) the application (Table 3).

The systolic BP of individuals in both groups decreased significantly after application compared to before application (OSH group: $p=0.004$; placebo group: $p=0.008$), whereas

diastolic BP and HR did not change (Table 4). However, no difference was found between the groups in terms of systolic BP, diastolic BP, and HRs before, immediately after, and 20 min after application (Table 5).

The α -amylase level of the OSH group 20 min after application decreased significantly compared to pre-application ($p=0.028$), and cortisol levels increased significantly compared to pre-application ($p=0.009$). There was no significant change in the levels of placebo group α -amylase ($p=0.943$) and cortisol ($p=0.161$) levels (Table 6). However, due to the intensity of concentrations of saliva samples, cortisol (OSH ($n=2$), placebo group ($n=3$)) levels of some individuals could not be analyzed. Change in α -amylase and cortisol levels is shown in Figure 3.

Table 1: Demographic information.

	OG (n=19) X $\pm$ SD	PG (n=20) X $\pm$ SD	p
Age	16.6 $\pm$ 1.5	17.3 $\pm$ 1.4	0.121 ^b
Height, cm	166.8 $\pm$ 9.1	167.5 $\pm$ 9.0	0.822 ^a
Weight, kg	58.7 $\pm$ 13.7	60.8 $\pm$ 11.5	0.423 ^b
BMI, kg/m ²	20.9 $\pm$ 3.4	21.5 $\pm$ 2.9	0.522 ^a
Duration of education, year	11.5 $\pm$ 2.3	12.1 $\pm$ 2.0	0.312 ^b
Duration of sleep, h	7.1 $\pm$ 1.7	7.0 $\pm$ 1.7	0.777 ^a
Time of salivary collection	10:24 $\pm$ 0:20	10:33 $\pm$ 0:25	0.266 ^b

OG, osteopathy group; PG, placebo group; BMI, body mass index; ^at test.

^bMann–Whitney U test.

Table 2: Comparison of state of mood outcome measure between groups before application.

Before application	OG (n=19) X $\pm$ SD	PG (n=20) X $\pm$ SD	t	p ^c
TAI	59.9 $\pm$ 9.0	57.3 $\pm$ 7.1	1.003	0.322
BDI	33.4 $\pm$ 9.5	32.1 $\pm$ 7.4	0.504	0.617

OG, osteopathy group; PG, placebo group; TAI, Trait Anxiety Inventory; BDI, Beck Depression Inventory; ^ct test.

Table 3: Comparison of SAI scores within and between groups.

SAI	OG (n=19) X $\pm$ SD	PG (n=20) X $\pm$ SD	t	p ^d
Before application	49.2 $\pm$ 9.3 ^a	49.8 $\pm$ 8.7 ^a	−0.205	0.839
After application	39.7 $\pm$ 9.6 ^b	43.7 $\pm$ 8.5 ^b	−1.35	0.185
20 min after application	40.4 $\pm$ 10.6 ^b	44.5 $\pm$ 10.0 ^b	−1.246	0.221
p ^c	<0.001	0.001		

SAI, State Anxiety Inventory; OG, osteopathy group; PG, placebo group; SD, standard deviation. The same letters in the row mean no significant difference (the Bonferroni correction). ^cRepeated measures for one-way analysis of variance (ANOVA). ^dt test.

Discussion

The level of α -amylase as a marker of sympathetic system activity [17] decreased and the level of cortisol as a marker of

Table 4: Intragroup changes in autonomic responses.

		BA X $\pm$ SD	AA X $\pm$ SD	20M X $\pm$ SD	p ^c
OG	Systolic BP	115.3 $\pm$ 13.8 ^a	112.3 $\pm$ 15.0 ^{a,b}	108.5 $\pm$ 13.5 ^b	0.004*
	Diastolic BP	73.5 $\pm$ 12.2	70.0 $\pm$ 7.7	69.3 $\pm$ 9.1	0.085
	Heart rate	80.5 $\pm$ 13.2	77.7 $\pm$ 12.6	78.5 $\pm$ 14.1	0.455
PG	Systolic BP	112.1 $\pm$ 11.3 ^a	109.9 $\pm$ 11.0 ^{a,b}	107.6 $\pm$ 10.0 ^b	0.008*
	Diastolic BP	69.4 $\pm$ 8.2	67.4 $\pm$ 8.2	67.7 $\pm$ 6.7	0.282
	Heart rate	81.4 $\pm$ 15.3	77.7 $\pm$ 13.7	78.3 $\pm$ 10.7	0.183

BP, blood pressure; OG, osteopathy group; PG, placebo group; BA, before application; AA, after application; 20M, 20 min after application; SD, standard deviation. The same letters in the row mean no significant difference (the Bonferroni correction). ^cRepeated measures for one-way analysis of variance (ANOVA). * $p<0.05$ statistically significant.

Table 5: Intergroup comparison of autonomic responses.

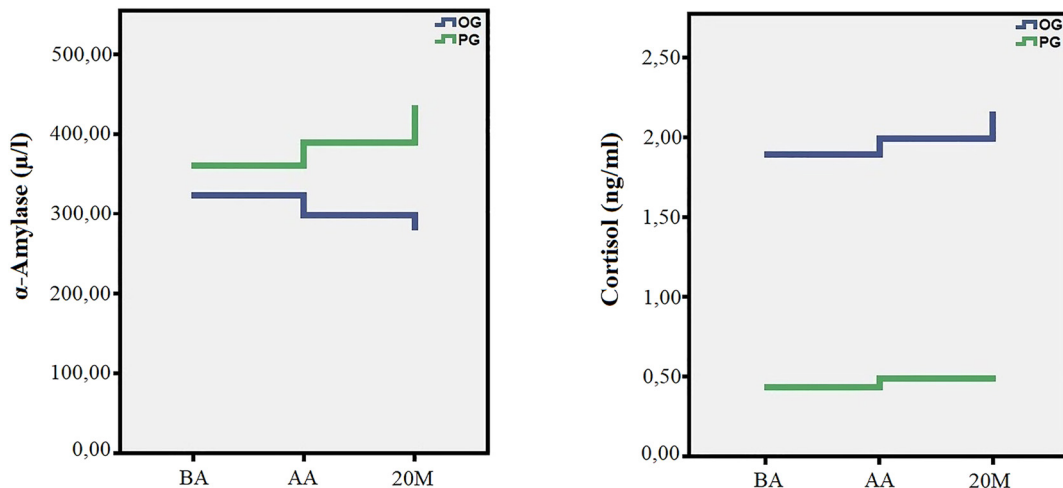
		OG (n=19) X $\pm$ SD	PG (n=20) X $\pm$ SD	t	p ^a
BA	Systolic BP	115.3 $\pm$ 13.8	112.1 $\pm$ 11.3	0.797	0.431
	Diastolic BP	73.5 $\pm$ 12.2	69.4 $\pm$ 8.2	1.248	0.22
	Heart rate	80.5 $\pm$ 13.2	81.4 $\pm$ 15.3	−0.18	0.858
AA	Systolic BP	112.3 $\pm$ 15.0	109.9 $\pm$ 11.0	0.575	0.569
	Diastolic BP	70.0 $\pm$ 7.7	67.4 $\pm$ 8.2	0.999	0.324
	Heart rate	77.7 $\pm$ 12.6	77.7 $\pm$ 13.7	0.009	0.993
20M	Systolic BP	108.5 $\pm$ 13.5	107.6 $\pm$ 10.0	0.243	0.809
	Diastolic BP	69.3 $\pm$ 9.1	67.7 $\pm$ 6.7	0.614	0.543
	Heart rate	78.5 $\pm$ 14.1	78.3 $\pm$ 10.7	0.069	0.945

BP, blood pressure; OG, osteopathy group; PG, placebo group; BA, before application; AA, after application; 20M, 20 min after application; SD, standard deviation. ^at test. $p<0.05$.

Table 6: Intragroup change of biochemical parameters.

		BA X±SD	AA X±SD	20M X±SD	p ^c
OG (n=17)	α-amylase, μ/L	324.2±99.0 ^a	299.3±71.2 ^{a,b}	280.9±80.8 ^b	0.028*
	Cortisol, ng/mL	1.9±0.6 ^a	2.0±0.7 ^{a,b}	2.2±0.6 ^b	0.009*
PG (n=17)	α-amylase, μ/L	360.5±99.5	389.6±160.0	432.2±268.8	0.943
	Cortisol, ng/mL	0.4±0.2	0.5±0.2	0.5±0.2	0.161

BA, before application; AA, after application; 20M, 20 min after application; SD, standard deviation. The same letters in the row mean no significant difference (the Bonferroni correction). ^cFriedman test. *p<0.05 statistically significant.

**Figure 3:** Changes in α-amylase and cortisol levels (BA, before application; AA, after application; 20M: 20 min after application).

HPA axis activity [20] increased after the application of OSH in this study.

Bennett et al. [37] reported that 90 min of traditional Thai massage did not cause a significant difference in salivary cortisol levels. Henderson et al. [36] reported that the rib raising technique did not affect cortisol levels and thus the HPA axis. These studies, which examine the effect of manual techniques applied to different regions on cortisol levels, have shown that the cortisol response did not change. However, we think that this may be related to the fact that these studies have involved only healthy individuals. Kovanur-Sampath et al. [38] presented moderate evidence about the effectiveness of spinal manipulation on cortisol levels in their systematic review and meta-analysis. Although there was no change in the placebo group in their study, there was a significant increase in cortisol levels in the OSH group 20 min after application. The HPA axis responds to stress by increasing the cortisol level if the stressor persists or cannot be coped with, and then this pattern is reversed and the cortisol level gradually decreases [39]. Therefore, this increase in cortisol level in the study may be important in terms of coping with chronic stress.

The prevalence rate of depressive episodes in representative samples of youth from the general community has been found to be approximately 2.7 % for the age group 8–15 years and 7.5 % for the age group 13–18 years [40]. The sample group in this study includes late adolescents (or youth), in whom depression is more prevalent. By 15 years of age, females are approximately twice as likely as males to have experienced an episode of depression [41]. In the present study, in the groups stratified by gender, the ratio of females to males with depression was just over 2:1. With respect to age and gender distribution, the sample group of the study reflects the distribution of the general population.

Anxiety is a state of arousal that enables the person to protect themselves against possible threats for survival. The literature supports a history of childhood anxiety being correlated with youth depression and predicts youth depression. However, mechanisms underlying this relationship remain unexplored [42]. Because anxiety is a conditionally altering emotion, it was appropriate to evaluate anxiety within the scope of this study. The change in anxiety levels after the application was examined with the SAI. SAI scores decreased immediately after and 20 min after

the application (although more pronounced in the OSH group) compared to the baseline values. However, OSH applications did not cause a more significant change than placebo applications. The placebo application also made similar positive changes in anxiety levels. This may have resulted from the positive effects of touching. In this situation, it must be mentioned that there are two different limitations. The first limitation is that mechanoreceptors sensitive to touch and pressure will be stimulated, creating the same input to the cortex, in both groups. The second limitation is that any intervention to the ribs may affect the sympathetic chain. Although the placebo intervention was performed away from the costovertebral joints, the ribs may have been mobilized, which is a limitation of this study. To overcome this effect, study designs with a third group with control intervention that does not involve touching are needed in the future.

de Araujo et al. [43] reported that passive intervertebral mobilization did not affect HR. Cardoso-de-Mello-E-Mello-Ribeiro et al. [44] investigated the effect of the fourth ventricle compression (CV-4) on the autonomic nervous system (ANS) in their study, which showed a decline in plasma catecholamines, BP, and HR after application, but no significant differences between the groups. These findings are similar to our results.

Although α -amylase, a digestive enzyme, is not a byproduct of the SNS, it has been found at high levels under stress. The levels of α -amylase in stress conditions are reported to correlate with the level of norepinephrine [45, 46]. Therefore, the saliva α -amylase level was utilized to measure the activity of the SNS in this study. Twenty minutes after the application, the amylase level decreased in the OSH group but did not change in the placebo group. The responses received from youths diagnosed with MDD were consistent with the results of the study by Henderson et al. [36]. Thus, OSH applications decreased amylase levels and consequently SNS activity in youths diagnosed with MDD.

In the literature, there are few studies examining the relationship between the SNS and HPA axis in chronic stress, and they mentioned the asymmetry between α -amylase and cortisol response [47–49]. HPA axis activity is associated with a lower SNS response. It was even reported that the ratio of cortisol to amylase is a better stress marker than either cortisol or amylase alone [46]. Another study reported that higher amylase and lower cortisol levels during an interaction task were associated with abstinence behaviors in children [50]. Similarly, victims of Hurricane Katrina were found to have lower basal cortisol levels and higher amylase levels [49]. These results reveal the asymmetry between the two systems. In this study, it was observed that there was an

asymmetrical change between these two systems, and while the amylase level of the individuals decreased, the cortisol levels increased after the OSH application. These results show that OSH applications may stimulate autonomic responses via the SNS. However, the immediate effect of a one-time OSH application was examined in the present study, which yielded limited data about the therapeutic effect of osteopathic applications on depression.

OMT shows potential effects on the normalization of the vegetative system in conjunction with changes in sensorimotor cortical areas and ANS activity [51]. It has been reported that cranial techniques, especially CV4, may have hyper-parasympathetic and anti-inflammatory effects that may contribute to the normalization of the vegetative state [52]. Further research is needed to fully understand the mechanisms and clinical consequences of these effects. It is important to note that the efficacy of OMT on the ANS is a topic of ongoing research and discussion.

In this study, Kuchera's techniques relating to the SNS were applied because of the integration between the SNS and the HPA axis [35]. The primary aim was to stimulate stress response centers; therefore, techniques that would stimulate the parasympathetic nervous system/craniosacral (PNS) were not preferred. In the future, there is a need for randomized controlled trials investigating the effect of utilizing a combination of SNS and PNS techniques rather than utilizing SNS techniques only on stress responses.

Conclusions

A single session of OSH application in youths with MDD reduces sympathetic activity, increases HPA axis activity, and has no effect on the level of anxiety. There is a need to determine the optimum number and frequency of sessions for therapeutic effects in which OSH applications are made in individuals with MDD in future studies.

Research ethics: This study was performed in line with the principles of the Declaration of Helsinki (as revised in 2013). Approval was granted by the Ethics Committee of Ankara Numune Training and Research Hospital (Date: 16.11.2016/ No: E-16-1106).

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: OOP conceived the idea for the study. OOP and SC contributed to the design and planning of the research. EG, MG and GMG diagnosed clinical depression according to the DSM-5 criteria and informed the participants about the study and obtained their consent.

OOP, GMG and MG were involved in data collection. OOP applied the OSH application. AS and BA analysed the biochemical data. OOP and SC wrote the first draft of the manuscript. SC coordinated funding for the project. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: The raw data can be obtained on request from the corresponding author.

Trial registration: This study was retrospectively registered at ClinicalTrials.gov (NCT04840043).

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