The investigation of ischemia modified albumin, sialic acid and malondialdehyde levels in cupping blood of interscapular area in women with headache, backache and low back pain

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**Aim:** Ischemia modified albumin (IMA), sialic acid and malondialdehyde (MDA) are the most frequently used biomarker of oxidative stress in many health problems. In this study, it was aimed to investigate the relationship between IMA, sialic acid and MDA levels and visual analogue scale (VAS) pain scores in the cupping blood of the interscapular region in women with headache, low back pain, and backache.

**Materials and Methods:** This study was performed on 96 women between the ages of 18-55. Participants were divided into three groups according to complaints of backache (30 women, group 1), low back pain (31 women, group 2) and headache (35 women, group 3). IMA, sialic acid and MDA levels were measured spectrophotometrically in the cupping blood of the interscapular region.

**Results:** There was no significant difference between IMA, sialic acid and MDA levels in the cupping blood of the interscapular region of groups. There was a statistically significant positive correlation between MDA levels of group 1, group 2 and group 3 and VAS values (p <0.01). In addition, there was a statistically significant positive correlation between sialic acid levels and VAS values of group 1 and group 3 (p <0.01). In addition, statistically significant positive correlation was found between IMA levels and VAS values of group 3 (p <0.01).

**Conclusion:** Our study was the first study in the literature investigating the MDA, IMA and sialic acid levels in the cupping blood in the differential diagnosis of different region pain types. The levels of these parameters were not found to be different in distinguishing different region etiologies of pain in the obtained findings. However, according to our VAS scores, there was a decrease the pain of the patients after cupping therapy and the relationship between these values and oxidative parameters was also determined.

**Introduction**

The method of sucking some blood by making millimetric incisions on the skin with a regional vacuum at specific points of the body is called bloodletting or cupping therapy [1,2]. Cupping is one of the oldest treatment modalities, and it is used as a complementary treatment practice in different regions of the world [1,2]. Although cupping has been practiced for a long time, its mechanism is not known completely and it is continued to conduct many clinical studies about it [1-3]. There are clinical studies showing that cupping has analgesic effects, especially in the treatment of pain, by increasing the release of opiates such as endorphins and enkephalin at the level of the spinal cord and cerebral cortex, and by inhibiting pain transmission [4].

Pain has been defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage" [5]. Pain is multidimensional, and therefore, non-physical components should be considered in addition to physical assessment when evaluating pain [5]. The pain threshold and the response to stimuli that may cause pain vary from person to person due to many factors such as genetics, race, and social-cultural environment [5]. Low back pain, which is characterized by the requirement of expen-
sive treatment and impaired quality of life, is a common musculoskeletal disorder with an increased incidence in industrialized cultures [6]. Backache is lower than low back pain due to its less mobility. Backache may arise entirely from the back region or may emerge like a complication of other diseases [7]. Headache is a neurological symptom that occurs as a result of the involvement of anatomical structures sensitive to cranial pain for various reasons, and that repeats frequently.

In recent years, Oxidative stress has become a commonly used term since it is considered to play a role in the etiopathogenesis of many diseases [8,9]. Ischemia-modified albumin (IMA), sialic acid, and malondialdehyde (MDA) are among the most commonly used biomarkers of oxidative stress in many health problems [11-13]. It is possible to encounter publications from the past years investigating the relationship between pain and oxidative stress. Studies have reported that there was an increase in oxidative stress parameters before and after the pain in people suffering from pain [8-10]. However, IMA and sialic acid, which are considered oxidative stress parameters, were been studied before in our target study group. However, unlike many previous studies, our study aimed to investigate the levels of IMA, sialic acid, and MDA in cupping blood of the interscapular area of women with headache, backache, and low back pain, rather than looking at these parameters in venous blood.

Materials and Methods

Establishment of working groups

The study was conducted on 96 women between the ages of 18 and 55 who applied to the Traditional and Complementary Medicine Center due to headache, back pain, and low back pain. There were 120 female patients who applied to the traditional and complementary medicine center of Necmettin Erbakan University Meram Faculty of Medicine and they had headache, back and low back pain. however, out of 24 patients It was 15 patients had cardiovascular and diabetes mellitus, 5 patients had chronic kidney disease and 4 patients had liver failure disease were excluded from the study. Thus, the sample size of the patient group was determined as 96 patients. Simple random sampling method was used in the study. 96 female patients between the ages of 18-55 who were eligible to participate voluntarily were selected by simple random sampling method. Patients selected in simple random sampling were composed of female patients who met the inclusion and exclusion criteria. Participants were divided into three groups according to complaints of backache (30 people, group 1), low back pain (31 people, group 2), and headache (35 people, group 3).

The group with low back pain was composed of patients diagnosed with 94% of the herniated lumbar disc, the group with headache diagnosed with 98% of migraine, and the group with backache diagnosed with 96% of fibromyalgia. Cupping blood and venous blood samples of the interscapular area were taken from the participants. In the participants' cupping blood, IMA, sialic acid, MDA measurements and in their venous blood, hemoglobin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), sodium (Na) and potassium (K) measurements were made. After coagulation, blood samples were centrifuged at 1500 g for 10 minutes, their serum was separated, and the separated samples were stored at -80 °C until the time of analysis. In addition, to evaluate the efficiency of the sedative effect on blood pressure during the cupping procedure, the blood pressures of participants were measured before and after the cupping process.

Approval of ethics committee of Necmettin Erbakan University, Meram Faculty of Medicine (Resolution number: 2018/1316) and informed consent from the patients were obtained for the study.

The criteria for inclusion of volunteers were determined as follows; those with a hemoglobin value above 9.5 mg/dL, those with complaints such as headache, backache, herniated disc, or low back pain. The criteria for exclusion of volunteers from the study are as follows: those who take treatment of antioxidant, vitamin, element supplement, those with diabetes, cardiovascular disease and hyperlipidemia, chronic kidney disease, liver failure, those who smoke and drink alcohol, those who are pregnant or in their menstrual cycle, those with infectious diseases (HIV, Hepatitis B), those with iodine allergy those who have delayed wound healing problems, those who take any medication such as blood thinners (salicylic acid, coumadin).

Calculation of visual analogue scale pain score

Visual analogue scale (VAS) pain scores of the participants before and after cupping were calculated. In a general manner, the cupping process was completed within 45 minutes. Therefore, VAS values were calculated in accordance with the time slice of 1st minute before cupping and the 45th minute after cupping. The pain severity of women in all groups was rated by using the VAS, from 0 (no pain) to 10 (unbearable pain). VAS is one of the most widely used pain measurement methods due to its ease of use and simple structure, and pain averages ranging from 0 to 10 are given in the evaluation. Accordingly, "0" indicates no pain, while a mean VAS value of 1-4 indicates mild pain, 5-6 indicates moderate pain, and 7-10 indicates severe pain [14].

Measurement of IMA

Measurement of IMA levels in serum were performed spectrophotometrically according to the method developed by Bar-Or et al. (2000). For the determination of serum IMA levels, 50µL of cobalt chloride was added to 200µL patient serum and incubated for 10 minutes. After incubation, 50µL dithiothreitol (DTT) was added to the measuring cuvette and mixed to determine the cobalt unbound to albumin. Due to this process, dithiothreitol was allowed to form a colored complex with cobalt unbound to albumin. The reaction was stopped by adding 1 mL of 0.9% NaCl solution two minutes after the addition of DTT. Color development was determined spectrophotometrically at 470 nm wavelength. The same process was conducted without DTT and accepted as blank, and the results obtained after the sample was zeroed against the blank were given as absorbance unit (ABSU).
Table 1. Demographic characteristics and values of clinical measurement of the groups (All values, Mean ± Standard deviation).

<table>
<thead>
<tr>
<th>Parameter Group 1 (n = 30)</th>
<th>Group 2 (n = 31)</th>
<th>Group 3 (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>49.7 ± 12.6</td>
<td>50.0 ± 12.7</td>
<td>46.8 ± 11.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.4 ± 13.3</td>
<td>77.1 ± 13.4</td>
<td>71.5 ± 11.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 ± 4.7</td>
<td>29.6 ± 5.3</td>
<td>27.5 ± 5.7</td>
</tr>
<tr>
<td>Systolic blood pressure before cupping (mmHg)</td>
<td>11.2 ± 0.7</td>
<td>11.1 ± 0.4</td>
<td>11.0 ± 0.8</td>
</tr>
<tr>
<td>Diastolic blood pressure before cupping (mmHg)</td>
<td>7.2 ± 0.5</td>
<td>6.9 ± 0.3</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>Systolic blood pressure after cupping (mmHg)</td>
<td>10.7 ± 0.7</td>
<td>10.5 ± 0.5</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>Diastolic blood pressure after cupping (mmHg)</td>
<td>6.8 ± 0.5</td>
<td>6.7 ± 0.5</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>VAS (before cupping)</td>
<td>6.9 ± 2.3</td>
<td>7.1 ± 2.7</td>
<td>6.6 ± 2.6</td>
</tr>
<tr>
<td>VAS (after cupping)</td>
<td>3.6 ± 1.7</td>
<td>4.3 ± 2.4</td>
<td>3.5 ± 2.0</td>
</tr>
<tr>
<td>VAS score, p values</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

VAS, Visual Analogue Scale; BMI, body mass index.

Measurement of sialic acid

The total sialic acid level in serum was measured by the Warren method [16]. The principle of the method is based on the formation of colored products as a result of periodate oxidation of sialic acid units in a strong acid environment and the extraction of these products with cyclohexanone. Serum levels of sialic acid were given in mmol/mL.

Measurement of MDA

MDA levels were measured by applying the method of Draper and Hadley (1990), based on the principle of measuring the color absorbance generated by MDA with thiobarbituric acid in an acidic environment at 535 nm. Within this context, 200 µL of serum was mixed with 2 mL solution, containing 15% (w/v) trichloroacetic acid, 0.38% (w/v) thiobarbituric acid, and 0.25N of hydrochloric acid (HCl). The mixture was heated at 100 °C for 30 minutes, and the absorbance was measured at 535 nm after centrifugation. The total MDA content of serum samples was determined by the difference in absorbance between test and standard samples using MDA solution as standard. Results are expressed as µmol/L.

Analysis of other analytes

Serum TC, TG, HDL, LDL, VLDL, Na, and K levels were measured by using available kits based on routine methods in the Synchron LX system (Beckman Coulter, Fullerton CA). Complete blood count was measured on Abbott Cell-Dyn Ruby hematology analyzers.

Statistical analysis

Statistical evaluation of the findings was made using the SPSS 16.0 package program. The appropriate citation for the SPSS program was searched. Statistical analysis was carried out using SPSS statistical software package version 16.0 (IBM, Armonk, NY, USA). The distribution of data was analyzed with the One-Sample Kolmogorov-Smirnov test. Student’s t and Mann-Whitney U tests were used to compare parametric and nonparametric variables, respectively. Kruskal – Wallis test (post-hoc analysis Mann-Whitney U) and One-Way Anova (post-hoc analysis LSD or Games-Howell) were also performed comparison of multiple groups. Correlations were evaluated by Spearman correlation analysis. P<0.05 was considered as statistically significant.

Study groups were tested through a one-way analysis of variance (ANOVA). ANOVA is short for "Analysis of variance" analysis of variance. The one-way ANOVA test is used to test whether it is statistically significant between the means of independent groups. Tukey’s HSD (Tukey’s honestly significant difference) test, one of the post-hoc tests, was used between the groups found to be significant. An Independent two-sample t-test was used to compare the VAS values of the groups. Parameters were expressed as mean ±SD and compared with One-way ANOVA analysis (post-hoc analysis with LSD or Tamhane’s T2 tests), they were evaluated with Bonferroni correction and the level of significance was taken as p<0.05), #: Parameters were expressed as median (min-max) and compared with Kruskal – Wallis test (post-hoc analysis Mann-Whitney U), they were evaluated with Bonferroni correction and the level of significance was taken as p<0.05). The mean values of the data were given together with ± standard deviation (SD). P<0.05 was considered significant in all tests. Parameters were expressed as mean ±SD, #: Parameters were expressed as median (min-max) and compared with Mann-Whitney test. Table 1 demographic characteristics and values of clinical measurement of the groups (All values, Mean ± Standard deviation). Table 2 values of the biochemical parameters of the groups (All values Mean ± Standard deviation). Table 3 oxidative Parameter values in the cupping blood of the groups (All values Mean ± Standard deviation).

The parametric method was used to model the relationships between the variables. The statistical significance
Table 2. Values of the biochemical parameters of the groups (All values Mean ± Standard deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 30)</th>
<th>Group 2 (n = 31)</th>
<th>Group 3 (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.2 ± 0.9</td>
<td>13.5 ± 1.0</td>
<td>13.2 ± 0.7</td>
<td>0.342</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>207.1 ± 48.3</td>
<td>216.1 ± 37.9</td>
<td>207.0 ± 44.5</td>
<td>0.732</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>124.2 ± 63.5</td>
<td>159.0 ± 98.0</td>
<td>133.1 ± 66.1</td>
<td>0.325</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>24.8 ± 12.7</td>
<td>28.3 ± 11.5</td>
<td>26.5 ± 13.5</td>
<td>0.693</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>54.7 ± 13.1</td>
<td>58.1 ± 11.0</td>
<td>56.1 ± 14.3</td>
<td>0.723</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>127.4 ± 42.8</td>
<td>128.1 ± 34.8</td>
<td>124.2 ± 39.7</td>
<td>0.941</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>139.9 ± 1.9</td>
<td>135.6 ± 1.9</td>
<td>139.8 ± 2.4</td>
<td>0.105</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.4 ± 0.3</td>
<td>4.7 ± 1.1</td>
<td>4.5 ± 0.4</td>
<td>0.478</td>
</tr>
<tr>
<td>Sialic Acid (mg/dL)</td>
<td>0.19 ± 0.1</td>
<td>0.17 ± 0.1</td>
<td>0.23 ± 0.2</td>
<td>0.494</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>7.47 ± 3.3</td>
<td>6.90 ± 2.4</td>
<td>7.54 ± 2.7</td>
<td>0.617</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.95 ± 0.5</td>
<td>0.97 ± 0.4</td>
<td>1.01 ± 0.4</td>
<td>0.899</td>
</tr>
</tbody>
</table>

Table 3. Oxidative Parameter values in the cupping blood of the groups (All values Mean ± Standard deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 30)</th>
<th>Group 2 (n = 31)</th>
<th>Group 3 (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>0.447*</td>
<td>0.457*</td>
<td>0.417*</td>
<td></td>
</tr>
<tr>
<td>Sialic acid</td>
<td>0.405</td>
<td>* 0.236</td>
<td>0.380*</td>
<td></td>
</tr>
<tr>
<td>IMA</td>
<td>* 0.187</td>
<td>0.343</td>
<td>0.393*</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 and Table 3, no statistically significant difference was found between hemoglobin, total cholesterol, triglyceride, VLDL, HDL, Na, K, MDA, IMA, and sialic acid levels of the groups. The correlations between the MDA, sialic acid, and IMA levels of the groups and the VAS values before cupping are given in Table 4. As seen in Table 4, a statistically significant positive correlation was found between MDA levels of group 1 (p<0.05), group 2 (p<0.01), and group 3 (p<0.05) and VAS values before cupping. Besides that, a statistically significant positive correlation was found between IMA levels and VAS values (p<0.05) of group 3. However, no statistically significant correlation was found between the sialic acid levels of group 2 and the IMA levels and VAS values of group 1 and group 2.

Discussion

The result of the imbalance between free radical formation and antioxidant defense mechanism, which leads to tissue damage, is defined as 'oxidative stress' [15]. The natural antioxidant system consists of antioxidant enzymes and many antioxidant compounds, and this system protects functional and structural molecules from the effects of reactive oxygen species. Oxidative stress may lead to changes in any stimulus that causes pain by local and spinal oxidant mechanisms [16]. Similarly, reactive oxygen species generated in reaction to oxidative stress due to tissue damage and inflammation may increase the stimulation of sensory neurons involved in pain transmission [17]. Furthermore, increased production of reactive oxygen species is considered responsible for the increase in circulating levels of proinflammatory cytokines, adipokines, and other inflammatory markers [18]. MDA is one of the most commonly used indicators of lipid peroxidation [11]. Free radicals and reactive species emerging during pain may accelerate lipid peroxidation of cell membranes, which may increase MDA levels. In our study, we could not find any significant difference between the MDA levels in the cupping blood of the groups with pain complaints in different regions. Nevertheless, we found a significant positive correlation between MDA levels and VAS values of cupping blood of women in all three groups who participated in our study. This result supports the abovementioned relationship between oxidative stress and pain. In their study,
In conclusion, our study is the first one in the literature which investigated the value of cupping blood’s levels of MDA, IMA and sialic acid in the differential diagnosis of different types of pain, and in the light of the results, the level of these parameters was not found to be valuable in different pain locations. Nonetheless according to our evaluation of VAS scores, a decrease in pain after cupping was found and the relationship between these values and oxidative parameters was also revealed. More scientific research is needed on cupping, which is a complementary treatment practice all over the world.

Ethics approval

Ethical approval for this study was obtained from Necmettin Erbakan University Meram Faculty of Medicine (Decision no: 2018/1316).

References