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Heavy metal and miRNA analysis in venous and wet-cupping blood taken simultaneously from female migraine patients

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Today, population growth, urbanization and industrialization have increased the contact of allliving things with heavy metals.With this study, we believe that the laboratory partial of the treatment with wet-cupping in migraine disease will be clarified. The aim of this study is to measure the levels of heavy metal and micro RNAs that are thought to play a act in migraine pathophysiology in female migraine patients and to reveal their differences compared to the control group.This study was carried out on a group of healthy women that were volunteer migraine patients who had applied to xxx university, Traditional / Complementary Medicine Center and on a control group. This study was conducted in 2018- Necmettin Erbakan University Konya Meram MedicalFaculty GETAT Center. This is a cross-sectional study. Group 1: control group women venous blood; Group 2: migraine women venous blood group; Group 3: women control group wet-cupping: Group 4: women with migraine group wet-cupping.In our study, Pb, Hg andNi wet-cupping blood rates were higher in venosus blood. The plasma miR-21 level of Group 2 was significantly higher than that of group 3 ($p< 0.05$). This result shows that giving venous blood is insufficient for heavy metals removal; wet-cupping is more effective. Prolong shield to Pb is happening at the same time with headaches. A closelylink between migraines and low Mg has been discovered. Low Mg, which might inhibit the production of nitricoxide, increases the distribution of substance P, so causing the narrow of cerebral vessels.

Keywords: Heavymetals, Migraine, miRNA, Women

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Equilibrium in the human body depends on harmony between systems and organs. Diseases occur when physiological balance deteriorates. It is believed that this balance can be recovered by wet cupping, which aims to eliminate potential harmful substances that cause disease[1,2]. Several researches have informed that negative pressure in trophy treatment increased blood circulation and provided flexibility in the muscles and nerves. Furthermore, the treatment of acupuncture points indicates that treatment by cupping has a therapeutic effect by creating hyperemia and hemostasis in the skin. The importance of nitric oxide (NO) in wound healing is well-known[(1,2).] The disease of migraine is a serious health problem that influences greater 10% of the general public in whole global in primary headache which influences the personal satisfaction[3]. In Turkey, in primary headache where the time during frequency of migraine is 16%, the rate by gender is 10.9% in men, but 21.8% in women[4]. Recently, microRNAs have been studied as potential biomarkers in migraine disease. MicroRNAs have been identified in various neurological conditions such as Alzheimer's illness or autism and have been suggested that potential biological markers. These regulatory RNAs could also be useful in the diagnosis of migraine[5-7]. Today, population growth, urbanization and industrialization have increased the contact of all living things with heavy metals[8]. Some heavy metals in particular are used very often and they can be more damaging[9]. More than sixty elements can be given as examples of heavy metals, the most common and best known being mercury (Hg), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), arsenic (As), chromium (Sn), lead (Pb), silver (Ag) and selenium (Se)[10,11,14.] The main

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causes of the toxic effects they have in the body are the disorders they cause in intracellular metabolic processes. These disorders are DNA damage, oxidative protein degradation due to increased oxidative stress, mitochondrial damage and induction of apoptosis, and autoimmune illness (ulcerative colitis, Crohn's disease, rheumatism), some organic diseases (renal illnes, allergy, eczema, asthma, etc.), and neurological illness (depression, migraine, Alzheimer's illnes, Parkinson's illness)[14,15.] Some miRNA's were changed in response to cesium (CS) metal toxin[16,17] and subsequently 21 new miRNAs have been identified[18.] With this study, we believe that the laboratory partial of the treatment with wet-cupping in migraine disease will be clarified. However, we think that the relevant miRNA and heavy metal levels will be value for the identification and therapy of migraine illness and will conduce to a good knowing of the etiopathogenesis of related diseases[12]. In some migraine cases, heavy metal load is thought to increase migraine attacks. In the literature, there are microRNAs related with migraine. The purpose of this research is to the quantity the amount of hazardous wastes and micro RNAs that are thought to perform a function in migraine development in female migraine patients and to reveal their differences compared to the control group.

Participants / Methods

This research was performed on a group of healthy women that were volunteer migraine patients who had applied to Konya XXX University Meram Medical Faculty Traditional and Complementary Medicine Center and on a control group. This study was conducted in 2018- Necmettin Erbakan University Konya Meram Medical Faculty GETAT Center. This is a cross-sectional study. The participants' interscapular region as well as venous blood were collected and used for the study. Blood materials were stocked at -80°C. During taking part in the study, written approvals were obtained and verbal information was provided. In our study, the grouping was as follows: Group 1: control group women venous blood; Group 2: migraine women venous blood group; Group 3: women control group wet-cupping: Group 4: women with migraine group wet-cupping. MicroRNAs targeted in participants' blood samples (let-7b, miR-21, miR-22, miR-27-b, miR-34a-5p, miR-126-5p, miR-155-5p, miR-382-5p) and heavy metals (mercury, manganese, cobalt, nickel, arsenic, chromium, lead, and silver) were studied. Trace element levels were measured in the ICP-MS (Inductively Coupled Plasma - Mass Spectrometer). For microRNA measurements, RNA isolation from blood was determined by using a special commercial kit, measures of related microRNAs were made using the Flexsix Dynamic Array method. The following were the inclusion and exclusion criteria applied to volunteers in this study. Inclusion criteria: hemoglobin (anemia) above 9.5 mg/dl (for patient group) and people with complaints such as fibromyalgia, chronic fatigue, back pain, neck hernia and lumbar hernia. Exclusion criteria: usage of antioxidants, vitamins, element supplemental therapy, diabetes, cardiovascular disorders, chronic kidney disease, hepatic insufficiency, smoking and alcohol intake, pregnancy, infectious diseases (HIV, Hepatitis B), iodine allergy, wound healing problem, use of blood thinners (antiaggregants, salicylic acid, coumadin), any drug use. Wet-cupping therapy was carry outdoing single use suction plop on acupuncture point regions: the Du-14(Dazhui) location on the back side plane, in under the process spinosus of the 7th neck spine; the Ub-42 (Pohu) location symmetry on the pointed projection, 3.0 cun on the side of to the undersharper of the the 3rd thorasic back bone; the Ub-46(Geguan) locationsymmertry on the pointed projection, 3.0 cun on the side of to the under sharper of the spinosus point of the 7ththorasic spine, and interscapular region, on the back side plane between the 3rd and 7th thorasic vertebra; the BL-12-15 points near the interscapular region for wet-cupping; and also some significant back-shu points, such as the heart back-shu point, which is widely used in Chinese medicine hypothesis is popular These points are associated to the lungs, pericardium and heart meridians in the traditional medicine are used to support, arrange, so grow stronger this tissues[(16,19).] Every wet-cupping practice took about 20 min and was directed in 5 stages:

1. Principal vacuum: The cups are put on the chosen place and the weather is withdrawn from inside the glass by hand vacuum. The cups are located on the derm and vacuumed for 3 minutes.
2. Sterilization: The picked locations are sterility with antisepticin advance the process.
3. Small superficial scrathes: Epidermic layer drawing, 2-3 mm in deep and 3-5 mm in long are produced. Only the epidermis layer of the skin is scratched with a lancet
4. Seconder vacuum: Again the cups put on the regions. Interscapular region blood samples and

venosus blood samples were taken to 2 each tubes with the help of injector.

5. Disconnection so shutting: The cup stuffed with hema is removed and destroyed as medicinal matter. A dressing with aseptic sponge is shutted.

Statistical data

Statistical tests for trace elements were performed using SPSS 16.0 program. In order to compare the differences between the groups, veril Independent-Samples T test med was applied for the data obtained in the evaluation we made in the evaluation of our data (copper, chrome, manganese). Two indepentent-samples (Mann-Whitney U test) test was performed to those who were suitable for nonparametric testing (iron, zinc, selenium, nickel, cobalt, molybdenum, cadmium). Statistical analysis for microRNAs Qiagen was performed using the miScript PCR Data Analysis online program. In our study, p <0.05 was considered significant. Certificate for study was given by Ethics Committee of Necmettin Erbakan University (no: 2018/1175 date:19.1.2018)

Results

The demographic characteristics and biochemical data of groups 1 and 2 are showed in Table 1. As seen in Table 1, the BMI values of group 1 were found to be statistically meaningful higher ($p< 0.05$) when compared with group 2. In addition, no statistically sense difference was discovered in age, weight, glucose, total cholesterol, triglyceride, HDL and LDL values between group 1 and group 2. The miRNA levels of the groups are shown in Table 2. As seen in Table 2, according to the ANOVA test results, for plasma miR-21 (p:0.037), miR-22 (p:0.023), miR-27-b (p: 0.041) and plasma miR-34a-5p (p: 0.020), statistically meaningful differences between levels were observed. The plasma miR-21 level of Group 2 was meaningfully higher than that of group 3 ($p< 0.05$). Also, the plasma miR-22 levels of group 2 was found to be statistically higher from that of group 3 ($p< 0.05$), while the plasma miR-22 levels of group 1 were meaningfully lower than those of group 2 ($p< 0.05$). The plasma miR-27-b levels of group 2 were meaningfully higher than those of groups 1 and 3 ($p< 0.05$). The plasma miR-34a-5p levels of group 2 were meaningfully higher than those of group 3 ($p< 0.05$).The heavy metal levels of each group are given in Table 3. According to the ANOVA test performed, the results of which are seen in Table 3, there was a statistically significant difference between the groups' plasma Co (p:0.001), Pb (p:0.041) and Hg (p: 0.030) levels. The plasma Co levels of group 1 were meaningfully higher than those of groups 2 and 4 ($p< 0.01$ for both). Also, the plasma Co levels of group 3 were meaningful higher than those of group 2 ($p< 0.01$). The plasma Pb levels of group 1 were meaningfully lower than those of group 4 ($p< 0.05$), and the plasma Hg levels of group 3 were meaningfully lower than those of group 4 ($p< 0.05$). According to the Pearson correlation test results, there was no correlation between the groups' miRNA levels and their heavy metal levels. Furthermore, a statistically significant positive correlation was found between plasma miR-34a-5p (r : 0.528, p: 0.01), miR-155-5p (r : 0.519, p : 0.011) and miR-382 (r : 0.517, p) and miR-21 levels in group 1. Likewise, the miR-21 levels of group 2 and plasma miR-34a-5p

Table 1 — Demographic characteristics and biochemical data of Group 1 and Group 2

Parameter	Group 1 (n=30)	Group 2 (n=30)	p
Age (years)	37.7 ± 4.8	39.4 ± 8.5	0.364
Weight (kg)	70.7 ± 11.0	65.7 ± 9.8	0.087
BMI (kg / m2)	26.5 ± 4.2	24.2 ± 3.4	0.034
Glucose (mg / dL)	92.3 ± 9.8	93.4 ± 19.6	0.877
Total cholesterol (mg / dL)	200.0 ± 31.3	192.0 ± 53.8	0.723
Triglycerides(mg/ dL)	102.0 ± 39.0	88.1 ± 44.8	0.533
HDL (mg / dL)	62.0 ± 11.2	56.7 ± 21.4	0.533
LDL (mg / dL)	117.7 ± 33.9	117.6 ± 45.8	0.999

Table 2 — Plasma miRNA levels of groups

miRNA	Group 1(n=30)	Group 2(n=30)	Group 3(n=30)	Group 4(n=30)	P
miR-21	2.30 ± 0.6	3.24 ± 1.1[a]	0.36 ± 0.1	1.41 ± 0.3	0.037
miR-22	20.46 ± 818.9[b]	579.4 ± 260.0[a]	25.6 ± 1.2	408.6 ± 123.0	0.023
miR-27b-3p	0.27 ± 0.05 [b]	1.73 ± 0.7[a]	0.17 ± 0.07	1.08 ± 0.2	0.041
miR-34a-5p	2.20 ± 0.5	4.12 ± 1.2[a]	0.62 ± 0.2	2.54 ± 0.3	0.020
miR-126-5p	0.27 ± 0.08	0.65 ± 0.2	0.18 ± 0.06	0.50 ± 0.1	0.130
miR-155-5p	7.87 ± 1.8	37.25 ± 20.4	4.57 ± 0.8	15.16 ± 3.9	0.168
let-7b	3.60 ± 0.5	2.72 ± 0.9	1.41 ± 0.4	1.59 ± 0.3	0.068
miR-382-5p	17.29 ± 3.9	18.11 ± 5.1	6.10 ± 2.5	12.13 ± 2.1	0.105

Table 3 — Plasma heavy metal levels of groups

Plasma heavy metal	Group 1(n=30)	Group 2(n=30)	Group 3(n=30)	Group 4(n=30)	P
Cr (µg/L)	2.86 ± 0.2	2.07 ± 0.2	2.99 ± 0.3	2.58 ± 0.3	0.053
Mn (µg/L)	8.73 ± 3.0	24.94 ± 17.0	47.49 ± 3.1	19.74 ± 13.8	0.521
Co (µg/L)	6.56 ± 0.8[a,b]	2.33 ± 0.4	7.64 ± 1.5 [a]	2.33 ± 0.4	0.001
Ni (µg/L)	33.91 ± 7.7	62.66 ± 24.4	44.62 ± 10.6	91.98 ± 55.6	0.601
As (µg/L)	8.88 ± 0.9	7.90 ± 0.7	9.42 ± 1.0	10.27 ± 2.1	0.648
Ag (µg/L)	51.97 ± 15.5	0.76 ± 0.01	91.60 ± 48.9	0.17 ± 0.01	0.824
Pb (µg/L)	3.30 ± 0.6[c]	3.85 ± 0.7	54.78 ± 22.5	66.93 ± 28.6	0.041
Hg (µg/L)	8.55 ± 1.8 [c]	16.51 ± 11.1	26.14 ± 5.3 [c]	161.6 ± 77.5	0.030

a; Compared to Group 2 ($p < 0.01$), b; Compared to Group 4 ($p < 0.01$),
c; Compared to group 4 ($p < 0.05$).

(r: 0.867, *p*: 0.001), let-7b (r : 0.522, *p* : 0.005) and miR-382 (r : 0.515, *p* : 0.005) showed a statistically significant positive correlation. Moreover, miR-27b-3p (r :0.852, *p*: 0.001), miR-34a-5p (r : 0.663, *p*: 0.001) found statiscally significant positive correlation with the miR-21 levels of group 4.Again in group 4 miR-126-5p (r : 0.371, *p*: 0.044), miR-155-5p (r : 0.461, *p*: 0.01), let-7b (r :0.760, *p*:0.001) and miR-382 (r : 0.743, *p*:0.001), were found to have a statistically significant positive correlation. No correlation was found between the miRNA levels of group 3.

Discussion

To our knowledge, the previous literature has not addressed wet-cupping and miRNA. From ancient times, complementary and traditional treatments have been of great importance for human health. There have been studies on the effectiveness of wet-cupping for ailments such as headache and migraine[19,20] and as effective for many diseases, primarily pain-related, for example; low back pain, skelalgia, fibromyalgia, generalized pain, infection pain (herpes zoster), neuralgia pain (headache and sciatica), cough or asthma, acne, common cold and urticaria[21.] However, few researches have examined the therapy of wet-cupping in migraine therapy.

Wet-cupping treatment is one of the oldest medical techniques (Macedonia, circa 3300 BC). The mechanism of wet-vacuum therapy (or *al-hijamah* in Islamic culture) raises capillary vascular leakage, leading to the topical gathering of leakage fluids, or lymph, subtilizes chemical matter, inflammatory mediators and nociceptive matters, washes nerve endings in gathered fluids, cuts system cohesions and decreases pain[23.] The skin is punctured during wet-cupping, and the removal of the derm triggers endogenous opioid (β-endorphin), stimulating adrenocortical hormones to enter the bloodstream. Endothelin-1, a pain negatiator produced by solid surface keratinocytes upon surface drawing, can also produce analgesia via the release of β-endorphin from keratinocytes[25,26.] Migraines are characterized by frequent crises of primerheadache.They are associated with autonomic symptoms, and they also limit daily activities, impair professional and educational performance, and affect family and social activities [27]. The neuropeptides associated with headaches contain substance P, and vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) are value negatiator[24]. Many medications used to control migraine attacks are generally pharmacological agents: 5-HT antagonists, nonsteroidal anti-inflammatory drugs, beta blockers, serotonin re-uptake inhibitors, and topiramate[28.] Preventive therapy makes treatment of acute attacks more effective. Complementary alternative medicine methods are also widely used globally[29,30.] In one study, after wet-cupping therapy, patients' headache and migraine pain decreased by 66% (31). In other study, a joint of cupping / acupuncture was made, and a favourable answer to therapy was accounted in 94% of illness[22.] Ahmadi et al., in their researches of patients with migraine and tension-type headaches, found that both sexes and all age groups found wet-cupping therapy equally effective[31.] Benli et al., who evaluated migraine treatment according to the phase of the moon, showed that wet-cupping was powerful in the therapy of migraine; moreover, the effect on the visual analogue score and the number of attacks was significantly better when the treatment was applied in the second half of the month when compared to those applied in the in the first half[20.] In our study, we aimed to look at changes in microRNA (let-7b, let-7, miR-21, miR-22, miR-27-b, mir-34a-5p, miR-155, miR-382-5p) expression levels during migraines. This

was relevant because miRNAs have been found to show abnormal expression in vascular diseases, particularly miRNA-21, 31, 146, 221 and 222[(ref. 32).]

In that research, it was observed that miR-21 protected cultured VSMCs from H2O2-dependent apoptosis and death[33.] In our study, the plasma miR-21 levels of group 2 were meaningfully higher than those of group 3 ($p< 0.05$). Therefore, we have found that miR-21 was meaningfully up-regulated in patients with migraines, which is not surprising, given that miR-21 performs an crucial value in vascular illness. Furthermore, the plasma miR-22 levels of group 2 were found to be statistically significant higher when compared to group 3 ($p< 0.05$). Pandley *et al.*[37] suggested that miR-22 might exhibit a supplemental integrity connect between migraine and cardiovascular illness, at least among females. Our findings were also compatible with the literature. In truth, it has been showed that miR-22 efficient oestrogen pathways by clearly targeting the estrogenic receptor (ER)-α[37.] It is thought that there is a functional connect between miR-22 and oestrogen receptor-α, as well as between cardiovascular system diseases and migraine in women. It may also explain the occurrence of migraine more frequently in the population of women.

Tafuri *et al.* stay on that miR-27b levels were meaningful higher in migrain patients than in controls[38.] Epigenetic changes may put on an act in migraine formation. Interestingly, Alp R et al in one study, broad correlation analyses associated miRNAs[36.]

In our study, miR-21, miR-22, miR-27-b, miR-34a-5p, miR-126-5p, miR-155-5p and miR-382-5p levels increased in both migraine groups (2 and 4), but let-7b levels increased only in group 4. This may be related to migraine having aura. In another experimental study, it was determined that miR-let-7b increased the current through the receptor in medulla spinalis. That study elucidated a direct nociceptive effect of miR-let-7b in vivo, documenting the release of miR-let-7b from nociception neurons upon activation[35.] It is thought that endothelial dysfunction and migraine pathogenesis share a common development process. Let-7a and let-7-b have a protective effect on endothelial dysfunction, such as atherosclerosis. Decreasing Let-7 may facilitate the emergence of vascular diseases such as migraine.

The following miRNAs have been found to show abnormal expression in vascular diseases: miRNA-21, 31, 146, 221 and 222[(ref. 32)]. As in the literature, we found that mirRNA-21 is closely related to the presence of migraine. In an experimental study, it was shown that miR-21 protected the surrounding muscle structure from H_2O_2-dependent death [(33).] In our study, the plasma miR-21 levels of group 2 were meaningfully higher than in group 3 ($p< 0.05$), which is in line with previous findings[33.] Also, the plasma miR-22 levels of group 2 were found to be statistically significant higher when compared to group 3 ($p< 0.05$). A large part of the heavy metals that cannot be excreted from the body accumulate in biological organisms. When these reach a certain level, they result in serious diseases (such as thyroid, neurological problems, autism and infertility) and can bring organisms close to death. It is possible to excrete heavy metals from the body by wet-cupping. Indeed, headaches have also been stay on to be related with hazard metals[40.] Moreover, continuous exposure to Pb is concomitant with headaches[39]. A close relation between migraines and depressed Mg has been viewed[40.] Depressed Mg, which might hinder the production of nitric oxide, increases the release of substance P, thus reasoning the narrow of cerebral vessels. Known evacuation systems may be inadequate to reduce heavy metal toxicity. Increased toxicity, decreased magnesium levels can trigger the occurrence of painful conditions such as migraine and even cause it to become chronic. Though there have been many problems with amalgams, no studies have addressed this[8.] Mercury can be taken into the body through fish consumption and amalgam fillings. In addition, it can be responsible for ambiguous-pain. In the present study, we have found that Pb and Hg were significantly up-regulated in patients with migraine. Hg plays an important role in migraine. In our study, Pb, Hg and Ni wet-cupping blood rates were higher in venosus blood. This result shows that giving venous blood is insufficient for heavy metals removal; wet-cupping is more effective.

In another study, on acute migraine attacks, the serum levels of Cu ($p< 0.001$), Mg ($p< 0.001$) and Zn ($p< 0.001$) were significantly lower in patients with acute migraine attack (AMA) compared to controls. Cu, Zn, Mg may play a preventive role in pain. The serum levels of Cd ($p =:0.005$), Fe ($p< 0.001$), Mn ($p < 0.001$) and Pb ($p< 0.001$) were higher in the cases compared to the controls[34].The serum levels of Ni, Pb and Hg were higher in the cases than in the controls. The levels of Pb in patients with migraine were

consistent with the literature. Heavy metals such as Pb, Hg and Ni can only be removed by detox treatment such as wet-cupping. Several clues suggest that Pb exposure may affect the frequence of acute migraines. High Pb levels may lead to unalterable injury to the central nervous system[34]. Repeating wet-cupping detox periodically for ongoing heavy metal exposure can remove re-formed deposits. In the literature, no studies have investigated let-7, let-7b, miR-21, miR-22, miR-27-B, miR34-5p, miR-126, miR-155 or miR-382-5p levels. In conclusion, we believe that our study contributes to current knowledge and will be helpful in defining the biological activity of microRNAs in a different treatment method, that of "wet-cupping."

Conclusion

We have found that miRNAs and hazard wastes may play a act in the cause of migraine in a significant number of illness. These findings may help foresee a new therapy method. Moreover researches, with multiple centres and large case series, are required to glow the acts of miRNAs and hazard wastes in migraine. Heavy metals are absorbed into the organism through the mouth, respiration and skin, and most of them cannot be excreted through the body's excretory pathways (kidney, liver, intestine, lung, skin) without special support. Therefore, a lot of the hazard wastes collect in tissues. As a result of collection, these metals, which are concentrated in the body of living things, can cause serious diseases (such as thyroid, neurological, autism and infertility) and even death when they reach effective doses. In heavy metal excretion, hijama (wet-cupping) is seen as an effective treatment method. The wet-cupping detox procedure explains the cause of painful conditions such as migraine and looks promising in the treatment of pain.

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Conflict of Interest

We confirm that the manuscript was not influenced by any primary or secondary interest, and there is no conflict of interest.

Author Contributions

While YH was included in the design and ethics committee approval of the study, UB contributed to the collection of the data of the study, and AH contributed to both the preparation and the writing of the study.

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