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By Hayriye ALP
Investigation of the levels of heavy metal in the blood and venous blood and microRNA levels in women with migraine.

Introduction

The balance of the human body depends on the harmony between systems and organs. Diseases occur when the physiological balance deteriorates. It is believed that this balance is recovered by wet-cupping the elimination of potential harmful substances that cause the disease(1,2). In the studies, it was reported that the negative pressure in the trophy treatment increased the blood circulation and provided flexibility in the muscles and nerves. Furthermore, the treatment of acupuncture points indicates that the treatment of cupping forms a therapeutic effect by creating hyperemia and hemostasis in the skin. The importance of nitric oxide (NO) in wound healing is known(1,2).

It is stated that decreasing NO level in diabetes patients may increase with cupping treatment. In a study of patients with rheumatoid arthritis, a dramatic reduction in the number of sensitive and swollen joints and disease activity scores was found in patients who received cupping treatment as well as pharmacological treatment. Laboratory parameters such as ESR, CRP, IL2 and RF have also decreased. Immunomodulatory effect in the immune system has been reported to reduce the side effects of pharmacological treatment(1,2). There are studies on the effectiveness of specific neck pain, chronic osteoarthritis, acute trigeminal neuralgia, headache and migraine. Significant improvement was observed in the pain of patients whose applied wet-cupping with non-specific lumbar pain and no side effects were observed. It was reported that the blood taken by wet-cupping could regulate the coagulation and anticoagulation by decreasing the fibrinogen level in which the blood had different contents from the venous blood, and increased the blood flow and oxygen transfer by lowering the
hematocrit. Drug metabolism caused by the metabolism of the excretion of the drugs, reducing the side effects of drugs are stated to reduce (1,2).

Migraine is a serious health problem which affects more than 10% of the general population in primary headache which affects the quality of life (3). In Turkey, where 16% of the lifetime prevalence of migraine, 10.9% in men, this rate was found to be 21.8% in women (4). The pathogenesis of migraine remains uncertain. Increasing study results suggest that the pathophysiology of migraine is related to primary neuronal mechanisms, but is still an issue that is not fully elucidated. As there is no single biological indicator for migraine, drug abuse problems and side effects of drugs are important problems in case of medication. It can be said that migraine is the most common disease at the beginning of the disease therapies by wet-cupping. In all patients with chronic migraine, preventive treatment should be applied, but only 3-13% of patients use prophylactic drugs in community-based studies. Preventive therapy makes treatment of acute attack more effective. Since their discovery, MicroRNAs have promised great hopes in various clinical practice sequences. The use of miRNAs as novel diagnostic markers in some cases may help to respond to diagnostic dilemmas that gene expression analysis or other types of analysis cannot satisfactorily respond to (5,7). In other examples, it is easy to perceive some miRNAs as targets of the original treatments, which downregulated pathway by targeting a single miRNA. It is unclear when this concept will reach maturity or reach maturity. Scientists agree that this area of biology has many advantages over exciting, highly promising and experiments with other biomolecules (5,7). Recently, microRNAs have been studied as potential biomarkers in migraine disease. Micro-RNAs have been identified in various neurological conditions such as Alzheimer's disease and autism and have been suggested as potential biological markers. It is possible that these regulatory RNAs may also be useful in the diagnosis of migraine (5,7). MiR-342-5p and miR-382-5p were found to be increased during migraine.
attacks. In addition, many microRNAs associated with migraine as a result of our literature search have been included in the literature: let-7, let-7b, miR-21, miR-22, miR-27-b, miR-34a-5p, miR-126, miR-155, miR-382-5p.

Today, increasing population, urbanization and industrialization have increased the heavy metal contact of all living things. Heavy metals are the most harmful environmental pollutants due to their widespread use. More than sixty elements can be given as examples of heavy metals, the most common and most known are 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). The effects of heavy metals on the body depend on the concentration of heavy metal as well as on the structure, solubility, chemical structure of the metal ions, the ability to form redox and complex, the frequency and the way of getting into body. The main cause of the toxic effects they form in the body is the disorders they form in intracellular metabolic processes. These disorders: DNA damage, oxidative protein degradation due to increased oxidative stress, mitochondrial damage and induction of apoptosis, autoimmune diseases (ulcerative colitis, crohn's disease, rheumatism, etc.) can count organic diseases (kidney disease, allergy, eczema, asthma, etc.) and neurological disorders (depression, migraine, Alzheimer's disease, Parkinson's disease).

Recent studies have shown the role of miRNA genes in heavy metal tolerance of plants. For example, in rice, the expression of miR390 decreased and the expression of the target gene, OsSRK gene, increased due to Cd stress. In transgenic rice where the expression of miR390 is increased, otherwise, the expression of OsSRK decreases, the tolerance of Cd decreases compared to the wild species and the accumulation of Cd in the plant is increased. In a study conducted in Arabidopsis thaliana, it was observed that 18 miRNAs, including miR157, miR160, miR165, miR168, miR171, miR319, miR397 and miR403, were changed in
response to cesium (CS) metal toxin(17). In addition, Cs toxin was found to destroy the mechanisms of pri-miRNA processing and AGO1-mediated gene silencing. In another study carried out in Medicago truncatula, a new generation sequencing technique known to be sensitive to aluminum (Al) stress and 21 new miRNAs have been identified(18).

With this study, we believe that the laboratory foot of the treatment with hammam in migraine disease will be clarified. However, we think that the relevant miRNA and heavy metal levels will be important to the diagnosis and treatment of migraine disease and will contribute to a better understanding of the etiopathogenesis of related diseases.

Material Metod

This study was carried out on healthy women who were volunteer migraine patients who applied to Konya Necmettin Erbakan University Meram Medical Faculty Traditional and Complementary Medicine Center and control group. The participant’s interscapular region and the venous blood collected from the participants was used for study. Blood samples were stored at -80 °C until the working day. In addition, the participants were not asked for anything. Before taking part in the study, written approvals were obtained and verbal information was given. In our study grouping; 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) have been 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 special commercial kit and related microRNAs were made by Flexsix Dynamic Array method. Inclusion Criteria of the volunteers; Hemoglobin (anemia)
should be above 9.5 mg/dl (for patient group) and people with complaints such as fibromyalgia, chronic fatigue, back pain, neck hernia and lumbar hernia.

Criteria for exclusion of volunteers in research; Antioxidant, vitamin, element supplemental therapy, having diabetes, having a cardiovascular disorder, chronic kidney disease, hepatic insufficiency, smoking and alcohol intake, being pregnant, infectious diseases (HIV, Hepatitis B), iodine allergy, wounds have a late recovery problem, blood thinner (antiaggregant, salicylic acid, coumadin) any drug use.

Wet-cupping therapy was performed using disposable vacuum cups on acupuncture point locations; Du-14(Dazhui) point on the posterior median line, in the depression below the processus spinosus of the 7th cervical vertebra; Ub-42 (Pohu) points bilateraly on the back 3.0 cun lateral to the lower border of spinous process of the 3rd thoracic vertebra; Ub-46(Geguan) points bilateraly on the back, 3.0 cun lateral to the lower border of spinous process of the 7th thoracic vertebra, and interscapular region; on the posterior median line between 3rd and 7th thoracic vertebra. BL-12-15 points near the interscapular region for wet-cupping. Also some significant back-shu points, such as the heart back-shu point which according to Chinese medicine hypothesis is popular for supporting the full size in the area. Points BL-13 to 15 are related to the lungs, pericardium and heart respectively and in acupuncture hypothesis are used to support, arrange, and grow stronger this organs(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 selected regions are disinfected with povidone iodine before the procedure.
3. Scarification; superficial incisions, 2-3 mm in depth and 3-5 mm in length are made on the skin using a number of 11 sterile surgical blade.

4. Seconder sucking; Again the cups placed on the sites. (Until filled with blood from the capillary) Interscapular region blood samples and venous blood samples were taken to 2 each tubes with the help of injector.

5. Disconnection and dressing; The cups filled with blood are remove and destroy as medical waste. A dressing with sterile sponge is applied.

Statistical analysis; Statistical analysis for trace elements was performed using SPSS 16.0 program. In order to compare the differences between the groups, veril Independent-Samples T test me was applied for the data obtained in the evaluation we made in the evaluation of our data (copper, chrome, manganese). 2 Independent-Samples (Mann-Whitney U test) test was applied 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.

Approval for study was granted by Ethics Committee of Necmettin Erbakan University (no: 2018/1175 date:19.1.2018)

Measurement of microRNA expression levels

RNAs isolated from plasma samples by using High Pure microRNA Isolation Kit (Roche Life Science, Mannheim, Germany). RNA samples converted to cDNA by using miScript II RT Kit (Qiagen, Hilden, Germany). cDNA samples are PreAmplified by using miScript Microfluidics PreAMP Kit (Qiagen, Hilden, Germany). qRT-PCR analysis performed by using miScript microRNA Assays (Qiagen, Hilden, Germany) with Dynamic
Array 96.96 (Fluidigm, South San Francisco, CA, USA) on BioMark System (Fluidigm, South San Francisco, CA, USA).

1 Measurement of serum trace elements levels

An ELAN DRC-e (Perkin Elmer SCIEX Inc., Ontario, Canada) inductively coupled plasma mass spectrometry (ICP-MS) system was used for the measurements of the levels of trace elements (Hg, Mn, Co, Ni, As, Cr, Pb, Ag, Se) in serum and using an multielement standard solution for IV and ICP (PerkinElmer Pure Plus, PerkinElmer Life and Analytical Sciences, USA). The MARS microwave digestion system (CEM Corporation, 3100 Smith Farm Road, Matthews, NC 28105-5044, USA) was employed for mineralisation of serum samples. The operating conditions for the ICP-MS are summarized in Table 1.

5 Measurement of other analytes

Serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-cholesterol), LDL-cholesterol and blood glucose was measured by commercially available kits based on routine methods by the Abbott Architect C16000 auto-analyzer (Architect C16000 auto-analyzer; Abbott Laboratory, Abbott Park, IL, USA).

Results

Demographic characteristics and biochemical data of group 1 and group 2 are given in table 1. As seen in table 1, the BMI (p <0.05) values of group 1 were found to be statistically significant when compared with group 2. In addition, no statistically significant difference
was found between age, weight, glucose, total cholesterol, triglyceride, HDL and LDL values of group 1 compared to group 2.

The miRNA levels of the groups are shown in Table 2. As shown in Table 2, according to the ANOVA test results, plasma miR-21 (p = 0.037), miR-22 (p = 0.023), miR-27-b (p = 0.041), and plasma miR-34a-5p (p <0.05), p = 0.020) statistically significant difference was found between the levels.

The plasma miR-21 level of Group 2 was significantly higher than group 3 (p <0.05). Also, plasma miR-22 level of group 2 was found to be statistically significant when compared to group 3 (p <0.05).

Plasma miR-22 levels of group 1 were significantly lower than group 2 (p <0.05). Plasma miR-27-b levels of group 2 were significantly higher than group 3 (p <0.05). Plasma miR-27-b levels in Group 1 were significantly lower than group 2 (p <0.05). Plasma miR-34a-5p levels of group 2 were significantly higher than group 3 (p <0.05).

On the other hand, the heavy metal levels of the groups are shown in Table 3. According to the ANOVA test performed as shown in Table 3, a statistically significant difference was found between the group’s plasma Co (p = 0.001), Pb (p = 0.041) and Hg (p = 0.030) levels.

Plasma Co levels of group 1 were significantly higher (p <0.01) compared to group 2, and plasma Co levels of group 1 were significantly higher (p <0.01) compared to group 4.

Plasma Co levels of group 3 were significantly higher than group 2 (p <0.01). Plasma Pb levels of Group 1 were significantly lower than group 4 (p <0.05). Plasma Hg levels of group 3 were significantly lower than group 4 (p <0.05).
According to the Pearson correlation test results, there was no correlation between miRNA levels and heavy metal levels of the groups. Furthermore, plasma miR-34a-5p ($r = 0.528 \ p = 0.01$), miR-155-5p ($r = 0.519 \ p = 0.011$) and miR-382 ($r = 0.517 \ p$) with miR-21 levels of group 1. A statistically significant positive correlation was found between. The miR-21 levels of group 2 and plasma miR-34a-5p ($r = 0.867 \ p = 0.001$), let-7b ($r = 0.522 \ p = 0.005$) and miR-382 ($r = 0.515 \ p = 0.005$) statistically significant positive correlation was found. Again, miR-27b-3p ($r = 0.852 \ p = 0.001$), miR-34a-5p ($r = 0.663 \ p = 0.001$) with miR-21 levels of 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 statistically significant positive correlation. No correlation was found between miRNA levels of Group 3.

Discussion

In our literature research we haven’t seen the study of wet-cupping and miRNA. From ancient times, complementary and traditional treatment has a great importance for human health. These applications, which have been the method of treatment with blood taking throughout history, have been accepted as the most effective treatment. In recent years, the method of taking blood from the body, which is a complementary and traditional method of treatment, has been started to be used again by coming popular. It is method of cleaning dirty and excess blood in the body which is applied to different parts of body. A lot of dirty blood and excess blood in the body causes many diseases. Excessive and dirty blood must be removed from the body to remove these diseases. Wet-cupping plays a role in the elimination of drug metabolites, heavy metal, chemical and endogenous toxic substances in the body. There are studies about the effectiveness of wet-cupping in diseases such as headache and migraine. There are studies in the literature that have reported that wet-cupping therapy is effective in many diseases, primarily pain-related diseases such as low back pain, skelalgia, fibromyalgia, generalized pain, infection pain(herpes zoster), neuralgia pain (headache and sciatica) and cough or asthma,
acne, common cold, and urticaria.(21) However, there are a few studies on the use wet-
cupping in migraine treatment.

Wet-cupping treatment is one of the oldestest medical techniques (Macedonia circa 3300 BC).
The mechanism of wet-vaccum therapy (Al-hijamah name is used at Islamic culture); rises
capillary vascular leakage, topical gathering of leakage fluids, lymph, subtilizes chemical
matter, inflammatory mediators, nociceptive matters, washes nerve ending in gathered fluids,
cuts system cohesions, decreases pain(23). The skin is punctured during wet-cupping, the
derm take away the trigger of endogenous opioid (β-endorphin) stimulates adrenocortical
hormones into blood flow. Endothelin-1 a pain mediator synthesized by normal skin
keratinocytes upon skin injury, can also produce analgesia via releasing of β-endorphin from
keratosites(25,26)

Migraine headache is a disease that has been known for thousands of years and usually seen
with attacks. The prevalence of chronic migraine worldwide is around 1-3 %, although it
varies according to different studies. In all patients with chronic migraine, preventive
treatment should be applied, but only 3-13% patients use prophylactic drugs in community-
based studies. Migraine is characterized by frequent crisis of acceptable to vigorous density. It
is associated with autonomic symptoms and also limits daily activities, impairs Professional
and educational performance, and affects activities in the family and society(27). The
neuropeptides involved in the pathogenesis of headache include substance P, vasoactive
intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) are important mediators.
(24) In medication to control migraine attacks are used many pharmacocigal agents: 5-HT
agonists, nonsteroidal antiinflammatory drugs, beta blockers, serotonin re-uptake inhibitors,
and topiramate(28). Preventive therapy makes treatment of acute attack more effective.
Complementary alternative medicine methods are widely used in the World (29). These methods
are: hot/cold therapy, diet, vitamin supplements, acupuncture, chiropractice methods and
relaxation/medication and cupping therapy (30). After wet-cupping therapy decreased by 66% in headache and migraine pain (31).

In other study, a combination of cupping and acupuncture was used and a positive response to treatment was reported in 94% of patients (22). Ahmadi et al. all of patients with migraine and tension-type headaches in their study conducted in both sexes and all age groups as wet-cupping therapy equally effective (31). Benli et al. in his study, evaluated according to the phase of moon in migraine treatment, show that wet-cupping is effective in the treatment of migraine, and the effect on visual analog score and the number of attacks was significantly better when the application was made in the second half of the month compared to those made in the first half (20).

miRNAs are RNAs with an average of 18-24 nucleotides in length, which do not encode proteins that regulate post-transcription gene expression. miRNAs suppress expression of target genes, leading to a decrease in the amount of protein. The genes in our body are thought to be controlled by miRNAs. MiRNAs are of great importance in the formation of diseases. In addition, miRNAs play a role in oxidative stress, mitochondrial damage, telomerase shortening and many more events that trigger cellular aging. In our study, we aimed to look at microRNAs (let-7b, let-7, miR-21, miR-22, miR-27-b, mir-34a-5p, miR-155, miR-382-5p) changes in expression levels in migraine. MiRNAs found to show abnormal expression in vascular diseases; miRNA-21, 31, 146, 221, 222 (32). A miRNA profiling of rat VSMCs treated with 200 μM H2O2 for 6 hours revealed an upregulation of miR-21 (33). In this study, it was found that miR-21 protects cultured vascular muscle cells (VSMCs) from H2O2-dependent apoptosis and death. In our study, the plasma miR-21 level of Group 2 was significantly higher than group 3 (p < 0.05). In the present study, we have found miR-21 was significantly upregulated in patients with migraine, miR-21 plays an important role in vascular diseases. Also, plasma miR-22 level of group 2 was found to be statistically significant
when compared to group 3 (p <0.05). Pandley et al suggested that miR-22 might represent an additional intriguing link between migraine and cardiovascular diseases, at least in the female sex. In fact, it has been demonstrated that miR-22 influences the estrogen pathway by directly targeting the estrogen receptor (ER)-α (37). It is thought that there is a functional link between Mir-22 and estrogen receptor-α as well as between cardiovascular system diseases and migraine. miR-22 influences the estrogen pathway by directly targeting the estrogen receptor (ER)-alpha (37). The fact that ER-alpha mutations are associated with both migraine and cardiovascular diseases suggests a functional link between miR-22, migraine, and cardiovascular events (44,45).

Tafuri et al. found that miR-27b levels were significantly higher in migraineurs when compared with controls (crude P value < 0.001), whereas levels of miR-181a (crude P value < 0.001), let-7b (crude P value < 0.001), and miR-22 (crude P value = 0.004) were significantly lower (38). They found that the expression of four miRNAs was significantly different in migraine without aura patients versus controls: miR-27b was up-regulated while miR-181a, let-7b, and miR-22 were down-regulated.

Interestingly, extensive correlation analyses revealed that 4 miRNAs (miR-296-5p, -532-3p, -361-3p and -30d) were positively correlated with CRPS-associated pain level, miR-150 was correlated with the occurrence of migraine within the CRPS patient cohort and an extensive array of miRNAs was found to correlate with the levels of circulating cytokines (36). In our study miR-21, miR-22, miR-27-b, miR-34a-5p, miR-126-5p, miR-155-5p, miR-382-5p levels were increased both of them group migraine (group 2 and 4) but let-7b level was increased in only group 4. In an experimental study, it was determined that miR-let-7b increased the current through the receptor in medulla spinalis (35). In this study elucidated a direct nociceptive effect of miR-let-7b in vivo, documented that miR-let-7b is released from
nociception neurons upon activation. It is thought that a common development process between endothelial dysfunction and migraine pathogenesis is observed. Let-7a, let-7-b has a protective effect on endothelial dysfunction such as atherosclerosis.

miRNAs are RNAs with an average of 18-24 nucleotides in length, which do not encode proteins that regulate post-transcription gene expression. miRNAs suppress expressions of target genes, leading to a decrease in the amount of protein. The genes in our body are thought to be controlled by miRNAs. miRNAs are great of importance in the formation of diseases. In addition miRNAs play a role in oxidative stress, mitochondrial damage, telomerase shortening and many more events that trigger cellular aging. Claudio T et all. In their study serum levels of miR-34a-5p and MiR-382-5p were remarkably high along migraine crisis. When comparing migraine sufferers in pain-free periods to the healthy control group, MiR-382-5p was significantly more expressed ($p < .01$). The authors conclude that migraine involves serum changes in miRNA expression not only during the attacks but also in the pain-free intervals, suggesting that microRNAs can play a role in the development of migraine.

miRNAs found to show abnormal expression in vascular diseases; miRNA-21, 31, 146, 221, 222. In an experimental study, it was shown that miR-21 protects the surrounding muscle structure from H2O2-dependent death. In our study: The plasma miR-21 level of Group 2 was significantly higher than group 3 ($p < 0.05$). These study findings support our findings. Also, plasma miR-22 level of group 2 was found to be statistically significant when compared to group 3 ($p < 0.05$).

The term ‘heavy metal’ is often expressed in the case of metal or semi-metals (metalloids) associated with contamination and potential toxicity. In Medicine the heavy metal, the atomic weights of elements are defined as metals with toxic properties. More than 60 elements can be
given to heavy metals. However, the most common and well-known heavy metals are Mercury(Hg), Manganese(Mn), Iron(Fe), Cobalt(Co), Nickel(Ni), Copper(Cu), Zinc(Zn), Cadmium(Cd), Arsenic(As), Chromium(Cr), Lead(Pb), Silver(Ag), and Selenium(Se). Heavy metals are very toxic in very low concentrations. Heavy metals are taken to the human body through the mouth, respiration and skin. Most heavy metals cannot be discharged through the body’s excretion pathways (kidney, liver, intestine, lung, skin) without special support. A large part of the heavy metals which cannot be excreted from the body accumulate in biological organisms and as a result of accumulation heavy metals that increase within living things when they reach effective doses result in serious diseases (such as thyroid, neurological, autism and infertility) and almost death. It is also possible to excrete heavy metals from the body by wet-cupping. Low levels of Mg in patients with migraine were noted, and headaches were also found to be associated with toxic metals.(40) Continuous exposure to Pb is concomitant with headaches.(39) A close association between migraines and Mg has been observed.(41) Low Mg might inhibit the production of nitric oxide (42). Low levels of Mg increase the release of substance P, thus causing the constriction of cerebral vessels.

There have been a lot of problems in the amalgams. However, there are no studies so far. (8). Mercury can be taken into the body through fish consumption and amalgam fillings. In addition, it is held responsible for non-pain. In the present study we have found Pb,Hg were significantly upregulated in patients with migraine. Hg plays an important role in migraine. In our study, Pb, Hg, Ni wet-cupping blood rates were higher in venous blood. This result shows that we cannot get rid of enough heavy metals by giving venous blood. Wet-cupping is more effective in removing heavy metals.

In other study with acute migraine attacks 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 the controls. 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. In our study levels of Co and Ag were lower with migraine patients compared to the controls. Serum levels of Ni, Pb, Hg were higher in the cases compared to the controls. The levels of Pb levels in patients with migraine was consistent with the literature. There are several clues suggesting that Pb exposure may affect the prevalence of acute migraines. High Pb levels may cause irreversible injury to the central nervous system. (34). Serum levels of Mn (group 2) were higher than control groups (group 1) but levels of group 4 lower group 3. Gönällü et al. found significantly higher Mn levels in patients with migraines compared to healthy controls. They had only studied venous blood samples. Further studies are required to elucidate the exact role of Mn in migraine.

As a result of our literature review, the wet-cupping blood and venous blood of migraine patients were compared and were found to be associated with heavy metal levels and migraine. In literature no studies investigating let-7, let-7b, miR-21, miR-22, miR-27-B, miR34-5p, miR-126, miR-155, miR-382-5p, levels. In conclusion we believe that our study will contribute to the current knowledge and it will be helpful to define the biological activity of microRNAs in a different treatment method 'wet-cupping'.

Conclusion

miRNAs and heavy metals may have a role in the genesis of considerable migraine patients. These levels may help provide a new treatment regimen. Further studies with multiple centres and large case series are required to illuminate the roles of miRNAs and heavy metals.
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