MATERIALS AND METHODS

The present study was adopted under Department of Physiology, Subharti medical college and hospital, Meerut in the year of 2011 from the month of April to August 2014. 150 patients who had complained of migraine were selected for this study. The age groups of subjects were between 10-55 years were screened by physician according to International headache society (IHS) criteria for migraine as well as excluding and including criteria of the study. Subjects were obtained from OPD of Chhatrapati Sivaji Subharti hospital, Meerut and adjoining clinics and hospitals in Meerut.

Inclusion criteria:

- Patients already diagnosed migraine without aura.
- Patients of either sex.
- Patients of all religions.
- Subjects between 10 to 55 years.
- Subjects agree to give consent for participating in the study.
- Subjects agree to comply with the protocol.

Exclusion criteria:

- Patients receiving other therapy for the treatment of migraine for last one month.
- Pregnant women.
• Any significant disease or disorder, which, in the opinion of the therapist, may either put the subject at risk because of participation in the study, or may influence the results of the study, or the subjects’ ability to participate in the study.

• Subjects not considered capable, as judged by the therapist, of following instructions of the study, e.g. because of a history of alcohol or drug abuse or any other reason.

• Subjects below 10 years and above 55 years.

• Subject does not agree to give consent for participating in the study. Patients unlikely to comply with the trial protocol.

All subjects were explained about the procedures to be undertaken, convinced them about the procedure of treatment mode and written informed consent was obtained. Parent’s informed consent was obtained in case of minor age group patients.

The protocol of the study was approved by the institutional Ethical committee, i.e. the research ethical committee of Subharti Medical College and Hospital, Meerut U.P.

Patients were instructed to maintain one diary for a period of six month, within which they recorded frequency, pain intensity and duration of migraine for each week. They were informed about triggering factors, types
and causes of migraine. Each participant also filled a personal detail form which was based on measurement of frequency and duration of migraine attack. Average record of frequencies and duration of headache of a week before and after treatment phase were collected.

Thirty patients were randomly assigned in each different group. Patients were treated according to group wise allotted treatment mode for six months.

<table>
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<th>Group</th>
<th>Treatment mode</th>
<th>NUMBER OF SUBJECT</th>
</tr>
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<td>30</td>
</tr>
<tr>
<td>B</td>
<td>Treated with homeopathy therapy alone</td>
<td>30</td>
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<tr>
<td>C</td>
<td>Treated with conventional medical therapy combined with yoga</td>
<td>30</td>
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<tr>
<td>D</td>
<td>Treated with combined homeopathy therapy combined with yoga</td>
<td>30</td>
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<tr>
<td>E</td>
<td>Treated with Yoga therapy alone</td>
<td>30</td>
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</table>

Treatment phase lasted for six month (April 2012 to September 2012). Patients were contacted twice a month in six month to get their feedback. All Patients were filled standardized questionnaires of Hospital anxiety and depression scale (HADS), McGill pain questionnaire (short-form), Pittsburgh
sleep quality index, The migraine disability assessment test (MIDAS) for the assessment of anxiety, depression, pain, sleep and migraine status before and after treatment. They also underwent experiments like blood cortisol estimation, recorded blood pressure, pulse rate and average frequency and duration of migraine before and after treatment

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Experiments</th>
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<tr>
<td>1</td>
<td>Cortisol hormone assessment&lt;sup&gt;108,109&lt;/sup&gt;</td>
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<td>2</td>
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<td>8</td>
<td>Migraine frequency and duration&lt;sup&gt;8&lt;/sup&gt;</td>
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Group-A (Treated with conventional medical therapy alone)

Patients were received conventional medical therapy from physician (prescribed by physician only) for six months. For acute attack most commonest drugs used were Sumatriptan, Naratriptan, Zolmitriptan, Almotriptan, Eletriptan, Ergotamine, NSAID’s-Aspirin, Paracetamol, Diclofenac, Naproxenetc. For prophylaxis Propranolol, Methysergide, Cyproheptadine, Flunarizine, Clonidine, Tricyclic antidepressants, Pizotifen were used.
Group-B (Treated with homeopathy therapy alone)

Patients were received homeopath therapy according to one homeopath physician for six months (Different dose and potency were prescribed by homeopath physician only according to patient’s individual need). Sulphur, Thujaoccidentalis. Tuberculinum, Medorrhinum, Lachesis mutans. Silicea. Sepia and Lycopodium clavatum Natrum muriaticum,Calcarea carbonicicum were commonly used.

Group-C (Treated with conventional medical therapy combined with yoga)

Patients were received yoga therapy combined with conventional medical therapy. They received conventional medical therapy from the same physician of group A. One professional yoga expert taught integrated yoga set including Asana, breathing practices, Pranayam (yoga breathing), relaxation practices and meditation for 5 days a week for 60 minutes. Kriya (cleansing process) was taught once in a week with deep relaxation. For better knowledge, perception and make them comfortable with the procedure participants were given handouts of techniques to practice at the prodromal stage of migraine, patients were instructed not to practice during headache, resolution, and postdrome stage. Integrated yoga set was prescribed to practice as follows:-
• **Asana** 8,114
  
  1. Tadasana - 5 rounds per day
  2. Pavanmuktasana- 5 rounds per day
  3. Bhujangasana- 5 rounds per day
  4. Makarasana- 5 rounds per day
  5. Savasana- minimum 10 min, after completion of all asanas.

• **Pranayam** 8,114
  
  1. Nadishodona- 10 rounds per day
  2. Bhramari- 10 rounds per day

• **KRIYA** 8,114
  
  1. Jalaneti- Once in a week.
  2. Kapal bhati- Once in a week

• **Meditation** 8,114
  
  Omkar dhyana- 5 min per day.

• **Yoga Asana** 8,118
  
  Asanas focus mainly on stretching of neck, shoulder, back muscles followed by relaxation, toning, strengthening, and flexibility.

• **Tadasana** 8,114
  
  1. Stand erect, legs together, hands by the side of the thighs, Gaze in front.
2. Raise your hands straight in front up to arms. Palms facing each other.
3. Bring the hands up straight towards sky, fingers pointing upwards.
4. Now slowly raise your heels and stand on toes. Raise heels as much as you can. Stretch body up as much as possible.
5. While returning to the original position, bring your heels on the ground first.
6. Slowly bring down your hands also.

- **Pavanmuktasana**

  1. In lying position, Fold both legs, bind knees over stomach with hands and raise head.

- **Bhujangasana**

  1. Lie on your chest with the palms down, and finger tips in line with the shoulder.
  2. Applying minimum pressure on your hands, raise your head high and bend back like a snake.
  3. Breathe immediately.
  4. Then lower your head slowly.
  5. Hold for 15 seconds and repeat 2-3 times.
• **Makarasana**

1. Take prone lying position, hands by the side of the thighs.
2. Slowly spread out both the legs. The toes should remain out and heels inwards.
3. Slowly fold the left hand at elbow bringing it from below the armpit, place it in the right shoulder. Fold the right hand at the elbow and place it on the left shoulder.
4. Place your hand on the triangle made by both the elbows.

• **Savasana**

1. Lie on your back, with the legs 12 inches apart.
2. Rest your hands by your side, palms up.
3. Close your eyes gently.
4. Relax the entire body, as you think about each part joints, the hips, abdomen, chest, hands, face.
5. Breathe normally.
6. The body should like motionless as if in death, for 3-5 min. relaxed body, alert mind.
7. This is essential closure to your active asana practice.
Breathing and Pranayama\textsuperscript{8,114}

Conscious breathing is recognized to have a calming effect on emotions (reducing fear and anxiety, for example) and on the nervous system. It also helps diminish tension accumulated around the areas of pain (forehead, temples, neck, and shoulders).

Nadi Shodhana Pranayama\textsuperscript{8,114}

Nadi means channel and shodhana means purification. When the breathing exercise Nadishodhana Pranayam is done there is purification of energy channels in the body. It comprise of breathing from left nostril and then right nostril breathing in the stipulated format. It synchronizes the cerebral hemisphere by balancing the energy channel.

Bhramari Pranayama\textsuperscript{8,114}

It is one of the calming breathing yogic exercise in which humming sound is produced during expiration, while thumbs are used to close the ears, index finger over forehead and remaining fingers closes the eyes. The act is performed in sitting position.

Kriya\textsuperscript{8,114}

Jalaneti (nasal water cleansing) followed by Kapalbhati (forced exhalations) goes further in stimulating the nerves, glands, and organs of the entire nasal and cranial area including the eyes, sinuses, ears, and cranium. Patients were
guided to practice breathing techniques, relaxation postures, and deep relaxation techniques in the prodromal stage.

• **Jalaneti**

The shuddi kriya for the cleaning of the nasal path is known as neti. The aim of the process is to purify the breathing path right from the nostrils to the throat. If the aim is achieved using water, the process is known as Jalaneti.

1. Patients were instructed to fill the neti pot, with warm salt mixed with water and then place the nose cone of the neti pot into the right nostril, which seals it to the nostril (by slight pressure).
2. Then opening the mouth and breath through the mouth.
3. They were instructed to bend slowly in the forward direction and keeping the nose cone fully sealed into the right nostril such a way that water does not come out, They continued mouth breathing while the water flows. After few seconds water ran out through the left nostril.

• **Kapalbhati**

1. Patients were instructed to sit straight and crossed legs. Then take a deep breath and exhale quickly and suddenly which arouse a puffing sound, They were asked to focus on exhaling forcefully and not on inhalation.
2. They were also told to draw the abdominal muscles inward during exhalation.

3. Simultaneously abdomen was also noticed for rising during inhalation and fold during exhalation.

- **Meditation**\(^{8,114}\)

Omkar chanting a sound of Om is said to be source of energy. Omkar chanting cleanses the mind and controls the emotions.

**Group-D (Treated with combined homeopathy therapy combined with yoga)**

Patients were received treatment from same homeopath therapist of group-B and yoga treatment from the same yoga therapist of group- C.

**Group-E (Treated with Yoga therapy alone)**

Patients were supposed to receive treatment of yoga therapy alone from the same yoga therapist of group- C, but after a long search no patients were convinced to join group-E. Although group-E was included in this clinical trial design but due to non-availability of patients, this group was removed later.

**Outcome measurements**

All the outcome parameters were assayed from each group before and after treatment and considered as pre treatment group and post treatment group.
1) **Cortisol hormone assessment**\(^{108,109}\)

Qualitative cortisol assessment was performed. The DRG cortisol ELISA kit, EIA-1887, DRG instruments GmbH, Germany was used.

**Principle of the test**

The DRG cortisol ELISA kit solid phase enzyme-linked Immunosorbent assay based on the principle of competitive binding.

The microtiter wells were coated with a monoclonal antibody directed towards an antigenic site on the cortisol molecule. Endogenous cortisol of a patient sample competes with a cortisol-horse-redish peroxidase conjugate for binding to a coated antibody, after incubation the unbound conjugates is washed off. The amount of bound peroxidase conjugates is inversely proportional to the concentration of cortisol in the sample. After addition of the substrate solution, the intensity of colour developed in inversely proportional to the concentration of the cortisol in the patients.

**Sample collection-Serum**

There is a fluctuation of cortisol achieving the highest level in the morning and lowest in the night. 2ml Blood was collected by venipuncture in morning from each patient allowed to clot and separated serum by centrifugation at 3000rpm (revolution per minute) for 30 min. at room temperature. Samples were collected from each patient of each group before and after treatment.
**Standardization**

Blood samples also were collected from 30 normal healthy individual (do not have migraine) of same age group as stated in this study and were converted into serum, considered as control group.

**Specimen storage-**

Specimens were capped and stored upto 5 days at 2-8° prior to assaying.

**Assay procedure-**

Kit instructions were followed. (DRG Cortisol ELISA.EIA-1887.Germany). assay was done in central lab. Shubharti Medical College Meerut.

a. Secured the desired number of Microtiter wells in the holder.

b. Dispensed 20 µL of each Standard, Control and samples with new disposable tips into appropriate wells.

c. Dispensed 200 µL Enzyme Conjugate into each well.

d. Thoroughly mixed for 10 seconds. It is important to have a complete mixing in this step.

e. Incubated for 60 minutes at room temperature (without covering the plate).

f. Briskly shake out the contents of the wells.
g. Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

h. Added 100 µL of Substrate Solution to each well.

i. Incubated for 15 minutes at room temperature.

j. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.

k. Read the OD at 450±10 nm with a micro titer plate reader within 10 minutes after adding the Stop Solution.

Calculation of Results

A. Calculated the average absorbance values for each set of standards, controls and donor samples.

B. Constructed a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

C. Used the mean absorbance value for each sample determined the corresponding concentration from the standard curve.

2) Blood pressure measurement

Mercury Sphygmomanometer and Stethoscope were used. Blood pressure was measured in morning.
The relaxed subject sat on a chair with the lower arm supported as before. The blood pressure cuff was placed on the subject's right arm, allowing 1 inch between the bottom of the cuff and the crease of the elbow. The diaphragm was placed over the brachial artery in the space between the bottom of the cuff and the crease of the elbow. At this point no sounds should be heard. The cuff pressure was inflated quickly to a pressure about 30 mm Hg higher than the systolic pressure determined by the method of palpation. Then the air was let out of the cuff at a rate such that cuff pressure was decreased at a rate of about 5 mm Hg/sec. At some point the person was listening with the stethoscope began to hear sounds with each heartbeat.

This point marked the systolic pressure. The sounds were called Korotkoff sounds. As the pressure was lowered further, the character of the Korotkoff sounds was changed. At some point, the sounds were disappeared. The pressure reading at this point gave the diastolic pressure.

3) **Pulse rate**

Radial pulse was palpated and the pulse rate was counted for 60 seconds, Time was measured by a digital wrist watch. Pulse rate was measured in morning.
4. Hospital anxiety and depression scale (HADS)\(^8\)

The HADS scale is a self-assessment instrument of 14 items intended to evaluate anxiety and depression in physically ill population. Seven questions given for anxiety and depression separately. Each item has 4 possible answers, scored from 0 to 3. The score of clinical significant for the 2 subscales is 10 and over. HADS scale is given below.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Status of anxiety and depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>Normal</td>
</tr>
<tr>
<td>8-10</td>
<td>Border line normal</td>
</tr>
<tr>
<td>11-21</td>
<td>Abnormal</td>
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</tbody>
</table>

5) Short form McGill pain questionnaire (short-form MPQ)\(^8\)

A short form of the MPQ was developed by Melzack (1987) to assess different components of reported pain. The main component of the SF-MPQ consists of 15 descriptors (11 sensory, 4 affective), which are rated on an intensity scale as 0 = none, 1 = mild, 2 = moderate, or 3 = severe. Three pain scores are derived from the sum of the intensity rank values of the words chosen for sensory, affective, and total descriptors. The SF-MPQ also includes the present pain intensity (PPI) index of the standard MPQ and a visual analogue scale (VAS). The PPI index utilizes a 1 to 10 intensity scale. The questionnaire is used by patients to specify subjective pain experience.
6) **Pittsburgh sleep quality index**\textsuperscript{111,112}

The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989) was developed to measure sleep quality during the previous month and to discriminate between good and poor sleepers. The PSQI is composed of 19 self-rated questions and 5 questions. The PSQI generates seven scores that correspond to the domains listed previously.

Each component score ranges from 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (Range of 0–21). A PSQI global score >5 is considered to be suggestive of significant sleep disturbance.

7) **The migraine disability assessment test (MIDAS)**\textsuperscript{113}

MIDAS Disability, defined as the consequences of illness on the ability to work and function, is measured using the Migraine Disability Assessment Score (MIDAS). Derived from the Headache Impact Test, MIDAS is a seven-item questionnaire that assesses severity of migraine status based on obtained grade. The MIDAS (Migraine Disability Assessment) questionnaire was put together to measure the impact of headaches. The definition of various grades is mentioned below.
Patients were asked to record the frequencies of their headache days, in each week for 6 month in their diary, they were also instructed to record the duration (in hours) of headache in each week for 6 month. Average record of frequencies and durations of headache of a week before and after treatment phase were collected.

**Statistical analysis of Data**

1. Graph pad was used in this study and paired t-tests were performed to compare and analyze the data between pre and post treatment subgroup of the each same group.

2. Difference between pre and post treatment group from each group was calculated, converted it into percentage value.
3. \{(Pre treatment value - post treatment value/pre treatment value) \times 100\}

formula was used to calculate percentage difference. All the negative sign was ignored.

4. All the percentage value of each group was compared by using one way ANOVA.

**Sample size calculation**

From a pilot study it is estimated that for 95% confidence interval, and significance level \( \alpha = 1\% \), \( P = 70\% \), \( Q = 30\% \), allowable errors is 30%, required sample size was 30.

**Ethical committee approval**

All subjects were explained about the procedure to be undertaken, and written informed consent was obtained. Ethical clearance was received for this proposed research from the ethical committee of Subharti Medical College & Hospital, Meerut. UP. One twenty screened and voluntary patients of migraine were selected, consent was taken from their parents who were below 18 years.