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Serum Calcitonin Gene Related Peptide (CGRP) Levels in Migrane: A Study on its Clinical Correlation and Diagnostic Efficacy

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ABSTRACT

BACKGROUND: Migraine is a primary headache disorder marked by recurrent unilateral headache episodes. Calcitonin gene related peptide (CGRP) plays major role in migraine pathophysiology. CGRP is multifunctional, and its vasodilating activity within the central and peripheral blood vessels is one in all its primary functions. The intention is to prove serum calcitonin gene related peptide (CGRP) as an early diagnostic tool for migraine and the novelty is to correlate it with characteristics of migraine so that it helps in early initiation of treatment.

METHODS: 100 subjects including 90 patients with migraine and 10 with non-headache (NH) age-matched controls were prospectively recruited in our current study. The subjects were aged from 15-50 years. The clinical assessment was made every month for the three months after the start of therapy. The subjects were compared based on the serum CGRP values. Serum CGRP concentrations were measured by using CGRP ELISA kit.

RESULTS: Out of total subjects selected, the maximum (23.3%)subjects were between age 26-30 years and least effected age group was 46-50 years (4.44%). Females' predominance with 82% than

males with 18%.Stress was major trigger occurring in 57% of cases. Throbbing pain with elevated CGRP levels 130.44±114.22 and p value (p = 0.01). The average CGRP levels was higher in test group 149.00±93.86 compared to control 61.30±24.37 with P value (p= 0.02).

CONCLUSION: The serum CGRP levels were statistically more in migraine patients correlated with characteristics like throbbing type of pain, stress and inadequate sleep. Hence, the serum CGRP levels estimation can be considered as a diagnostic tool for migraine when the clinical character's overlap or early in the course of migrane when all criteria for diagnosis are not yet fulfilled.

Keywords: Migraine, CGRP, Neuralgia, ICHD, Photophobia, Phonophobia, ELISA.

INTRODUCTION

Migraine is a primary headache disorder marked by unilateral recurrent headache episodes with various proposed patho-physiological mechanisms. Migraine can be with or without aura in its mild form may have nausea, giddiness and inflated sensitivity to light and sound. Headaches presents as episodic attacks of variable frequency that disturbs the conventional daily activities , taking a toll on physical, mental and social well being and has severe economic consequences. There are triggers that increase the frequency or severity of headache like stress and anxiety, alkaloid or alcohol intake, inadequate sleep, inadequate food, environmental changes, changes in women throughout menstrual cycle, food that contains nitrates (meats, hot dogs), aminoalkanoic acid (cheese, smoke-cured fish), flavouring, sweetener. The identification of headache is supported by international headache society criteria. Migrane can be classified as with aura and without aura [1].

Migraine without Aura is termed as common headache

- Fiveattacks fulfilling criteria B-D.
- Headache attacks lasting 4-72 60 minutes (untreated or unsuccessfully treated).
- Headache has 2 of the subsequent four characteristics:Unilateral location, beating quality, moderate or severe pain intensity, aggravation by or inflicting rejection of routine physical activity (e.g.,walking or ascension stairs).
- Throughout headache one in all the following: Nausea and/or instinctive reflex, photophobia and acousticophobia E. Not higher accounted for by another ICHD-3 identification.

Migraine with Aurais termed as classic headache

- Two attacks fulfilling criteria B.
- One or a lot of of the subsequent absolutely reversible aura symptoms: Visual sensory, speech and/or language, motor, brainstem, retinal.
- Three of the below characteristics: One aura symptom spreads step by step over ≥5 minutes. Two or a lot of aura symptoms occur in succession. Every individual aura symptom lasts 5-60 minutes. One aura symptom is unilateral.5.One aura symptom is positive. 6.The aura is accompanied, within 60 minutes by headache.

Calcitonin gene related Polypeptide (CGRP) is 37 amino acid neuropeptide . It has two forms, αCGRP and β-CGRP.αCGRP is present across the central and peripheral nervous system. β-CGRP is positioned within the enteric system and therefore has the endocrine role. CGRP is multifunctional, and its vasodilating activity within the central and peripheral blood vessels is one in all its primary functions. CGRP has several essential physiological functions. The CGRP receptor, as characterised by International union of basic and clinical pharmacology (IUPHAR), is a convolution between thyrocalcitonin receptor-like receptor (CLR) and receptor activity-modifying supermolecule one (RAMP1). It is the primary receptor to be recognized as a convolution between a G supermolecule coupled receptor (GPCR) and a RAMP. Various mechanisms are proposed to be involved in the release of CGRP in migrane from the Trigeminal Ganglia. The most accepted theory is of a state of Neuroinflammation. Hence aim of this study was to estimate the serum CGRP levels in migraine patients and the objective is to correlate the levels with certain characteristics of migraine.CGRP levels estimation could facilitate in assessing its role as diagnostic tool and if the serum CGRP level shows correlation with the characteristics, it should facilitate in early diagnosis which in turn helps in initiation of treatment. The patients are enrolled based on ICHD diagnostic criteria, taking under consideration the patient's complete history so as to grasp details on previous medications or diseases which may be the reason for headache [2].

First-Line medications

NSAIDs: NSAIDs are acceptable first-line medical aid for mild to moderate head ache provided taken during early phase of headache cycle. How ever severe attacks to headache tend to be less responsive to NSAIDS. isobutylphenyl propionic acid for moderate to severe migraines in 200-mg and 400-mg doses were effective for short pain relief, the 400-mg dose conjointly aid to alleviate the symptoms like photophobia and acousticophobia. Acetaminophen and Naproxenare also commonly used Nsaids to terminate an acute attack.

Triptans: Triptans are specific medicine for head ache that bind to serotonergic receptors. They're prescribed as first-line medical aid for moderate to severe headache, or mild to moderate attacks unresponsive to nonspecific analgesics.

Combination: An established dose combination of sumatriptan-85mg/ naproxen-500mg (Trexima) is associate possibility for acute treatment. The mixture of NSAIDs with β blocking agents orCa – channel blocking agents are usually considered in dual therapy.

MATERIALS AND METHODS

Our study is a prospective and observational study with sample size 100 subjects presenting with headache episodes satisfying the IHC criteria with study duration of six months conducted at neurology out-patient department and Central research laboratory, Owaisi hospital and research center, Hyderabad. This study has been approved by Institutional review board (IRB), on 27

November, 2019 at Deccan college of medical sciences and allied hospital, Hyderabad, Telangana [3].

Study criteria

Inclusion criteria

- Patients presenting with migraneous headache.
- Patients ensuing ICHS guidelines
- Newly diagnosed Episodic migraine patients.
- Patients above 18 & less than 50 years of age.

Exclusion criteria

- Subjects unwilling to participate in the study.
- Pregnant women.
- Patient with comorbidities (Seizures, CVA, stroke).

Study procedure

We prospectively recruited 100 adult patients with 90 migraineurs and 10 normal controls at Owaisi hospital and research center from August 2019 to April 2020.The study subjects were aged from 15 – 50 years. Patients we assessed at the Out patient Services of Neurology department. A detailed history was taken to assess the type, pattern, severity and duration of headache. Those matching our study criteria were included. Patients presenting with Episodic Migrane were included. Steps were taken to avoid including those individuals who have overlap features with other types of headaches, those who have taken any prophylactic medication or other medications so as to strengthen the quality of study and its analysis. The diagnosis of migraine was based on the International classification of headache disorder 3rd edition beta version(ICHD-3beta).After detailed history taking and examination , Patients satisfying the criteria of study were selected and an informed consent was taken. 4ml blood samples were collected within 24hours of the Headache attack and CGRP levels were estimated by using commercially available ELISA kit with quantitave analysis. The process was done at Research Lab at our institute. The kit is based on principle of Double antibody sandwich ELISA technique in which Precoated antibody is anti human CGRP-monoclonal antibody and detection antibody is biotinylated polyclonal antibody.The kit has various components like the plate, precoated antibody solution, enzyme solution, diluents, reagent and pipette. It has a high sensitivity of detecting CGRP as low as 5pg/ml with a range of 15.6-1000pg/ml and highly specific with no cross reaction to any other factors. Inter test precision of test is <8% and the kit has to be stored at 4C. Follow up of patients was done either on phone or with a personal visit to opd. Percieved stress scale was used for assessment of stress and Head ache was assessed using a detailed questionnaire. PSS is a classic stress assessment scale It has a series of 10 questions with each answer having points of

0-4 with the total score adding upto 40. It is graded as Mild when score is 0-13, Moderate 14-26, Severe 27-40 [4].

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0). Unpaired t test was used to find out the significant differences between two groups which were illustrated as mean and standard deviation. t is the test statistics that measures the statistical relationship or association between two continuous variables. It gives information about the magnitude of the association, or correlation as well as the direction of the relationship. Multivariate analysis was included to consider correlation between various factors.

RESULTS

Age distribution

Out of 90 subjects selected, 23.3% patients between age 26-30 years are affected with migraine irrespective of gender, followed by age group 21-25 years with 23.3 % and age group 46-50 years with least 4.44% of patients affected. Mean age is (Mean±SD) 30.51±8.64 (Table 1and Figure 1).

Table 1: Representing Details of age distribution.

Age groups	Number of subjects	Percentage
15-20	13	14.4
21-25	17	18.8
26-30	21	23.3
31-35	16	17.7
36-40	12	13.3
41-45	7	7.77
46-50	4	4.44

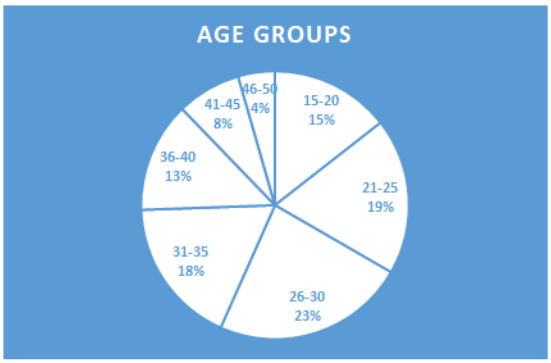

Figure 1: Representing Age distribution in study subjects on graph.

Gender distribution

Among all the subjects there was female predominance with 82% compared to male subjects with 18% (Figure 2).

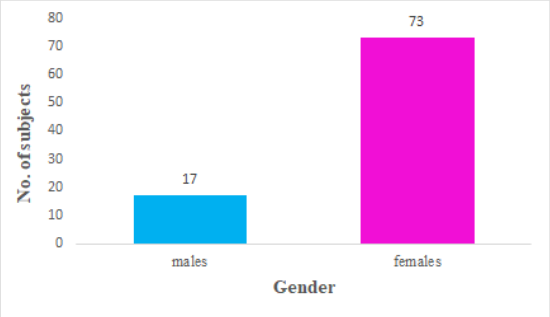

Figure 2: Representing Gender distribution in study subjects on graph.

Triggers causing migraine

Among all the subjects, stress was found to be the major trigger in migraine patients occurring in 57% of cases, while workload was only 12% trigger in subjects (Figure 3).

Figure 3: Representing percentage of triggers on graph.

In the table above we compared the CGRP levels with clinical manifestations. It was observed that patients with throbbing pain were found with high CGRP levels 130.44±114.22 compared to that of absence of throbbing pain.88.37±73.42.The p value was found to be significant (i.e.,p = 0.01). Moreover, CGRP levels were observed to be elevated in patients with stress 137.05±86.34 than patients without stress 86.07±53.62.The p value was found to be significant (i.e., p= 0.03). Similarly, CGRP levels were increased in patients with inadequate sleep 192.23±102.34 than the one's with adequate sleep 108.77± 79.31. The difference was statistically significant (p=0.05) (Table 2).

Table 2: Representing details of association between CGRP levels and clinical manifestations.

VARIABLES	POSITIVE	MEAN & SD VALUE	NEGATIVE	MEAN & SD VALUE	t	P VALUE

TYPE						
Throbbing	44	130.44±114.22	46	88.37±73.42	2.55	0.01*
Frequent	68	151.05±99.61	22	114.62±109.99	1.19	0.24
TRIGGER						
Stress	48	137.05±86.34	42	86.07±53.62	2.26	0.03*
Workload	11	120.60±111.77	79	127.50±81.05	0.14	0.89
Perfumes	11	102.50±82.58	78	135.25±100.69	0.8	0.43
Inadequate sleep	13	108.77±79.31	76	192.23±102.34	1.9	0.05*
LOCATION						
Unilateral	21	121.57±110.48	68	110.48±48.63	0.12	0.72
Bilateral	53	135±87±127.62	36	127.62±58.36	0.87	0.42
Holocranial	47	122.68±62.38	42	108.58±42.89	0.82	0.19

The comparison of CGRP between test and control subjects

The average CGRP levels in test group were 149.00±93.86 whereas in control it was 61.30±24.37. The difference was statistically significant (p= 0.02) (Figure 4).

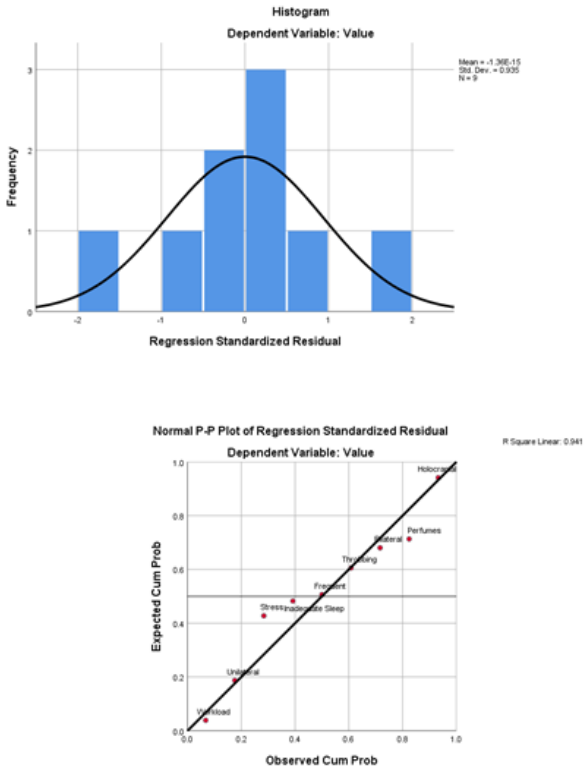

Figure 4: The average CGRP levels in test group were 149.00±93.86 whereas in control it was 61.30±24.37. The difference was statistically significant (p= 0.02).

Multivariate regression analysis suggestive of significant correlation between factors like, Throbbing type of pain, Stress, Inadequate sleep, sensitivity to perfumes and pattern of headache with higher CGRP values (Figure 5).

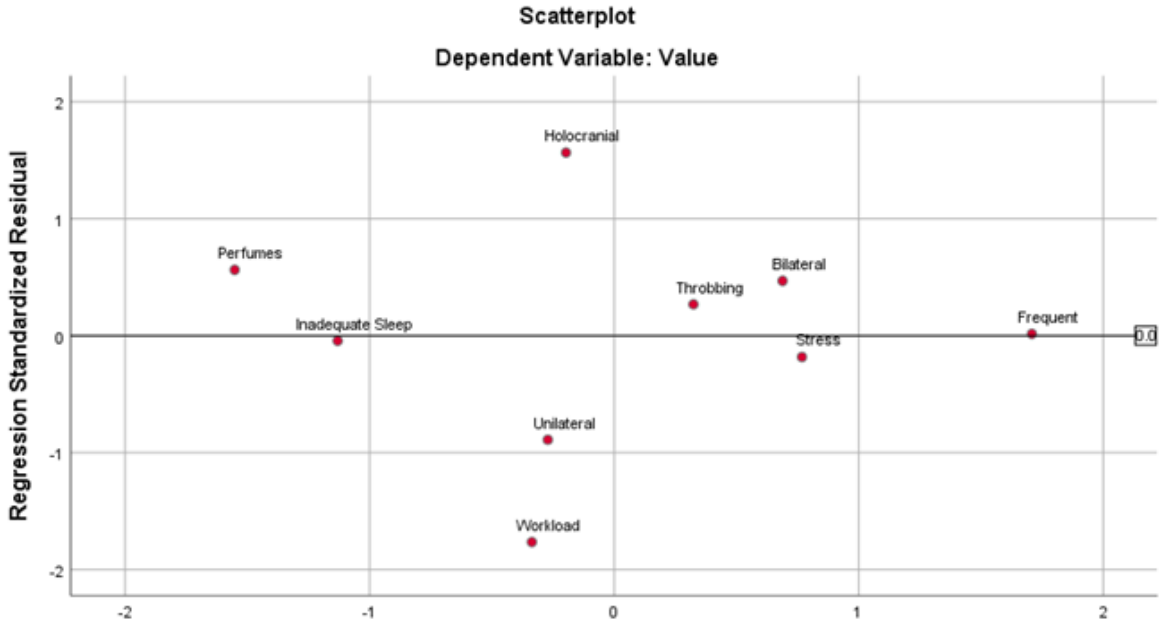

Figure 5: Inadequate sleep, sensitivity to perfumes and pattern of headache with higher CGRP values.

DISCUSSION

There is no effective diagnosing tool for chronic and acute migraine, CGRP levels can help in differentiating migraine and other types of headache. A state of neuroinflammation is thought to precipitate the release CGRP which in turn leads to vasodilatation causing headache. However many hypothesis are proposed as precipitating factors for inflammation but none have been proven yet. As the previous studies proved that the CGRP plays an integral role in migraine pathophysiology as its levels increases in migraine estimating CGRP in the serum may help in early diagnosis of migraine. Previous studies have also proved plasma CGRP as a potential biomarker for migraine, but in our present study we have taken serum CGRP levels of adult migraine patients into consideration. The prediction of serum CGRP levels and the novel idea of correlating it with the migraine characteristics is the core purpose of our study. We have enrolled 100 subjects in our study, which are divided as 90 migraine patients and 10 normal controls (without headache or migraine symptoms).The selection of subjects was based on ICHD 3 beta guidelines, drug therapy was given to the patients and clinical assessment was done every month for about three months. The blood samples were collected and the centrifuged for separation of serum from blood and serum CGRP levels were estimated by using commercially available CGRP ELISA kit. Among total of 90 subjects, 17 were male and 73 were female patients, indicating female predominance, consistent with previous studies. It is thought that probably hormonal fluctuations and varied receptor sensitivity in females is responsible for more female preponderance.Similarly if there is a difference of CGRP sensitivity and levels between either sexes, has to be assessed in a separate study. Majority of patients i.e.,23.3% affected with migraine were in between age 26-30 years and age group 45- 50 years was the least effected i.e.,4%. It has been reported by Leslie Kelman that there is age related decline in triggers of migraine like stress,

photophobia, phonophobia throbbing and pressure due to headache. CGRP is thought to exert its vasodilating effect through nitric oxide (NO) and also an antiproliferative effect. As age progresses the levels of NO decrease which might to an extent explain the age related variation of CGRP, which inturn explains reduction in migrane.The estimated CGRP values were compared for different parameters like symptoms, triggers and treatment response.No studies have been published to compare the CGRP levels in relation to various triggers and clinical features. The comparison of CGRP levels with clinical manifestations revealed the statistical significance, as patients with throbbing pain were found with elevated CGRP levels 130.44±114.22 with p value 0.01. A study conducted by Chou CH et al concluded that an increase in intracranial pressure is responsible for initiating throbbing pain also low intracranial pressure can in some cases accentuate throbbing pain(29). Moreover, CGRP levels were increased in patients with triggers like stress with high PSS score 137.05±86.34 where as other patients without stress or low PSS score 86.07±53.62, p= 0.03. Stress is associated with various hormonal changes and also elevation of inflammatory markers. These factors might inturn elevate the CGRP levels. Similarly, CGRP levels were increased in patients with inadequate sleep 192.23±102.34 than patients with adequate sleep 108.77± 79.31(p=0.05). This shows the statistical significance for stress and inadequate sleep which indicates that stress along with sleep deprivation are the high-risk factors for migraine. Stress ratings, duration of previous nights' sleep, and headache severity were evaluated using a series of linear mixed models with random effects in a study conducted byTimothy T. Houle et al to observe individual differences. High stress and inadequate sleep are major risk factors for predicting the severity of headache. The average CGRP levels in test group were 149.00±93.86 which were higher than the controls, it was 61.30±24.37. The difference was statistically significant (p= 0.02). Previous studies have shown doubtful role of CGRP as a biomarker or diagnostic tool in migrane.In our study a statistically significant value signifies its role as a diagnostic tool however we propose its role depends on the phase of headache during which it is assessed .CGRP levels in adult patients are higher than those in healthy controls at the baseline. During migraine attacks, plasma CGRP levels are elevated in adults and their changes shows relation with headache intensities. Hence, serum CGRP levels were statistically correlated with characteristics like throbbing pain, stress and inadequate sleep. Which means that throbbing pain, inadequate sleep along with stress are the risk factors for migraine. Patients were either prescribed monotherapy or dual therapy after evaluation of symptoms on the very first visit to the hospital. Drugs used in mono therapy: Naproxen 500mg (3-5 days) along with Sibelium or Flunarizine 5/10mg (30 days). Whereas, the dual therapy includes Naproxen 500 mg (35days) along with Flunarizine 5mg/10mg (30 days) and Tab. Inderal la (Propranolol) 40mg-1 month(or) Tab. Topamac (Topiramate) 25mg-1 week (or) Tab. Amicon (Amitryptiline) 10mg-1month. The patients were assessed clinically every month for three months. Patients who showed no response to monotherapy, the drug therapy was changed to dual. Like all studies there are some limitations in this study too. Firstly, the patient’s volume is less (i.e., 100). Large sample size will provide more details about the statistical parameter. Secondly a larger study with measurement of serum CGRP at baseline and ictal or interictal values may provide more information to include CGRP as biomarker in diagnostic criteria in future. Till date no studies have been done to predict the correlation between serum CGRP levels and migraine characteristics hence we provide the data and correlation between various factors [5].

CONCLUSION

From our study we concluded the serum CGRP levels are elevated in migraineours.Patients with high stress, inadequate sleep and throbbing pain had significantly higher CGRP levels when compared to the control group, indicating that these are the major risk factors in migraine. Thus, serum CGRP levels estimation by ELISA can be used as a diagnostic tool for migraine which will help in early start of treatment.

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