ASSOCIATION OF VITAMIN D AND ZINC WITH INFLAMMATORY MARKERS AND OXIDATIVE STRESS IN MIGRAINE PATIENTS

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TO MY BELOVED FAMILY

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ABSTRACT

Migraine is the most common neurological condition seen in primary care. Globally its affects 2% of population (out of which 1.5% are female and 0.5% are male). Migraine is an episodic and complex sensory processing disorder characterized by a range of symptoms, with headache being the hallmark feature, typically localized to one side of the head. Numerous studies have investigated the role of biochemical parameters in migraines to establish a clear cause-and-effect relationship. This study is an attempt to contribute towards studies that are trying to establish exact cause for prevalence of migraine. The objectives of the study were to compare the biochemical parameters such as Vitamin D, Zinc, IL-10, IFN-Gamma, CRP, SOD & MDA. This was a case control study and ethical approval was taken from Bahria University Health Sciences Karachi (BUHSCK). Migraine patients between age 20-40 years were included in cases and healthy participants were included in controls. The calculated sample size of 246 subjects were divided into two groups, out which 123 were cases and 123 were controls. Venous blood sample was taken for measuring different parameters like Vitamin D, Zinc, IL-10, IFN- Gamma, CRP, SOD & MDA. The obtained results were statistically analyzed by SPSS version 23. Descriptive statistics were presented in terms of frequency with percentages and mean with standard deviation and Median with Interquartile range (IQR). Independent sample t-test was used to compare the mean of baseline characteristics of controls and migraine patients. Mann Whitney U test was used to compare the median of skewed and not normally distributed parameters between two study groups. Pearson Correlation was used to check the relationship. Binary Logistic regression analysis was used to estimate the risk estimation of migraine. P-values less than 0.05 were considered statistically significant. The mean comparison of baseline characteristics between controls and migraine patients, in control group mean Age (years) was 33.6±4.7, mean BMI (kg/m2) was 27.7±5.1, mean SBP was 122.2±9, mean DBP was 81.8±4.4, mean Temperature was 98.1±0.2, whereas in migraine patients mean Age (years) was33.2±4.6, mean BMI (kg/m2) was 28.4±4.8, mean SBP was 121.5±10, mean DBP was 81.9±4.7, mean Temperature was 98±0.1, Independent sample t-test showed significant mean difference in temperature between controls and migraine patients (p=0.027), all other

characteristics were found statistically insignificant ($p>0.05$). The comparison results of Vitamin D, Zinc, SOD and MDA between controls and migraine patients, showed in control group samples median for Vitamin D (ng/ml) was 14(IQR=7), median for Zinc (μg/dL) was 88(IQR=11), median for SOD (nm/ml) was 41(IQR=29), and median for MDA (U/mL) was 2(IQR=1), whereas in migraine patients median for Vitamin D (ng/ml) was 20(IQR=15), median for Zinc (μg/dL) was 65(IQR=7) median for SOD (nm/ml) was 10(IQR=4), and median for MDA (U/mL) was 4(IQR=1). Mann Whitney U test gave significant difference in the median of vitamin D, Zinc, SOD, and MDA between controls and migraine patients ($p<0.01$). The comparison of CRP, IL-10 and IFN Gamma between control and migraine patients, results showed among controls median for CRP level (mg/dL) was 1(IQR=2), median for IL-10 level (pgm/ml) was 301(IQR=1313), and median for IFN gamma (ngm/ml) was 141(IQR=203), whereas in migraine patients median for CRP level (mg/dL) was 2(IQR=4), median for IL-10 level (pgm/ml) was 243(IQR=114), and median for IFN gamma (ngm/ml) was 63(IQR=108). Mann Whitney U test gave significant difference in the CRP levels, IL-10 and IFN gamma between controls and Migraine with $p<0.05$. Our study concludes that migraine patients exhibit deficiencies in vitamin D and zinc, accompanied by reduced superoxide dismutase (SOD) levels and elevated malondialdehyde (MDA), indicating a role for oxidative stress in migraine pathogenesis. Increased CRP levels, along with reduced IFN-gamma and IL-10, point to a complex inflammatory response. The relationship between these biomarkers suggests that vitamin D and zinc deficiencies may exacerbate oxidative stress and inflammation, leading to neuronal damage and enhanced vasodilation, thus contributing to migraine development. These novel insights will aid healthcare professionals in refining the management approaches, allowing for targeted treatments that address underlying nutritional deficiencies, oxidative stress, and inflammation in migraine patients.

Key words: migraine, BMI, vitamin D, zinc, SOD, MDA, IL-10, IFN-gamma, CRP

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LIST OF ABBREVIATIONS

VDR	Vitamin D receptor
NO	Nitric Oxide
CGRP	Calcitonin gene-related peptide
CRP	C reactive protein
SOD	Superoxide dismutase
MDA	Malondialdehyde
Zn	Zinc
NMDA	N-METHYL-D-Aspartate
GABA	Gamma-aminobutyric acid
ROS	Reactive oxygen species
RNA	Reactive nitrogen species
GPx	Glutathione peroxidase
DNA	Deoxyribonucleic acid
IFN-γ	Interferon gamma
IL-10	Interleukin 10
CNS	Central nervous system
PNS	Peripheral nervous system
Hs-CRP	High sensitivity CRP
ER	Endoplasmic reticulum
Th	T-helper cells
APC	Anaphase-promoting complex
MHC	Major histocompatibility complex
MAPK	Mitogen-activated protein kinase
ERK	Extracellular signal-regulated kinase
PI3K/Akt	Phosphoinositide-3-kinase
JAK/STAT	Janus kinase/signal transducers and activators of transcription
IFNGR	Interferon gamma receptor
BBB	Blood brain barrier

NSC	Neural stem cell
5HT	5-hydroxytryptamine
MSG	Monosodium glutamate
CYP27B1	Cytochrome P450 family 27 subfamily B member 1
Vitamin D	1,25-dihydroxycholecalciferol
TGF	Transforming growth factor
PACAP	Pituitary adenylate cyclase-activating polypeptide
BMI	Body mass index
SBP	Systolic blood pressure
DBP	Diastolic blood pressure

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Migraine is known as a most common neurological condition reported in primary care. According to the latest survey by Global Burden of Disease, migraine remains the leading cause of disability among young women and ranks second globally**.** Because of hormonal influences, migraine is reportedly more common in women (Wimalawansa, 2019).

The 2% of the world's population is effected by migraine out of which 18% are women and 6% are men. Its pain is very burdensome for migraine sufferers, their families, and society all together. It is a complicated sensory disorder which is episodic in nature that is associated with other symptoms, but still headache is the most striking feature. Migraine can be classified based on the presence of an aura and the frequency of headaches. Migraines with a visual aura include visual side effects that typically begin before the headache and last for at least five minutes (Tullet et al., 2017).

The visual aura often manifests as an enlarging blind spot or as scintillating objects in the field of vision. It is not enough to have blurred vision to diagnose aura. Other aura characteristics include reversible word finding issues, aphasia (the inability to express words or comprehend words), sensory phenomena like tingling that extends to the face, motor issues like weakness, brainstem issues like unsteadiness, and characteristics of cranial nerve dysfunction. These aura symptom usually last 5 to 60 minutes (Kambe et al., 2021).

Available literature suggests that an activated intracranial network increases the sensitivity of the trigemino-vascular system, releases inflammatory markers, and triggers an inflammatory response similar to a meningeal reaction, leading to headaches. Genetic factors may significantly influence an individual's susceptibility to migraines. Activation of vascular networks results in headache by causing meningeal vasodilation and inflammation and. Modulation of pain coming from disturbed neural systems is a key component of migraine

pathophysiology. Researches have demonstrated that the trigeminovascular system, have the trigeminal nucleus caudalis and efferent neurons that supply vascular networks and afferent neurons that feed information, is controlled by the diencephalic nuclei and brain stem. Due to the activation of these networks, head pain is interpreted as meningeal inflammation and vasodilation (Gibson et al., 2016).

In the past few years vitamin D insufficiency has been identified as a major public health problem worldwide. In healthy people, vitamin D insufficiency is present between 30% and 50% of the time. Despite having a lot of sun exposure, the highest rates of vitamin D insufficiency in the world is middle east. It is well known that a vitamin D deficiency and chronic pain are related. The pathogenic role of vitamin D in neuroinflammatory, neuropsychological and neurodegenerative illnesses such as headache, Alzheimer's disease, cognitive decline psychosis, autism and depressio has received a lot of attention recently. Well-known neurosteroid vitamin D has a crucial function in both developing and adult brains (Groves et al., 2014). The existence of a potential link between vitamin D and migraines caused a lot of alarm. It was shown that vitamin D plays a part in the pathways responsible for migraine pathogenesis, including pain sensitivity, inflammation, and immunological dysfunction (Hussein et al., 2019). Although randomized trials were unable to validate vitamin D's impact on indicators of systemic inflammation, it may primarily influence migraines through its anti-inflammatory activity. Instead of being a cause of disease or its clinical progression, vitamin D deficiency may be an effect of inflammatory processes (Yin & Agrawal, 2014).

Vitamin D has been shown to play a non-skeletal function in inflammation, immunology and the metabolism of neurotransmitters, and the brain contains a large number of vitamin D receptors. Furthermore, vitamin D is related to severe headaches and is thought to be a possible preventative medication or adjuvant in the treatment of migraine. Low levels of physical activity and lengthy work hours are said to increase the incidence of headaches or migraines. Migraineurs prefer to avoid sunshine owing to photophobia during an attack. It has been recognized that the risks of vitamin D deficiency and migraine change with latitude, indicating the necessity to look into the vitamin D insufficiency and migraine frequency relationship (Mottaghi et al., 2013). At the molecular level it has been discovered that vitamin D receptor (VDR) gene polymorphisms may raise the danger for migraine

without aura. Different brain areas expressing VDR is an indirect indicator of vitamin D activity (Ouyang & O'Garra, 2019).

Vitamin D may have a role in the growth and development of neuronal stem cells, according to the broad expression in the neuroepithelium. A further indication of the importance of vitamin D in cognitive role and memory in the hippocampus and limbic system is the manifestation of VDR. Additionally, VDR is found in the substantia nigra, confirming the association between vitamin D and dopamine, and the sensory cortex, signifying its character in the somatosensory system. The data also proof that vitamin D is also synthesized and metabolized in the brain, in addition to the liver and kidneys, which carry out the typical vitamin D metabolism. The vitamin D also have anti-inflammatory properties, which may alter the neuroinflammation associated with migraine, may also explain the association between vitamin D deficiency and migraine that has been found. Numerous studies demonstrated that vitamin D can inhibit the synthesis of proinflammatory cytokines such tumor necrosis factor- and interleukin-6 at physiological levels. The anti-inflammatory cytokine interleukin-10 production is also increased by vitamin D. Additionally, nitric oxide synthase, can be inhibited by vitamin D in its active form, 1,25(OH)2D due to which nitric oxide is not formed. Calcitonin gene-related peptide (CGRP), which is produced and released by trigeminal ganglion neurons, in turn increases the production of NO. In light of this, there may be a chance of a positive feedback loop, which might intensify and perpetuate inflammatory processes (Wang et al., 2017). The adverse relationship between C-reactive protein and vitamin D levels further supports the theory that inflammation shows a role in the development of migraine and vitamin D insufficiency. The processes behind migraine and migraine frequency connections with vitamin D are yet unknown. However, here are a few hypotheses: First, the brain contains Vitamin-D-binding protein and the enzyme responsible for the synthesis of the active form of vitamin D that is vitamin D receptors 1-hydroxylase. Vitamin D promotes brain cell differentiation, governs axonal development, and directly affects the signaling of calcium in the brain. Its deficiency also influences the generation of certain neurotrophic factors and reactive oxygen species in brain. In the periosteum the central and peripheral sensitization of neurons in the results in headache is also controlled by vitamin D. Second, Zinc is a key player in vitamin D production and metabolism. Decreased blood Zinc levels are often related with decreased vitamin D levels.

Third, neurological illnesses connected with vitamin D, such as depression and fibromyalgia, are also linked to migraine (Makrani AH, *et al* 2017). Zinc operates as a cofactor for vitamin D because it is required by the transcriptional activity of genes dependent on vitamin D to carry out pleiotropic effects such mineral ion regulation. Zinc homeostasis may be regulated by zinc transporters when vitamin D is present. When combined, zinc and vitamin D support overall health, including the musculoskeletal system. However, deficiencies in any of these minerals can lead to a variety of diseases that impact nearly all bodily systems (Amos & Razzaque, 2022).

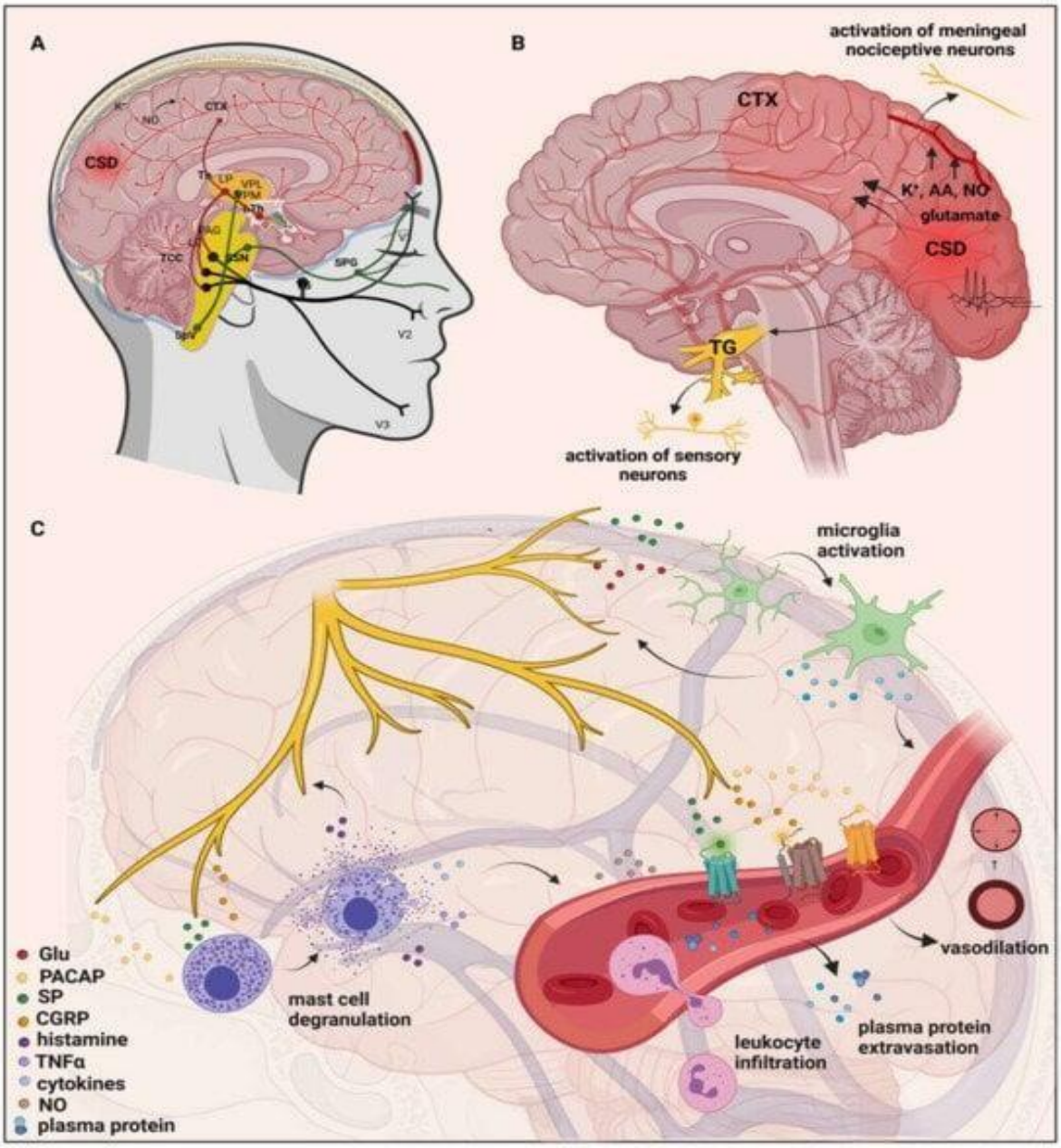

Fig 1.1: pathogenesis of migraine (Spekker et al., 2023)

Zinc is an indispensable trace element that plays a vital role in our body. It has powerful antioxidant and anti-inflammatory properties, helping to protect our cells from damage. Zinc also acts as a cofactor for enzymes that fight oxidative stress and is crucial for proper neural signaling. A moderate prevalence of zinc insufficiency was found in few observational studies of migraine sufferers. Neurological issues such as attention deficit disorder, tiredness, memory loss, and learning difficulties can result from severe zinc deficiency (Ahmadi et al., 2020). Low blood zinc levels have also been demonstrated to positively correlate with migraine episodes. Through its actions on the neurological system, zinc may raise the threshold for migraine episodes and lower their frequency. This substance could prevent the hippocampus from releasing glutamate. The N-Methyl-D-aspartate (NMDA) receptor, an essential receptor in the beginning of migraine episodes, is stimulated by glutamate. Zinc may also act as an NMDA receptor competitive antagonist. Additionally, zinc may block the NMDA receptor by raising levels of the neurotransmitter gamma-aminobutyric acid (GABA), which has an NMDA-inhibitory action (Krall et al., 2020).

Trigeminal ganglion neurons serve as the link between the central nervous system and the peripheral areas. They link the meninges, blood vessels and the primary afferent fibers of the trigeminal ganglion to the central terminals of the trigeminal nucleus caudalis. Large amounts of the neuropeptide CGRP, which is also synthesized inside the trigeminal ganglion and released from the peripheral and central nerve terminals, are produced by trigeminal ganglion neurons. (Iyengar et al., 2019). Nitric oxide production is raised and the trigeminal nerves become more sensitive as a result of CGRP being released from peripheral terminals. The CGRP released by the trigeminal ganglion work together with surrounding neurons and satellite glial cells to preserve peripheral sensitization and can trigger central sensitization in second-order neurons. The transition from activity-dependent to activity-independent central sensitization may reveal a mechanism that causes the progression from episodic migraine to chronic migraine. One of the main factors producing migraines is CGRP. Migraine treatments that causes CGRP inhibition in the peripheral trigeminal system may be useful for reducing the pain frequency and intensity of migraine and its related symptoms. It could also be able to halt a migraine before it begins by preventing the trigeminal nerve from getting sensitized by decreasing CGRP activity in the region. This method could also be beneficial for cluster headaches (Wattiez et al., 2020).

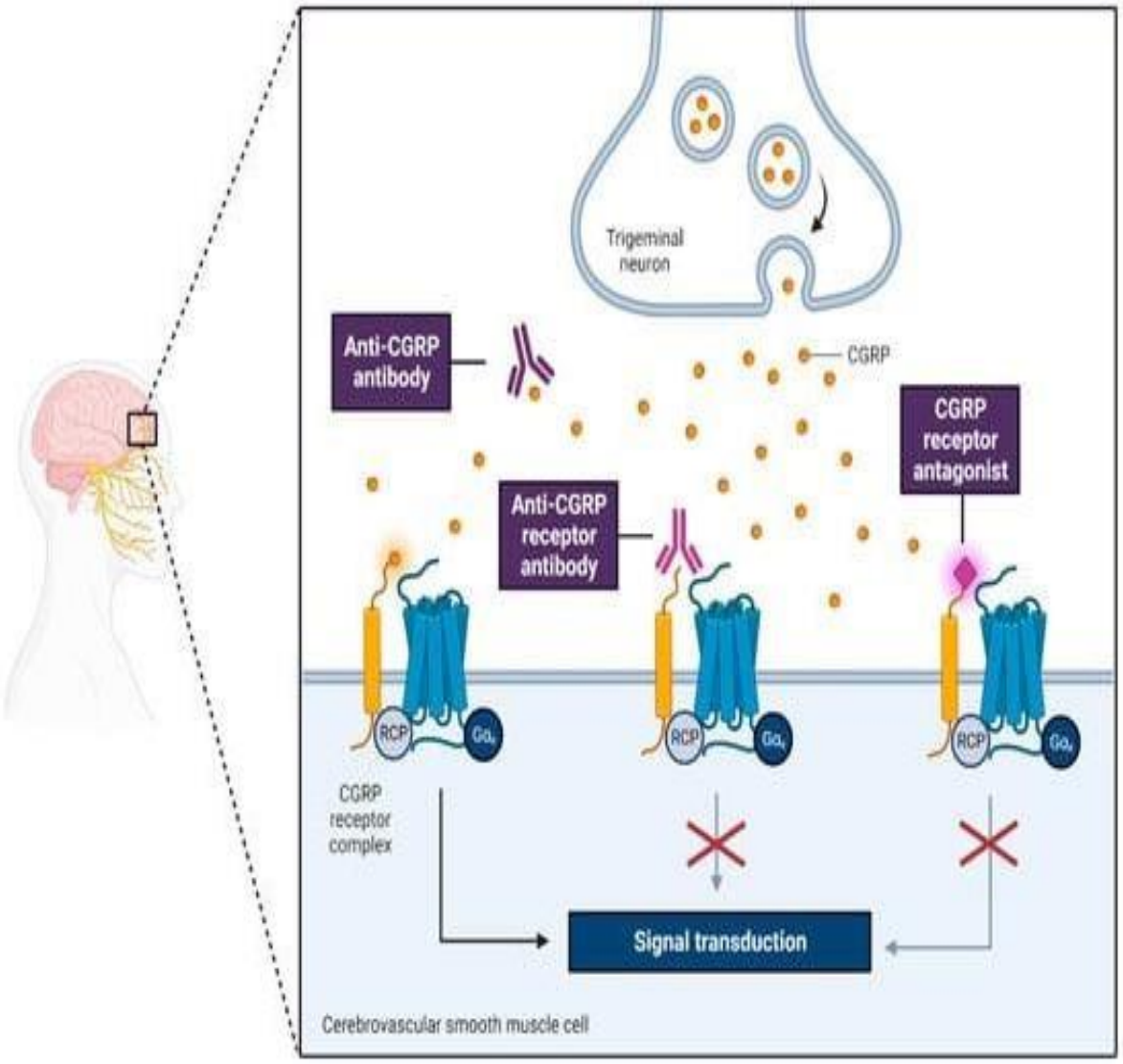

Fig 1.2 CGRP- targeted therapy for Migraine (Horia Pleș et al., 2023)

When the body's levels of beneficial antioxidants and damaging oxidants are out of balance, oxidative stress results. Studies indicate that this imbalance is linked to a considerable increased danger of a number of illnesses, including diabetes, cancer liver problems, CVS diseases, and neurological disorders. Disruptions in cellular metabolism can lead to raised levels of reactive oxygen species and nitrogen species (ROS and RNS) which can make cells more susceptible to environmental oxidative chemicals and weaken their antioxidant defenses. Oxidative stress arises from various factors, including changes in cellular energy metabolism, the release of nitric oxide (NO) and inflammatory mediators, and the presence of increased levels of PUFA polyunsaturated fatty acids in cell membranes, which escalate the risk of lipid peroxidation. (Alves de Lima et al., 2020).

The body's antioxidant system, which helps prevent the release of harmful oxidants like RNS and ROS, consists of glutathione (nonenzymatic agent) and some vitamins like vitamins A, C, and E and some enzymatic mediators like catalase (CAT) superoxide dismutase (SOD) and glutathione peroxidase (GPx). When ROS and RNS levels increase, they can damage intracellular molecules like proteins and DNA, leading to oxidation. This oxidation can disrupt receptors, signaling pathways, and transport systems, and cause dysfunction in cellular organelles and structures. Research indicates that people who suffer from migraines tend to have higher levels of nitric oxide (NO) metabolites and malondialdehyde (MDA), a byproduct of lipid oxidation, compared to those without migraines. Migraine sufferers may experience increased oxidative and nitrosative stress, as evidenced by elevated serum levels of MDA and NO, both of which are associated with migraine development. Furthermore, a decreased overall non-enzymatic antioxidant capacity and a lessened antioxidant enzyme system may indicate a lesser level of antioxidant defense in them. Additionally, oxidative stress indicators are anticipated to increase along with a possible decrease in antioxidant enzyme levels and total antioxidant capacity as the number of headache days each month rises (Andreou AP et al, 2019).

Oxidative stress happens when the body's antioxidant defenses are overpowered, resulting in damage to cellular components like proteins, lipids, and DNA. Many migraine triggers and aggravating factors might share a common thread: oxidative stress. Fasting, missing meals, physical activity, stress, and seemingly unrelated stimuli such as hormonal fluctuations, weather variations, odor, alcohol, loud sound and bright light, and loud may all

be linked to alterations in mitochondrial metabolism and thus causes oxidative stress (Gross et al., 2021).

The transient receptor potential TRP channels that are present in brain in the nerve terminals of meninges are activated by oxidative, nitrosative, and nitric oxide stress. This activation suggests a mechanism via which frequent migraine triggers that generate oxidative stress might cause migraine discomfort. (Gross EC, et al 2019). According to an epigenetic theory, the buildup of damage from harmful oxidation of biomolecules brought on by the (ROS) which are highly reactive free radicals that the cells make as a result of the essential utilization of oxygen results in ageing. The ageing is a process which leads to the steady functional deterioration and maturity that continues throughout life, which in turn results in chronic diseases (Marsman, 2018). The cellular senescence shows increased production & release of pro-inflammatory molecules, which includes interferon (IFN-γ), reactive oxygen species (ROS). ROS within organ and immune cells acts as mediators of signal communication, thus speed up inflammaging. The defect in immunosenescence leading to over activity, inflammation, and autoantibody production has predictable health repercussions. (Duggal et al, 2019)

Since migraine is associated with inflammation, inflammatory markers are usually found raised in migraine. Neuroinflammation is defined as an adaptive response caused by unpleasant stimuli like as infection, damage, or tissue stress, and it is crucial in pathogenesis is of many central nervous system illnesses. Neurogenic neuroinflammation is described in the context of migraine as inflammatory responses in both CNS & PNS Trigeminovascular system components in response to neuronal activity (Gilhus NE, et al 2019;). Depending on the biological circumstances, tissue homeostasis, cell types involved, and inflammatory components vary, because inflammation in the CNS can be both harmful and protective. The immune system is important in migraine pathophysiology. Several cytokines, including Interferon Gamma (INF-γ) have been linked to inflammation, pain regulation, sensitivity of trigeminal nerve fiber and migraine precipitation. According to several lines of evidence, during migraine, a variety of proinflammatory cytokines are actively produced and subsequently participate in a complicated sensitization of nerve terminals in the meninges, increasing or intensifying the sensation of pain (Biscetti et al., 2021).

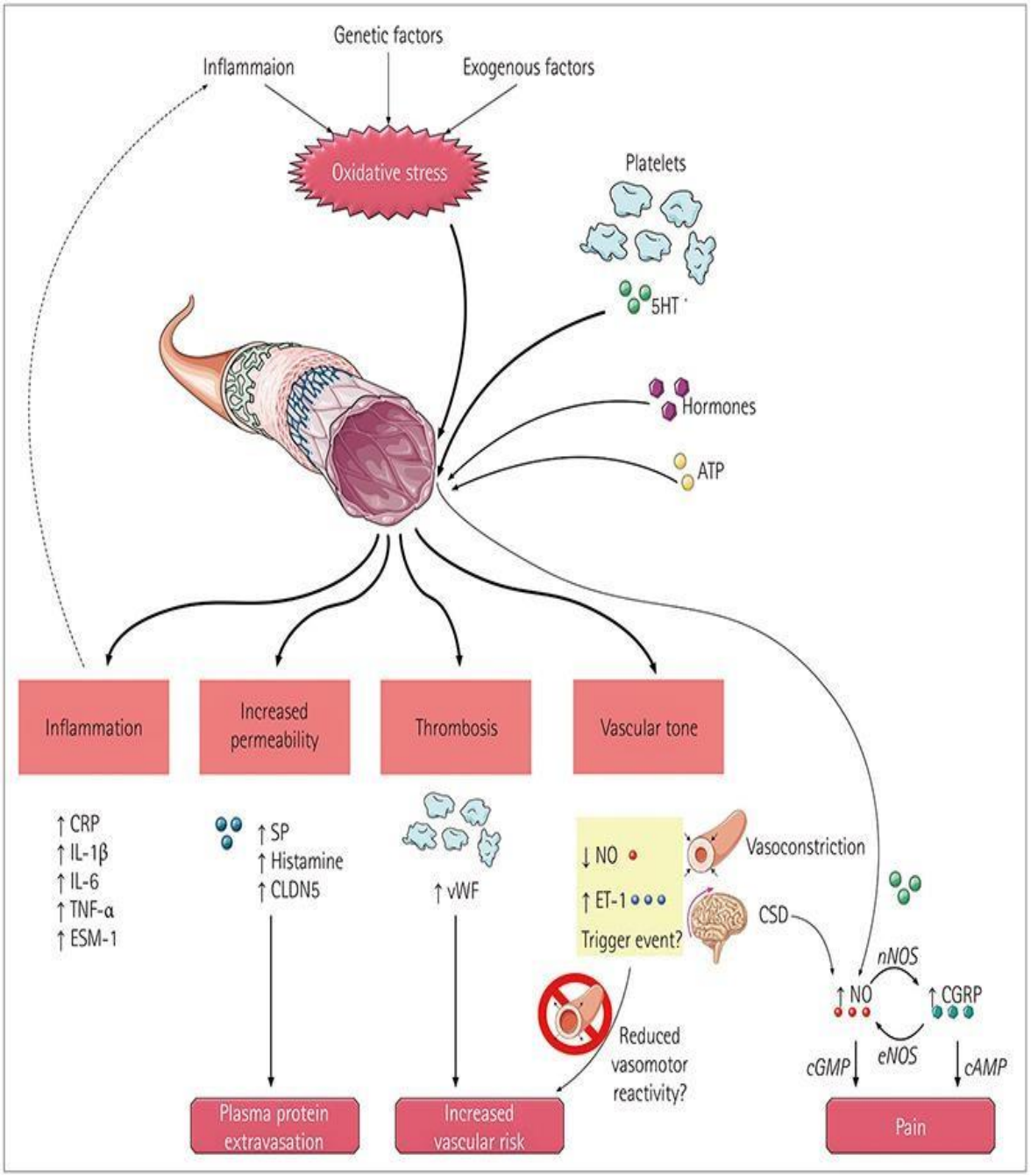

Fig 1.3 Endothelial dysfunction in migraine (Paolucci et al., 2021)

The zinc is a necessary mineral that has a significant connection to vitamin D. Additionally, zinc operates as a cofactor for vitamin D since it is necessary for the transcriptional activity of genes that are dependent on vitamin D to perform pleiotropic tasks, such as regulating mineral ions. Zinc transporters may potentially be induced by vitamin D in order to control zinc homeostasis (Amos & Razzaque, 2022). When present in sufficient quantities, zinc and vitamin D work together to support the health of the musculoskeletal system and other body systems. Conversely, deficiencies in either nutrient can lead to a variety of diseases that impact nearly every system in the body. About 90% of the 2-3 g of zinc in the body is contained in muscle and bone. It is attached to plasma proteins such transferrin, metallothionein, and albumin. Due to the lack of a real zinc storage mechanism in human bodies, daily zinc intake is required. The GIT and the excretory systems performs a major role in maintaining the equilibrium of zinc. Zinc homeostasis is mostly controlled by the gastrointestinal tract through excretion in the faeces. It is momentarily retained in intestinal cells before being sloughed off and eliminated. When zinc is necessary in the gastrointestinal system, it is absorbed; otherwise, it is excreted in faeces (Krebs, 2000).

The zinc is an important trace element which is fundamental for both overall human health and a healthy immune system. Zinc is significant for many biological processes in the human body, including those that impact cell division, proliferation, and apoptosis, all of which have an impact on an organism's ability to grow (Costa et al., 2023). When one takes into account bioinformatics studies that have identified around 3000 human proteins that are thought to bind zinc, the significance of zinc becomes immediately clear. Among them, the divalent cation is especially needed as a stabilizer for zinc fingers and domains that include zinc fingers. Since zinc is required for the functioning of several metalloenzymes, it is also necessary for other catalytic and regulatory needs. Zinc is known to be the second most prevalent micronutrient in humans after iron. Numerous studies have been conducted since zinc's significance for human health was originally discovered. These days, it is recognized that zinc, and particularly zinc deficiency, is linked to a number of metabolic and chronic illnesses, such as diabetes, cancer (such as esophageal, hepatocellular, breast, and colon cancer), and neurological disorders (Maywald & Rink, 2022). Zinc is mostly distributed intracellularly between the nucleus (~30–40%) and certain zinc-storage organelles known as zincosomes (~50%). The cytoplasm and other cell organelles contain the remaining zinc.

Zincothioneins (MT), zinc-chelating proteins, bind zinc mostly in the cytoplasm. Zinc storage or release from organelles, such as zincosomes, the endoplasmic reticulum (ER), or the Golgi apparatus, influences the availability of cytosolic zinc. Zincosomes are zinc-storing granules that resemble vesicles; they are commonly visible with zinc-binding probes. In order to preserve optimal zinc homeostasis, the body must replenish its lost zinc. The human body does not have a specific area for storing zinc, thus the trace mineral has to be consumed every day in enough amounts together with meals. Zinc loss can come from both intestinal and nonintestinal sources, such as loss of hair, nails, and desquamated skin, as well as excretions of zinc from the faeces and urine, perspiration, menstrual flow, and semen. Furthermore, unique life situations that require individualized adjustments to the anticipated zinc loss must be considered. Examples of these situations include pregnancy, breastfeeding, early infancy, and young children still in development. (Gibson et al., 2016)

Interleukin-10 (IL-10) is a critical anti-inflammatory cytokine that helps regulate immune responses by inhibiting the activation of immune cells and the production of pro-inflammatory cytokines in various innate immune cells. Deficiency in IL-10 signaling can lead to severe inflammatory conditions, such as life-threatening inflammatory diseases in humans. IL-10 is the first member of a family of different interleukin cytokines which contain IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29. (York et al., 2024). Research has demonstrated that neuroinflammation and endothelial dysfunction are intricately linked to the pathogenesis of migraines. Migraine sufferers exhibit an imbalance in the regulation of pro-inflammatory and anti-inflammatory cytokines, alongside the activation of immune cells such as mast cells and T cells, contributing to the development and persistence of migraine symptoms. (Faraji et al., 2021). IL-10 plays a multifaceted role in the immune system. It is primarily known for counteracting the effects of pro-inflammatory cytokines. Additionally, it promotes the activation of B cells and stimulates the proliferation of natural killer cells, while also enhancing the production of interferon-γ. These functions underscore its importance in regulating immune responses and maintaining immune balance. (Qin et al., 2016). IL-10 plays a crucial role in migraine pathophysiology due to its anti-inflammatory properties. As a regulatory cytokine, IL-10 helps suppress the activity of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are known to contribute to the neuroinflammation seen in migraine. Studies have shown that migraines are associated with

an imbalance between pro-inflammatory and anti-inflammatory cytokines, and reduced levels of IL-10 in migraine patients suggest a loss of this anti-inflammatory regulation. This deficiency in IL-10 may lead to increased inflammation within the trigeminovascular system, which plays a key role in migraine development. Furthermore, the dysregulation of IL-10 could exacerbate endothelial dysfunction and oxidative stress, further contributing to the progression and intensity of migraine attacks. Thus, IL-10's neuroprotective and anti-inflammatory functions make it a potential target for therapeutic interventions aimed at modulating immune responses in migraine patients. (Maria Grazia Vittorini et al., 2024). Interleukin-10 (IL-10) plays a critical role in regulating immune responses and has been shown to act as an anti-inflammatory cytokine in various conditions, including migraine. Its neuroprotective properties are linked to its ability to suppress excessive immune activation, which can mitigate neuroinflammation—a key factor in migraine pathophysiology. Research suggests that the imbalance between pro-inflammatory and anti-inflammatory cytokines, such as IL-10, is associated with increased neuroinflammation in migraine sufferers. Elevated levels of IL-10 can reduce the inflammatory response by inhibiting the release of pro-inflammatory cytokines, thereby limiting neuronal damage and potentially alleviating migraine symptoms. Studies indicate that lower levels of IL-10 during migraine attacks contribute to sustained inflammation and oxidative stress, exacerbating the pain and other symptoms associated with migraines. (Carlini et al., 2023)

The IL-10 is vital for maintaining homeostasis and preventing neuronal damage caused by infection or inflammation. It has been linked to improved neuronal survival and regulation of adult neurogenesis. By modulating NF-κB signaling and involving STAT3, administering IL-10 has been found beneficial for axon regeneration. IL-10 also reduces the susceptibility of neurons to CNS damage and ischemia. Inactive astrocytes respond to IL-10 by expressing IL-10R to have an immediate impact on CNS cells. Numerous studies highlight the pro-repair functions of IL-10, traditionally associated with its anti-inflammatory properties. Due to its wide range of anti-inflammatory effects, therapeutic modification of IL-10 has attracted significant attention. (Ouyang & O'Garra, 2019)

Interferon-gamma (IFN-γ) plays a crucial role in the immune response, including its involvement in neuroinflammatory processes linked to migraine. IFN-γ is a pro-inflammatory cytokine produced mainly by T cells and natural killer cells, which helps

regulate immune responses by activating macrophages and promoting antigen presentation. In the context of migraine, increased levels of IFN-γ have been associated with neuroinflammation, which may contribute to the pathogenesis of migraines by promoting inflammatory signaling in the brain. Some studies suggest that alterations in IFN-γ signaling could exacerbate inflammatory responses during migraine attacks, affecting vascular reactivity and nociceptive pathways. IFN-γ has been linked to endothelial dysfunction, which plays a key role in the vascular component of migraine, as it can promote the release of pro-inflammatory cytokines, chemokines, and nitric oxide, leading to enhanced pain signaling and vasodilation, common features of migraine. (Biscetti, De Vanna, et al., 2021)

Interferon-gamma (IFN-γ) plays a significant role in the pathophysiology of migraine, primarily through its pro-inflammatory effects and interaction with immune responses. Research indicates that migraine patients exhibit elevated levels of IFN-γ, suggesting a potential link between this cytokine and migraine attacks.

1. **Pro-Inflammatory Role**: IFN-γ is known to promote inflammatory responses, which may contribute to the neuroinflammation observed in migraine. Increased levels of IFN-γ during migraine attacks can lead to the activation of inflammatory pathways, enhancing pain perception and potentially triggering migraine episodes.
2. **Cytokine Interaction**: Studies show that IFN-γ can influence the production of other cytokines, such as IL-6 and TNF-α, which are also elevated in migraine patients. The interaction between these cytokines and IFN-γ suggests a network of inflammatory mediators that may play a critical role in the migraine pathogenesis.
3. **Migraine Phases**: Research indicates that the levels of IFN-γ may vary between the ictal (attack) and interictal (between attacks) phases of migraine. Elevated IFN-γ during the attack phase suggests its involvement in the acute phase of migraine, while its levels might normalize or decrease in the interictal phase
4. **Therapeutic Implications**: Understanding the role of IFN-γ in migraines could lead to novel therapeutic approaches. Targeting IFN-γ or its signaling pathways might help in managing migraine attacks by modulating the inflammatory responses associated with the condition. (Musubire et al., 2023)

In migraines, there is an elevation in inflammatory markers such as interferon-gamma (IFN-γ) and C-reactive protein (CRP), alongside a decrease in anti-inflammatory cytokines like interleukin-10 (IL-10). This imbalance stimulates the trigeminovascular system, leading to the release of calcitonin gene-related peptide (CGRP) and substance P, which in turn enhances vasodilation and neuroinflammation, ultimately resulting in migraine pain episodes. IFN-γ is a potent pro-inflammatory cytokine that can amplify inflammatory responses. Elevated levels of IFN-γ have been observed during migraine attacks, contributing to neuroinflammation. This cytokine facilitates the activation of microglia and macrophages in the central nervous system, which release additional pro-inflammatory mediators, perpetuating the cycle of inflammation and pain. IFN-γ interacts with other cytokines, such as IL-6 and TNF-α, creating a complex network of inflammatory signals. These interactions can lead to an increased production of pain-inducing mediators, enhancing the overall inflammatory milieu associated with migraines. The dysregulated network is believed to play a significant role in the pathophysiology of migraine.

(Takizawa et al., 2024)

The mucosal epithelial cells, T cells, natural killer cells and macrophages produce the soluble cytokine IFN-γ. This cytokine binds to the IFN-γ receptor (IFN-γR), activates the JAK-STAT pathway, and promotes the production of IFN-γ-stimulated genes. IFN-γ plays a crucial role in both innate and adaptive immunity, and anomalies in its expression or activity have been associated to several human illnesses. IFN-γ/IFN-γR signaling may, however, have contradictory effects on the development of cancer, as its pro- or anti-tumorigenic effects depend on the tissue microenvironments. The two IFN-γR1 and IFN-γR2 chains that make up the IFN-γR protein each have subunits that, depending on the situation, have distinct functions. This review evaluated the expression, polymorphisms, and roles of IFN-γR in the onset and course of several human illnesses, whether they were IFN-γ dependent or independent. In order to support the continued use of IFN-γR, this review also included the tumor. The microenvironment, microbial infections, and significant components in IFN-γ upstream signaling can impact IFN-γR production, drug resistance, and potential drug targets. IFN-γ, a soluble cytokine released by various immune and mucosal epithelial cells, binds to IFN-γR1/2 receptors, stimulating the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. This pathway regulates numerous cellular

functions, including immune response, leukocyte transport, cell division, apoptosis, and both pro- and anti-tumor effects. Thus, IFNγR is essential for the biological actions and signaling of IFN-γ. As an antiparallel dimer peptide, IFN-γ binds to the IFNγR receptor complex, interacting with the extracellular domain of the IFNγR1 subunit in the classic IFN-γ/IFNγR/JAK/STAT pathway. The intracellular carboxyl termini of IFNγR1 and IFNγR2 contain non-receptor tyrosine kinases, JAK1 and JAK2, which phosphorylate the receptors upon ligand binding. The IFNγR2 subunit then transmits intracellular signals, leading to the endocytosis of the IFNγ-IFNγR1 complex and the movement of the IFNγR1 extracellular domain into the intracellular domain. This shift causes JAK2 to move from IFNγR2 to IFNγR1 due to a change in binding affinity. The activated JAK1 and JAK2 then phosphorylate the IFNγR1 cytoplasmic domain, creating binding sites needed to activate STAT proteins, particularly STAT1 activated by STAT1 binds to gamma-activated sequence sites on major response genes, controlling gene transcription and expression after moving into the cell nucleus. Variations in IFNγR1 polymorphisms can impact immune responses to infectious diseases by altering the receptor's affinity for its ligand. For instance, a variation in the IFNγR1 promoter has been linked to increased vulnerability to hepatitis B virus infection over a long period. Additionally, IFNγR1 polymorphism is related with a greater danger of cancer. For example, the IFNγR1-56C/T polymorphism has been identified as a factor contributing to the development and early incidence of gastric cancer. (Ding et al., 2022)

Long known for their functions in immunity, cytokines also perform a chief role in the central nervous system (CNS) in non-immune ways, such as regulating behavior and preserving homeostasis. Many studies have concentrated on cytokine signaling on immune cells in the central nervous system, such as microglia, due to their historical significance as immune mediators; nevertheless, cytokines may also directly affect neurons and are necessary for significant neuron-intrinsic activities. Few things are known about how cytokines penetrate the CNS parenchyma and communicate with neurons due to the CNS's distinct isolation and protection from the outside world. Recent research suggests that the immune system can interact with and assist the central nervous system (CNS) under normal settings. The identification of immunological niches around the CNS and lymphatic system highlights the significance of cytokine signalling in neurones, namely the IFN-γ/STAT1 pathway. IFN-γ,

the sole type II interferon, is a pro-inflammatory cytokine generated predominantly by immune cells in the adaptive immune system. It can stimulate neurones expressing IFN-γ receptors (IFNGR) (Kulkarni et al., 2016).

STAT1 plays a non-canonical role in CNS homeostasis, disease, and promoting IFN-γ-induced immune responses. The IFNGR is a heterodimer composed of two IFNGR1 and two IFNGR2 subunits. When IFN-γ binds to IFNGR1, it alters IFNGR2's structure, leading to autophosphorylation of Janus Kinase 2 (JAK2). JAK1 phosphorylates IFNGR1, while JAK2 most likely phosphorylates STAT1 following attaching to IFNGR1. Activated STAT1 dimerises and goes to the nucleus, where phosphorylated homodimers connect to chromatin at IFN-γ activation site GAS, promoting the transcription of canonical IFN-γ-stimulated genes (ISGs) that are pro-inflammatory, antiviral, and anticancer. Typically, this pathway is quickly activated and suppressed in peripheral immune and non-immune cells to rapidly combat infections and avoid unnecessary inflammation. However, recent research suggests that IFN-γ/STAT1 signaling in neurons may differ from these traditional signaling characteristics, showing non-cytolytic viral clearance and prolonged but muted STAT1 signaling. This could be due to the unique constraints neurons face, such as their distinctive shape and irreplaceable nature (Clark et al., 2022).

Under normal settings, the CNS generates minimal quantities of IFN-γ. However, production rises after infection. The BBB protects the CNS, thus it's unclear how IFN-γ directly activates parenchymal neurons. Although little is known about cytokine flow into and out of the CNS, the lymphatic system and immune compartments in the CNS periphery are potential sources of IFN-γ. IFN-γ/STAT1 signaling promotes neural stem cell proliferation and differentiation, while also maintaining appropriate neuronal excitability. It also prevents neurotropic infections. Excessive IFN-γ/STAT1 signaling in neurons has been linked to neuronal diseases, including abnormal neural activity and gene expression, affecting neurodevelopmental and neurodegenerative disorders. The mechanisms of IFN-γ/STAT1 signaling that lead to such different consequences in neurons are not fully known, but may be impacted by complex morphology, timing, and intensity of signaling, as well as the diverse structure of neurons. (Lemmens et al., 2019)

C-reactive protein (CRP) is a significant biomarker of inflammation, increasingly recognized for its involvement in migraine pathophysiology. Elevated CRP levels in

migraine patients indicate systemic inflammation, often triggered by cytokines such as interleukin-6 (IL-6). Research has demonstrated that individuals experiencing migraines, particularly chronic migraines, exhibit significantly higher CRP levels, which correlate with increased attack frequency and severity. This suggests a potential link between inflammation and the intensity of migraine episodes. CRP may also play a role in neuroinflammatory processes, potentially activating the complement system and recruiting immune cells to inflammatory sites, which exacerbates migraine symptoms. Understanding CRP's role could lead to targeted therapeutic approaches aimed at reducing inflammation to alleviate migraine pain. (Hagen et al., 2020)

Migraine is characterized by alterations in the neurovascular system, including cortical spreading depression (CSD) and activation of the trigeminovascular system. CSD is a wave of neuronal and glial depolarization that can trigger the release of inflammatory mediators, including calcitonin gene-related peptide (CGRP) and substance P. These neuropeptides contribute to vasodilation and increased permeability of the blood-brain barrier, leading to neurogenic inflammation. Elevated CRP levels may indicate systemic inflammation that exacerbates these neurovascular changes, creating a cycle of pain and inflammation. (Kofi Frimpong-Manson et al., 2024). Genetic predisposition and environmental triggers contribute to the pathophysiology of migraine. Certain genetic factors may influence the inflammatory response and CRP production. For instance, polymorphisms in genes related to inflammation may affect an individual's susceptibility to migraines, potentially influencing CRP levels. Additionally, lifestyle factors such as diet and stress can modulate inflammation and CRP levels, further impacting migraine susceptibility CRP serves as a key inflammatory marker in migraine pathophysiology, reflecting systemic and neurogenic inflammation that may contribute to the initiation and exacerbation of migraine attacks. Ongoing research continues to explore the complex interactions between inflammatory pathways, genetic factors, and neurovascular changes in understanding and managing migraine. (Horia Pleș et al., 2023)

The pentamer of human CRP, consisting of five identical subunits, binds to phosphocholine (PCh) in a way that is reliant on Ca2+ (Pathak & Agrawal, 2019). Immune-suppressive properties of C-reactive protein (CRP), an acute-phase protein mostly produced in the liver in response to IL-6 activation. It is a member of the pentraxin protein family and interacts with bacteria and dead cells by binding to lysophosphatidylcholine, which triggers

the complement system and encourages phagocytosis (Kinoshita et al., 2021). Long CRP has been employed as a sensitive serum biomarker to monitor the progression of inflammatory disorders. Moreover, low-grade inflammation has been found to raise the risk of cardiovascular disease in recent years (Lawler et al., 2020). According to recent research, elevated CRP levels can be an indicator of vascular inflammation. A crucial part of the pathophysiology of vascular disorders is inflammation. Additionally, CRP levels are a strong indicator of metabolic syndrome and cardiovascular disease. Furthermore, a noteworthy correlation has been documented between migraine and raised CRP. Elevated CRP is indicative of systemic inflammation (Lopez-Candales et al., 2017). Pentameric CRP (pCRP) is a protein that belongs to the highly evolutionarily conserved pentraxin class. It is made up of five identical protomers organized in a pentameric configuration. Crucially, every CRP protomer has a phosphocholine (PC) binding site that enables pCRP to attach to PC that is exposed on bacterial cell walls and inflammatory, apoptotic cells. On the CRP pentamer's "binding" face, the PC binding pockets of pCRP include two essential residues that mediate CRP-PC interactions. The connections between the methyl group and the positively charged nitrogen of PC are mediated by these residues, Phe-66 and Glu-81, respectively (McFadyen et al., 2020). The CRP pentamer's "effector" face is the opposite face. CRP can regulate innate immune responses by interacting with the Fcγ receptor and the globular head of the complement C1q subunit. Interestingly, it is now understood that pCRP is not an inflammatory molecule, but rather a precursor form of the circulating "CRP system." It has been demonstrated that when healthy human volunteers are given pure pCRP, no pro-inflammatory effects are elicited. (McFadyen et al., 2018).

Malondialdehyde (MDA) is a significant marker of oxidative stress and has been implicated in the pathophysiology of migraine. Elevated MDA levels indicate increased lipid peroxidation, which can lead to cellular damage and neuroinflammation. In migraine patients, higher MDA levels have been associated with more severe oxidative stress, contributing to the activation of pain pathways and the release of pro-inflammatory cytokines that play a crucial role in migraine episodes. Research suggests that oxidative stress, as indicated by elevated MDA levels, correlates with the frequency and intensity of migraine attacks. This relationship highlights the potential for oxidative stress as a target for therapeutic intervention, as antioxidants may alleviate symptoms and reduce the frequency

of attacks. Additionally, MDA can exacerbate neuroinflammation, which is a critical component of migraine pathophysiology, suggesting that managing oxidative stress could mitigate migraine severity and frequency. (Karsan & Goadsby, 2021)

Malondialdehyde (MDA) concentration has long been measured and utilised as a lipid peroxidation marker in oxidative stress and redox signaling research. In general, lipid peroxidation is the process by which oxidants, such as free radicals, target lipids that have one or more carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs). Lipid peroxidation has been extensively documented in the last forty years, demonstrating its significance for human health and cell biology (Ayala et al., 2014). Apolar and Polar are the two traditional groups into which lipids are classified. Mammals normally store the majority of their energy in adipose tissue (fat), but they may also store triglycerides (apolar) in other types of cells. Polar lipids are structural components of cell membranes that help create lipid bilayers, which act as permeability barriers for cells and subcellular organelles. Glycerol-based phospholipid is the primary lipid type that differentiates this bilayer in nearly all membranes. The fact that lipids may change a membrane organelle's biophysical properties, like as polarity and permeability, to control its physiological state emphasizes. The significance of the membrane lipid physical (phase) condition. In biology, lipids play a vital role in signalling molecules. Uncontrolled oxidative damage can harm cells, tissues, and organs. Oxidative stress is defined as a prooxidant-antioxidant imbalance that favours prooxidants. High amounts of reactive oxygen species (ROS), also known as free radicals, have been shown to directly damage lipids. Peroxisomes, endoplasmic reticulum, mitochondria, and the plasma membrane are the primary sources of endogenous ROS generation. These ROS are generated through processes such as enzymatic reactions and the autooxidation of substances like hydroquinone and catecholamines. Various external stimuli, such as UV rays, ionizing radiation, smoke, tobacco, infections, environmental toxins, and herbicide/insecticide exposure, can also trigger ROS production in vivo. Among the ROS, hydroperoxyl (HO•2) and hydroxyl radical (HO•) are the most impactful on lipids. The hydroxyl radical (HO•) is one of the smallest, most reactive, and highly mobile forms of activated oxygen that is water-soluble. This short-lived molecule can be produced from O2 under different stress conditions and during cell metabolism. Each cell generates about fifty hydroxyl radicals per second, resulting in roughly 4 million hydroxyl radicals daily, which

can attack or neutralize biomolecules. Hydroxyl radicals indiscriminately target biomolecules within a few nanometers of their formation site, causing oxidative damage to cells and contributing to diseases such as neurodegeneration, CVD and cancer.

MDA is created by the enzymatic or non-enzymatic degradation of arachidonic acid and bigger PUFAs. Although the enzymatic synthesis of MDA is widely recognised, its biological activities and possible dose-dependent dual roles are poorly understood. Despite this, MDA is less hazardous than 4-HNE and methylglyoxal (MG) and has a higher chemical stability and membrane permeability than ROS. Some studies show that MDA may affect gene expression and behave as a signaling messenger.

1. Recent research suggests that MDA primarily controls islet glucose-stimulated insulin secretion (GSIS) via the Wnt pathway. Moderate dosages of MDA (5 and 10 μM) activated islet GSIS, elevated cytosolic Ca2+ levels and ATP/ADP ratios, and influenced the expression of key GSIS regulator genes, protein synthesis, and activity.
2. MDA stimulated collagen gene expression in hepatic stellate cells by upregulating Sp1 and Sp3 proteins, as well as the Sp1 gene. Sp1 and Sp3 can interact with and recruit proteins involved in the transcription initiation complex, histone-modifying enzymes, and chromatin remodelling complexes, indicating that they play important roles in chromatin remodelling and gene expression control. Despite their potential therapeutic utility, the processes behind nonenzymatic MDA generation remain poorly understood. MDA is hypothesised to be formed in response to stress and has a high affinity for forming adducts with other biomolecules such as proteins and DNA. Excessive MDA production has been associated to several clinical conditions (Cordiano et al., 2023).

The intracellular antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) controls the baseline levels of oxidative stress caused by the generation of superoxide ($O_2^{\cdot -}$) in the cytosol and mitochondria. SOD1 is highly abundant in the cytosol, unlike the other two human superoxide dismutases: extracellular superoxide dismutase (SOD3), which is linked to the extracellular matrix, and Mn superoxide dismutase (SOD2), which is found exclusively in mitochondria (Hayyan et al., 2016). It efficiently converts $O_2^{\cdot -}$ to O_2 and H_2O_2 through oxidation and reduction, facilitated by the redox cycling of Cu^+ within the two active sites of the SOD1 homodimer. SOD1 regulates signal transduction pathways involving reactive

oxygen species (ROS) and promotes cytoprotective mechanisms, such as initiating gene transcription in response to neurotoxic stimuli. (Trist et al., 2020)

Superoxide dismutase (SOD) is a crucial antioxidant enzyme that plays a significant role in mitigating oxidative stress, a factor implicated in the pathophysiology of migraine. SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide, thereby protecting cells from oxidative damage. Research has demonstrated that individuals with migraine often exhibit reduced levels of SOD, which may lead to increased oxidative stress and subsequent neuronal damage. This deficiency could exacerbate inflammatory processes, as oxidative stress is known to trigger the release of pro-inflammatory cytokines and neuropeptides, such as calcitonin gene-related peptide (CGRP), which are central to the migraine attack mechanism (Lee et al., 2020).

Elevated oxidative stress can contribute to neuronal excitability and central sensitization, making the nervous system more susceptible to migraine triggers. Moreover, studies have shown that antioxidant therapies, including those targeting SOD levels, might provide therapeutic benefits for migraine sufferers. By enhancing the antioxidant defense system, these therapies may help reduce the frequency and severity of migraine attacks, highlighting the potential for SOD as a biomarker and therapeutic target in migraine management. (Younus, 2018)

Superoxide dismutase (SODs) are ubiquitous enzymes found in all oxygen-containing life forms. They act as catalysts to convert superoxide into hydrogen peroxide and oxygen. Superoxide anions are produced as a result of various metabolic activities, such as mitochondrial respiration, and are intentionally produced by certain signaling enzymes. SOD enzymes regulate a range of reactive oxygen species (ROS) and reactive nitrogen species, limiting the potential toxicity of these molecules and controlling many aspects of cellular life governed by their signaling roles. (Wang et al., 2018)

1.2 Research Gap / Rationale:

The rationale for conducting this study is based on the emerging evidence suggesting potential links between migraine and biological parameters such as vitamin D, zinc levels, inflammatory biomarkers and oxidative stress. These associations have important implications for understanding the underlying mechanisms and developing targeted

interventions for migraine prevention and management. Furthermore, addressing the contextual gaps, such as population diversity, environmental influences, healthcare disparities, cultural and lifestyle factors, and patient perspectives, is essential for ensuring the relevance and applicability of the findings in diverse populations and real-world settings. By investigating the role of vitamin D, Oxidative stress & inflammatory biomarkers in migraine, this study aims to contribute to the knowledge base and provide evidence-based strategies for optimizing prevention and treatment approaches.

1.2.1 Theoretical Gap

The specific mechanisms underlying the relationship between vitamin D, zinc, oxidative stress inflammatory biomarkers in migraine, are still not fully understood. Further research is needed to elucidate the molecular pathways through which these factors interact and influence each other. The optimal vitamin D levels for migraine prevention and reduction of oxidative stress are still debated. Determining the ideal range of vitamin D levels that may provide the most benefit requires more research. Additionally, identifying whether there is a threshold effect or a dose-response relationship between vitamin D levels and migraine and oxidative stress outcomes is important.

1.2.2 Contextual Gap

Most studies investigating the relationship between vitamin D, zinc, oxidative stress inflammatory biomarkers & migraine have been conducted in specific populations or regions. There may be variations in the prevalence of vitamin D deficiency, migraine subtypes, and inflammatory markers across different ethnicities, geographical locations, and socioeconomic groups. Therefore, it is important to expand research to diverse populations to ensure the generalizability of the findings. Environmental factors such as sun exposure is a primary source of vitamin D synthesis in the body, and it can vary significantly depending on geographical location, climate, and season. The impact of environmental factors on vitamin D, zinc levels, inflammatory biomarkers, and migraine prevalence should be

considered. Additionally, exposure to environmental pollutants or toxins may influence the relationship between these variables and could be an important contextual factor to explore.

1.2.3 Methodological Gap

Migraine is often episodic in nature, and their relationship with vitamin D, zinc and oxidative stress or inflammatory biomarkers may change over time. Longitudinal studies with repeated measures can provide more insights into the temporal associations and help establishing the causal relationships. Genetic predisposition can be added in the study for the understanding of proper pathophysiology at molecular level.

1.3 Problem Statement

Despite emerging evidence suggesting a potential role of vitamin D, zinc, oxidative stress and inflammatory biomarkers in migraine, there is a lack of comprehensive understanding regarding the causal relationships, underlying mechanisms, and optimal interventions. Additionally, contextual factors such as population diversity, environmental influences, healthcare disparities, cultural and lifestyle factors, and patient perspectives have not been adequately addressed. Therefore, there is a need for further research to bridge these knowledge gaps and provide a solid foundation for effective prevention and management strategies of migraine.

1.4 Research Question/ Hypothesis of Study

A) Null Hypothesis:

Vitamin D, Zinc, Oxidative stress and Inflammatory biomarkers are not associated with migraine

B) Alternate Hypothesis:

Vitamin D, Zinc, Oxidative stress and Inflammatory biomarkers are associated with migraine

1.5 Objectives

1. To estimate the levels of vitamin D, Zinc, Oxidative stress and Inflammatory markers in migraine patients.
2. To determine the association of vitamin D, Zinc, Oxidative stress and inflammatory markers in migraine.

1.6 Significance of Study

Migraine is a debilitating neurological condition that significantly impacts the quality of life. Recent research suggests that the deficiency of certain vitamins and minerals, as well as imbalances in oxidative stress and inflammatory markers, play a crucial role in the onset and severity of migraines. This study aims to investigate the relationship between migraine and various biological parameters, including Vitamin D, zinc, malondialdehyde (MDA), superoxide dismutase (SOD), interleukin-10 (IL-10), and interferon-gamma (IFN-γ).

By understanding these connections, we hope to highlight the importance of early detection and treatment of deficiencies in these parameters. Addressing Vitamin D deficiency, along with supplementing zinc and other antioxidants, may reduce the frequency and severity of migraines. Moreover, managing oxidative stress and inflammation through targeted interventions could lead to significant improvements in the overall wellbeing of individuals suffering from migraines. Notably, the prevalence of migraine in Pakistan is estimated to be 22% (Herekar AA, 2017), underscoring the urgent need for effective preventive measures.

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CHAPTER 2

LITERATURE REVIEW

A prevalent chronic illness, migraine is categorized by frequent pain, migraines cause incapacitating headache symptoms and episodes for instance aura. While few monogenic variations exist in this multifactorial condition, it is often comorbid with myocardial infarction, depression, epilepsy, and stroke. Aura is likely triggered by spreading depolarization, which can activate trigeminal sensory pathways and lead to headaches. Contrary to popular belief, vasoconstriction is not necessary for antimigraine medications to be effective; vasodilation is merely a secondary effect. Mild episodes are treated with analgesics or NSAIDs, while moderate to severe attacks are managed with 5HT(1B/1D) receptor agonists or triptans. (Ferrari et al., 2022)

Promising acute migraine therapies include lasmiditan, a selective 5HT1(F) receptor agonist, and CGRP receptor antagonists, also known as "gepants." For chronic migraine sufferers (those with migraines more than 15 days a month), intramuscular onabotulinumtoxin A can be beneficial. Additionally, monoclonal antibodies directing towards CGRP or its receptor, along with two gepants, shown to be effective and well-tolerated for preventing migraine attacks. Various neuromodulation techniques are also suggested for preventive and acute migraine management. These developments highlight the promising future of migraine therapy (GDaIIaP, 2016).

Migraine is a severely unbearable primary headache, affecting around 15% of the general population annually. The Global Burden of Disease Study states that migraine is considered as the second most common neurological condition globally and causes more debility than the other neurological conditions together (Feigin et al., 2019) Clinically, migraine is characterized by recurring headache episodes and various associated symptoms. Around one-third of sufferers practice acute neurological abnormalities, called as migraine

aura, that can occur before, during, or after a migraine attack. It is widely accepted that both central and peripheral activation of the trigeminovascular system shows a vital part in producing the pathophysiology of migraines, particularly in chronic migraine, where episode frequency increases. The underlying neurophysiological basis of migraine aura is presented as cortical spreading depression. However, there are currently few mechanism-based therapeutic options, and much about the specific pathogenic processes remains unclear. (Ashina et al., 2021)

Personalized dietary treatments for many illnesses, including metabolic syndrome, are being designed through the expanding field of precision nutrition. It's interesting to note that migraine has been associated with metabolic disorders or metabolic endocrine disorders. An exclusion diet approach has been established in the area due to the recognition of the role that dietary components play in the aetiology of headaches. It is well acknowledged that certain food elements can cause migraine episodes and that migraines are sensitive to diet. There are extensive lists of possible food triggers, but the area is still debatable. There have been reports of issues with citrus fruits, choclates, ice cream, nuts, onions, dairy products, alcohol, tomatoes, coffee, caffeine, nitrites, sucralose, histamine, tyramine, and monosodium glutamate (MSG). A patient's reaction to a particular food trigger may vary depending on a number of parameters, including the time and quantity of exposure. High doses of certain foods or substances, such as MSG or aspartame, can trigger headaches, as experiments have shown. Some foods, like coffee, might cause headaches, while others can induce headaches when consumption is stopped. Certain foods or chemicals may only produce headaches in certain patient populations, such as those with coeliac disease or those who have diet-related immunological responses, including those who test positive for IgG antibodies. As a result, determining food triggers might be difficult. Certain serological tests and food diaries have been used to pinpoint triggers for particular individuals. (Razeghi Jahromi et al., 2019)

Several nutrients, especially vitamins and minerals, seem to be linked to migraines. Therefore, dietary, behavioral, and lifestyle changes become viable options for preventing migraines, and vitamin supplementation, particularly riboflavin (vitamin B2), is recognized as a helpful additional treatment. Vitamin D, a fat-soluble vitamin, is derived from animal sources and obtained through skin contact to sunshine. The active form of vitamin D, 1-25 dihydroxyvitamin D, is not a good indication of blood vitamin D levels since it is produced

in the kidneys by hydroxylation. Instead, vitamin D levels are assessed using its precursor form, 25-hydroxyvitamin D (25(OH)D), which is created when the liver hydroxylates the vitamin acquired via food or produced by the skin (Donmez et al., 2018).

The vascular hypothesis of migraine involves the dilation of meningeal arteries and the production of the potent vasodilator calcitonin gene-related peptide (CGRP). A potential connection between vitamin D deficiency and migraines could stem from calcium's role in the contraction of smooth muscle cells in artery walls, which leads to vasoconstriction. Moreover, the vitamin D receptor (VDR) is extensively dispersed throughout the brain and has numerous physiological and neural functions. Vitamin D has already been associated with various neurological conditions, suggesting a possible link between vitamin D deficiency and migraines. (Conte, et al 2020)

Vitamin D is a fat-soluble substance that has hormone-like properties. UVB radiation on 7-dehydrocholesterol (provitamin D3) can cause it to be eaten or generated in the epidermis, where it is converted into 1,25(OH)2D, an active form of vitamin D synthesised in the liver and kidney by hydroxylation. Sun exposure produces around 90-95% of vitamin D in humans. Only fatty fish and sunlight-grown mushrooms contain vitamin D. Endogenous vitamin D production is controlled by environmental factors such as sun exposure, season, and latitude (Institute of Medicine, 2011).
Vitamin D has several roles, one of which is enhanced calcium absorption. Calcium/phosphorus equilibrium for bone mineralisation, prevention of numerous autoimmune illnesses, chronic diseases, endothelial dysfunction, immunological dysfunction, and oxidative stress. Oxidative stress occurs due to reactive oxygen species (ROS) which results in cellular damage. Under normal conditions, however, there is a balance of antioxidant & oxidants activity. Superoxide dismutase, Glutathione peroxidase and numerous vitamins, including vitamin D, A & E & several other substances, are part of the antioxidant defense system. Vitamin D, in particular, has been shown to alleviate oxidative stress by positively regulating cellular glutathione and superoxide dismutase. (Renke et al, 2023)

Vitamin D is an important regulator of inflammation. Its deficiency is associated with increased expression of inflammatory markers such as CRP and IFN-γ. Migraine is related with Vitamin D deficiency and raised inflammatory markers. Migraine is a neurologic illness

defined as a recurrent headache syndrome accompanied by numerous signs of neurologic dysfunction in varied admixtures or an episodic headache. It is unilateral and throbbing in nature, and it is linked with nausea, vomiting, photophobia, and phonophobia. Patients suffering from migraine might be classified as having resistance or refractory migraines. On the basis of types, migraine is classified as: 1. migraine with aura, 2. migraine without aura 3. chronic migraine, and 4. episodic migraine. It can cause very negative impact on both physical and mental health, including poor quality of life, diminished functioning, co-occurring psychiatric problems, tension-type headaches, anxiety, depression, and sleeplessness. The majority of migraine patients suffer from significant disabilities, resulting in impaired financial status, social and professional life, and decreased productivity, accounting for about 3% of disability, making it the eighth most burdensome sickness and the second largest contributor to disability globally-adjusted life years (Sacco S *et al*, 2022). One of the main regulators of mitochondrial respiratory function, oxidative stress, and systemic inflammation—and therefore, the ageing process in humans—is vitamin D. Consequently, 1,25(OH)2D inhibits oxidative stress, tissue and cell damage, and the ageing process through molecular and cellular mechanisms. Conversely, hypovitaminosis D increases oxidative stress and systemic inflammation while impairing mitochondrial functioning. The interaction of 1,25(OH)2D with its intracellular receptors alters vitamin D-dependent gene transcription and activates vitamin D-responsive elements, resulting in the activation of various second messenger systems. Sufficient vitamin D levels reduce oxidative stress and enhance mitochondrial and endocrine function, lessening the risk of diseases including autoimmune, infections, metabolic irregularities, and poor DNA repair. All of these benefits contribute to a healthy and graceful ageing process. Vitamin D is also a powerful antioxidant that supports proper mitochondrial function and protects against DNA damage, lipid peroxidation, and protein oxidation caused by oxidative stress. Following normalisation of serum 25(OH)D and tissue 1,25(OH)2D concentrations, new insights into vitamin D-related improvements in metabolomics, transcriptomics, and epigenetics, as well as its potential to manage oxidative stress in conjunction with micronutrients, vitamins, and antioxidants, are likely to promise more affordable and improved clinical outcomes in humans (Wimalawansa, 2019).

Numerous variables influence the metabolism and activities of vitamin D. A growing body of research demonstrates the biological connections between vitamin D and enhanced physical and mental health as well as lower risk of illness. The topic is quickly increasing, with new findings in the biology and metabolism of vitamin D, its impact on the vitamin D axis and gene polymorphisms, and the physiology of vitamin D-vitamin D receptor (VDR) interactions. While 1,25(OH)2D is involved in a variety of intracellular genomic activities, as well as biochemical and enzymatic reactions, 25(OH)D levels are critical for combating inflammation, eliminating invasive bacteria and parasites, reducing oxidative stress after repeated exposure to toxic agents, and slowing the ageing process. The expression of the nuclear factor erythroid-2 (Nf-E2)-related factor 2 (Nrf2) and Klotho, a phosphate-regulating hormone and antiaging protein, is both elevated in the presence of a physiological 25(OH)D concentration. Additionally, it makes protein stabilisation easier. Moreover, Klotho controls the cellular signalling pathways that result in the production of antioxidants (Tullet et al., 2017).

Vitamin D, which is well recognized for its character in bone health, has emerged as an important regulator of immunity to microbial danger. In immune cells such as macrophages, immune-specific inputs induce the expression of CYP27B1. At the site of infection, the hydroxylation of the 25-hydroxyvitamin D takes place which results in synthesis of hormonal 1,25-dihydroxyvitamin D (1,25D) an active form of vitamin D. Among the physiological roles of vitamin D expression of CYP27B1 in immune cells is critical in signaling immune system regulation. Cyp27b1 expression is inducible by interferon gamma (IFN-γ), a T cell cytokine generated by pro-inflammatory cytokines SASP senescent-associated secretory phenotype is a pro-inflammatory phenotype that includes inflammatory factors, chemokines, cytokines, interleukins, growth factors, and extracellular matrix proteases, and it accumulates consistently as senescent cells multiply in diverse organs. In the ageing process, there is an imbalance between inflammatory and immunological processes, which lowers the efficacy of immune responses and causes immunosuppression (Feehan, 2021). Inflammatory mediators enhance myelopoiesis and increase immunosuppressive cells, particularly regulatory T (Treg) cells and M2 macrophages that release immunosuppressive molecules such as IFN-γ, ROS. Vitamin D (1,25-dihydroxycholecalciferol) release is also known to be promoted by zinc. Zinc

furthermore serves as a vitamin D cofactor. For the vitamin D-dependent genes transcriptional activity zinc is required to perform pleiotropic activities such as mineral ion control. Zinc transporters are activated by vitamin D, by which zinc homeostasis is regulated. In proper quantities, zinc and vitamin D support the maintenance of a healthy physiological system; however, deficiencies in any of these nutrients can result in a variety of illnesses impacting practically all body systems. Zinc is required for the biological activities of 1,25-dihydroxycholecalciferol because zinc finger also selectively bind with vitamin D receptor (VDR) as it is bind to other steroid receptors. Zinc fingers are found in numerous transcriptional factors. Zinc also alters the DNA binding domain of 1,25-dihydroxycholecalciferol, making it unable to generate normal VDR structural conformation in the absence of zinc. As a result, zinc is required for the activation of vitamin D-dependent genes, making it an essential cofactor for vitamin D activity. Further study is needed to determine the specific chemical links between zinc and vitamin D. Understanding zinc's involvement in the body's physiologic processes, as well as its relationship to vitamin D, is critical for promoting preventive medicine and reducing disease burden (Amos, et al 2022).

Vitamin D reduces antigen presentation and the generation of interferon-gamma, while increasing the production of IL-10, an anti-inflammatory cytokine. In general, local production of 1,25(OH)2D by monocytes/macrophages resulted in a substantial shift in immunological status from pro-inflammatory to anti-inflammatory. It promotes a shift of immune profile by suppressing the expression of pro-inflammatory cytokines such as IFN-γ while inducing the expression of anti-inflammatory cytokines such as IL-10. Vitamin D is a major regulator of systemic inflammation, oxidative stress, as well as the ageing process. In turn, 1,25(OH)2D's molecular and cellular effects decrease oxidative stress, cellular damage, and the ageing procedure. Hypovitaminosis D, causes the opposite effects in the body by increasing the cellular damage increase oxidative burden and accelerate aging process & systemic inflammation. Many intracellular oxidative stress-related functions are downregulated when vitamin D levels are adequate. Suboptimal serum 25(OH)D levels also fail to moderate oxidative stress, resulting in increased intracellular oxidative damage and death. The vitamin D-activated enzyme glucose-6-phosphate dehydrogenase reduces nitrogen oxide (NOx), a major precursor to the production of ROS that convert oxygen to H2O2, while increasing superoxide dismutase (SOD), which regulates glutathione formation.

These vitamin D-related actions work together to lower the quantity of intracellular ROS. Activated vitamin D is required for the mitochondrial respiratory chains to continue working correctly and contribute in energy generation. Furthermore, 25(OH)D influences the expression of the uncoupling protein, which controls thermogenesis and is connected to the inner membrane of mitochondria. Chronic vitamin D deficiency reduces mitochondrial respiration capacity by causing changes in nuclear mRNA. Another mechanism that increases the risk of cancer is a decrease in adenosine triphosphate (ATP) through downregulation of complex I of the electron transport chain (Berridge, et al 2018). Even in otherwise healthy people with normal serum 1,25(OH)2D levels, vitamin D deficiency—defined as serum 25(OH)D concentrations less than 30 ng/mL—is linked to an increased risk of illnesses and disorders, as well as an increase in all-cause mortality. Some of vitamin D's important roles include reduced chronic inflammation and oxidative stress, as well as the preservation of mitochondrial respiratory capability. Vitamin D has considerable therapeutic benefits on lowering oxidative stress, inflammation, and energy metabolism due to its focused mitochondrial activity and numerous ROS-suppressing pathways. Vitamin D can enhance the anti-inflammatory cytokine IL-10 while lowering the pro-inflammatory cytokines TNF-, IL-1, IL-6, IL-8, and IL-12 production. (Wyckelsma, et al 2017)

Zinc function is important for immunity and human defense mechanism particularly in cancer cell. This is partially due to the tumor's ability to grow faster than the body's defenses can, as well as the fact that the tumor's produced molecules, for example transforming growth factor (TGF), interleukin 10 (IL-10), block the effects of cytotoxic agents. Interferon (IFN-γ) IL 1 & IL 6, are pro-inflammatory cytokines, is prevented from being produced by the anti-inflammatory cytokine IL-10. (Mungunsukh, et al 2021). The production of the pro-inflammatory cytokines rises in cases of zinc deficiency. And with optimal levels of zinc production of anti-inflammatory cytokine IL-10 takes place. Zinc also known to have a very potent antioxidant property like Superoxide dismutase (SOD 1, SOD3), as zinc converts free reactive radicals to water. This prevents the production of additional toxic free radicals and their derivatives (Wessels, et al 2013). Migraine severity and frequency is known to highly associated vitamin D deficiency. There might be neurogenic inflammation exacerbated by vitamin D deficiency, leading to increased migraine intensity. Furthermore, neuronal hyperexcitability in hypocalcemia has been identified. (Khan M. et al,

2019) The significance of vitamin D in the comorbidity of migraine is postulated, as the following elements;

(1) Central dopaminergic dysfunction is one of the commonly recognized pathogenic pathway is dopaminergic disturbance. Furthermore, dopaminergic nucleus malfunction may induce or worsen migraine by enhancing trigeminal nerve complex discharge, and dopamine receptor agonists are beneficial in lowering migraine attack frequency (S., et al 2019).

(2) Vitamin D has been shown in studies to enhance dopamine or its metabolites in the brain, as well as up-regulate glutathione and neurotrophin synthetase levels by blocking nitric oxide (NO) generation, which protects dopaminergic neurons. Consequently, low vitamin D levels can lead to dopaminergic issues and increase nitric oxide (NO) levels, which exacerbate migraine headaches (Nowaczewska M, et al 2020).

(3) The dural inflammation constantly stimulates the trigeminal neurovascular system, resulting in peripheral sensitization, which subsequently spreads to the trigeminal nucleus and even the thalamic neurons. Furthermore, the basal ganglia and/or descending dopaminergic circuits may be involved. Vitamin D works as an anti-inflammatory and analgesic by suppressing T-cell responses and lowering the production of pro-inflammatory cytokine (Togha M, et al 2018).

Previous study has linked migraine to higher levels of inflammatory biomarkers. Excessive pro-inflammatory cytokines and lipid mediators are linked to the pathophysiology of organ dysfunction in the early stages of inflammation. Cytokines and chemokines are important pain and molecular mediators in neurovascular inflammation. Following migraine episodes, several proinflammatory cytokines levels rose (R., 2018). Furthermore, cytokines may play an important role in the start and maintenance of pain by stimulating nociceptive sensory neurons. IL-1, IL-2, IL-4, IL-6, IL-10, TNF- IFN-γ are the most important cytokines associated with migraines. Recent research on migraine neuroinflammation highlight the participation of the inflammasome via inflammasome complex players such as IL-1 and IL-18 (Kursun O, et al 2021). When the body's antioxidant capacity is surpassed, oxidative stress occurs, which causes damage to cellular components such as proteins, lipids, and DNA. The majority of migraine trigger and aggravating factors may have one thing in common: oxidative stress. While a clear connection to energy homeostasis appears. While some of the more "metabolic" triggers, such as fasting/skipping meals, physical exercise, stress, and

relaxation, are obvious, the majority of the seemingly unrelated triggers, such as ovarian hormone changes, weather changes, alcohol, strong odours, intense light, and loud noises, may share a common denominator: changes in mitochondrial metabolism and/or oxidative stress. TRP channels, present in meningeal nociceptive nerve terminals, can be activated by oxidative, nitrosative, and nitric oxide stress. Electrophilic stress provides a method by which established migraine trigger variables that increase oxidative stress might cause migraine discomfort (Gross EC, et al 2019).

Neuroinflammation is hypothesized to alter the activation of the trigeminal nerves as well as the production of vasoactive neuropeptides, both of which are associated with migraine onset. Furthermore, vasoactive neuropeptides like CGRP and pituitary adenylate cyclase-activating polypeptide (PACAP) may induce trigeminovascular activation, arterial vasodilation, and mast cell degranulation, all of which are hypothesized to be involved in the neuro-inflammatory state linked to migraine. In contrast, after central trigeminal system activation, in addition to the release of NO and serotonin, CGRP and other components such as substance P (SP) and neurokinin A may be secreted. Finally, these chemicals may exacerbate inflammatory responses in the trigeminovascular system, specifically by increasing arterial vasodilation and mast cell degranulation. Furthermore, migraineurs may experience oxidative stress, which can damage cell membranes, nucleic acids, proteins, lipids, and extracellular components (such as collagen and proteoglycans). This may worsen CNS dysfunction in migraineurs (Dodick, 2018).

Research suggests that migraineurs have higher levels of pro-inflammatory cytokines (e.g., CRP, TNF-a, IL1-β, and IL-6), neuro-inflammatory factors (e.g., NO, CGRP, SP, histamine, vasoactive intestinal peptide (VIP), PACAP, neurokinin A), and oxidative stress biomarkers (e.g., malondialdehyde, thiobarbituric acid reactive substances, and total oxidant status) compared to healthy individuals (Yigit et al., 2018).

The pharmacological treatment of migraines comprises prophylactic medications (such as tricyclic antidepressants, beta-blockers, calcium channel blockers, serotonin–norepinephrine reuptake inhibitors (SNRIs), ergot derivatives, and nonsteroidal anti-inflammatory drugs (NSAIDs)) as well as medications that relieve acute attacks. A correct diagnosis and careful consideration of the best preventive medications for migraine treatment with the fewest side effects could significantly improve migraine symptoms because

headaches, and migraines in particular, are frequently progressive disorders that can affect patients for the rest of their lives and even interfere with daily functioning. Thus, novel approaches to therapy are needed to improve the characteristics of migraine attacks, stop them from getting worse, lessen the consequences and handicap associated with them, and improve the quality of life for those who suffer from them. One of the most crucial ways to get past the obstacles in migraine treatment is to use the right preventive or acute medications, eliminate or modify the suggested risk factors of migraine development or progression through various strategies, such as applying dietary interventions, treating comorbidities and sleep disturbances, and so on. Among these dietary supplements that may be helpful for migraines is vitamin D. Numerous review studies that discuss the role of dietary variables in headaches provided a concise explanation of the link between vitamin D and headaches (Lukacs et al., 2017)

It has been established that there is a negative correlation between the level of vitamin D and inflammation. Supplementing with this vitamin has been shown to lower levels of proinflammatory/oxidative agents, such as hs-CRP, CRP, IFN-g, IL-1β, IL-6, TNF-α, IL-17, and MDA; conversely, vitamin D administration has been shown to increase total antioxidant capacity and the secretion of anti-inflammatory factors, such as IL-4, IL-5, and IL-10. (Sepidarkish et al., 2019)

Research has shown that vitamin D, a crucial neurosteroid vitamin, possesses anti-inflammatory and antioxidant properties. Therefore, the endothelium system's performance, immunological responses, and cell proliferation may be impacted by this anti-inflammatory hormone. It's important to remember that a vitamin D shortage may affect the inflammatory processes in the brain, which may put the brain at risk for these pathophysiological events. Significantly, given the critical role the hypothalamus plays in migraine pathogenesis, the presence of vitamin D and its metabolites in this area increases the vitamin's significance in migraine. Thus, it stands to reason that treating a vitamin D deficit may help prevent the onset or progression of migraines. Therefore, it appears that vitamin D may be used as a therapeutic agent in addition to current migraine medications. (Anjum et al., 2018)

With anti-inflammatory and antioxidant qualities, zinc is a necessary trace element. It is recognized as a cofactor for antioxidant enzymes and is essential for neural signaling (Lewandowski et al., 2018) There is a moderate prevalence of zinc insufficiency among

migraine sufferers, according to some observational studies. Severe zinc deficiency can lead to neurological issues such as attention deficit disorder tiredness, memory impairment, and learning difficulties. Moreover, it has been demonstrated that migraine episodes are strongly correlated with low blood zinc concentrations. Zinc may be beneficial for migraine episodes because of the inflammatory nature of migraine and its role in oxidative stress.

OPERATIONAL DEFINITIONS

1,25(OH)2D: It is the most active form of vitamin D**.** (Feehan, 2021)

Migraine: Migraine is a neurologic illness defined as a recurrent headache syndrome accompanied by numerous signs of neurologic dysfunction in varied admixtures or an episodic headache. (Sacco S, 2022)

Oxidative Stress: Cellular damage due to activation of ROS. Or a misbalance between oxidant and anti-oxidant activity. (Renke, 2023)

ROS: They are oxygen-containing reactive species (Renke, 2023)

Neuroinflammation: Inflammatory response within brain and spinal cord (Kursun O, 2021)

Inflammasome: Multiprotein oligomers of the innate immune system found in the cytosol that are responsible for the activation of inflammatory reactions. (Kursun O, 2021)

Inflammatory biomarkers: these are inflammatory cytokines (IFN-γ) and acute phase proteins (CRP) (Kursun O, 2021)

Hyperexcitiblity: A propensity to overreact to stimuli, often happening during a manic episode. (Khan M., 2019)

CGRP receptor antagonists: Calcitonin gene-related peptide (CGRP) is a protein found in the brain and nervous system. Medications that block CGRP can help manage or treat migraines. (Edvinsson & Warfvinge, 2017)

Nitrosative: Oxidative/nitrosative stress happens when there are more harmful oxidants than protective antioxidants in the body. This imbalance can disrupt cell functions and cause molecular damage. (Ahsan, 2013)

Substance P (SP): Substance P plays a role in causing inflammation in the dura mater, the outer membrane of the brain, which is believed to be a source of migraine pain. (Carmody et al., 1996)

Neurokinin A: NK1 receptors are a type of G protein-coupled receptor that gets activated by Substance P, which is released from small sensory nerve fibers. When these receptors are activated, they cause a prolonged change in cell membrane voltage and increase the levels of calcium inside the cell. (Yaksh, 2014)

Neurosteroid: Neurosteroids affect the activity of GABA-A and NMDA receptors, and they may play a role in the development of migraines. (Koverech et al., 2019)

N-Methyl-D-aspartate (NMDA) receptor: The NMDA receptor is a protein in the brain that acts as a channel for ions. It becomes active when two substances, glycine and glutamate, attach to it. (Furukawa et al., 2005)

Nuclear factor erythroid-2(Nf-E2)-related factor 2(Nrf2): Nrf2 signaling helps reduce oxidative stress caused by reactive oxygen species (ROS), decreases neuroinflammation, and improves mitochondrial function. (Basu et al., 2022)

Metallothionein: Metallothioneins (MTs) are a family of proteins known for their high content of heavy metals and cysteine. They are believed to play important roles in protecting and repairing the nervous system. (Thirumoorthy et al., 2011)

Zincosomes: Zinc has important regulatory roles in cells, so its levels are tightly controlled. One way cells manage zinc is by storing it in and releasing it from special vesicles called zincosomes. (Gerd Wellenreuther et al., 2009

CHAPTER 3

METHODOLOGY

3.1 Study Design

Case Control Study

3.2 Subjects

Participants who fulfilled the inclusion criteria.

3.3 Place of sample collection/ Setting

This study was conducted in Medicine OPD PNS Shifa, hospital, Karachi

3.4 Inclusion Criteria:

- Both genders with age between 20 to 40 years
- Patient who reported in Medicine OPD of PNS Shifa Hospital with the history of migraine were included in the study
- Age matched healthy controls were added

3.5 Exclusion Criteria:

- Participants with any other type of headache
- Participants with any inflammatory disease
- Participants taking vitamin D & zinc supplements
- Participants taking any anti-oxidants and immunity boosting medications

3.6 Duration of Study

- 06 months

3.7 Sample Size

- Group A: 123 cases of Migraine
- Group B: 123 age matched controls

Calculated by OpenEpi, Version 3. 0% confidence interval and 5% confidence limit. Sample size was drawn by taking in consideration with the prevalence of migraine in Pakistan. (Herekar AA, 2017)

Sample Size for Unmatched Case-Control Study

For:

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	1
Hypothetical proportion of controls with exposure	40
Hypothetical proportion of cases with exposure:	22.68
Least extreme Odds Ratio to be detected:	0.44

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	113	112	123
Sample Size - Controls	113	112	123
Total sample size:	226	224	246

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19

CC = continuity correction
Results are rounded up to the nearest integer.

3.8 Sampling Technique

- Convenient Sampling

3.9 human subjects and consent

Atttached as annex

3.10 Materials

3.10.1 Questionnaire

Participants were given a questionnaire that comprise two sections in which Appendix I contain questions regarding demographic and general characteristics of the participants while Appendix II comprises question assessing, identification of migraine using ID-Migraine scale and impact of headache that will be assessed using HIT-6 scale.

3.10.2 Culture Media

N/A

3.10.3 Drugs

N/A

3.10.4 Equipment

3.10.4.1 SPIRIT SWAB

They are frequently employed to clean and disinfect the skin prior to administering an injection.

3.10.4.2 WEIGHING MACHINE

- A calibrated digital scale (ZT-120) was used to collect weight measurements. The scale was tested for accuracy and recalibrated prior recording each measurement.
- The participants of the study were asked to remove shoes, heavy outerwear, and any objects from their pockets before the measurement. They were instructed to stand upright in the center of the scale platform with their weight evenly distributed on both feet.

3.10.4.3 HEIGHT MACHINE

- Height measurements were obtained using a stadiometer (ZT-120). Before each set of measurements, the stadiometer was adjusted and leveled. Each participant was instructed to take off their shoes and stand barefoot against the stadiometer with their feet flat on the floor, their backs straight, and their heels together.
- Participants were instructed to keep their heads in the Frankfurt horizontal plane and look straight ahead.
- The participant's height was recorded to the closest 0.1 centimeter after which it was converted to meter by dividing the length in centimeters by 100.

3.10.4.4 BLOOD PRESSURE MACHINE

- Blood pressure was measured using a calibrated mercurial sphygmomanometer (Yamasu Model-600, Japan) in accordance with the American Heart Association (AHA) recommendations. 51
- The participants sat comfortably, with their backs supported, and feet flat on the floor. The cuff size was determined by the participant's arm circumference.
- The cuff was worn on the upper arm, 2.5 cm above the antecubital fossa. The arm of the subject was supported and positioned at the level of heart.
- The pressure in the cuff was gradually increased until it exceeded the anticipated systolic pressure. The pressure was gradually released, and the pressure gauge was observed while listening to the Korotkoff noises using a stethoscope.
- The systolic blood pressure was measured by the appearance of clear, repetitive tapping noises (Korotkoff Phase I). The diastolic blood pressure was measured as the complete cessation of noises (Korotkoff Phase V).

3.10.4.5 TORNIQUET

A tourniquet is a tool that doctors use to momentarily prevent blood from flowing to a particular region of the body. During blood collection (phlebotomy), a tourniquet is used to make veins stand out and make venipuncture easier. By putting a tourniquet around the area where the needle will be inserted, blood flow is briefly cut off which makes the veins fill up and easier to reach.

3.10.4.6 SYRINGE

It is particularly useful to draw small quantities of blood for laboratory analysis. In our study phlebotomy was performed using a 5cc syringe.

3.10.4.7 VACUTAINER

A vacutainer is a blood collection tube system brand that is commonly used in medical settings for blood sampling and specimen collection. Its primary function is to aid in the safe and efficient collection, storage, and transportation of blood samples for diagnostic purposes. A yellow top vacutainer of 5 ml was used for blood collection. In this type of

vacutainer, the gel separator is placed. After centrifugation, a gel barrier is formed in the tube that separates the serum from the cellular components of the blood. It aids in the preparation of clear, separated serum for laboratory examination. The clot activator encourages coagulation, allowing the blood sample to clot prior to centrifugation.

3.10.4.8 MICROPIPETTE

A micropipette is a typical device used in laboratories that is used to measure small quantities of liquids with a volume range between 1 and 1000µl. In addition to this, a micropipette may be used to move an exact quantity of liquid from one container to another. We used micropipettes of 100µl to dispense the analyte, standards and substrate solution.

3.10.4.9 PIPETTE TIPS

Pipette tips are autoclavable disposable tools for transferring liquids with a pipette. The yellow pipette tips were used to accurately pipette liquids with volumes between 10 and 200

3.10.4.10 EPPENDORF TUBES

Eppendorf Tubes are polypropylene tubes that can be used only once for storing and transporting solid and liquid samples and reagents, as well as for mixing, centrifuging, and transporting them. A 1.5 ml eppendorf tube was used to prepare serum aliquots after centrifugation.

3.10.4.11 ULTRA LOW TEMPERATURE FREEZER

Long-term sample preservation is possible in ultralow-temperature upright and chest freezers because of their superior insulation and cabinet- and door-mounted gaskets. (Gumapas & Simons, 2013). Samples were stored in a VWR® ULT Upright Freezers (40086A-VWR International, USA) which is set at –86 °C to maintain their integrity and stability for a long term sample storage. Serum aliquots were appropriately labeled and securely sealed in eppendorf tubes prior to placement in the cabinet inside the freezer. They were placed in the designated storage boxes within the ultra-low temperature freezer.

3.10.4.12 CENTRIFUGE MACHINE

Centrifuge machine is a device that generates centrifugal forces required for the purpose of separation of various substances on the basis of their density. The samples were centrifuged at 3500rpm set at 20°C for 3 minutes prior to biochemical analysis using a centrifuge.

3.10.4.13 VORTEX

A vortex is a piece of machinery that enables for the consistent mixing of different substances. Prior to biochemical analysis through ELISA machine the serum sample taken inside the Eppendorf tubes was homogenized using a vortex.

3.10.4.14 LABORATORY DRYING OVEN

Thermostatic lab dry oven is a device designed for low-temperature thermal treatments like heating, drying and testing at low temperatures in a setting with air flow.

3.10.4.15 ELISA MICROPLATE READER

ELISA plate reader is a device which detects and measure the changes in color in each well of the plate. They perform spectrophotometry, which is the measurement of the amount of light absorbed and reflected by an item, such as a protein. After addition of stop solution to the wells, the readings were obtained using microplate reader.

3.10.5 KIT USED

- **Human IL-10 ELISA KIT**

Cat.No:	E0102Hu
Standard Curve Range:	5-1500pg/ml
Sensitivity:	2.59pg/ml
Storage:	Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

- **Human IFN- γ ELISA KIT**

Cat.No:	E0105Hu
Standard Curve Range:	1-400ng/ml
Sensitivity:	0.49pg/ml
Storage:	Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

- **Human CRP**

Cat.No:	BPE193
Standard Curve Range:	15-1000pg/ml
Sensitivity:	2.92pg/ml
Storage:	Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

- **Human Vitamin D**

Cat.No:	E3339Hu
Standard Curve Range:	3-900ng/L
Sensitivity:	1.29ng/L
Storage:	Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

- **Zinc**

Cat.No:	330 001
wavelength:	546nm
linearity:	61.2 mmol/L

Storage: Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

- **Superoxide Dismutase**

1. Alkaline pH, superoxide anion of oxygen causes oxidation of adrenaline to adenochrome

2. 0.15ml of choloform (ice chilled) + 0.75ml of ethanol mixed with 0.1 ml of serum centrifuge at 3000rpm for 15 mins

t

3. Add 1.0 ml of 0.1M carbonate bicarbonate buffer (pH 10.2) 0.5 ml of EDTA (0.6mM) and epinephrine (1.8mM)

t

4. Absorbance recorded at 480nm

- **Malondialdehyde**

1. Add 0.1 ml serum with 3.0cm³ of glacial acetic acid and 3.0cm³ of thiobarbutyric acid (TBA) (1%) in 2%NaOH

t

2. the mixture will be boiled for 15 mins and allow to cool

t

3. Absorbance will be recorded at 532 nm

3.10.5.1 INTENDED USE

The Sandwich kit is for the accurate quantitative detection of in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

3.10.5.2 ASSAY PRINICPLE

Human IL-10

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL10 antibody. IL10 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL10 Antibody is added and binds to IL10 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL10 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL10. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Human IFN- γ

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre- coated with Human IFNG antibody. IFNG present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IFNG Antibody is added and binds to IFNG in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IFNG antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IFNG. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Human Vitamin D

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human CYP24A1 antibody. CYP24A1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human CYP24A1 Antibody is added and binds to CYP24A1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CYP24A1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human CYP24A1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Human CRP

1. **Sample and Detection Antibody**: Add 50 µL of the standard or sample into each well. Immediately add 50 µL of the Biotinylated Detection Antibody working solution to each well. Incubate the plate for 90 minutes at 37°C.
2. **Aspirate and Wash**: Remove the contents of the wells and wash the plate three times.
3. **HRP Conjugate**: Add 100 µL of the HRP conjugate working solution to each well. Incubate the plate for 30 minutes at 37°C. After incubation, aspirate the contents and wash the plate five times.
4. **Substrate Reaction**: Add 90 µL of the substrate reagent to each well and incubate for 15 minutes at 37°C.
5. **Stop Reaction**: Add 50 µL of Stop Solution to each well to halt the enzymatic reaction.
6. **Reading**: Immediately read the absorbance at 450 nm using a microplate reader.
7. **Standard Curve**: Use the absorbance values from the standard wells to create a standard curve. This can be done by plotting the known concentrations of the standards on the x-axis and their corresponding absorbance values on the y-axis.
8. **Sample Concentration**: Using the standard curve, determine the concentration of the samples by finding where their absorbance values fall on the curve.
9. **Final Calculation**: Apply any necessary dilution factors to get the final concentration of the analyte in the samples.

3.10.5.3 REAGENTS

Human IL-10 ELISA KIT

- Standard solution (1600 pg/ml): 0.5 ml
- Pre-coated ELISA plate: 12 strips of 8 wells
- Standard diluent: 3 ml

- Streptavidin-HRP: 6 ml
- Stop solution: 6 ml
- Substrate solution A: 6 ml
- Substrate solution B: 6 ml
- Wash buffer concentrate (25x): 20 ml
- Biotinylated Human IL-10 antibody: 1 ml
- User instruction: 1 copy
- Plate sealer: 2 pieces

Human IFN- γ ELISA KIT

- Standard solution (480 ng/ml): 0.5 ml
- Pre-coated ELISA plate: 12 strips of 8 wells
- Standard diluent: 3 ml
- Streptavidin-HRP: 6 ml
- Stop solution: 6 ml
- Substrate solution A: 6 ml
- Substrate solution B: 6 ml
- Wash buffer concentrate (25x): 20 ml
- Biotinylated Human IFN-γ (Interferon Gamma) antibody: 1 ml
- User instruction: 1 copy
- Plate sealer: 2 pieces

Human Vitamin D ELISA KIT

- Standard solution (1,25-dihydroxyvitamin D(3) 24-hydroxylase): 0.5 ml
- Pre-coated ELISA plate: 12 strips of 8 wells
- Standard diluent: 3 ml
- Streptavidin-HRP: 6 ml

- Stop solution: 6 ml
- Substrate solution A: 6 ml
- Substrate solution B: 6 ml
- Wash buffer concentrate (25x): 20 ml
- Biotinylated Human CYP24A1 (1,25-dihydroxyvitamin D(3) 24-hydroxylase) antibody: 1 ml
- User instruction: 1 copy
- Plate sealer: 2 pieces

Human CRP ELISA KIT

- Micro ELISA Plate (Dismountable): 96T: 8 wells × 12 strips
  Storage: 2-8°C for 1 month
- Reference Standard: 96T: 2 vials
  Storage: Discard unused reconstituted standard and dilutions
- Reference Standard & Sample Diluent: 1 vial, 20 mL
  Storage: 2-8°C
- Biotinylated Detection Antibody Working Solution: 1 vial, 6 mL
  Storage: (not specified in your list)
- HRP Conjugate Diluent: 1 vial, 14 mL
  Storage: (not specified in your list)
- Concentrated Wash Buffer (25×): 1 vial, 30 mL
  Storage: (not specified in your list)
- Concentrated HRP Conjugate (100×): 96T: 1 vial, 120 μL
  Storage: 2-8°C (Protect from light)
- Substrate Reagent: 1 vial, 10 mL
  Storage: (not specified in your list)
- Stop Solution: 1 vial, 10 mL
  Storage: 2-8°C
- Plate Sealer: 5 pieces

- Product Description: 1 copy
- Certificate of Analysis: 1 copy

3.10.5.4 OTHER MATERIALS REQUIRED

I. An incubator set to 37°C ± 0.5°C

II. Absorbent paper

III. Precision pipettes with disposable tips

IV. Clean test tubes

V. Deionized or distilled water

VI. A microplate reader equipped with a 450 nm ± 10 nm wavelength filter

3.10.5.5 PRECAUTIONS

- Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- This instruction must be strictly followed in the experiment.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don’t expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

3.11 Parameters of Study:

3.11.1 Anthropometric parameters

Age, Gender, Weight, Height, BMI

3.11.2 Lifestyle Parameters:

Smoking, Dietary patterns, Fish intake, Milk intake, Fizzy drink intake, Sunlight exposure, Working hours

3.11.3 Migraine scale:

Migraine scale is added in the study for the detection of intensity of headache.

- Intensity of migraine will be tested by Headache Impact Test (HIT-6) which was designed to provide a global measure of adverse headache impact (Ware JE, 2000).

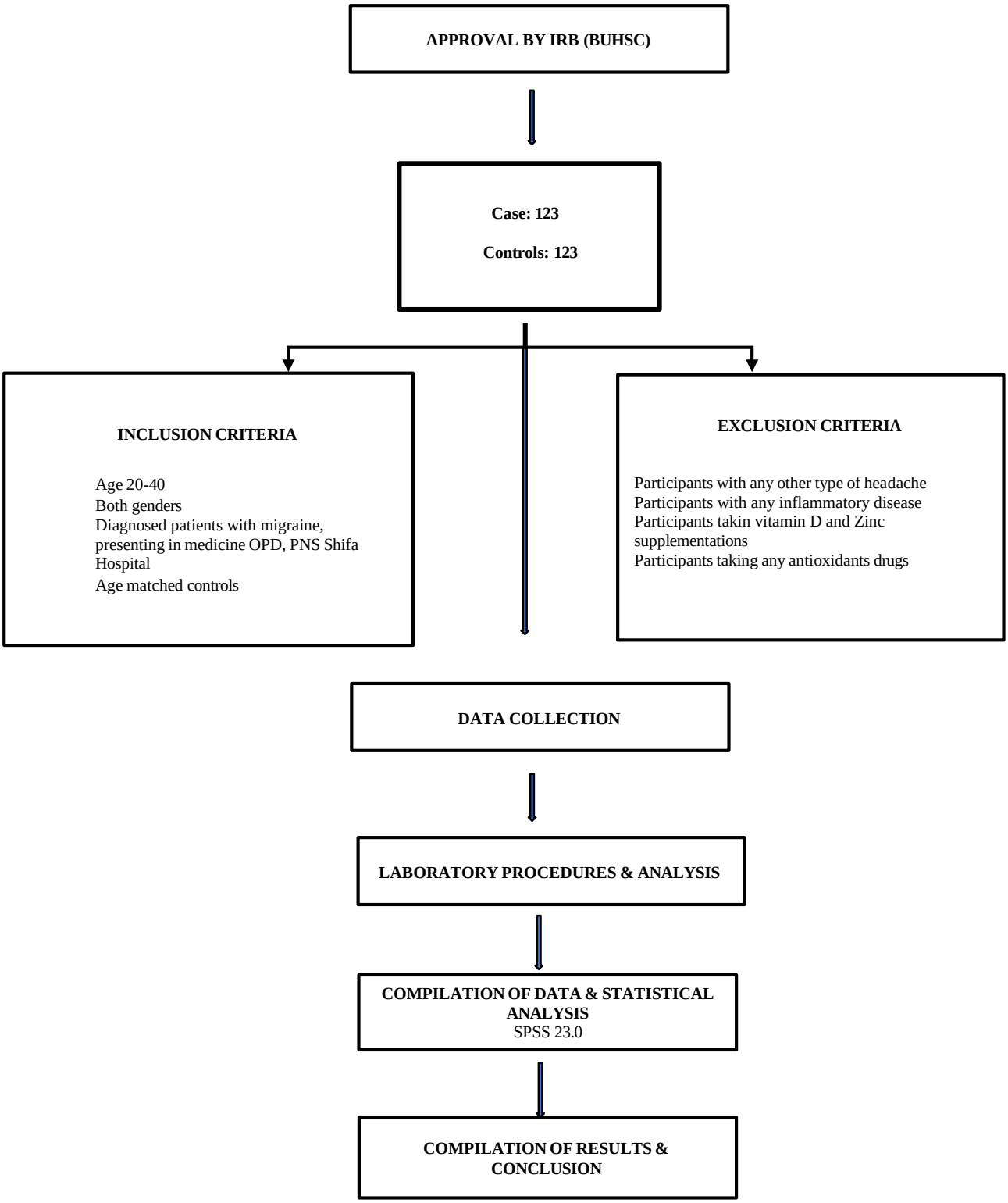
3.11.4 Biochemical Biomarkers:

Vitamin D, Malondialdehyde, Superoxide dismutase, Interferon Gamma (INF-γ), CRP, IL-10, Zinc.

3.12 Protocol of Study

- Ethical approval and informed consent was obtained
- General information of participants was documented
- Questionnaire and subject evaluation form was filled by participants
- Identification of migraine was done by ID-Migraine scale (Lipton RB, 1999,)
- Intensity of headache was assessed by HIT-6 scale (Ware JE, 2000)
- 5 ml of blood was taken from the subjects after ensuring the septic measures using 5cc syringe
- The samples were centrifuged at 3000rpm to separate serum from the whole blood for serum analysis of the biomarkers
- Samples were refrigerated at -40°c freezer

3.13 Algorithm of study



3.14 STATISTICAL ANALYSIS

- Data were analyzed using IBM-SPSS version 23.0;
- Counts with percentages were reported for gender, cast, House environment, Milk, Fish, and Fuzzy drink intakes of controls and migraine patients.
- Mean with standard deviation were given for age, weight, BMI, SBP, DBP, it is Body Temperature, and working hours per day of controls and migraine patients. Descriptive on migraine triggers, family history of migraine, caffeine intake, physical activity, co morbidity, self-medication, medicines used intensity of migraine, symptoms of headache, characteristics of headache, sleep hours per night and HIT-6 scales were also reported for migraine patients.
- Median with Interquartile range (IQR) for CRP, IL-10 levels, IFN Gamma, Vitamin D, Zinc, SOD, and MDA were also reported for two studied groups.
- Pearson Chi Square test was used to check the association of cast, gender, house environment, milk, fish and fuzzy drink intake between groups.
- Independent sample t-test was used to compare the mean of baseline characteristics of controls and migraine patients.
- Mann Whitney U test was used to compare the median of skewed and not normally distributed parameters between two study groups.
- Pearson Correlation was used to check the relationship of age, BMI, SBP, DBP. Temperature, CRP, IL-10, IFN Gamma, vitamin D, Zinc, SOD and MDA with each other among migraine, controls and overall samples.
- Binary Logistic regression analysis was used to estimate the risk estimation of migraine using demographic, baseline characteristics, diet intakes, CRP, IL-10, IFN

gamma, vitamin D, SOD and MDA, odds ratio with 95% confidence interval were reported in univariate model and multivariate model, multivariate models were adjusted with age and gender.

- P-values less than 0.05 were considered statistically significant.

CHAPTER 4

RESULTS

A total 246 participants were taken during this study period. These participants were divided into 2 groups, out of which 123 were migraine cases and 123 were controls. Biochemical parameters of these groups were compared in the study.

Table-4.1 reports the cast, gender and house environment among controls and migraine patients, in control group cast Balochi were (18.7%), Muhajir were (2.4%), Pathan were (19.5%), Punjabi were (37.4%), Sindhi were (11.4%), and Siraiki were (10.6%), for gender male were (19.5%), female were (80.5%), for house environment resident in house were (13%),bungalow were (5.7%), and apartment were (81.3%), whereas in migraine patients, Balochi were (17.1%), Muhajir were (5.7%), Pathan were (18.7%), Punjabi were (32.5%), Sindhi were (14.6%), Siraiki were (11.4%),male were (14.6%), female were (85.4%), resident in house were (8.1%),bungalow were (8.9%), and apartment were (82.9%). Pearson Chi Square test did not give any significant association of these cast, gender and house environment with migraine ($p>0.05$).

Table 4.1: Association of Cast, Gender and House Environment with Migraine

Characteristics		***Group***				***p-value***
		Control (n=123)		***Migraine (n=123)***		
		n	***%***	***n***	***%***	
Cast	Balochi	23	18.7	21	17.1	0.75
	Muhajir	3	2.4	7	5.7	
	Pathan	24	19.5	23	18.7	
	Punjabi	46	37.4	40	32.5	
	Sindhi	14	11.4	18	14.6	
	Siraiki	13	10.6	14	11.4	
Gender	Male	24	19.5	18	14.6	0.30
	Female	99	80.5	105	85.4	
House environment	House	16	13.0	10	8.1	0.31
	Bungalow	7	5.7	11	8.9	
	Apartment	100	81.3	102	82.9	
*p<0.05 was considered statistically significant using Pearson Chi Square test						

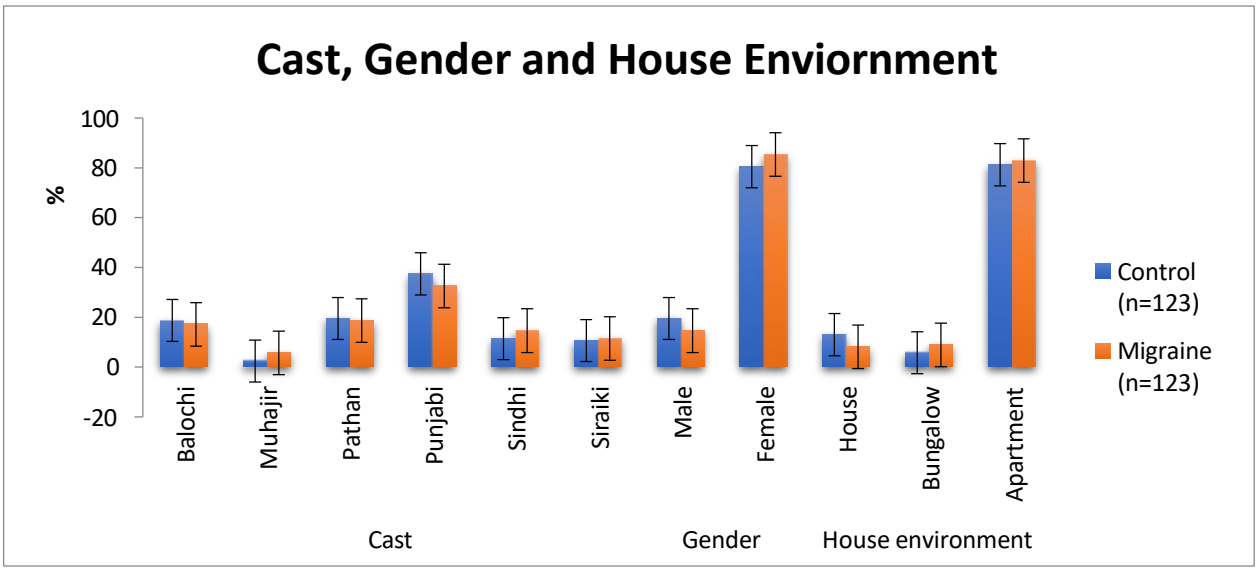


Figure 4.1: Association of Cast, Gender and House Environment with Migraine

Table-4.2 gives the mean comparison of baseline characteristics between controls and migraine patients, in control group mean age (years) was33.6 (SD=±4.7), mean weight (kg) was 68 (SD=±10), mean height (m) was 1.6 (SD=±0.1), mean BMI (kg/m2) was 27.7 (SD=±5.1), mean SBP was 122.2 (SD=±9), mean DBP was 81.8 (SD=±4.4), mean temperature was 98.1 (SD=±0.2), and mean working hours were 3.1 (SD=±4.2) units, whereas in migraine patients mean Age (years) was33.2 (SD=±4.6), mean weight (kg) was 69.4 (SD=±9.2), mean height (m) was 1.6 (SD=±0.1), mean BMI (kg/m2) was 28.4 (SD=±4.8), mean SBP was 121.5 (SD=±10), mean DBP was 81.9 (SD=±4.7), mean temperature was 98 (SD=±0.1), mean working hours was 2.5 (SD=±3.9) units. Independent sample t-test showed significant mean difference in temperature between controls and migraine patients ($p=0.027$), all other characteristics were found statistically insignificant ($p>0.05$).

Table 4.2: Mean Comparison of Baseline Characteristics between Migraine and Controls

Parameters	***Control (n=123)***		***Migraine (n=123)***		***p-value***
	Mean	***SD***	***Mean***	***SD***	
Age (years)	33.6	4.7	33.2	4.6	0.54
Weight (kg)	68.0	10.0	69.4	9.2	0.23
Height (m)	1.6	0.1	1.6	0.1	0.65
BMI (kg/m2)	27.7	5.1	28.4	4.8	0.21
SBP	122.2	9.0	121.5	10.0	0.57
DBP	81.8	4.4	81.9	4.7	0.87
Temperature	98.1	0.2	98.0	0.1	0.027*
Working hours	3.1	4.2	2.5	3.9	0.23
*p<0.05 was considered statistically significant using Independent sample t-test					

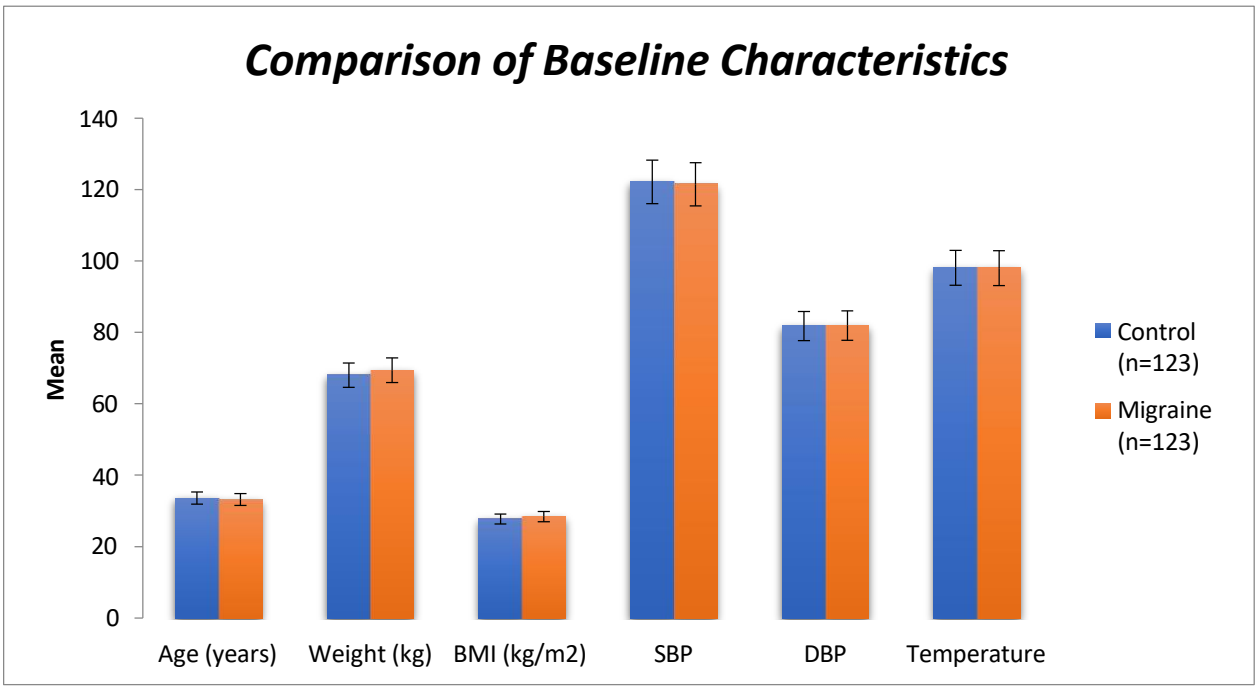


Figure 4.2: Mean Comparison of Baseline Characteristics between Migraine and Controls

Table-4.3 reports the association of diet intakes with migraine, in control group Samples with milk intake Yes were (67.5%), Occasionally milk intake were (4.9%), fish intake Yes were (52.8%), Occasionally fish intake were (9.8%), and fuzzy drinks intake Yes were (42.3%), Occasionally fuzzy drink intake were (26.8%), whereas among migraine patients milk intake Yes were (65%), Occasionally milk intake were (0.8%), fish intake Yes were (62.6%),Occasionally fish intake were (7.3%), and fuzzy drinks intake Yes were (33.3%),Occasionally fuzzy drink intake were (32.5%), Pearson Chi Square test did not showed any significant association of milk, fish and fuzzy intake drink between controls and migraine ($p>0.05$).

Table 4.3: Association of Milk, Fish and Fuzzy Drink with Migraine

Variables		***Group***				***p-value***
		Control (n=123)		***Migraine (n=123)***		
		n	***%***	***n***	***%***	
Milk in take	Yes	83	67.5	80	65.0	0.10
	Occasionally	6	4.9	1	0.8	
	No	34	27.6	42	34.1	
Fish intake	Yes	65	52.8	77	62.6	0.29
	Occasionally	12	9.8	9	7.3	
	No	46	37.4	37	30.1	
Fuzzy drink intake	Yes	52	42.3	41	33.3	0.33
	Occasionally	33	26.8	40	32.5	
	No	38	30.9	42	34.1	
*p<0.05 was considered statistically significant using Pearson Chi Square test						

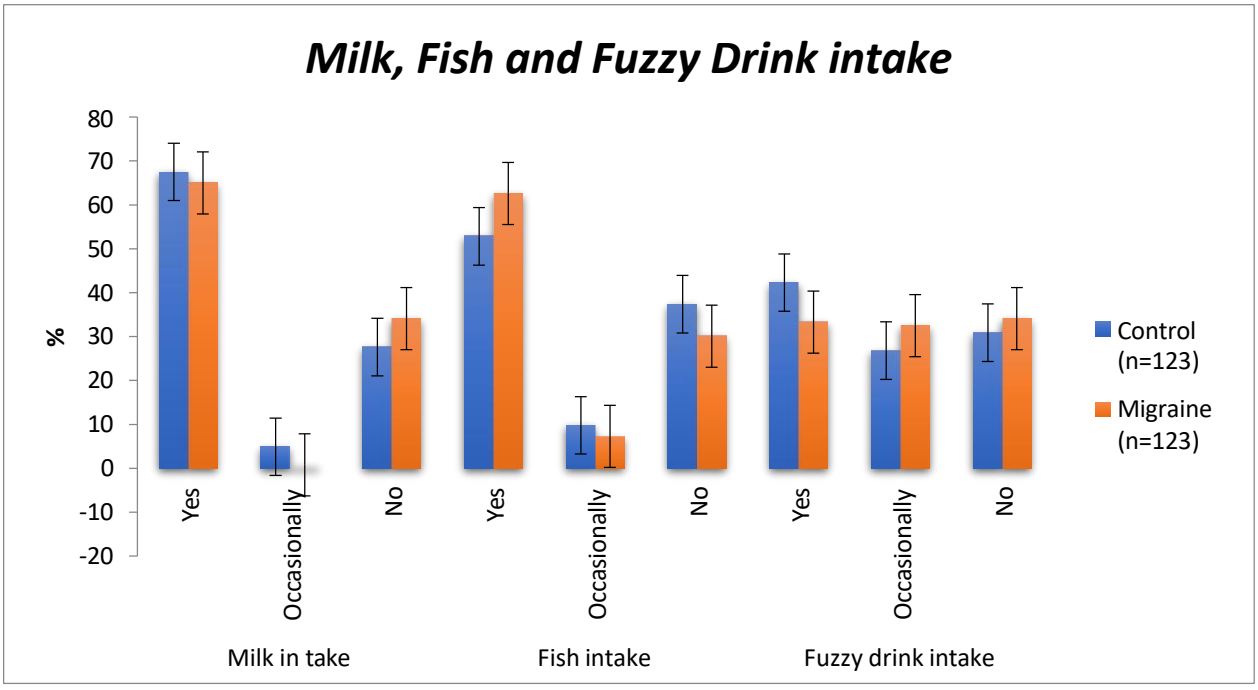


Figure 4.3: Association of Milk, Fish and Fuzzy Drink with Migraine

Table-4.4 reports the observed characteristics of migraine patients, in migraine triggers cases of Caffeine/ screen were (0.8%), Less sleep were (2.4%), Less sleep/screen were (17.1%), Physical activity were (0.8%), Physical activity/ less sleep were (35%), Physical activity/screen/ Less sleep were (17.9%),only Screen were (0.8%), and none were (25.2%), all were found with negative family history of migraine (100%), caffeine intake were (40.7%), physically active were (74.8%),with co morbidity were (16.3%), self-medicated were (57.7%), all were uses medicines Inderal/ topiramate (100%), with Adequate fluid consumption were (100%), cases with Moderate intensity of migraine were (21.1%), cases with Severe intensity of migraine were (78.9%), in symotoms of headache Nausea were (31.7%), Nausea/photophobia/ phonophobia were (29%), Nausea/vomiting were (0.8%), Nausea/vomiting/photophobia/phonophobia were (0.8%), Phonophobia were (0.8%), Photophobia were (24.6%), Photophobia/phonophobia were (0.8%), and None were (2.4%), in characteristics of headache Unilateral were (46.3%),Unilateral/pulsating were (18.7%),Unilateral/pulsating/throbbing were (2.4%),Unilateral/throbbing were (16.3%), and Unilateral/throbbing/pulsating were (16.3%), mean sleep hours were reported 7.6 (SD=±1.5) per night and mean HIT-6 scale of migraine patients was 69.5 (SD=±9.9).

Table 4.4: Observed Characteristics of Migraine Patients (N=123)

Characteristics		***n***	***%***
Migraine triggers	Caffeine/ screen	1	0.8
	Less sleep	3	2.4
	Less sleep/screen	21	17.1
	Physical activity	1	0.8
	Physical activity/ less sleep	43	35
	Physical activity/screen/ Less sleep	22	17.9
	Screen	1	0.8
	None	31	25.2
Family history of migraine	No	123	100.0
Caffeine intake	Yes	50	40.7
Physical activity	Yes	92	74.8
Co morbidity	Yes	20	16.3
Self-medication	Yes	71	57.7
Medicines used	Inderal/ topiramate	123	100.0
Fluid consumption	Adequate	123	100.0
Intensity of migraine	Moderate	26	21.1
	Severe	97	78.9
Symptoms of headache	Nausea	39	31.7
	Nausea/photophobia/ phonophobia	48	29
	Nausea/vomiting	1	0.8
	Nausea/vomiting/photophobia/phonophobia	1	0.8
	Phonophobia	1	0.8
	Photophobia	30	24.6
	Photophobia/phonophobia	1	0.8
	None	3	2.4
Characteristics of headache	Unilateral	57	46.3
	Unilateral/pulsating	23	18.7
	Unilateral/pulsating/throbbing	3	2.4
	Unilateral/throbbing	20	16.3
	Unilateral/throbbing/pulsating	20	16.3
Sleep Hours per night	*Mean (±SD)*	*7.6*	*±1.5*
HIT -6 Scale	*Mean (±SD)*	*69.5*	*±9.9*

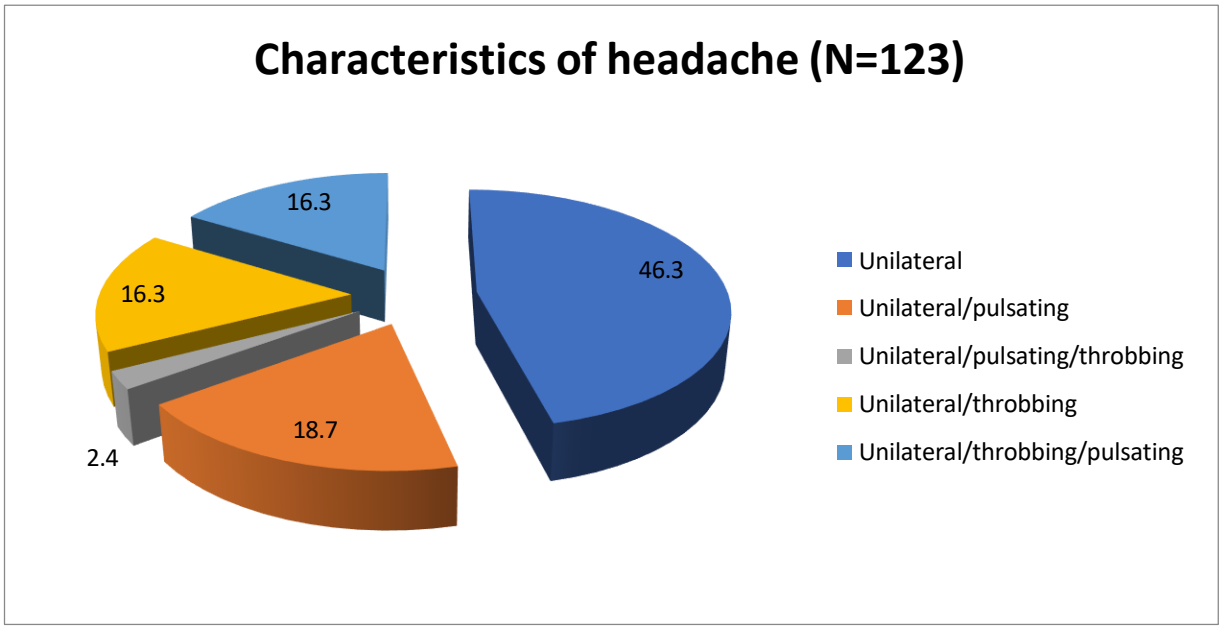


Figure 4.4 (a): Characteristics of headache (N=123)

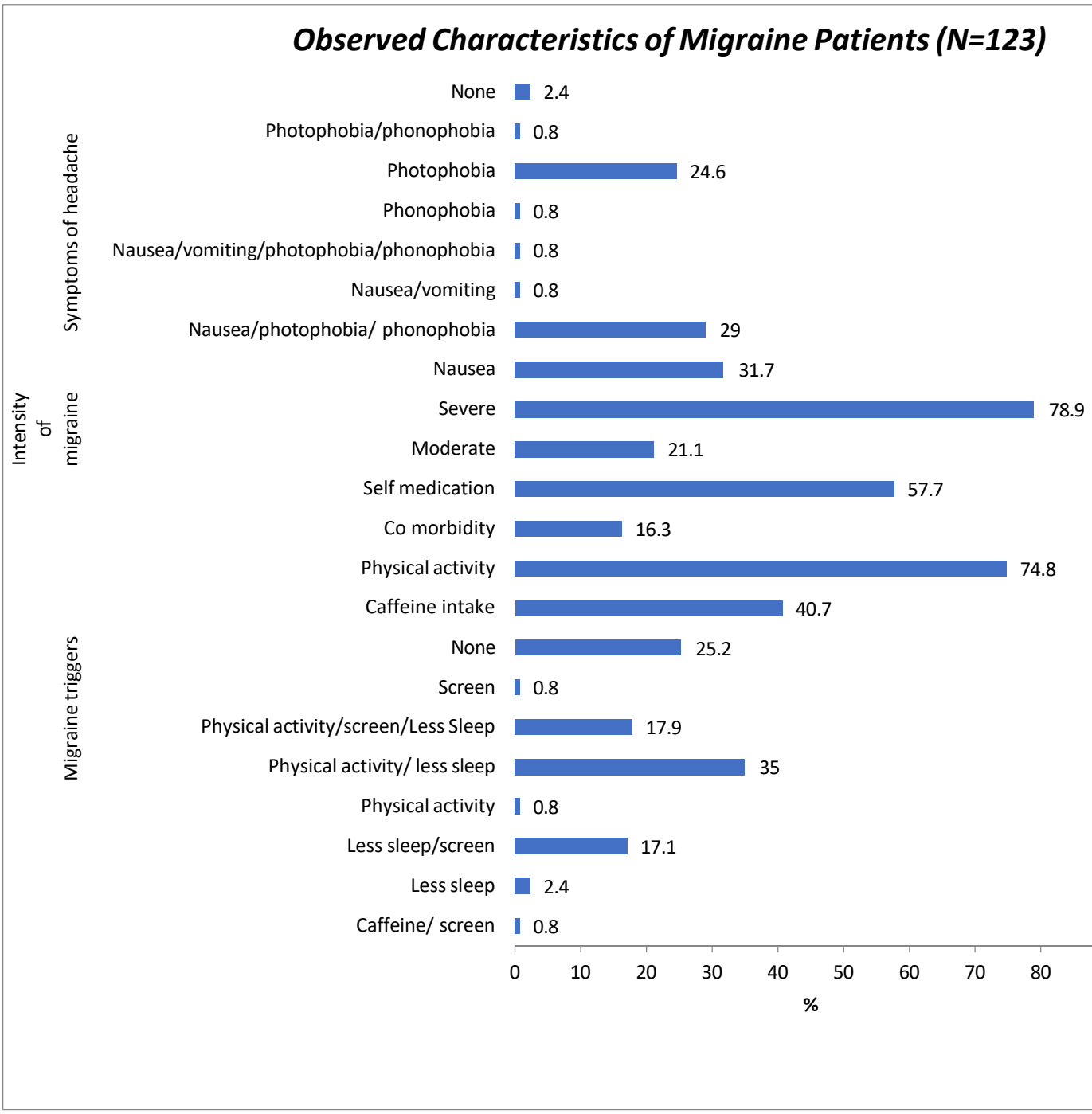


figure 4.4 (b): Observed Characteristics of Migraine Patients (N=123)

Table-4.5(a) reports the comparison of Vitamin D, Zinc, SOD and MDA between controls and migraine patients, results showed in control group samples median for Vitamin D (ng/ml) was 14(IQR=7), median for Zinc (μg/dL) was 88(IQR=11), median for SOD (nm/ml) was 41(IQR=29), and median for MDA (U/mL) was 2(IQR=1), whereas in migraine patients median for Vitamin D (ng/ml) was 20(IQR=15), median for Zinc (μg/dL) was 65(IQR=7),median for SOD (nm/ml) was 10(IQR=4), and median for MDA (U/mL) was 4(IQR=1). Mann Whitney U test did gives significant difference in the median of vitamin D, Zinc, SOD, and MDA between controls and migraine patients ($p<0.01$).

Table-4.5(b) reports the comparison of CRP, IL-10 and IFN Gamma between control and migraine patients, results showed among controls median for CRP level (mg/dL) was 1(IQR=2), median for IL-10 level (pgm/ml) was 301(IQR=1313), and median for IFN gamma (ngm/ml) was 141(IQR=203), whereas in migraine patients median for CRP level (mg/dL) was 2(IQR=4), median for IL-10 level (pgm/ml) was 243(IQR=114), and median for IFN gamma (ngm/ml) was 63(IQR=108). Mann Whitney U test did give significant difference in the CRP levels, IL-10 and IFN gamma between controls and Migraine with $p<0.05$.

Table 4.5(a): Comparison of Vitamin D, Zinc, SOD and MDA between Migraine and controls

Parameters	**Controls Median (Q3 – Q1)**	**Migraine Median (Q3- Q1)**	***p-value***
Vitamin D (ng/ml)	14(17-10)	20(26-11)	<0.001*
Zinc (μg/dL)	88(96-85)	65(68-61)	<0.001*
SOD (nm/ml)	41(55-26)	10(12-8)	<0.001*
MDA (U/mL)	2(2-1)	4(5-4)	<0.001*
*p<0.05 was considered statistically significant using Mann Whitney U test			

Table 4.5(b): Comparison of CRP, IL-10 and IFN Gamma between Migraine and Controls

Parameters	**Controls Median (Q3 – Q1)**	**Migraine Median (Q3- Q1)**	***p-value***
CRP level (mg/dL)	1(2-0)	2(4-0)	0.037*
IL-10 level (pgm/ml)	301(1523-210)	243(318-204)	0.002*
IFN gamma (ngm/ml)	141(233-30)	63(141-33)	0.002*
*p<0.05 was considered statistically significant using Mann Whitney U test			

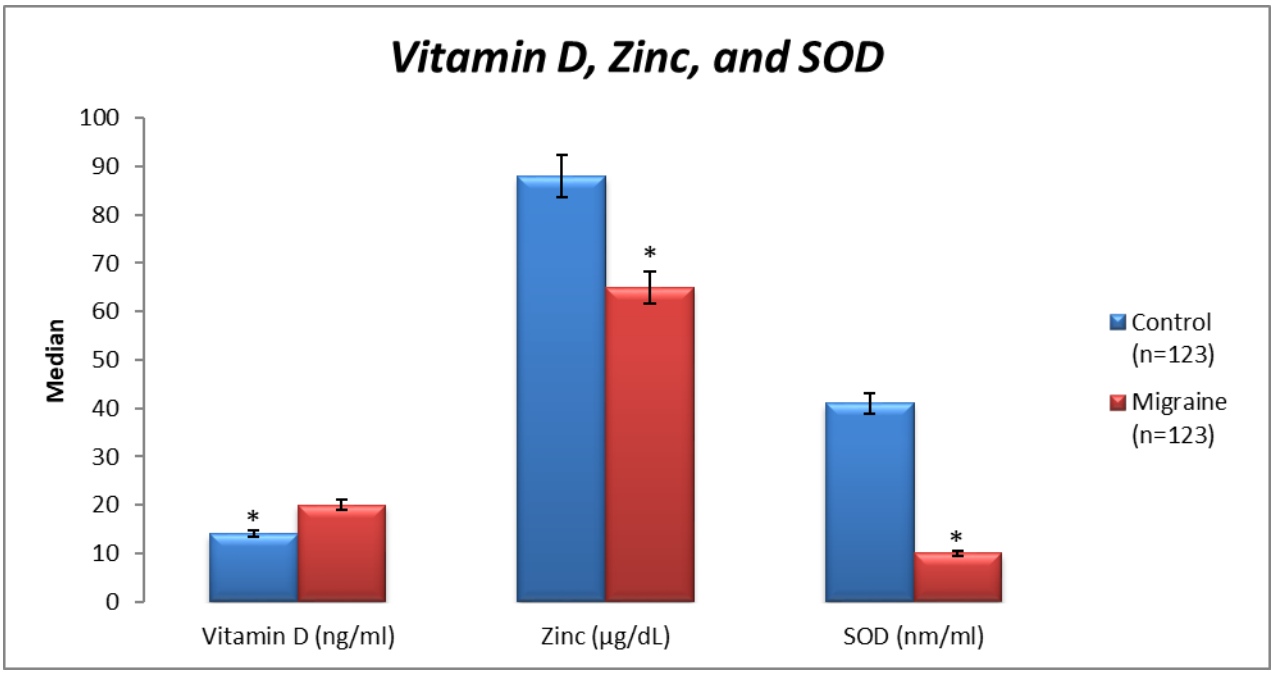


Figure 4.5(a): Comparison of Vitamin D, Zinc, SOD between cases and controls

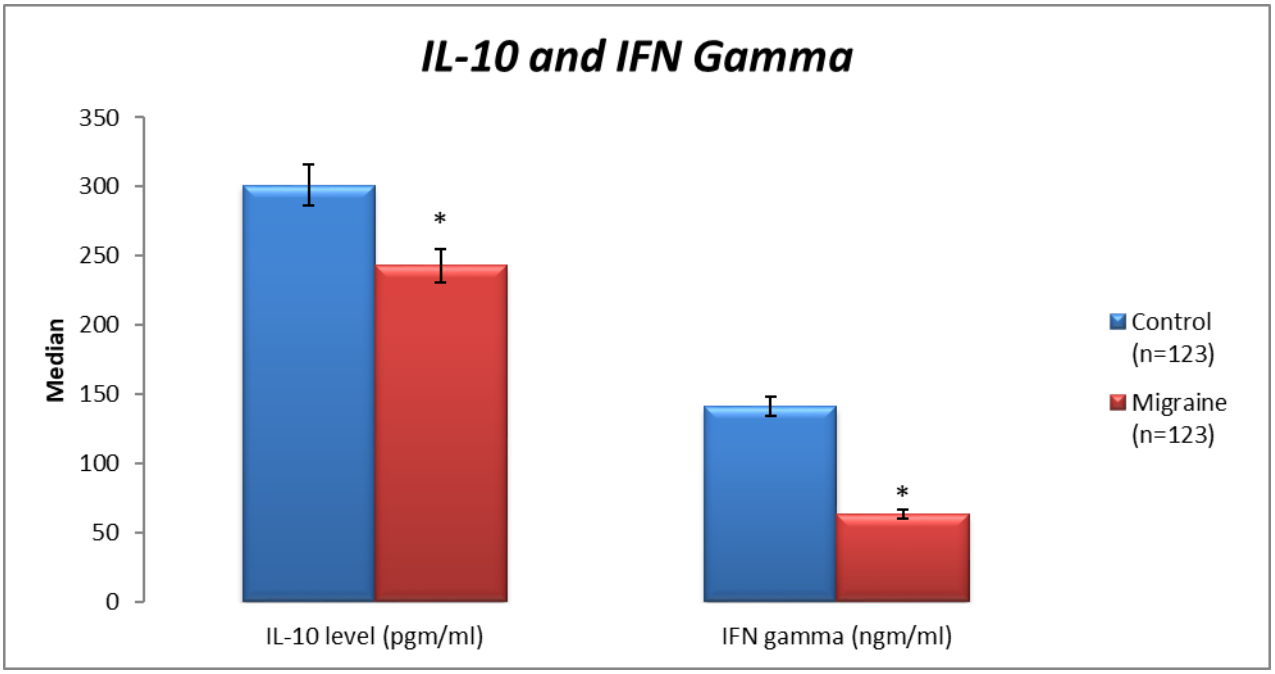


Figure 4.5(b): Comparison of IL-10 and IFN Gamma between Migraine and Controls

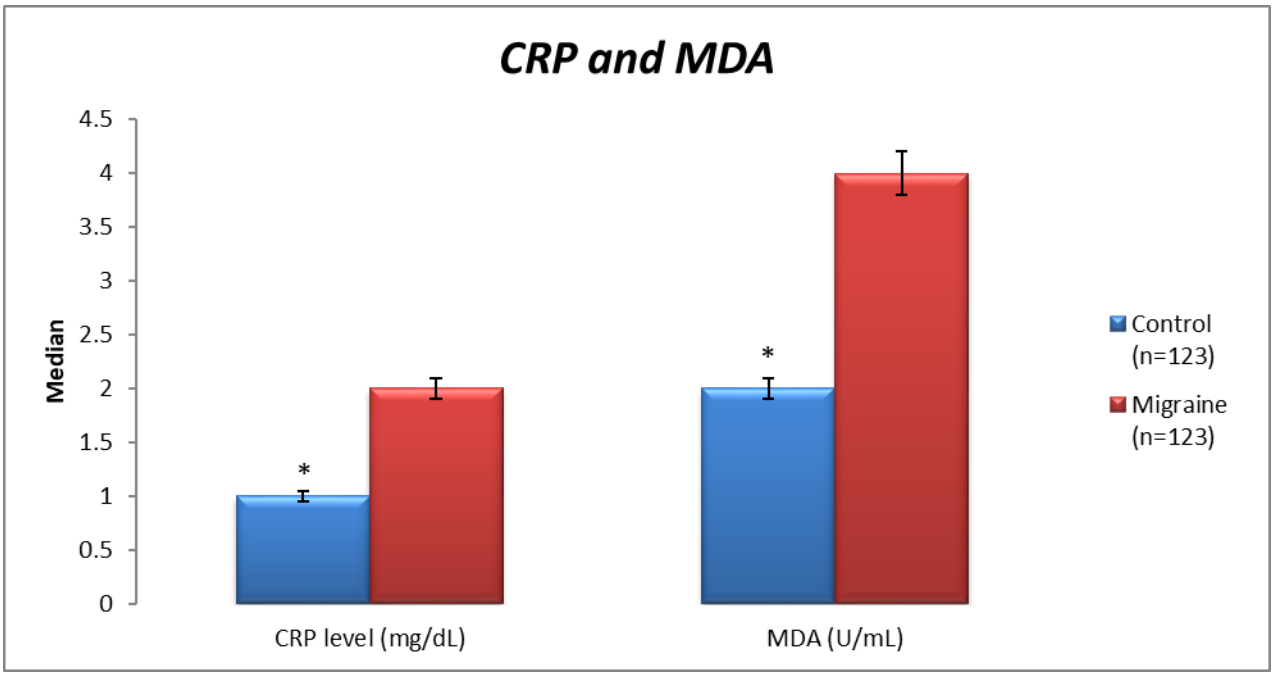


Figure 4.5(c): Comparison of CRP and MDA between Migraine and Controls

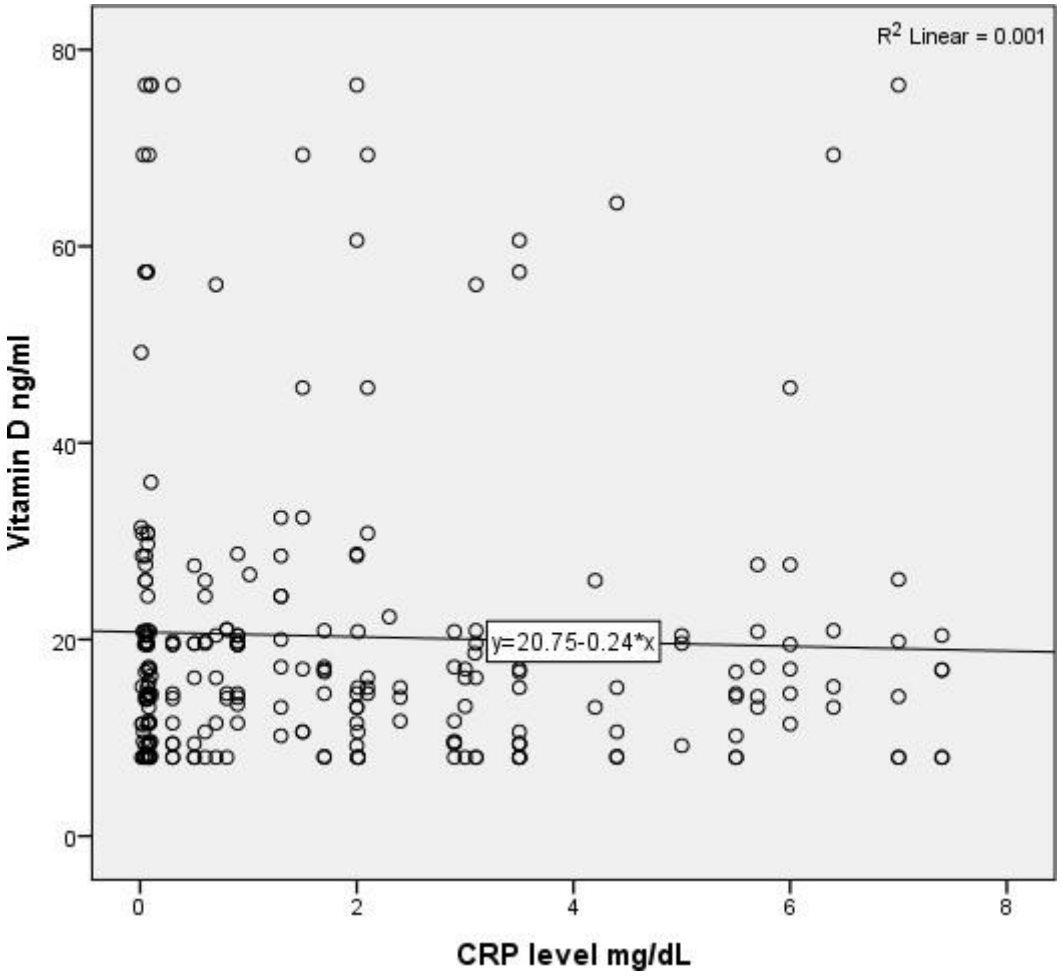


Figure 4.6: Vitamin D showed negative correlation with CRP among groups.

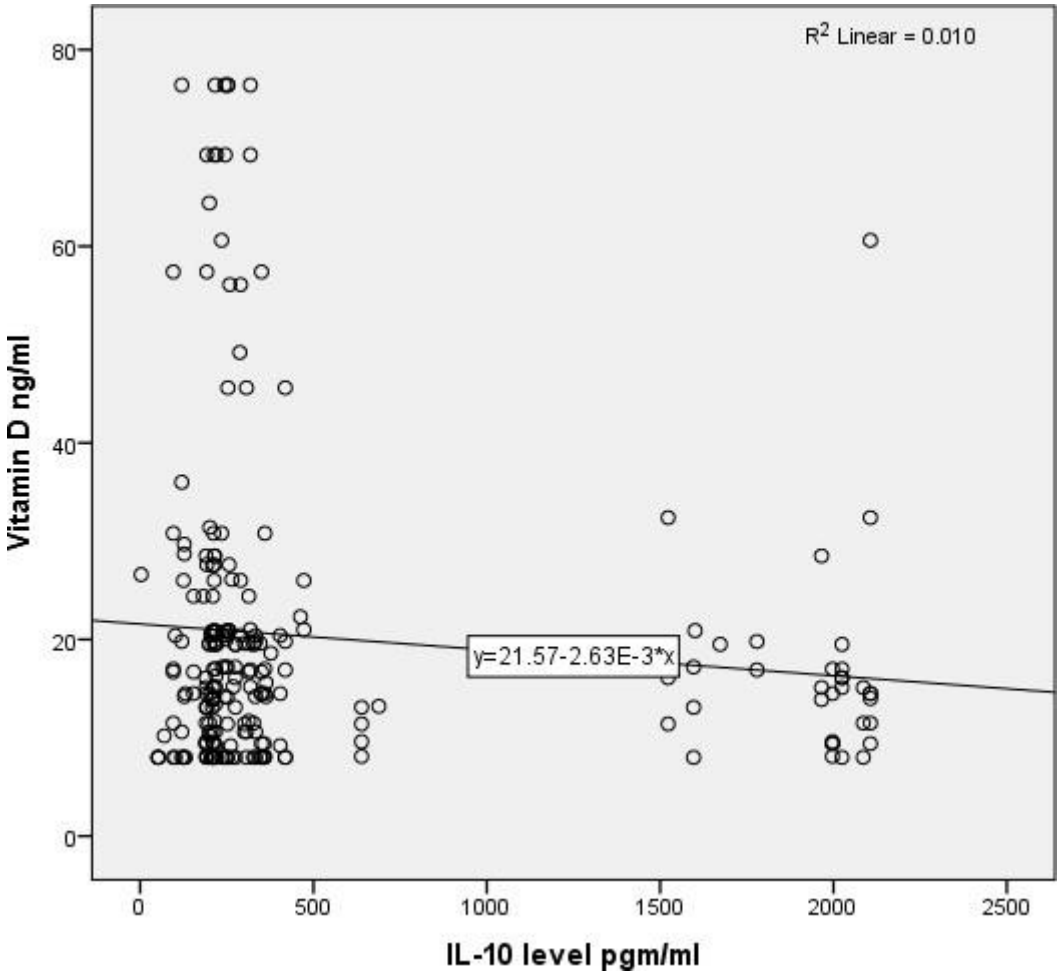


Figure 4.7: Vitamin D showed negative correlation with IL-10 among groups.

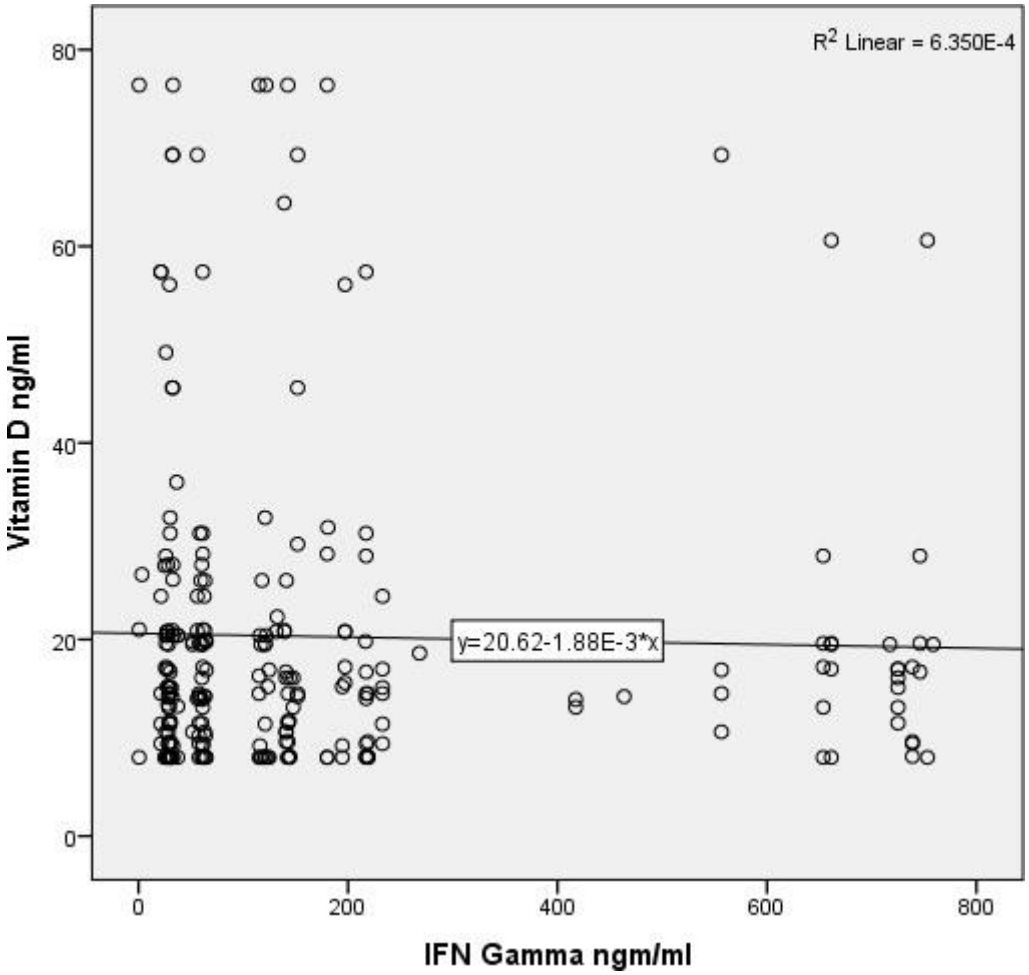


Figure 4.8: Vitamin D showed negative correlation with IFN among groups.

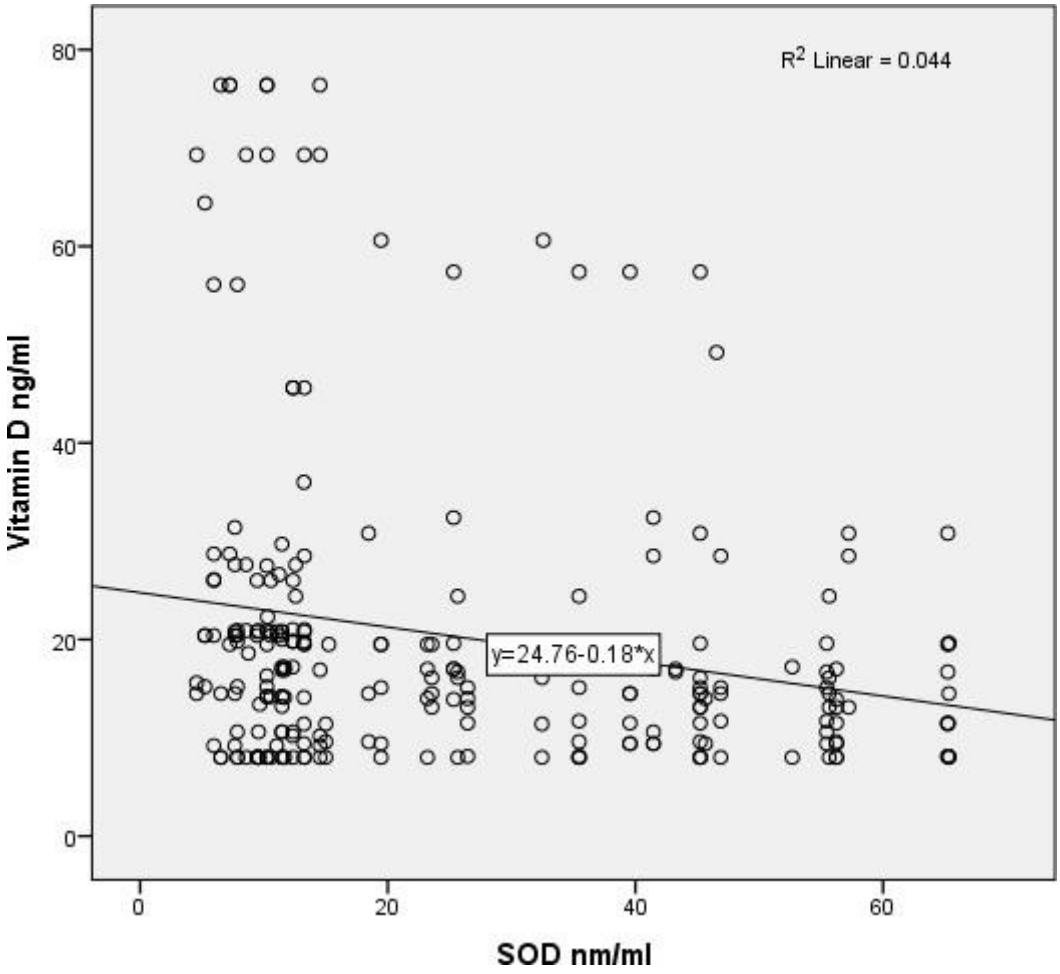


Figure 4.9: Vitamin D showed negative correlation with SOD among groups.

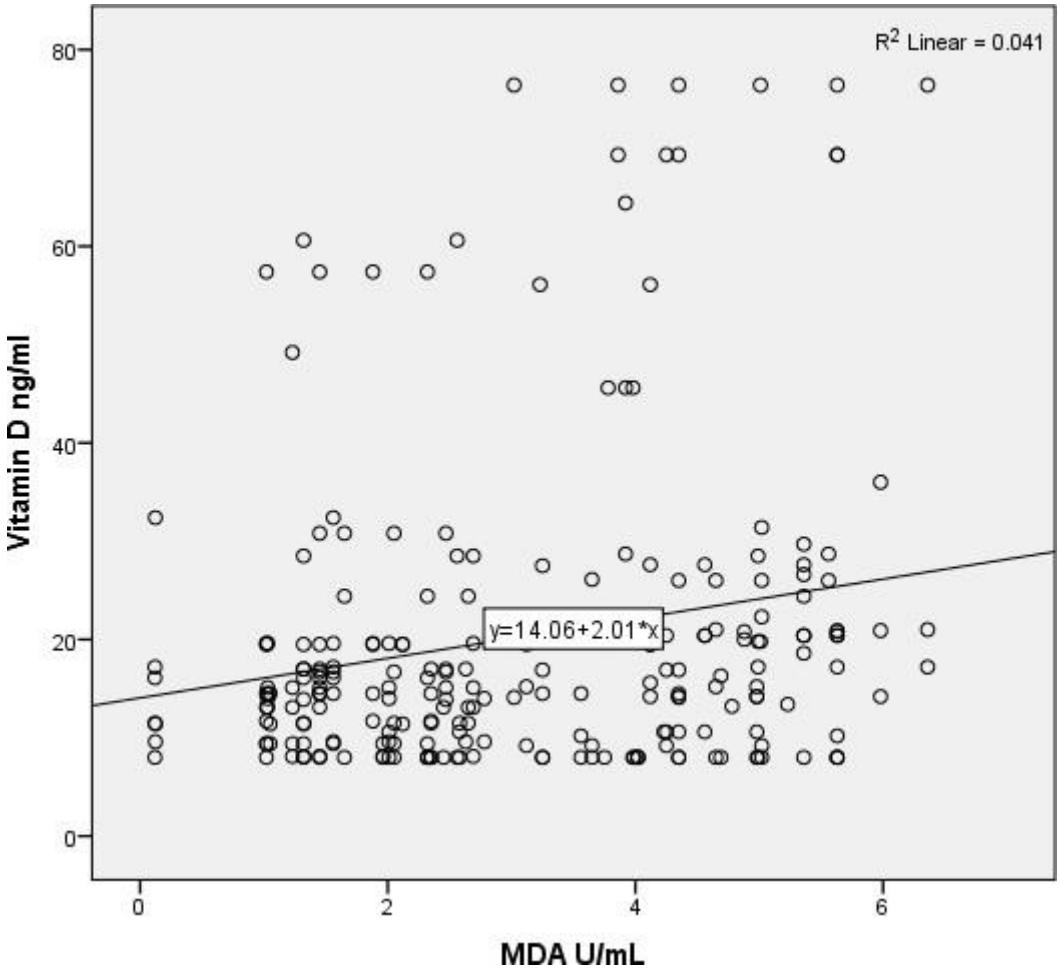


Figure 4.10: Vitamin D showed Positive correlation with MDA among groups.

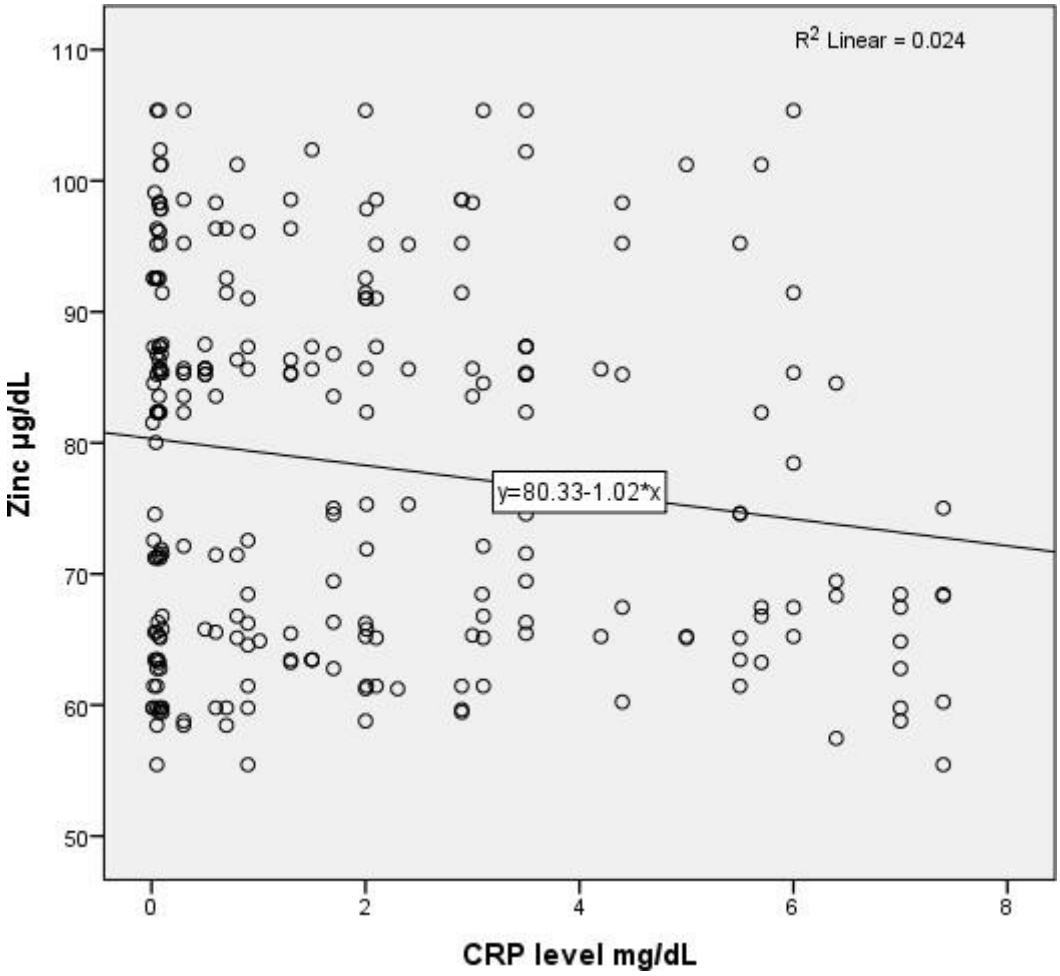


Figure 4.11: Zinc showed negative correlation with CRP among groups.

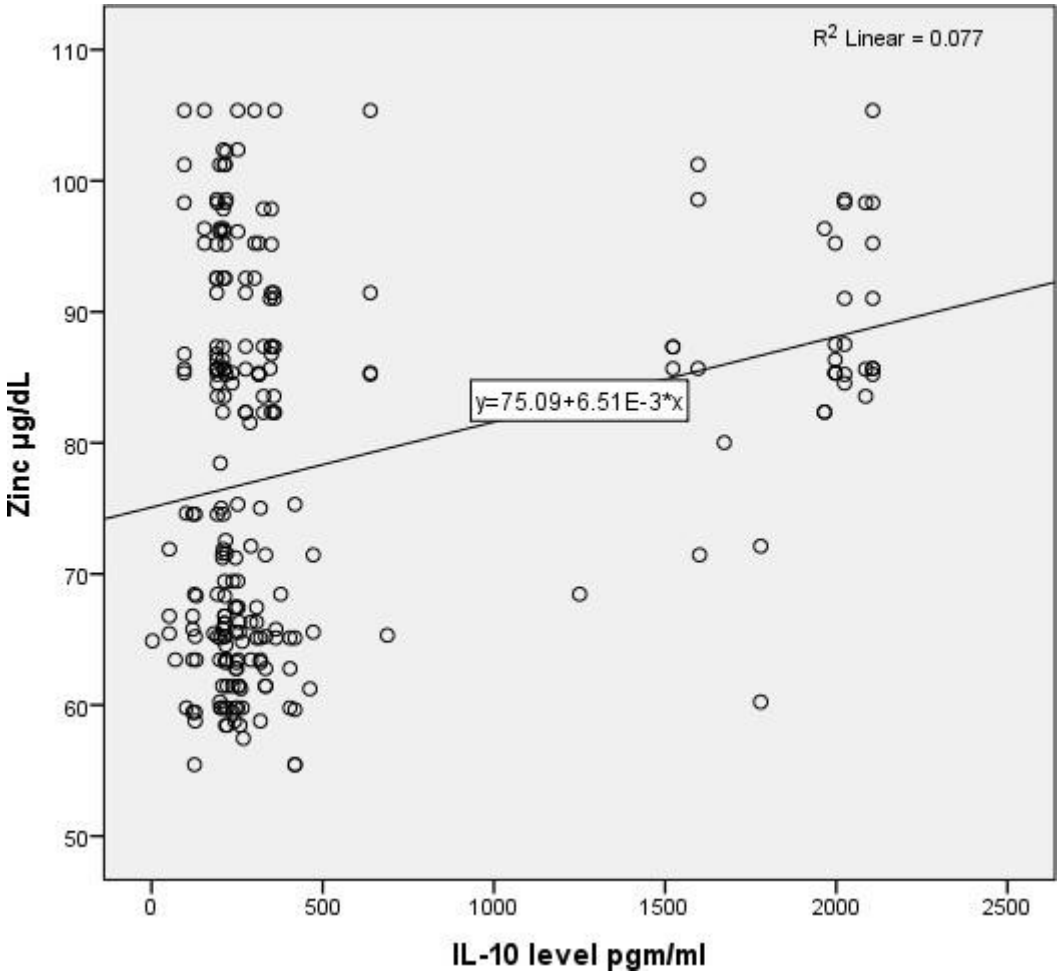


Figure 4.12: Zinc showed Positive correlation with IL-10 among groups.

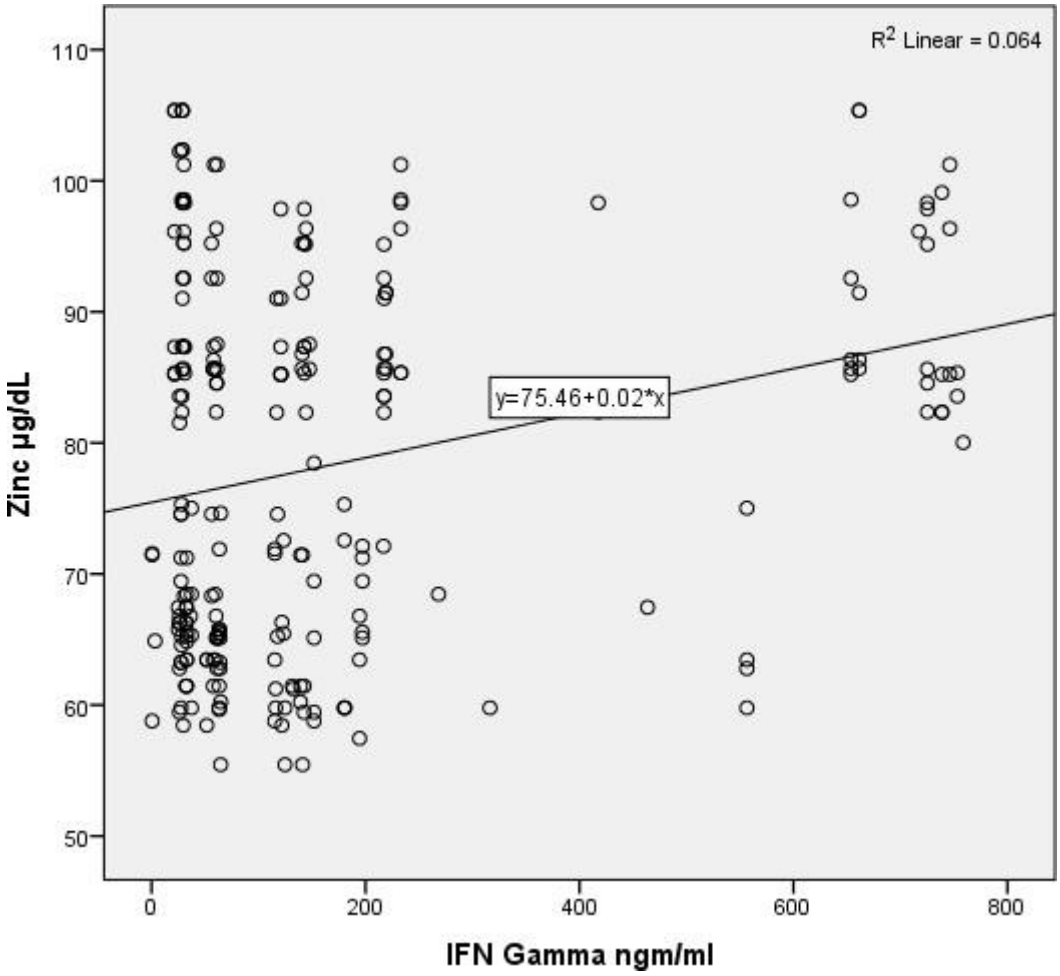


Figure 4.13: Zinc showed positive correlation with IFN among groups.

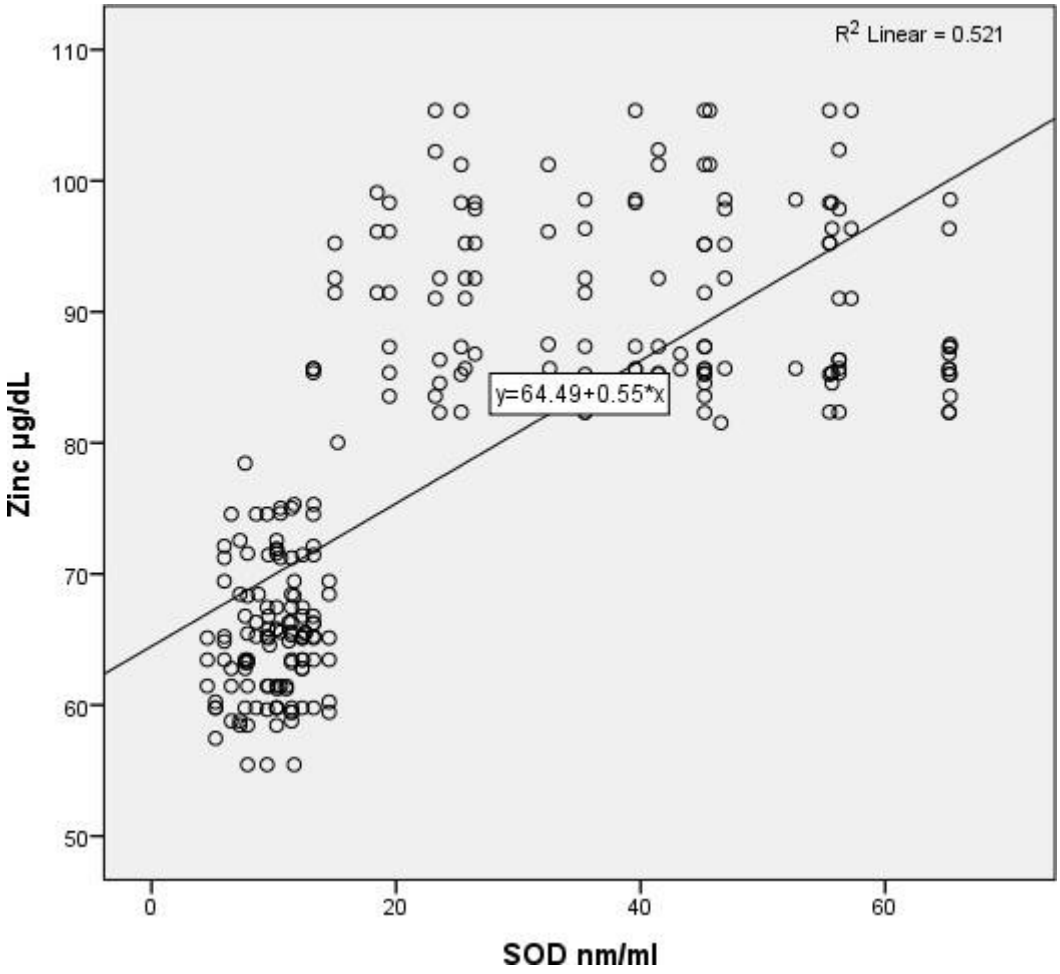


Figure 4.14: Zinc showed Positive correlation with SOD among groups.

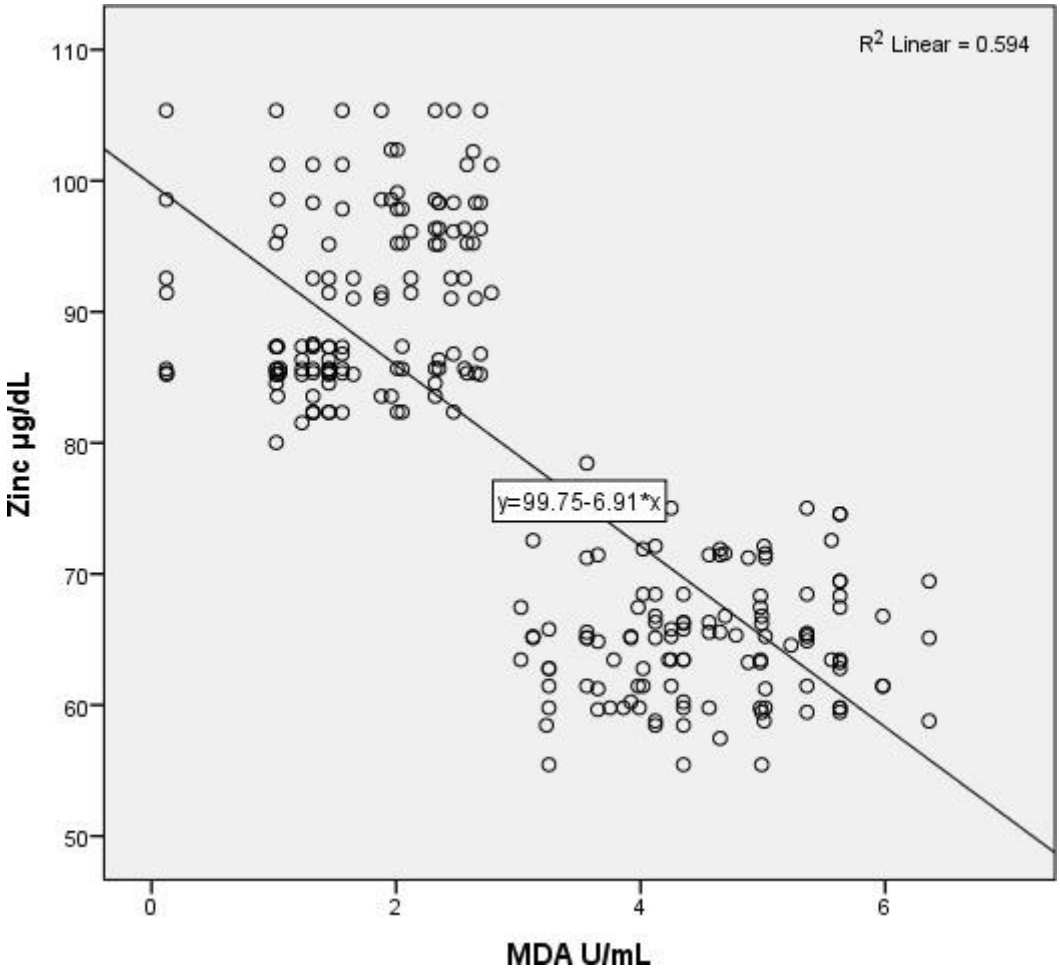


Figure 4.15: Zinc showed negative correlation with MDA among groups.

Table-4.6 reports the risk estimation for migraine with baseline characteristics using binary logistic regression analysis, in univariate model male sample gives negative association with migraine [OR=0.7,C.I(0.36-1.38)], residents from bungalow gives positive association with migraine [OR=2.51,C.I(0.73-8.63)], residents from apartment gives positive association with migraine [OR=1.63,C.I(0.70-3.76)], age (years) gives negative association with migraine [OR=0.98,C.I(0.93-1.03)], BMI (kg/m2) gives positive association with migraine [OR=1.03,C.I(0.98-1.08)], SBP negative association with migraine [OR=0.99,C.I(0.96-1.01)], DBP positive association with migraine [OR=1,C.I(0.95-1.06)], temperature gives significant negative association with migraine [OR=0.15,C.I(0.02-0.99)], and working hours per day also gives negative association with migraine [OR=0.96,C.I(0.90-1.02)], in multivariate model male gender gives negative association with migraine [OR=0.85,C.I(0.40-1.81)], residents from bungalow gives positive association with migraine [OR=2.23,C.I(0.62-7.96)], resident from apartment gives positive association with migraine [OR=1.47,C.I(0.62-3.49)], age (years) gives negative association with migraine [OR=0.98,C.I(0.92-1.03)], BMI (kg/m2) gives positive association with migraine [OR=1.03,C.I(0.98-1.09)], SBP gives negative association with migraine [OR=0.98,C.I(0.96-1.01)], DBP gives positive association with migraine [OR=1,C.I(0.94-1.06)],temperature gives negative association with migraine [OR=0.15,C.I(0.02-1.09)], and working hours per day also gives negative association with migraine [OR=0.97,C.I(0.90-1.04)].

Table 4.6: Risk Estimation for Migraine with baseline characteristics using Binary Logistic Regression Analysis

Parameters	***Univariate Model Odds Ratio (95% C.I)***	***Univariate Model Odds Ratio (95% C.I)***
Male	0.70 (0.36-1.38)	0.85 (0.40-1.81)
Bungalow	2.51 (0.73-8.63)	2.23 (0.62-7.96)
Apartment	1.63 (0.70-3.76)	1.47 (0.62-3.49)
Age (years)	0.98 (0.93-1.03)	0.98 (0.92-1.03)
BMI (kg/m2)	1.03 (0.98-1.08)	1.03 (0.98-1.09)
SBP	0.99 (0.96-1.01)	0.98 (0.96-1.01)
DBP	1.00 (0.95-1.06)	1.00 (0.94-1.06)
Temperature	0.15* (0.02-0.99)	0.15 (0.02-1.09)
Working hours per day	0.96 (0.90-1.02)	0.97 (0.90-1.04)
odds ratio considered statistically significant with $p<0.05$, Dependent variable: Migraine cases		

Table-4.7 reports the risk estimation for migraine with diet intake, results showed in univariate model Samples with Milk intake gives negative association with migraine [OR=0.78,C.I(0.45-1.34)], samples with Milk intake occasionally gives negative association with migraine [OR=0.13,C.I(0.01-1.17)], samples with Fish intake gives positive association with migraine [OR=1.47,C.I(0.85-2.53)], samples with Fish intake occasionally gives negative association with migraine [OR=0.93,C.I(0.35-2.45)], samples with Fuzzy drink intake gives negative association with migraine [OR=0.71,C.I(0.39-1.29)], samples with Fuzzy drink intake occasionally gives positive association with migraine [OR=1.09,C.I(0.58-2.07)], in multivariate model samples with Milk intake gives negative association with migraine [OR=0.75,C.I(0.43-1.32)], samples with Milk intake occasionally gives negative association with migraine [OR=0.12,C.I(0.01-1.09)], samples with Fish intake gives positive association with migraine [OR=1.47,C.I(0.84-2.56)], samples with Fish intake occasionally gives positive association with migraine [OR=1.03,C.I(0.38-2.80)], samples with Fuzzy drink intake gives negative association with migraine [OR=0.7,C.I(0.38-1.29)], and samples with Fuzzy drink intake occasionally gives positive association with migraine [OR=1.08,C.I(0.56-2.09)].

Table 4.7: Risk Estimation for Migraine with diet intake using Binary Logistic Regression Analysis

Parameters	**Univariate Model Odds Ratio (95% C.I)**	**Multivariate Model Odds Ratio (95% C.I)**
Milk intake yes	0.78 (0.45-1.34)	0.75 (0.43-1.32)
Milk intake occasionally	0.13 (0.01-1.17)	0.12 (0.01-1.09)
Fish intake yes	1.47 (0.85-2.53)	1.47 (0.84-2.56)
Fish intake occasionally	0.93 (0.35-2.45)	1.03 (0.38-2.80)
Fuzzy drink intake yes	0.71 (0.39-1.29)	0.70 (0.38-1.29)
Fuzzy drink intake occasionally	1.09 (0.58-2.07)	1.08 (0.56-2.09)
*odds ratio considered statistically significant with $p<0.05$, Dependent variable: Migraine cases		

Table-4.8 reports the risk estimation of migraine with CRP, IL-10 levels, IFN gamma, Vitamin D ,SOD and MDA, in univariate model it was found that Increase in CRP level (mg/dL) gives positive association with migraine [OR=1.21,C.I(1.07-1.37)], increase in IL-10 level (pgm/ml) gives negative association with migraine [OR=0.99,C.I(0.99-0.99)], increase in IFN gamma (ngm/ml) gives negative association with migraine [OR=0.99,C.I(0.99-0.99)], increase in Vitamin D (ng/ml) gives positive association with migraine [OR=1.03,C.I(1.01-1.05)], increase in SOD (nm/ml) gives negative association with migraine [OR=0.62,C.I(0.53-0.73)], and increase in MDA (U/mL) gives positive association with migraine [OR=97.4,C.I(20.2-468)], all these associations were considered statistically significant with $p<0.05$, whereas in multivariate model after adjusting for age and gender samples with increase in CRP level (mg/dL) gives positive association with migraine [OR=1.26,C.I(1.09-1.46)], increase in IL-10 level (pgm/ml) gives negative association with migraine [OR=0.99,C.I(0.99-0.99)], increase in IFN gamma (ngm/ml) gives negative association with migraine [OR=0.99,C.I(0.99-0.99)], increase in Vitamin D (ng/ml) gives positive association with migraine [OR=1.07,C.I(0.99-1.16)], increase in SOD (nm/ml) gives negative association with migraine [OR=0.8,C.I(0.67-0.94)], and increase in MDA (U/mL) gives positive association with migraine [OR=11.5,C.I(2.98-44.9)], in multivariate model association of CRP, IFN gamma, IL-10 and MDA with migraine was found statistically significant with $p<0.05$.

Table 4.8: Risk Estimation for Migraine with other studied parameters using Binary Logistic Regression Analysis

Parameters	**Univariate Model Odds Ratio (95% C.I)**	**Multivariate Model Odds Ratio (95% C.I)**
CRP level (mg/dL)	1.21* (1.07-1.37)	1.26* (1.09-1.46)
IL-10 level (pgm/ml)	0.99* (0.99-0.99)	0.99* (0.99-0.99)
IFN gamma (ngm/ml)	0.99* (0.99-0.99)	0.99* (0.99-0.99)
Vitamin D (ng/ml)	1.03* (1.01-1.05)	1.07 (0.99-1.16)
SOD (nm/ml)	0.62* (0.53-0.73)	0.80 (0.67-0.94)
MDA (U/mL)	97.4* (20.2-468)	11.5* (2.98-44.9)
*odds ratio considered statistically significant with $p<0.05$ Multivariate model was adjusted for age and gender Dependent variable: Migraine cases		

CHAPTER 5

DISCUSSION & CONCLUSION

5.1 SEQUENCE OF DISCUSSION EXPERIMENT

The discussion chapter has covered the elaborate analysis in light of the results of the present study. It has analyzed the parameters like age, and BMI based on height and weight. The role of the specific parameters has been discussed in the discussion section one by one. The various reviews which are taken from the literature from previous records to compare and contrast the present study output.

Migraine is one of the most common and disabling diseases globally, significantly disrupting patients' daily routines. It is known to be a primary neurovascular disorder with an unclear pathophysiology. In the Western world, about 6% of males and 18% of females suffer from migraines. Key nervous system structures thought to play a crucial role in triggering migraine pain include cranial blood vessels and the trigeminovascular system, along with its connections to the parasympathetic outflow. The most common sites of migraine pain are the frontal and temporal regions, but it can also present as referred pain in the parietal, occipital, and upper cervical regions. (Agosti et al., 2023)

Female sex hormones have long been recognized as important contributors in causing migraines throughout a woman's life. The increased occurrence of migraines in women begins after menarche, peaks around age 40, and declines after menopause. Many women report more frequent migraine episodes during their menstrual cycles, during the first trimester of pregnancy, and in the perimenopausal era (Szewczyk et al., 2023).

In our study, we found that migraines affect women more frequently than men. Previous epidemiological studies have shown a significant gender difference in the prevalence of migraine, with women being three times more likely to suffer from them than

men. This disparity is supported by findings from both structural and functional MRI studies (Delaruelle et al., 2018). Additionally, biological and psychological differences between males and females may contribute to the higher prevalence of headaches in women. In a research (Tonini, 2018) highlighted significant sex and gender differences in headaches, emphasizing that migraines predominantly affect women. Because pain intensity perception is subjective, and attack duration is an objective metric, some researchers claim that, with similar pain scores for men and women, the longer duration of assaults and recovery time may explain why women grade their pain intensity higher than men. Added to this is the widespread idea that, due to gender norms, males are less likely to rank their experience of pain intensity as high. On contrary (Bolay et al., 2014) reported that men are more likely than women to experience high-frequency migraines, defined as more than 10 migraine headache days (MHD) per month

Currently, migraine diagnosis relies on clinical evaluation, as no specific blood or radiological biomarkers have been identified. There is a strong genetic component to migraines, involving several gene-related contributing factors. (Sutherland, Albury, & Griffiths, 2019)

The standard criteria for diagnosing migraines globally are defined by the International Headache Society. According to these guidelines, a migraine includes an episodic headache lasting 4 to 72 hours with at least two of the following major symptoms: unilateral headache, throbbing headache, headache aggravated by movement, and moderate to severe pain intensity. Additionally, at least one of the following minor symptoms must be present: associated nausea and/or vomiting, sensitivity to light (photophobia), or sensitivity to sound (phonophobia). (International Headache Society, 2018)

In the current study it is found that mean age of migraine patients is 33.2years. A study conducted by (Straube & Andreou, 2019) showed that the peak age for migraine occurrence was between 30 and 39 years. Similarly, ((Fila et al., 2023)) studied the prevalence of migraines between 20-40years. Another study by (Takeshima et al., 2019) also reported that the average age of migraine sufferers was 30 years and above. All these studies align with the results of the present study. However, a study by (Wijeratne et al., 2019) described the peak age for migraines in girls to be in their teens, specifically between 14 to

17 years, and between 13 to 15 years in boys, which contrasts with the current study's findings.

The prevalence of overweight and obesity has surged dramatically over recent decades, becoming one of the top risk factors for various diseases and increased mortality worldwide. Total body obesity (TBO) is commonly assessed using body mass index (BMI). (The GBD 2015 Obesity Collaborators, 2017). In this study the mean BMI in migraine patients was 28.4 (kg/m²) (SD=±4.8). In a contrast study individual with a higher BMI (over 25) have a greater risk of experiencing chronic headaches (more than 14 days per month) compared to those with a normal weight. (Kristoffersen, Børte, Hagen, Zwart, & Winsvold, 2020) a small study has suggested that patients referred for obesity surgery had a higher prevalence of migraine compared with subjects with a BMI of < 25.0 kg/$m^{2.}$ Another study found that individuals undergoing surgery for obesity have a higher prevalence of migraines compared to those with a BMI of less than 25 kg/m². ((Mínguez-Olaondo et al., 2020). On contrary, the characteristics of migraine attacks were not related to BMI and showed no differences between obese and non-obese women experiencing active migraines. (Gelaye et al., 2017)

In this study, we found mean SBP 121.5, mean DBP was 81.9 which aligns with findings from other research studies. However, previous epidemiological studies on the relationship between blood pressure (BP) and migraines, have shown inconsistent results, making this topic still controversial. (Silva, Alencar Neto, et al., 2022) (Mazzacane et al., 2024)

Results from studies focusing on hypertension have found a positive correlation between headaches and blood pressure. Migraine frequency, indicated by the number of migraine days per month, appears to be linked to a genetic predisposition primarily in males. Additionally, a stronger family history of migraines is associated with an earlier age of onset, an increased number of days requiring medication, and the presence of migraine with aura. (Pelzer et al., 2018)

Our study found no relation between migraine and family history. However, we found contradictory studies by (Bron et al., 2021)

The beneficial role of dairy products in decreasing low-grade inflammation and maintaining gut microbiome balance has been documented. Therefore, it can be hypothesized

that higher consumption of these foods may help lower the likelihood of inflammation-related conditions like migraines. (Nieman et al., 2020). In our study we found reports the association of milk intake with migraine, in control group Samples with milk intake Yes, were (67.5%), Occasionally milk intake were (4.9%), whereas among migraine patients milk intake Yes were (65%), Occasionally milk intake were (0.8%), Pearson Chi Square test did not show any significant association of milk intake drink between controls and migraine ($p>0.05$).

The relationship between sleep disturbances and migraines is complex and not fully understood. Migraine sufferers often report poor sleep quality both before and during migraine attacks. Additionally, sleep is commonly cited as a therapeutic measure for alleviating the pain of an acute migraine episode. In our study, we found that 2.4% of patients reported insufficient sleep as a triggering factor for migraines, less sleep/screen were (17.1%), Physical activity were (0.8%), Physical activity/ less sleep was (35%), Physical activity/screen/ Less sleep were (17.9%). Among lifestyle factors, we found that infrequent exercise (less than three days a week for at least 30 minutes) and in our study, insufficient sleep and increased screen time were identified as the most significant triggering factors for migraines, with 17.1% of participants affected by insufficient sleep. Another study found that high screen time (over 6 hours a day) was linked to migraines. This is supported by (Rafi, Islam, Hasan, & Hossain, 2022). Other research articles also align with our findings. (Montagni, Guichard, Carpenet, Tzourio, & Kurth, 2016)

Poor hydration is linked to worsened migraine symptoms. A cross-sectional study found a negative dose-response relationship between migraine characteristics and daily water intake (Khorsha, Mirzababaei, Togha, & Mirzaei, 2020). Women who drank less water experienced greater migraine disability, higher headache frequency, more intense pain, and longer headache durations, which aligns with our research findings.

However, several other studies have shown a positive correlation between fluid intake and migraine relief (Nowaczewska et al., 2019). (Sommerfield et al., 2016) demonstrated that in migraine patients, the average headache frequency decreased by approximately half when water intake was higher compared to when it was low.

Nausea is a key characteristic in the classification of migraines. Clinically, it appears that the severity of a migraine attack correlates with the intensity of nausea and other associated symptoms such as photophobia and phonophobia (Imai & Yasuhiko Matsumori, 2024)
). Increasing nausea and also other associated symptoms are correlated with increasing migraine pain intensity (Yin et al., 2021). This aligns with our finding where severity of pain was present in 78.9% of patients. In symptoms of headache Nausea were (31.7%), Nausea/photophobia/ phonophobia were (29%), Nausea/vomiting were (0.8%), Nausea/vomiting/photophobia/phonophobia were (0.8%), Phonophobia were (0.8%), Photophobia were (24.6%), Photophobia/phonophobia were (0.8%).

The Headache Impact Test (HIT-6) was utilized to measure the impact of migraine headaches among migraine patients. The HIT-6, developed by (Kosinski et al., 2003) is a brief and user-friendly tool designed to measure the negative impact of headaches. It is used for screening and monitoring patients with headaches in both clinical settings and research. The test includes six questions and each question is rated on a five-point Likert scale from 6 (Never) to 13 (Always). The total score, ranging from 36 to 78, is calculated by adding up the scores from each question, with higher scores indicating a more severe impact of headaches. in our study mean HIT-6 scale of migraine patients was 69.5 (SD=±9.9). the result of our study coincides with other studies which shows The high frequency and intensity of headaches during migraine attacks resulted in severe migraine-related disability in individuals with migraines, as measured by the HIT-6 scale. (Shin, Park, Kim, & Lee, 2008), (Goldstein et al., 2019)

Low levels of vitamin D have been seen in migraine sufferers, as demonstrated by (Zandifar et al., 2014). In Greece, the northern and regions with lower average temperatures had higher headache prevalence and frequency than the southern region. Lower latitude residents have greater serum vitamin D levels, which is a major contributing cause to the low occurrence of headaches. Research indicates that headache episodes occur more frequently in the autumn and winter and less frequently in the summer.

In most studies, vitamin D deficiency, insufficiency, and sufficiency were defined as <20, ≥20 and <30, and ≥30 ng/mL of 25(OH)D, respectively. (Nowaczewska et al., 2020)
In our study, we observed that the central value vitamin D level in migraine patients was 20 ng/ml, while in the control group it was 14 ng/ml. These findings are consistent with some

previous research indicating that vitamin D levels do not significantly impact the occurrence of migraines. According to (Gazerani et al., 2018)

there were no discernible variations in the mean blood 25(OH)D levels between migraineurs (13.55 ng/mL) and controls (13.19 ng/mL). There's no connection between 25(OH)D levels and the intensity of headaches. There is no discernible link between the severity of a vitamin D deficit and migraine. Serum vitamin D levels and migraine were not found to be significantly correlated in a cross-sectional study involving 11,614 participants in the sixth Tromsø study (Kjaergaard, Eggen, Mathiesen, & Jorde, 2012). The percentages of migraineurs who were deficient, inadequate, or sufficient in vitamin D were 13.2%, 68.4%, and 18.4%, respectively. The proportion of migraineurs (6%) who had normal vitamin D levels compared to controls (37%).There is no discernible link between the level of blood vitamin D and migraine intensity (Ghorbani et al., 2019).

Zinc may possibly have a role in the pathophysiology of migraines. Zinc has a major role in glutamatergic neurotransmission modulation. vesicles to store glutamate. Both migraine episodes and vascular tone may be impacted by glutamate (Liu et al., 2023).

Zinc is an antagonistic co-transmitter on NMDA receptors and is stored in synaptic vesicles with glutamate. Zinc has the ability to prevent the NMDA receptor complex from working. The literature is ambiguous on Zn's precise function. The frequency of migraine attacks may be correlated with lower ionized zinc concentrations, and zinc supplementation may lessen the frequency of such attacks. (Gonullu et al., 2015) found lower zinc levels in migraine patients.

People with migraines have been found to have reduced blood zinc levels, and this may have therapeutic significance as zinc is vital for several different brain processes related to migraine. According to clinical research, zinc may help treat migraines. There was a decrease in migraine attack frequency and headache severity in an 8-week randomised clinical study (n = 80) with zinc sulphate (50 mg zinc daily). (Liu, Gale, Reynolds, Weiss, & Aizenman, 2021).

In a follow-up research, which was also a randomised clinical trial with 60 participants, the effects of 15 mg of zinc per day on migraine frequency and intensity were evaluated for a period of 12 weeks and the findings indicate that migraine patients have reduced plasma levels of essential minerals. Therefore, dietary interventions to ensure

adequate mineral intake should be considered as a potential therapeutic approach for managing migraines. (Silva et al., 2022).

In our study, we found that patients with migraine had low zinc concentrations, with an average of 65 μg/dL (61 to 68). In contrast, the control group had higher zinc levels, averaging 88 μg/dL (85 to 96). It is noteworthy that the normal range for serum zinc levels is between 80 and 120 μg/dL. The most significant metalloenzymes that shield cells from oxidative stress are superoxide dismutases (SODs). These enzymes are created when reactive oxygen species are created during vasoconstriction, vasospasm, and ischemia/reperfusion. Thiobarbituric acid reactive substances (TBARS), such as malondialdehyde (MDA), are formed when membrane breakdown produces highly reactive and unstable lipid peroxides due to inadequate SOD and other oxidoreductases. In our study we found that levels of SOD were significantly lower in migraine patients as compared to healthy controls. The central value concentration of SOD in migraine patients was 10(12-8) nm/ml, whereas in healthy controls it was 41(55-26) nm/ml. these results aligns with significantly elevated SOD activity levels in migraine patients ((Matschke et al., 2019). (Togha, Razeghi Jahromi, Ghorbani, Martami, & Seifishahpar, 2018) also showed lower levels of SOD in migraine patients as compared to controls (Sıdıka Genç et al., 2022) also found the same result.

Oxidants originate endogenously in the brain from various sources and can be harmful in excessive amounts. The brain contains ion channels that detect oxidative stress, triggering neurogenic inflammation. It is plausible that common migraine triggers share the capacity to increase oxidative stress. In our study we aim to find the link between MDA (an oxidant) and migraine and we found that concentration of MDA is higher in migraine patients as compared to controls. The central value of concentration of MDA in migraine patients was 4(5-4) U/ml, whereas in controls it was 2(2-1) U/ml. our results aligns with a research by (Karsan & Goadsby, 2021), MDA was found in migraineurs more often than in healthy patients, particularly during the ictal stage. found that the MDA level of patients in the migraine group was significantly higher than that in controls. On contrary, (Cordiano et al., 2023) found no appreciable variations in the MDA serum levels between migraine-affected women and controls.

Multiple lines of evidence now confirm that inflammation is a key factor in the development of migraines. During a migraine, various proinflammatory cytokines are

released, which contribute to the complex process of nerve ending sensitization in the meninges, thereby promoting or intensifying the sensation of pain (Hagen et al., 2020). C-reactive protein (CRP) has been strongly linked to several medical conditions, so, it is reasonable to explore whether CRP might also be epidemiologically or causally connected to migraines.

In our study we found slight increase of CRP levels in migraine patients 2(4-0) mg/dL as compared to controls 1(2-0) mg/dL. The result of our study aligns with other researches which shows increase levels of CRP in migraine patients. According to (Lippi, Mattiuzzi, & Cervellin, 2014) the median CRP level was significantly higher in the migraine patients. Also according to, (Horia Pleș et al., 2023) women with migraine had a substantially higher levels of CRP than women without migraine. In a very large cross-sectional study, (Nelson et al., 2010) studied the concentration of CRP in cases was found to be marginally but substantially higher than in controls. In a cross-sectional research, (Kinoshita et al., 2021) included 50 healthy matched controls and 62 migraine sufferers. The rate of elevated CRP readings was almost twice as high in cases as in controls. (Hamed, Hamed, Ezz Eldin, & Mahmoud, 2010) also found the same result.

The result of our study also contradict with other researches as according to (Silva, Alencar Neto, et al., 2022) cross-sectional study the CRP levels did not significantly differ between controls and migraineur patients. In a case-control research, (Rockett, Perla, Perry, & Chaves, 2013) included 29 healthy controls and 30 female migraineurs, An ANOVA revealed no statistically significant differences in CRP concentrations across the groups.

Migraine is thought to be a neuroinflammatory disorder in which pain receptors are activated by inflammatory mediators in the meninges sensory nerves. IL-10 is produced by Various subsets of CD4+ T-cells, macrophages, dendritic cells, and B cells and they are involved in limiting inflammatory responses by preventing the release of pro-inflammatory cytokines such TNF-α, IL-1, and IL-6. (Rutz & Ouyang, 2016)

Our study we found decreased level of IL-10 243(318-204) pgm/ml in migraine patients as compared to controls 301 (1523-210) pgm/ml this result aligns with other studies published by (Cowan et al., 2021), (Domínguez et al., 2017), (de et al., 2017).

No difference in IL-10 in cases and controls was found by, (Perini et al., 2005), whereas increased levels of IL-10 in cases were found by (Chaudhry et al., 2019). In female

migraineurs (Oliveira et al., 2017) found cytokine profile characterized by low levels of IL-10.

IFN- Gamma are produced by T-lymphocytes, more especially Th1 and NK T-cells, enhance antigen presentation and innate immune system activation, promote macrophage activation and inflammation, mediate antiviral and antibacterial immunity. In our study we found decreased levels 63 (141-33) ngm/ml of IFN-Gamma in migraine patients as compared to control group 141(233-30), this results contradicts with a study by (Lee et al., 2015) who found increased in IFN-Gamma in migraine patients than in healthy controls. According to (Cowan et al., 2021) there was no change in the blood levels of IFN-γ between migraine patients and healthy individuals.

In our study, we observed various correlations between different parameters. The results indicated a negative relationship between Vitamin D and Zinc, with Zinc explaining 6.3% of the variation in Vitamin D levels for migraine patients (R-square = 6.3%). Additionally, there was a negative relationship between MDA and SOD, where SOD accounted for 49.2% of the variation in MDA levels for migraine (R-square = 49.2%). We also found a negative relationship between CRP and IL-10 levels, with IL-10 explaining 0.5% of the variation in CRP levels for migraine (R-square = 0.5%). Conversely, a positive relationship was observed between CRP and IFN-gamma, with IFN-gamma explaining 0.01% of the variation in CRP levels for migraine (R-square = 0.01%). Similarly, a positive relationship was found between IL-10 and IFN-gamma, with IFN-gamma accounting for 3.4% of the variation in IL-10 levels for migraine (R-square = 3.4%). Additionally, there was a positive relationship between MDA and Vitamin D, where Vitamin D explained 4.1% of the variation in MDA levels for migraine (R-square = 4.1%). Lastly, a negative relationship between MDA and Zinc was observed, with Zinc explaining 59.4% of the variation in MDA levels for migraine (R-square = 59.4%).

In our study the risk estimation for migraine based on dietary intake showed the following results:

In the univariate model:

- Milk intake was negatively associated with migraine [OR=0.78, CI (0.45-1.34)].

- Occasional milk intake also showed a negative association with migraine [OR=0.13, CI (0.01-1.17)].
- Fish intake was positively associated with migraine [OR=1.47, CI (0.85-2.53)].
- Occasional fish intake had a negative association with migraine [OR=0.93, CI (0.35-2.45)].
- Fuzzy drink intake was negatively associated with migraine [OR=0.71, CI (0.39-1.29)].
- Occasional fuzzy drink intake was positively associated with migraine [OR=1.09, CI (0.58-2.07)].

In the multivariate model:

- Milk intake showed a negative association with migraine [OR=0.75, CI (0.43-1.32)].
- Occasional milk intake continued to show a negative association with migraine [OR=0.12, CI (0.01-1.09)].
- Fish intake remained positively associated with migraine [OR=1.47, CI (0.84-2.56)].
- Occasional fish intake showed a positive association with migraine [OR=1.03, CI (0.38-2.80)].
- Fuzzy drink intake was negatively associated with migraine [OR=0.7, CI (0.38-1.29)].
- Occasional fuzzy drink intake remained positively associated with migraine [OR=1.08, CI (0.56-2.09)].

The result of our study showed risk estimation of migraine in relation to CRP, IL-10 levels, IFN gamma, Vitamin D, SOD, and MDA revealed the following results:

In the univariate model:

- An increase in CRP levels (mg/dL) was positively associated with migraine [OR=1.21, CI (1.07-1.37)].
- An increase in IL-10 levels (pg/mL) showed a negative association with migraine [OR=0.99, CI (0.99-0.99)].

- An increase in IFN gamma (ng/mL) also exhibited a negative association with migraine [OR=0.99, CI (0.99-0.99)].
- An increase in Vitamin D (ng/mL) was positively associated with migraine [OR=1.03, CI (1.01-1.05)].
- An increase in SOD (nm/mL) showed a negative association with migraine [OR=0.62, CI (0.53-0.73)].
- An increase in MDA (U/mL) was strongly positively associated with migraine [OR=97.4, CI (20.2-468)].

All these associations were considered statistically significant with $p<0.05$.

In the multivariate model, after adjusting for age and gender:

- An increase in CRP levels (mg/dL) was positively associated with migraine [OR=1.26, CI (1.09-1.46)].
- An increase in IL-10 levels (pg/mL) continued to show a negative association with migraine [OR=0.99, CI (0.99-0.99)].
- An increase in IFN gamma (ng/mL) maintained its negative association with migraine [OR=0.99, CI (0.99-0.99)].
- An increase in Vitamin D (ng/mL) showed a positive association with migraine [OR=1.07, CI (0.99-1.16)].
- An increase in SOD (nm/mL) remained negatively associated with migraine [OR=0.8, CI (0.67-0.94)].
- An increase in MDA (U/mL) was positively associated with migraine [OR=11.5, CI (2.98-44.9)].

In the multivariate model, the associations of CRP, IFN gamma, IL-10, and MDA with migraine were found to be statistically significant with $p<0.05$.

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATION

Present study has established a relationship between serum levels of Vitamin D, Zinc, IL-10, CRP, IFN-Gamma, SOD and MDA and migraine in Pakistani population. Through careful analysis of patient's data collected from PNS SHIFA, it has been proved that migraine patients have increased levels oxidative stress and inflammatory markers and decreased levels of vitamin D, zinc, anti-inflammatory marker and anti-oxidant marker. Though, further studies are required to establish exact cause and effect relationship between Levels of these markers and migraine to further explore that whether decreased vitamin D, Zinc, anti-inflammatory marker and antioxidants have any effect on severity of migraine, however, it is considered that this research work has strong theoretical implication in establishing that increase CRP and MDA decreased levels of vitamin D, zinc and SOD as biomarker for diagnosis of Migraine

5.2.2 PRACTICAL IMPLICATIONS

It has a strong practical implication. Medical practitioner may advice Vitamin D, Zinc, IL-10, CRP, IFN-Gamma, SOD and MDA levels to be evaluated in the laboratory to diagnose migraine as the current study has proved that vitamin d, zinc, IL-10 and MDA levels are decreased in migraine patients. Moreover, these supplements can also aid medical practitioners for prevention of migraine.

5.2.3 POLICY IMPLICATIONS

Further studies are required to use results of this research work in policy formulation and implementation.

5. 3 LIMITATIONS & STRENGTHS OF STUDY

5.3.1 LIMITATIONS

• Self-funded study with no allocation of research funds from any institute or organization.

• Single centered study.

• Short duration of study of six months as part of MPhil degree requirement.

• Small sample size.

• Lack of relevant literature as no one has investigated this concept earlier in Pakistan

5.3.2 STRENGTHS

• Serum levels of these markers altogether are being detected first time in Pakistani population.

• New associations were discovered that could be useful in theory and practice.

• Sample size is recruited statistically in agreement with the prevalence of migraine in Pakistani population.

• Detailed statistical analysis provided a complete description of the data and the results.

5.4 FUTURE RESEARCH DIRECTIONS / RECOMMENDATIONS

• Future prospective studies are recommended to fully understand role of these markers in pathogenesis of migraine.

• Role of Vitamin D, Zinc and anti-oxidant marker should be explored as a putative therapeutic target.

• Precise threshold of levels of these markers to diagnose migraine or probability to develop migraine in future needs to be established

• Establish exact cause and effect relationship between the levels of these markers and migraine.

5.5 CONCLUSION

Our study concludes that migraine patients exhibit deficiencies in vitamin D and zinc, accompanied by reduced superoxide dismutase (SOD) levels and elevated malondialdehyde (MDA), indicating a role for oxidative stress in migraine pathogenesis. Increased CRP levels, along with reduced IFN-gamma and IL-10, point to a complex inflammatory response. The relationship between these biomarkers suggests that vitamin D and zinc deficiencies may exacerbate oxidative stress and inflammation, leading to neuronal damage and enhanced vasodilation, thus contributing to migraine development. These novel insights will aid healthcare professionals in refining the management approaches, allowing for targeted treatments that address underlying nutritional deficiencies, oxidative stress, and inflammation in migraine patients.

Overall, the present study has significant theoretical and practical implications, as these levels are being reported together for the first time.

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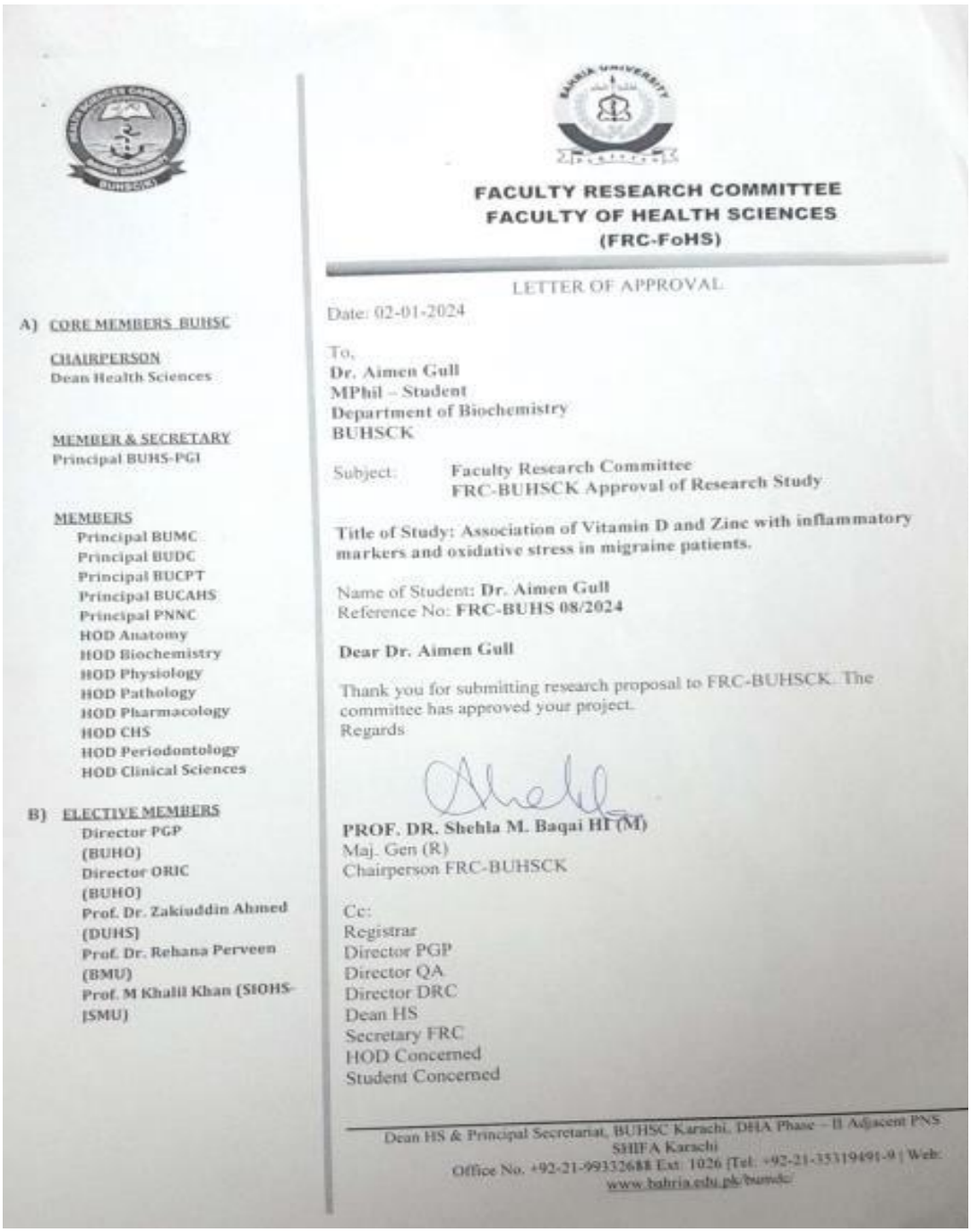
A



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FACULTY OF HEALTH SCIENCES
(FRC-FoHS)

LETTER OF APPROVAL

Date: 02-01-2024

To,
Dr. Aimen Gull
MPhil – Student
Department of Biochemistry
BUHSCK

Subject: **Faculty Research Committee**
FRC-BUHSCK Approval of Research Study

Title of Study: Association of Vitamin D and Zinc with inflammatory markers and oxidative stress in migraine patients.

Name of Student: **Dr. Aimen Gull**
Reference No: **FRC-BUHS 08/2024**

Dear Dr. Aimen Gull

Thank you for submitting research proposal to FRC-BUHSCK. The committee has approved your project.
Regards

PROF. DR. Shehla M. Baqai HI (M)
Maj. Gen (R)
Chairperson FRC-BUHSCK

Cc:
Registrar
Director PGP
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B

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Ms. Abida Rasool	Member
Dr. Nayyar Sabir	Member

BUHS-IRB # R-034/23
Date: 07/06/2024
Name of PI: Dr. Aimen Gull
Affiliation & Department: MPhil Candidate Biochemistry,
Address-BUHS,C Sailor Street, DHA Phase 2, Karachi

Subject: **APPROVAL OF YOUR RESEARCH PROPOSAL:**

Title of the research
Association of vitamin D and zinc with inflammatory markers and oxidative stress in migraine patients.

Dear Dr. Aimen Gull

I am writing this letter at your request to confirm that we support the research project **"Association of vitamin D and zinc with inflammatory markers and oxidative stress in migraine patients".** I know the research will determine the role of vitamin D and oxidative stress on migraines so that its deficiency can be detected and managed early, or prevention of migraines can be made through proper vitamin D and antioxidants supplementation. The IRB will support the project under the proposed guidelines in the IRB application for six months.

Suppose any unanticipated problems or adverse advents occur. In that case, it is up to Dr. Aimen Gull to report these events to the IRB as promptly as possible. This research will connect the dots between vitamin D, inflammatory markers in oxidative stress, and migraine patients in this region. We will be happy to support this endeavour.

Sincerely,

Thaver

Prof. Dr. Inayat H. Thaver
Chair, Institutional Review Board.

IRB Office, BUHSC(K) Adjacent PNS SHIFA, Sailor Street, DHA Phase – II Karachi
Office No. +92-21-35319491-6 Ext: 1080 | Fax: +92-21-99332689

C

WRITTEN INFORMED CONSENT FORM OF PATIENT

You are giving your consent to participate voluntarily and at your own will in this research clinical project that aims to analyze the role of vitamin D, oxidative stress and inflammatory marker in migraine.

You have been explained in detail the nature and significance of participating in the project and you understand the provided explanation.

You have been told that findings of your disease and your data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

You have been explained that laboratory investigations will be conducted for the diagnosis of disease. For this purpose, you fully agree to give your blood samples at the beginning and end of study.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified to you that no incentive will be provided to you for participating in the study except the cost of lab investigations and drugs, whereas you do have the right to withdraw from the study at any time.

You are advised to contact Dr. Aimen Gull on mobile number: 0319-4943069 or visit Shifa hospital in case of any query/ emergency related to your disease.

Name of Patient: _____

S/D/ W/o _____

Signature / Thumb impression of patient: _____

Name of Researcher: _____

Signature of Researcher: _____

مریض کی تحریری باخبر رضامندی کا فارم

آپ اس تحقیقی کلینیکل پروجیکٹ میں رضاکارانہ طور پر اور اپنی مرضی سے حصہ لینے کے لیے اپنی رضامندی دے رہے ہیں جس کا مقصد درد شقیقہ اور آکسیٹیٹیو تناؤ پر وٹامن ڈی اور سوزش مارکر کے کردار کا

تجزیہ کرنا ہے۔ آپ

کو پروجیکٹ میں حصہ لینے کی نوعیت اور اہمیت کے بارے میں تفصیل سے بتایا گیا ہے اور آپ فراہم کردہ

وضاحت کو سمجھتے ہیں۔ آپ کو

بتایا گیا ہے کہ آپ کی بیماری کے نتائج اور آپ کے ڈیٹا کو سختی سے خفیہ رکھا جائے گا اور صرف

کمیونٹی، اشاعتوں اور کاغذی پیشکشوں کے فائدے کے لیے استعمال کیا جائے گا۔ آپ کو سمجھایا گیا ہے کہ

بیماری کی تشخیص کے لیے لیبارٹری تحقیقات کی جائیں گی۔ اس مقصد کے لیے، آپ

مطالعہ کے آغاز اور اختتام پر اپنے خون کے نمونے دینے پر مکمل اتفاق کرتے ہیں۔ آپ تمام متعلقہ معلومات کو مکمل طور پر اور اپنی بہترین معلومات کے مطابق محقق کو دینے سے بھی اتفاق کرتے ہیں۔ آپ کے لیے واضح کیا جاتا ہے کہ آپ کو مطالعہ میں حصہ لینے کے لیے کوئی ترغیب نہیں دی جائے گی سوائے لیب کی تحقیقات اور ادویات کے اخراجات کے، جب کہ آپ کو کسی بھی وقت مطالعہ سے

دستبردار ہونے کا حق حاصل ہے۔ آپ کو

مشورہ دیا جاتا ہے کہ ڈاکٹر ایمن گل سے موبائل نمبر: 0319-4943069 پر رابطہ کریں یا اپنی بیماری سے متعلق کسی بھی سوال/ایمرجنسی کی صورت میں شفا ہسپتال تشریف لائیں۔

مریض کا نام:_____ ____

S/D/ W/o

مریض کے دستخط / انگوٹھے کا نشان:_____

محقق کا نام:_____

محقق کے دستخط:_____

D

SUBJECT EVALUATION FORM

Demographic Parameters	
Serial No	
Date	
Name	
Father's/ Husband's Name	
Sex	
Age	
Ethnicity	
General Physical Examination	
Height	
Weight	
Blood Pressure	
Body Temperature	
Body Mass Index	
Participant's History	
Smoking History	Yes/ No
Drug history	Yes/ No
History of fracture	Yes/ No
Frequency of infections	
Dietary Patterns	
Milk intake	
Fish intake	
Fizzy drinks intake	
Sunlight Exposure	
Resident of	Apartments/Bungalow
Working hours	
Laboratory Investigations	
Serum Vitamin D levels	
CRP Levels	

QUESTIONNAIRE

Appendix I

Name _____ Father/ Husband Name _____

Age _____Years Gender: Male / Female

Weight _____kg Height _____m BMI _____

Native (City, town, village)

Ethnicity (Punjabi, Sindhi, Balochi, Saraiki, Pashtun, Muhajir, Kashmiri, hazara others)

1. Did you have two or more headaches in the last 3 months? (Yes/No)
2. How many hours of sleep do you typically get per night?
3. Do you find that lack of sleep or poor sleep quality triggers your migraines? (Yes/No)
4. How much time do you typically spend using electronic devices, such as smartphones, tablets, or computers?
5. Do you find that prolonged screen time triggers your migraines or exacerbates your symptoms? (Yes/No)
6. Family History of Migraine/headache. (Yes/No)
7. Smoking habits (Yes/No)
8. Fast Food intake (frequency per week)
9. How much caffeine do you consume daily?
10. What are your main sources of caffeine? (Coffee, tea, energy drinks, or soda)
11. Do you find that caffeine consumption triggers your migraines or exacerbates your symptoms? (Yes/No)
12. Have you ever tried reducing your caffeine intake to see if it helps to manage your migraines? (Yes/No)
13. How often do you engage in physical activity or exercise? (30 min per day normal)

14. Do you find that physical activity triggers your migraines, or helps to alleviate them? (Yes/No)
15. Any co – morbidities or psychological illnesses? (Yes/No)
16. Did you ever self-medicate for migraine / headache? (Yes/No)
17. What medications do you use for headaches?
18. Do you consume enough fluids, such as water, daily?
19. What was the Intensity of headache during the attack?

    0 = no headache 1 = mild headache 2 = moderate headache 3 = severe headache)
20. What symptoms do you feel during an attack? (Nausea, Vomiting, Photophobia, others)
21. What are the Characteristics of your headache? (Unilateral, bilateral, pulsating, and throbbing)

Appendix II

HIT – 6 Scale

Please rate how frequently you experience the following symptoms when you have a headache:

	NEVER	RARELY	SOMETIMES	VERY OFTEN	ALWAYS
1. When you have headaches, how often is the pain severe?					
2. How often do headaches limit your ability to do usual daily activities including household work, work, school, or social activities?					
3. When you have a headache, how often do you wish you could lie down?					
4. In the past 4 weeks, how often have you felt too tired to do work or daily activities because of your headaches?					
5. In the past 4 weeks, how often have you felt fed up or irritated because of your headaches?					
6. In the past 4 weeks, how often did headaches limit your ability to concentrate on work or daily activities?					

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