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Plasma Levels of Cyclooxygenase-2 (COX-2) and Visfatin During Different Stages and Different Subtypes of Migraine Headaches

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Background: The aim of this study was to determine the plasma levels of cyclooxygenase-2 (COX-2) and visfatin in different stages and different subtypes of migraine headaches compared to a control group to elucidate the pathological mechanisms involved.

Material/Methods: We recruited a case-control cohort of 182 adult migraine patients and 80 age-matched and gender-matched healthy controls. The migraine patients were divided into two groups: the headache-attack-period group (Group A, n=77) and the headache-free-period group (Group B, n=105). The two groups were further divided into subgroups according to whether they had aura symptoms. Solid phase double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure the plasma levels of COX-2 and visfatin. Statistical analysis was performed using SPSS 17.0.

Results: The plasma levels of COX-2 and visfatin in the headache-attack-period group were significantly higher than in the headache-free-period group and the control group; there were no significant differences between the headache-free group and the control group. There were no significant differences in plasma levels of COX-2 and visfatin between the subgroups: headache-attack-period with aura subgroup and the headache-attack-period without aura subgroup.

Conclusions: COX-2 and visfatin participated in the pathogenesis of migraine headaches. The presence of aura had no effect on the serum levels of COX-2 and visfatin.

MeSH Keywords: **Cyclooxygenase 2 • Inflammation • Migraine Disorders • Nicotinamide Phosphoribosyltransferase**

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Background

Migraine headache is a common clinical disease, the term is mainly used to describe recurrent unilateral or bilateral pulsating headaches. Although the pathogenesis of migraines remains unclear, the trigeminovascular theory is generally recognized by most scholars. This theory suggests that the trigeminal vascular system (TVS) is influenced by a variety of factors that influence a series of inflammatory substances to cause aseptic neurologic inflammation, which causes the migraine headache [1,2]. In our earlier research, we published several studies based on this theory [3–5].

COX-2 is a key enzyme in the synthesis of prostaglandins (PG) which are known to participate in a variety of inflammatory reaction processes. In addition, previous studies have reported high levels of expressions of COX-2 in animal brains [6], but there is no published report on the relationship between human plasma levels of COX-2 and migraines. Likewise, there have been no reports on the relationship between the plasma level of visfatin and migraines. Visfatin is a cytokine secreted by fat cells and is also participates in the inflammatory process.

This study explore the connection of COX-2 and visfatin with migraines through the detection of the plasma levels of these compounds in different headache periods and different types of migraine patients, and discusses possible strategies for migraine prevention.

Material and Methods

The study object

From March 2013 to January 2015, 182 patients with migraine headaches were recruited from the outpatient department, emergency department, and inpatient department of Neurology, at the People's Hospital of Liaoning Province. There were 77 patients who were in a headache-attack period, 105 patients who were in a headache-attack interval (free period), 56 patients with aura, and 126 patients without aura. At the same time, 80 healthy people were selected as the control group. There were no significant differences in age, sex, race, occupation, and BMI among patients in the headache-attack-period group, patients in the headache-free-period group, and the control group. The International Headache Society (ICHD-II) criteria [7] was used to identify patients with migraine headaches; and patients' disorders were diagnosed by two different neurologists. All the patients gave their written informed consent for the examination. This study was conducted in accordance with the Declaration of Helsinki and approved by our hospital ethics committee.

Inclusion and exclusion criteria

The inclusion criteria was as follows: 1) age 18–65 years; and 2) able to understand and cooperate with the needs of the study.

The exclusion criteria was as follows: 1) BMI <18 kg/m² >30 kg/m²; 2) more than 15 headache attacks per month; 3) taking any pain medication such as triptans or opioids 72 hours prior to blood sample collection; 4) taking any preventive medication for migraines in the last month; 5) history of cerebrovascular or cardio disease, diabetes, hypertension, hyperlipidemia, severe renal or hepatic disease; 6) abnormalities in routine blood tests, erythrocyte sedimentation rate, or rheumatoid factor abnormalities; (7) history of rheumatoid arthritis, systemic lupus erythematosus, or other autoimmune rheumatic diseases; 8) pregnant and/or breastfeeding.

Groups

All patients diagnosed with migraines were divided into either the headache-attack-period group (Group A, n=77) or the headache-free-period group (Group B, n=105), and these two groups were further divided into four subgroups, with and without aura: the headache-attack-period with aura group (A1, n=22), the headache-attack-period without aura group (A2, n=55), the headache-free-period with aura group (B1, n=34) and the headache-free-period without aura group (B2, n=71). There were 80 healthy volunteers selected as the control group (Group C, n=80).

Research method

General data acquisition

Using a unified self-made investigation form, the study participants were assigned a number and their demographic details recorded (name, gender, age, race, occupation, cultural degree, height, weight, contact telephone number), as well as headache-related information (location of headache, nature, sustainable time, induced and mitigating factors, originating age, frequency and extent, with or without aura, family history, past medical history, treatment history, and auxiliary examination).

Specimen detection

Group A blood samples were collected during a headache attack. The fasting blood samples of Group B and Group C were collected at 7–8 am: 2 mL peripheral venous blood samples were collected into a heparin sodium tube, and centrifuged at 3,000 rpm for 15 minutes to obtain plasma samples, then stored at –80°C until needed. The plasma levels of COX-2 and visfatin were measured by enzyme-linked immunoassay (COX-2, visfatin; Yi Bang Shanghai Biological Technology Co, Ltd.) according

Table 1. Comparison of the general situation of the participants in each group.

Group	Number n	Gender		Work		Age (years)	BMI (kg/m ²)
		Male	Female	Mental worker	Physical worker		
Group A	77	37.66%	62.34%	66.23%	33.77%	29.98±8.62	22.4±2.1
Group A1	22	36.36%	63.64%	68.18%	31.82%	30.01±7.83	22.1±3.2
Group A2	55	38.18%	61.82%	64.10%	35.90%	29.60±8.87	22.7±2.3
Group B	105	36.19%	63.81%	64.76%	35.24%	27.42±6.28	21.5±3.1
Group B1	34	32.35%	67.65%	64.71%	35.29%	28.12±5.41	23.1±2.8
Group B2	71	38.02%	61.98%	64.79%	64.21%	26.77±7.22	20.7±2.5
Group C	80	35.00%	65.00%	66.67%	33.33%	30.21±8.39	23.3±3.4

Group A – Headache-attack-period group; Group A1 – headache-attack-period with aura group; Group A2 – headache-attack-period without aura group; Group B – headache-free-period group; Group B1 – headache-free-period with aura group; Group B2 – headache-free-period without aura group; Group C – the control group. There was no significant difference between three groups for gender, occupation, age, or BMI ($p>0.05$).

to the manufacturer's instructions. The experimental steps were as follows: place the COX-2 and visfatin kit at room temperature (20–25°C) for 30 minutes; set up the standard group, blank control group, and the sample group; dilute the samples and the fixed protein according to the experimental manual; place them in a 96-well plate at 37°C for 30 minutes; then wash them five times; add enzyme conjugate to the standard group and specimen group; incubate at 37°C for one hour; then wash the plate five times again; add chromogenic agent to each well for 15 minutes at 37 °C in the dark; add stop solution and determine the optical density with a microplate reader set to 450 nm; and calculate the concentration of each sample.

Statistical processing

We used SPSS 17.0 statistical software for statistical analysis of data with mean standard \pm deviation ($\bar{x}\pm s$). We used a 2-sample *t*-test for two-group comparisons and we used analysis of variance (ANOVA) for multi-group analysis; rates were compared with chi square test; $p<0.05$ was considered the level of significant difference.

Results

Patients with migraine headaches enrolled in this study included 182 cases; men accounted for 36.81% (67/182), women accounted for 63.19% (115/182); the sex ratio was 1: 1.72. The age of onset of migraine was mainly concentrated at 10 years to 30 years old (82.41% or 150/182); age of onset over 60 years was rare (1.11% or 2/182). "Mental" workers (versus "physical" workers) accounted for 66.10% (119/182 patients).

There were no significant differences among the headache-attack-period group, the headache-free-period group, and the control group in terms of gender, age, BMI, and professional work category ($p>0.05$), see Table 1.

Table 2 shows the median and range of plasma levels for the investigated parameters in the migraine patient groups and in healthy control group. Migraine patients had significantly higher COX-2 and visfatin plasma levels during migraine headache attacks (Group A) compared with headache-free intervals (Group B) and healthy controls (Group C) ($p<0.05$). We also noticed no statistically significant difference between the concentrations of COX-2 and visfatin with patients in the headache-free period (Group B) and healthy participants (Group C), $p>0.05$. Additionally, there were no statistically significant differences in the headache-attack-period with aura group compare to the headache-attack-period without aura group regarding the plasma levels of COX-2 and visfatin ($p>0.05$).

Discussion

Migraine headache is a common disorder seen in patients presenting to the neurological department, it presents with repeatedly and frequently occurrences that exhibit unilateral pulsating headaches. A survey showed that the world migraine morbidity ranged from 8.4% to about 28%, and the incidence rate of females was significantly higher than that of males [8]. The pathogenesis of migraines is not clear, but the trigeminovascular hypothesis is recognized by most scholars. This hypothesis suggests that the activation of the trigeminovascular system is closely related to migraines, and that

Table 2. Comparison of plasma COX-2 and lipid levels in each group.

Group	Number (n)	COX-2 (ng/mL)	Vistafin (pg/mL)
Group A	77	4.03±3.86 ^{*,**}	21.30±21.90 ^{*,**}
Group A1	22	4.12±5.76 [#]	20.08±31.33
Group A2	55	3.85±3.50 ^{##}	22.20±16.12
Group B	105	2.38±1.83 ^{***}	8.79±12.36
Group B1	34	2.19±2.38	9.98±10.46
Group B2	71	2.57±1.71	8.34±14.31
Group C	39	2.16±1.64	8.23±8.71

Group A – headache-attack-period group; Group A1 – headache-attack-period with aura group; Group A2 – headache-attack-period without aura group; Group B – headache-free-period group; Group B1 – headache-free-period with aura group; Group B2 – headache-free-period without aura group; Group C – the control group. * Statistically significant difference between Group A and Group B ($p<0.05$); ** Statistically significant difference between Group A and Group C ($p<0.05$); *** No statistically significant difference between Group B and Group C ($p>0.05$); # Statistically significant difference between Group A1 and Group B1 ($p<0.05$); ## Statistically significant difference between Group B1 and Group B2 ($p<0.05$).

aseptic neural inflammation is the pathophysiological basis of migraines. The acting trigeminovascular system can release calcitonin gene-related peptide (CGRP), P substance, neurokinin A, and so on, and that these substances can cause aseptic meningitis. A large number of inflammatory mediators, including IL-6, TNF-alpha, nitric oxide (NO), and prostaglandin E2 (PGE2) are released into the blood and activate pain receptors such that a headache-attack occurs [9,10]. Our previous studies have shown that 5-HT, NF-kappa B, ICAM-1, and PPARs are closely related to migraines [3–5].

COX is a rate-limiting enzyme in the synthesis of prostaglandins (PGs) that intervenes in the process of arachidonic acid metabolism. COX can be divided into two subtypes: COX-1 and COX-2. COX-1 is a structural enzyme that exists in normal tissue cells and participates in the regulation of vascular relaxing and platelet aggregation [11]. COX-2 is an inducible enzyme, which is expressed in normal tissue at a low level, but it can be activated to a high level of expression in certain tissues, such as monocytes, macrophages, and vascular endothelial cells through inflammation; the expression level of COX-2 is closely related to the severity of inflammation [12,13]. There are a number of studies that have shown the close relationship between COX-2 and migraines in animal models [6], but no study has been carried out in human beings.

In this study, we found that the plasma levels of COX-2 in patients with migraines in headache-free periods and the plasma levels in the healthy controls were both low in expression. By contrast, COX-2 levels were significantly higher in patients with migraines in the headache-attack period compared with patients in the headache-free period and healthy controls; this effect held true in both migraine patients with and without

aura. Those results showed that COX-2 was involve in the process of acute migraine headache attack and might promote the occurrence of headache attack. We speculate that the specific mechanism might be as follows: in the migraine attack period the expression levels of some specific proinflammatory factors, such as tumor necrosis factor and NF-κB are elevated [14,15], these proinflammatory factors can upregulate the expression of COX-2 [16,17], and the expression of prostaglandin will then be raised by the irritation of COX-2 [18]. High levels of prostaglandin can directly affect the cerebral vascular and platelet function, and promote the occurrence of a headache attack [19]. There may also be another way that COX-2 participates in an acute migraine attack: the increased expression of COX-2 may raise the expression of matrix metalloproteinase 9 (MMP-9) by inflammation factors, and those actions probably lead to injury of the blood brain barrier and edema, promoting the occurrence of acute migraine attack [20,21]. Many studies have confirmed MMP-9 has a close relationship with acute migraine attack [22].

Visfatin is a kind of pre-B cell clonal factor of lymphocyte secretion (pre-B cell colony enhancing factor or PBEF), which is highly expressed in adipose tissue, liver, kidney, brain, and muscle. Research on visfatin has mainly focused on metabolic and immune diseases; and the role of inflammatory mediators is being gradually recognized in such studies. Visfatin can induce the expression of proinflammatory cytokines such as IL-1, IL-6, and TNF-alpha, according to its dose; the activity of nuclear factor kappa B (NF-κB) can also be increased by visfatin, which then increases the expression of matrix metal protein-2 (MMP-2) and MMP-9 [23–25].

In this study, there were statistical differences in visfatin plasma levels among patients in the headache-attack-period group, headache-free-attack period group, and the healthy control group, but there were no significant differences between the headache-free-attack group and the control group. These results suggest that visfatin is involved in the process of migraine headache attacks and likely promotes the occurrence of headache attacks. The probable mechanism between a high level expression of visfatin and an acute migraine attack may be as follows: some special instance may elevate the expression level of visfatin during the acute migraine attack period, and this high level of visfatin may upregulate the transcriptional activity of NK- κ B, then some inflammation factors, such as adhesion molecules (VCAM-1, ICAM) and nitric oxide synthase (iNOS) may be elevated due to the irritation of NK- κ B and thus promote the occurrence of a headache attack [26,27].

Cortical spreading depression (CSD) has been considered as the pathophysiological equivalent of migraine aura. In this study, there was no statistical difference between patients with migraines with aura and without aura regarding plasma levels of

COX-2 and visfatin. We believe that COX-2 and visfatin may be involved in the inflammatory process of migraine headaches, but they have no effect on the excitability of the cerebral cortex.

In conclusion, COX-2 and visfatin may be involved in the process of acute migraine attacks. The upregulation of serum levels of COX-2 and visfatin probably causes acute migraine attacks, and the downregulation of serum levels of COX-2 and visfatin may prevent the occurrence of migraine attacks, which possibly provides a way to prevent the occurrence of migraine attack and better understand the mechanism of migraine headaches.

Conclusions

COX-2 and visfatin may be involved in the neurogenic inflammation pathophysiology of migraines and promote the occurrence of migraines. If we can find a way to downregulate the expression of COX-2 and visfatin, the occurrence of migraines could be prevented.

References:

- Miller S, Matharu MS: Migraine is underdiagnosed and undertreated. *Practitioner*, 2014, 258(1774): 19–24, 2–3
- Cutrer FM: Pathophysiology of migraine. *Semin Neurol*, 2010; 30(2): 120–30
- Qiu H, Huai-liang W, Xin-hua Z et al: The role of 5-HT₁ receptors agonists in neurogenic inflammation of dura mater of intravenous glyceryl trinitrate induced rates. *J Apoplexy Nervous Diseases*, 2007; 24(5): 520–22
- He Q, Lin X, Wang F et al: Associations of a polymorphism in the intercellular adhesion molecule-1 (ICAM1) gene and ICAM1 serum levels with migraine in a Chinese Han population. *J Neurol Sci*, 2014; 345(1–2): 148–53
- He-min Z, Guo-Rong B, Qiu H et al: Changes in plasma PPARs levels in migraine patients. *Med Sci Monit*, 2015; 21: 735–39
- Tassorelli C, Greco R, Armentero MT et al: A role for brain cyclooxygenase-2 and prostaglandin E₂ in migraine: effects of nitroglycerin. *Int Rev Neurobiol*, 2007; 82: 373–82
- International Headache Society (2004): The international classification of headache disorders: 2nd edition. *Cephalalgia*, 2004; 24(Suppl.1): 9–160
- Lipton RB, Stevan WF, Sinlon D: Medical consultation for migraine: Results from the American Migraine Study. *Headache*, 1998; 38: 91–96
- Edvinsson L, Villalón CM, Maassen Van Den Brink A: Basic mechanisms of migraine and its acute treatment. *Pharmacol Ther*, 2012; 136(3): 319–33
- Kim GM, Jin KS, Chung CS: Differential effects of corticosteroids on the expression of cyclooxygenase-2, tumor necrosis factor- α and matrix metalloproteinase-9 in an animal model of migraine. *Cephalalgia*, 2008; 28(11): 1179–87
- Simmons DL, Botting RM, Hla T: Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. *Pharmacol Rev*, 2004; 56: 387–437
- Dicken BJ, Graham K, Hamilton SM et al: Lymphovascular invasion is associated with poor survival in gastric cancer: An application of gene-expression and tissue array techniques. *Ann Surg*, 2006; 243: 64–73
- Masferrer JL, Leahy KM, Koki AT et al: Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res*, 2000; 60: 1306–11
- Fawzi MS, El-Shal AS, Rashad NM et al: Influence of tumor necrosis factor alpha gene promoter polymorphisms and its serum level on migrainesusceptibility in Egyptian patients. *J Neurol Sci*, 2015; 348(1–2): 74–80
- Greco R, Tassorelli C, Cappelletti D et al: Activation of the transcription factor NF- κ B in the nucleus trigeminalis caudalis in an animal model of migraine. *Neurotoxicology*, 2005; 26(5): 795–800
- Lee K M, Kang BS, Lee HL et al: Spinal NF- κ B activation induces COX-2 up regulation and contributes to inflammatory Pain hypersensitivity. *Eur J Neurosci*, 2004; 19: 3375–81
- Kim GM, Jin KS, Chung CS: Differential effects of corticosteroids on the expression of cyclooxygenase-2, tumor necrosis factor- α and matrix metalloproteinase-9 in an animal model of migraine. *Cephalalgia*, 2008; 28(11): 1179–87
- Simmons DL, Botting RM, Hla T: Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. *Pharmacol Rev*, 2004; 56: 387–437
- Dolkart O, Amar E, Shapira S et al: Protective effects of rosuvastatin in a rat model of lung contusion: Stimulation of the cyclooxygenase 2-prostaglandin E-2 pathway. *Surgery*, 2015; 157(5): 944–53
- Sun WH, Sun YL, Fang RN et al: Expression of cyclooxygenase-2 and matrix metalloproteinase-9 in gastric carcinoma and its correlation with angiogenesis. *Jpn J Clin Oncol*, 2005; 35: 707–13
- Badawi AF, Eldeen MB, Liu Y et al: Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor. *Cancer Res*, 2004; 64(3): 1181–89
- Gupta VK: CSD, BBB and MMP-9 elevations: Animal experiments versus clinical phenomena in migraine. *Expert Rev Neurother*, 2009; 9(11): 1595–614
- Matsui H, Tsutsumi A, Sugihara M et al: Visfatin (pre-B cell colony-enhancing factor) gene expression in patients with rheumatoid arthritis. *J Ann Rheum Dis*, 2008; 67(4): 571–72
- Lee WJ, Wu CS, Lin H et al: Visfatin-induced expression of inflammatory mediators in human endothelial cells through the NF- κ B pathway. *Int J Obes (Lond)*, 2009; 33(4): 465–72
- Adya R, Tanb K, Chen J et al: Nuclear factor kappa B induction by visfatin in human vascular endothelial cells: Its role in MMP-2/9 production and activation. *Diabetes Care*, 2008; 31(4): 758–60
- Adya R, Tanb K, Chen J et al: Pre-B cell colony enhancing factor(PBEF)/visfatin induces secretion of MCP-1 in human endothelial cells: Role in visfatin-induced angiogenesis. *Atherosclerosis*, 2009; 205(1): 113–19
- Sarchielli P, Floridi A, Mancini ML et al: NF- κ B activity and iNOS expression in monocytes from internal jugular blood of migraine without aura patients during attacks. *Cephalalgia*, 2006; 26(9): 1071–79