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# INFLUENCE OF MIGRAINE ON AXON REFLEX-MEDIATED AND ENDOTHELIAL-DEPENDENT VASODILATATION IN THE SKIN

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*The aim of the study was to evaluate the changes in skin blood flow as a result of local heating tests in migraine patients during the interictal period, measured by laser Doppler perfusion imaging (LDI). The aim of the study was also to estimate the correlations between the results of these tests and interleukin (IL)-8 levels. Twelve migraine patients during their interictal period were compared with twelve healthy control subjects. Only women were included in the study. Both groups were matched with regard of their age, body mass index and blood pressure. For the purpose of measuring cutaneous microvascular blood flow, heating (+44 °C) of the dorsal side of the palm as a response to the local LDI was used. IL-8 was measured in serum by ELISA method. The findings suggested that migraine patients have a cutaneous vasomotor dysfunction during the interictal period. The results showed a significant decrease in the initial peak of vasodilation and the second peak of vasodilation (plateau phase). Also there were significant changes observed in the length of the time interval required to reach the first and second vasodilation peak. It is known that migraine patients have a shorter time interval to reach the first perfusion peak (axonal reflex-mediated) and longer time interval to reach the second peak (endothelium-dependent). The results confirmed the correlation between proinflammatory chemokine IL-8 levels, and the time interval till the second peak of blood flow in all study subjects.*

**Key words:** *laser Doppler perfusion imaging, calcitonin gene-related peptide, nitric oxide, interleukin-8, cutaneous vasomotor dysfunction.*

## INTRODUCTION

Migraine constitutes a common painful neurovascular disorder affecting around 10% of population worldwide, involving 18% of females and 6% of males (Munno *et al*., 1998; Conti *et al*., 2019). The exact pathophysiological mechanism of migraine, with and without aura, is still unclear, but activation of the trigeminovascular system and cortical spreading depression are thought to play important roles (Kowalska *et al*., 2016). During a migraine attack, the unbalanced levels of neurotransmitters and neuromodulators in the synaptic clefts of the pain pathways activate the trigeminal afferents that release calcitonin gene-related peptide (CGRP), substance P, pituitary adenylate cyclase-activating polypeptide and neurokinin A, leading to vasodilatation and an increase of vascular permeability including degranulation of mast cells and excitation of dural veins, all of which cause also neurological inflammation (Kowalska *et al*., 2016; Yücel *et al*. 2016; Marics *et al*. 2017; Conti *et al*., 2019).

The plasma level of CGRP is significantly higher between attacks in migraine patients. CGRP is involved in the transmission of pain and the promotion of inflammation, including by stimulating the release of inflammatory cytokines, which play an important role in migraine (Kowalska *et al*., 2016; Yücel *et al*. 2016). Cytokines and other inflammatory mediators may increase sensitisation of meningeal nociceptors and thus induce activation of the trigeminovascular nociceptive pathway. Central sensitisation is an important pathophysiological mechanism in the development of migraine (Marics *et al*., 2017).

Persistent pain in migraine patients may be associated with activation of the immune and inflammatory systems (Conti *et al*., 2019). Studies confirm that peripheral circulation concentrations of proinflammatory cytokines, including proteininflammatory chemokines, e.g., interleukin (IL)-8, are increased during the ictal period (migraine attacks) and also during the interictal period (headache-free days) (Munno *et al*., 1998).

Endogenously produced nitric oxide (NO) is heavily implicated in migraine, and NO regulation is significantly altered in migraine pathogenesis (Amynah *et al*., 2018). NO not only causes excessive vasodilation by direct action to smooth muscle cells in the walls of blood vessels of migraine patients, but also causes an excessive release of CGRP from the perivascular sensory nerves. CGRP-induced vasodilation is partly dependent on NO release. Data that are already published confirm a significant role of both CGRP and NO on vascular changes observed during a migraine attack (de Hoon *et al*., 2006).

Some studies have tested the hypothesis that migraine patients are particularly sensitive to CGRP and NO signals in forearm blood flow, including at the cutaneous microvascular level. Vasomotor responses have been evaluated by various methods: ultrasound technique (de Hoon *et al*., 2006), plethysmography (Napoli *et al*., 2009), laser Doppler flowmetry (LDF; measures blood flow at a single point) (Edvinsson and Edvinsson, 2008), and laser Doppler perfusion imaging (LDI; areas are scanned) (Ibrahimi *et al*., 2017).

Capsaicin is known to activate transient receptor potential vanilloid 1 and thus causing the release of CGRP from peripheral sensory nerve endings (sensory axonal reflex). The LDI test with use of topical application of capsaicin showed that an CGRP-induced increase in dermal blood flow was higher in female migraineurs compared to healthy subjects (Ibrahimi *et al*., 2017). However, some other studies using other methods did not show significant vasomotor changes in migraine patients, such as LDF in combination with iontophoresis. Data showed that there is no change in the microvascular responsiveness of the subcutaneous microvasculature in migraine patients (Edvinsson and Edvinsson, 2008). Another study showed that (1) changes in forearm blood flow (FBF) during intrabrachial infusion of serotonin, sodium nitroprusside, and CGRP as measured by venous occlusion plethysmography, and (2) flow-mediated dilation of the brachial artery as measured by ultrasound method, suggested that neither NO nor CGRP increased vascular resistance in migraine patients (de Hoon *et al*., 2006). Of particular interest was another study that used plethysmography to assess FBF during infusions of vasoactive agents into the brachial artery and found that migraine patients have a severe vascular smooth muscle dysfunction (Napoli *et al*., 2009).

We hypothesised that the LDI local heating test data will reflect cutaneous vasomotor dysfunction, including significant changes in the sensory axonal reflex and endothelium-dependent vasodilation in migraine patients and that the results of this test will correlate with blood levels of proinflammatory chemokines IL-8.

LDI is a reliable technique for the measurement of cutaneous blood flow under physiological and pathophysiological conditions, including for measurement of thermoregulatory vascular responses (Lakatos *et al*., 2020). The skin blood flow response to local, rapid heating below the pain threshold (~ 43 °C) is different from the mechanisms that increase microvascular blood flow in response to an increased overall temperature of the body. There is a biphasic increase in the skin blood flow due to two different mechanisms. The initial phase shows a rapid increase in blood flow and is mediated by a sensory axon reflex. In this phase, temperature-induced activation of skin sensory C-fibber afferent neurons occurs, releasing CGRP and substance P. The secondary phase follows after a brief nadir and there is a slow increase in blood flow until it reaches a plateau. This phase is ~70% dependent on the vasodilatory effect of endothelial NO on smooth myocytes (Houghton *et al*., 2006; Low *et al*., 2020). Studies suggest that the release of CGRP and substance P may be partially dependent on NO (Houghton *et al*., 2006).

The aim of our study was to evaluate the changes in the skin blood flow caused by the LDI local heating test in migraine patients during their interictal period, as well as to estimate the correlations between the results of these tests and IL-8 levels.

## MATERIALS AND METHODS

**Study subjects.** During their interictal period, twelve migraine patients were compared with twelve healthy control subjects. Only women, not in the menstrual period, were included in the study. Both groups were matched with regard to their age, body mass index and blood pressure. Healthy subjects were excluded if they had first-degree relatives suffering from migraine. The baseline characteristics for both study groups are shown in Table 1.

Exclusion criteria were: history of cardiovascular disease, arterial hypertension, hyperlipidaemia, diabetes mellitus and smoking. Other exclusion factors were thyroid dysfunction, acute or chronic inflammatory state, renal or liver diseases, malignancies, and other diseases that are known to be associated with significant changes of cytokines. No regular

*Table 1*. The baseline characteristics

| | Control group n = 12 | Patient group n = 12 | *p* value |
|---|---|---|---|
| Age, years | 38.9 (3.1) | 41.4 (3.9) | 0.09 |
| BMI, kg/m$^2$ | 23.4 (3.4) | 23.7 (3.3) | 0.66 |
| Systolic blood pressure, mm Hg | 113 (10) | 117 (13) | 0.24 |
| Diastolic blood pressure, mm Hg | 78 (9) | 79 (10) | 0.29 |

Data are expressed as a number (n) and mean (SD).

medication was allowed, including the hormonal contraceptive method.

This study was carried out in accordance with the Declaration of Helsinki. All subjects gave their informed consent to the protocol, which was approved by the Medical Ethics Committee of the Rīga Stradiņš University for biomedical research.

**LDI local heating test.** The LDI (Moor Instruments, Devon, UK) was used to measure cutaneous microvascular blood flow on the dorsal side of palm as a response to the local heating (+44.0 °C). Measurements were conducted in a quiet, temperature-controlled room (22.0 ± 0.5 °C), with the subjects in the supine position. Microvascular measurements were obtained after 15 minutes of acclimatisation with the investigated hand at heart level. The baseline blood flow was registered for first 3 min and the microcirculatory measurements of local heating +44 °C for the next 25 minutes. In this LDI test, microvascular blood flow (in perfusion units, PU) and the time intervals till the first (initial peak) and second blood flow (plateau) peaks were measured (Gazerani *et al*., 2011). The obtained data were processed and analysed with the MoorLDI V5.0 Research Software.

**Laboratory assays.** Venous blood samples for all blood biomarker tests of this study were collected from the study subjects in the morning after overnight fasting, on the day of the LDI test. Blood samples were centrifuged, and sera were stored at –80 °C. IL-8 was measured in serum by ELISA method using a TECAN Infinite 200 PRO multimode reader (Tecan Group, Ltd., Mannedorf, Switzerland). Concentrations of lipids, glucose, and other routine blood biomarkers were analysed by standard methods.

**Statistical analysis.** Data distributions were estimated with the Kolmogorov–Smirnov, Lilliefors and Shapiro–Wilk's tests. The data were recorded as medians (Q1 — the lowest; Q3 — the highest point of the interquartile range). Statistical differences between two groups were analysed using the Mann–Whitney test, including cases with a normal data distribution. Correlation analyses were performed using one-factor linear regression analysis. All analyses were performed using STATISTICA 10 software (StatSoft Inc., USA).

## RESULTS

Skin temperature in the LDI measurement area and basal perfusion before local heating did not differ between migraine patients and healthy subjects (29.0 (26.6; 30.8) vs. 27.9 (26.5; 28.3) °C, $p > 0.05$ and 478 (425; 521) vs. 539 (472; 644) PU, $p > 0.05$).

Local heating of the skin region caused an increase in blood flow. The LDI heating test data show that patients had a statistically significant reduction in both the first (initial maximum) and second blood flow (plateau) peaks (478 (425; 521) vs. 539 (471; 644) PU, $p < 0.05$ and 526 (437; 582) vs. 582 (535; 638) PU, $p < 0.05$) (Fig. 1).

The time interval till the first and second peak blood flows was not only statistically significantly different, but also showed the opposite effects. During the LDL local heating test the time interval till the first maximum (peak) was statistically significantly reduced in migraine patients (360 (315; 420) vs. 510 (390; 645) s, $p < 0.05$), but the time interval till the second peak was prolonged in these patients (1275 (1200; 1305) vs. 1380 (1275; 1455) s, $p < 0.05$) (Fig. 2).

There was a close correlation between the first and second blood flow peaks ($r = 0.71$; $p < 0.0001$) (Fig. 3), taking into account the data of all control subjects and migraine patients (Fig. 3), but the time interval till the first and second peaks was not correlated.

Patients with migraine did not have significantly higher serum IL-8 levels compared to controls, but a correlation was found between IL-8 and the time interval till the second blood flow maximum ($p < 0.05$) (Fig. 4).

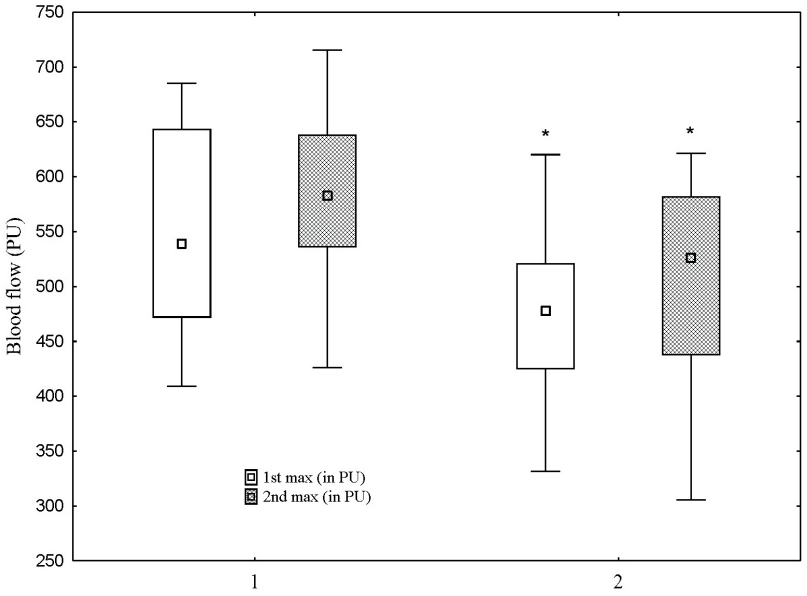


*Fig. 1.* Blood flow in healthy subjects (1) and migraine patients (2). Data are expressed as the medians (Q1; Q3). *$p < 0.05$ compared to the control group.

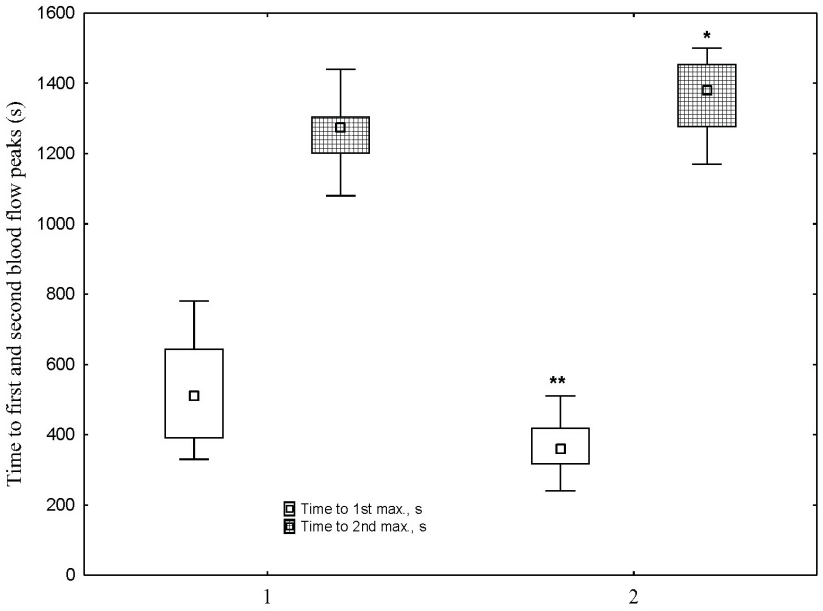


*Fig. 2*. Time interval till the first (initial peak) and second blood flow (plateau) peaks in healthy subjects (1) and migraine patients (2). Data are expressed as medians (Q1; Q3). *$p < 0.05$, **$p < 0.01$ compared to the control group.

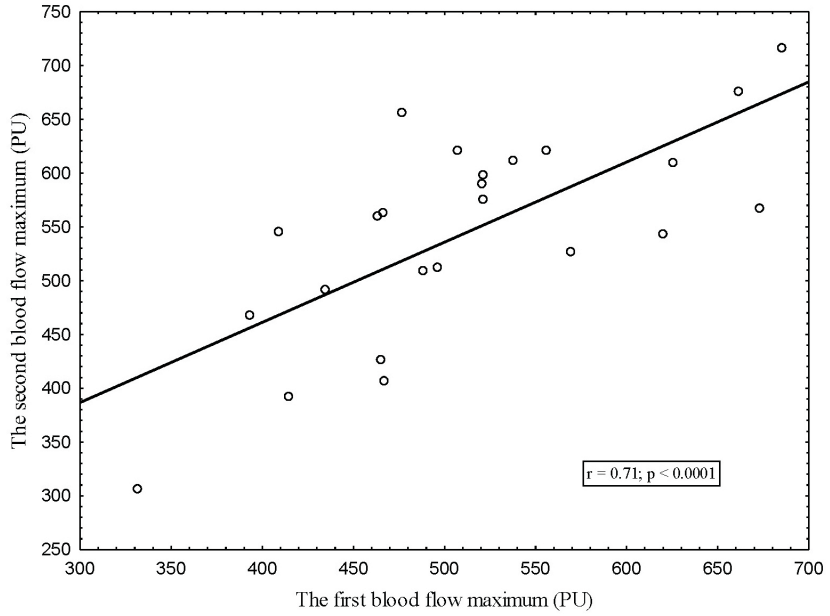


*Fig. 3*. Correlation of the first and second blood flow maximums in all study subjects (r = 0.71, $p < 0.0001$).

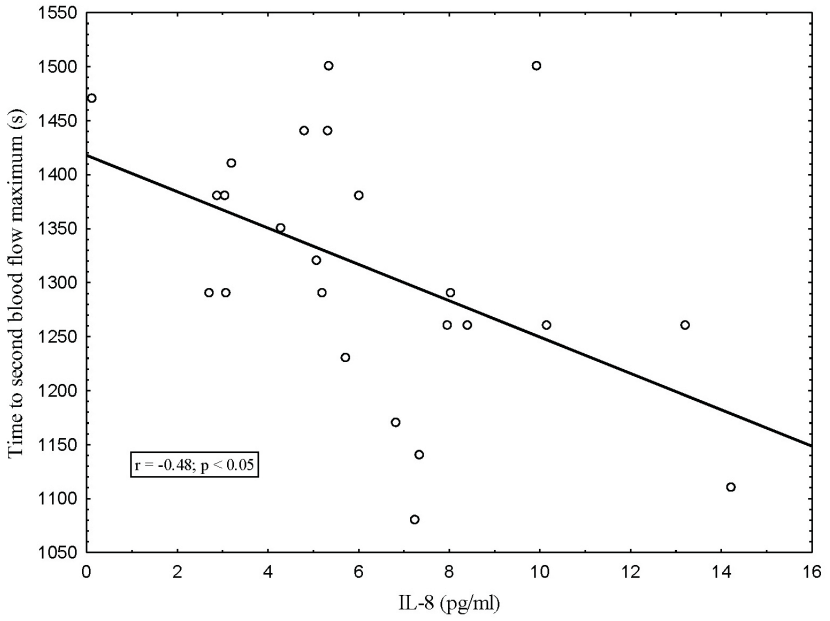


*Fig. 4*. Correlation of IL-8 and time interval till the second blood flow maximum in all study subjects (r = 0.48, $p < 0.05$).

## DISCUSSION

The results of this study confirmed that there was skin vasomotor dysfunction in migraine patients. In addition, this is consistent with our hypothesis that this dysfunction can be effectively reflected by the LDI local heating test. Thus, it confirms significant changes in the sensory axonal reflex-mediated and endothelium-dependent vasodilation mechanism in the skin of migraine patients.

To date, studies have mainly shown that migraines do not cause significant changes in skin microvascular function (de Hoon *et al*., 2006; Edvinsson and Edvinsson, 2008). The number of these studies is small and technologically different methods were used to assess vasomotor reactions in the skin. Since migraines are associated with increased secretion of CGRP and substance P (de Hoon *et al*., 2006), which are influenced by increased NO (Amynah *et al*., 2018), this suggests that vasomotor reactions in the skin will be more pronounced. With the exception of a few studies (Ibrahimi *et al*., 2017), this assumption has not been confirmed.

Moreover, the study provides evidence of a severe vascular smooth muscle dysfunction in migraine patients (Napoli *et al*., 2009). The LDI heating test data show that migraine patients have a significant reduction in both the first and second blood flow peaks. The first peak of vasodilation is related to CGRP and substance P exposure, while the second peak is related to NO exposure to smooth myocytes. This might be explained by development of some resistance to smooth myocytes to these vasodilators (CGRP, substance P, NO) in migraine patients.

There was a very close correlation between the first and second vasodilation peaks (Fig. 3). This means that these two vasodilation peaks are closely related, although each of them is determined by a different vasodilation mechanism. This indicates that both sensory axonal reflex-mediated and endothelium-dependent vasodilation have direct and pronounced response to local skin heating (+44.0 °C). This does not contradict the finding that the release of CGRP and substance P may be partly dependent on NO (Houghton *et al*., 2006).

We obtained surprising data on the length of time interval required to reach the first and second vasodilation peaks. Migraine patients need a shorter time interval to reach the first perfusion peak (sensory axonal reflex-mediated) and longer to reach the second peak (endothelium-dependent) (Fig. 2). Our finding that migraine patients require a shorter time interval to reach the first perfusion peak could be explained by the rapid release of vasodilators (e.g., P-substance, CGRP) from sensory C-fibre nerve endings, although vasodilation capacity is reduced. In turn, the fact that migraine patients need a longer time interval to reach the second peak is mainly due to the longer endothelial ability to release NO, although the vasodilatory capacity is reduced in this case as well.

The molecular mechanisms of the vasodilatory response caused by local heating have been examined in many studies (Patricia *et al*., 2014). The initial peak of vasodilation is mainly the effect of CGRP and substance P, as well as NO and endothelial-derived hyperpolarising factors (EDHF) on vascular smooth myocytes. In turn, the second peak of vasodilation (plateau phase) is ~60–70% dependent on NO, where various factors can modulate the bioavailability of NO in this phase, e.g., transient receptor potential vanilloid type-1, channels, adenosine receptors and reactive oxygen species. The remaining ~30–40% of the plateau is attributed to EDHF, e.g., epoxycatosrienoic acid, which promotes thermal hyperaemia of the skin (Houghton *et al*., 2006; Patricia *et al*., 2014). Currently, there are no studies that can accurately explain the effects of migraine on these vasodilation mechanisms of thermal hyperaemia, but the findings of the present study confirm a vasomotor dysfunction in the skin.

Of particular interest was the finding that serum IL-8 levels correlated only with the time interval till the second blood flow maximum (plateau phase) in all study subjects (Fig. 4). Some studies have shown that patients with migraine, including in their interictal period, have elevated levels of the proinflammatory chemokine IL-8 (Duarte *et al*., 2015; Oliveira *et al*., 2017). In addition, there is evidence that CGRP may induce activation of IL-8 gene expression (Sarchielli *et al*., 2004). But the authors of the present study did not find that migraine patients had significantly higher serum IL-8 levels. Other studies have confirmed an association between migraine and proinflammatory cytokines associated with endothelial dysfunction in migraine patients (Yücel *et al*., 2016). One of the signs of endothelial dysfunction is a reduced endothelium-dependent vasodilation. Thus, IL-8, an inflammatory cytokine, may contribute to the development of endothelial dysfunction.

## CONCLUSION

The findings suggest that migraine patients have cutaneous vasomotor dysfunction during their interictal period. This is confirmed by reduced sensory axonal reflex-mediated and endothelium-dependent vasodilation, which was assessed by the LDI local heating test. The results showed a significant decrease in the initial peak of vasodilation and the second peak of vasodilation (plateau phase). Also, there were significant changes observed in the length of time required to reach the first and second vasodilation peaks. Migraine patients have a shorter time interval to reach the first perfusion peak (sensory axonal reflex-mediated) and longer time interval to reach the second peak (endothelium-dependent). The results confirm the correlation between proinflammatory chemokine IL-8 levels and the time interval till the second peak of the blood flow (plateau phase) in all study subjects.

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*The authors declare that there is no conflict of interest regarding the publication of this paper.*

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## MIGRĒNAS IETEKME UZ AKSONA REFLEKSA MEDIĒTO UN ENDOTĒLIJA ATKARĪGO VAZODILATĀCIJU ĀDĀ


Pētījuma mērķis bija novērtēt izmaiņas ādas asins plūsmā, kuras tiek izraisītas ar lokālo sildīšanu un novērtētas ar lāzera Doplera attēldiagnostisko (LDI) testu migrēnas pacientiem interiktālajā jeb starplēkmju periodā, kā arī noskaidrot korelācijas starp šī testa rādītājiem un interleikīna (IL)-8 koncentrācijām serumā. Pētījumā tika iesaistīti 12 migrēnas pacienti un 12 veseli indivīdi. Tika iekļautas tikai sievietes. Abas grupas bija līdzīgas pēc vecuma, ķermeņa masas indeksa un asinsspiediena. Lai novērtētu vazomotorās atbildes plaukstas virspuses ādā, tika izmantots LDI lokālās sildīšanas (+44 °C) tests. IL-8 koncentrācija serumā tika noteikta ar ELISA metodi. Mūsu iegūtie rezultāti apstiprina ādas vazomotoro disfunkciju pacientiem ar migrēnu interiktālajā periodā. Migrēnas pacientiem tika konstatēts būtiski samazināts pirmais (iniciālais) vazodilatācijas pīķis un arī samazināts otrais asins plūsmas palielinājums (plato fāze), kā arī būtiski samazināts laiks līdz pirmajam pīķim (aksona refleksa-mediēts), bet pagarināts laiks līdz otrajam asins plūsmas palielinājumam (endotēlija atkarīgās vazodilatācijas izraisīts). Iegūtie rezultāti apstiprina korelāciju starp IL-8 koncentrāciju serumā un laiku līdz otrajam asins plūsmas palielinājumam veseliem indivīdiem un migrēnas pacientiem.