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The onset of the migraine attack

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Chapter 9.

Reduced trigeminovascular cyclicity in patients with menstrually related migraine

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Abstract

Objective
A case-control study to investigate the effect of the menstrual cycle on trigeminal nerve-induced vasodilation in healthy women and patients with menstrually-related migraine (MRM).

Methods
Using a laser-Doppler imager, we compared the vasodilator effects of capsaicin application and electrical stimulation (ES) on the forehead skin, a trigeminal nerve-innervated dermatome, in premenopausal MRM patients (n=22), healthy controls (n=20), and postmenopausal women without migraine (n=22). Blood samples were collected for female sex hormones measurements.

Results
Dermal blood flow (DBF) responses to capsaicin were higher in controls during days 1-2 than during days 19-21 of their menstruation cycle (Mean E_{max} ±SEM: 203±28 a.u. vs 156±27 a.u. (p=0.031) for 0.06 mg/ml capsaicin and 497±25 a.u. vs 456±24 a.u. (p=0.009) for 6.0 mg/ml capsaicin). In contrast, MRM patients demonstrated DBF responses without significant cycle-dependent variability (day 1-2 vs day 19-21: E_{max} 148±20 a.u. vs 154±20 a.u. (p=0.788) for 0.06 mg/ml capsaicin and 470±17 a.u. vs 465±20 a.u. (p=0.679) for 6.0 mg/ml capsaicin). DBF response to ES were not different between either MRM patients or controls, at either occasion. Estradiol levels on day 19-21 of the menstrual cycle were higher in healthy controls (Mean±SEM: 75±8 pg/ml) than in MRM patients (52±4 pg/ml, p=0.014). In postmenopausal women, DBF responses to capsaicin and ES, as well as estradiol levels at both visits, were all significantly reduced compared to MRM patients and controls (in all cases, $p<0.05$).

Conclusions
Our study provides evidence for a reduced menstrual cyclicity of both estradiol levels and the trigeminovascular vasodilator system in MRM patients.

Introduction

Migraine is twice as prevalent in women than in men [1]. Migraine incidence in women changes because of estrogen variations around menarche and menopause, but also during the menstrual cycle [2, 3]. Estrogen withdrawal increases migraine attack incidence migraine attacks;[4] a process that may be postponed by estradiol injections [5-7]. Migraine attacks associated with menstruation are generally perceived as more severe than attacks outside this period [8, 9].

The potent vasodilator calcitonin gene-related peptide (CGRP), a key mediator in migraine,[10] is released from primary afferents of the trigeminal ganglion, exerting its effects via the trigeminovascular system [11, 12]. Given the relationship between migraine attack incidence and hormonal fluctuations, an interaction between female sex hormones and CGRP seems likely [13, 14]. We have developed a human model to study trigeminal nerve-mediated vasodilatation, by applying capsaicin and electrical stimulation (ES) to the forehead skin, a trigeminal nerve-innervated dermatome [15]. Capsaicin activates the transient receptor potential vanilloid type 1 (TRPV1) channel, thereby enhancing CGRP release from trigeminal nerve terminals [15]. In contrast, ES appears to act directly on trigeminal nerve terminals, without the need for TRPV1 activation to evoke release of CGRP [15].

We investigated whether (i) varying levels of sex hormones during the menstrual cycle affect trigeminal nerve-mediated vasodilatation; and (ii) this pattern differs between menstrually-related migraine (MRM) patients and healthy controls. We hypothesized that (i) trigeminal nerve-mediated vasodilatory responses are increased preceding the menstruation, (ii) this increase is more pronounced in MRM patients than in controls, and (iii) trigeminal nerve-mediated vasodilatory responses are consistently low in postmenopausal women.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The Independent Ethics Committee of Erasmus MC, Rotterdam, The Netherlands, reviewed and approved the study protocol. All participants gave written informed consent after explanation of the study, which was conducted in accordance with local laws, the ethical principles of the Declaration of Helsinki, as well as the principles of Good Clinical Practice.

Design and procedures

For our case-control study design we compared MRM patients with age-matched healthy controls. We included postmenopausal women as a reference group (without menstrual cycle) for both the MRM patients and their age-matched healthy controls. MRM patients were recruited via a Dutch website inviting migraineurs to participate in research as part of the LUMINA project [16]. On the website, patients completed validated questionnaires based on the ICHD-III-b criteria to diagnose migraine and MRM [17]. MRM is classified as migraine without aura occurring on day 1±2 of the menstrual cycle with additional attacks of migraine at other times of the menstrual cycle [17]. Our MRM diagnosis was adapted from the ICHD-

III-b with a record of 3 menstrual cycles, of which 2 were prospective, and an interview. We recruited women without migraine via advertisement in local (Rotterdam, Netherlands) newspapers and flyers distributed in the Erasmus MC.

Data on medical history, medication, and information about the menstrual cycle or menopausal status were collected via an additional questionnaire. Information about headache frequency and severity of the MRM patients was acquired from the LUMINA database [16]. Only MRM patients not using migraine prophylactic treatment and consenting to refrain from the use of acute migraine therapy 48 hours prior to visits participated to prevent bias. MRM patients and healthy age-matched controls with a regular menstrual cycle, not using hormonal contraceptives, were eligible for inclusion.

Recruitment started March 2011 and continued until August 2012. Research was executed in the Internal Medicine Department of Erasmus MC. MRM patients did not have any medical condition besides migraine. Healthy age-matched controls and postmenopausal women were screened with a thorough interview checking for any (cardiovascular) disease or medication use. Non-smoking healthy women were included. For premenopausal women, one study visit was 19-21 days after the first day of menstruation and the second visit on day 1-2 of the subsequent menstruation (Figure 1). For postmenopausal women, two visits were scheduled 7-10 days apart. Weight, height and (supine) blood pressure were measured.

The first research visit was in July 2011 and the last one in September 2012. Migraine attack incidence was recorded from one month prior to the first research visit until two months later. Follow-up ended December 2012.

Forehead dermal blood flow (DBF) studies

Experiments were performed in a quiet, temperature-controlled room. Participants fasted for 3 hours before the measurements and both visits were during the same period of the day. After 15 min acclimatization, 3 electrodes containing a 0.5-ml reservoir were placed on the forehead (for details, see our model validation paper [15]). The electrodes were subsequently filled with three different types of solutions: normal saline, 0.06 mg/ml capsaicin, and 6.0 mg/ml capsaicin. A fourth electrode was placed in the neck region. An iontophoresis device (PeriIont 382b, Perimed, Sweden) was connected with the negative lead to the electrode containing saline and the positive lead to the electrode in the neck. DBF was measured with the PeriScan PIM-3 system (Perimed, Järfälla, Sweden).

DBF at the site of the electrodes on the forehead was continuously measured for 40 min. After 2 min baseline measurement, a current (0.2 mA) was applied for 1 min to the electrode containing saline. DBF was subsequently measured during 6 min. This process was repeated with increasing current intensities (0.4 mA, 0.6 mA, 0.8 mA), up to 1.0 mA.

Peripheral dermal blood flow response to ischemic stimulus

Post-occlusive reactive hyperemia (PORH) was measured at the volar site of the non-dominant forearm (area 1x1 cm). A blood pressure cuff was placed around the upper arm. After a 2-min baseline DBF measurement, the pressure in the cuff was quickly increased

to 200 mmHg, maintained for 5 min and subsequently released. PORH was continuously measured for 10 min.

CGRP measurements in saliva

Test subjects were given a cotton Salivette swab (Sarstedt AG & Co., Nümbrecht, Germany) to chew for 5 min. The swab was collected in the Salivette and stored at -80ºC. CGRP was determined by radioimmunoassay (Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA), according to the manufacturer's instructions. Total protein content was determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, Il, USA). Samples were measured in 2 blinded batches.

Estrogen and progesterone levels

Blood was collected via the cubital vein. Serum estradiol and progesterone levels were determined with the Coat-A-Count Estradiol and the Coat-A-Count Progesterone radioimmunoassay kits (Siemens Medical Solutions, Erlangen, Germany). Samples were measured in 2 blinded batches.

Statistical analysis

The number of cases and controls was based on the previously published validation study of our model[15]. Based on this study, a sample size of 20 is sufficient to be able to detect a 25% shift in the DBF response during the cycle and between the groups to 6 mg/ml capsaicin with an 80% power and 5% significance.

For each subject the maximal DBF (E_{max}) responses to capsaicin, and the ischemic stimulus were calculated. Similarly, for each subject the maximal DBF responses to each ES current intensity (0.2 mA-1.0 mA) was calculated. Individual data were analyzed in a blinded fashion. Group values are provided as mean values and standard error of the mean, except for the demographic data in Table 1, where standard deviations and ranges are presented as indicated. Differences between groups were analyzed by ANOVA and unpaired Student's t-test. Differences within groups were examined by Student's paired t-test. Repeated measurements ANOVA with multiple comparison tests were computed. Linear regression analysis was performed and Beta values with 95% confidence intervals are presented. The difference in DBF responses to capsaicin (Delta DBF) between groups and across the menstrual cycle were tested with the Mann-Whitney U test. A p-value <0.05 was considered to indicate statistical significance. No correction for multiple testing was applied.

Results

Subjects

Flow diagrams providing details on the recruitment are provided in Supplemental figure e-1. BMI, blood pressure, and heart rate were similar between groups (Table 1). Migraine attack incidence of the MRM patients was highest on day 1 of the menstrual cycle (Table 1).

Table 1. Demographics of the study population.

	Migraine patients	*Controls*	*Postmenopausal women*
Population, n	22	20	22
Age, years	37±7 (21-45)	33±9 (19-45)	60±5 (50-68)
BMI, kg/m²	22.9±3.9	24.0±1.6	23.8±2.3
BP, mmHg			
Systolic	105±17	109±9	118±9
Diastolic	66±7	64±6	71±6
HR , bpm	61±8	63±9	62±8
Age at migraine onset, years	18±7 (4-36)		
Disease duration, years	19±9 (6-41)		
Attack frequency, attacks per year			
1–2	1		
3–6	2		
7–12	5		
13–54	14		
Migraine incidence on day 1±2 of the menstrual cycle, No. of attacks			
Day 27	1		
Day 28	1		
Day 1	7		
Day 2	1		
Day 3	2		

BMI, body mass index. BP, blood pressure. HR, heart rate. Mean ± SD (Range)

Forehead DBF responses

In healthy controls, DBF responses to 0.06 mg/ml and 6.0 mg/ml capsaicin were significantly higher during day 1-2 of their menstrual cycle compared to day 19-21 (Figures 2A and 2B). In contrast, in MRM patients DBF responses to 0.06 mg/ml and 6.0 mg/ml capsaicin were similar throughout their menstrual cycle. The increase in DBF responses of healthy controls to 0.06 mg/ml capsaicin between visits (Delta: 47±20 a.u.) was significantly different from the slight decrease in DBF responses of the MRM patients (Delta: -6±24 a.u., p=0.040), whereas the slightly smaller increase in response to 6.0 mg/ml capsaicin (Delta: 41±14 a.u.) in healthy controls was not significantly different from that in MRM patients (Delta: 6 ±14 a.u., p =0.078). There was no association between DBF responses of MRM patients and the time to/since their migraine attacks. DBF responses in postmenopausal women were significantly lower than those in women with a menstrual cycle (controls and MRM patients) and were similar between visits. DBF responses to ES between visits did not differ for either of the groups (Figure 2C). Repeated measures ANOVA with a Greenhouse-Geisser correction revealed that mean DBF responses to ES differed significantly between stimulation intensities (0.2 mA-1 mA (F(2.017, 121.032): 414.878, p<0.0001). Repeated measures ANOVA revealed a group effect, with the lowest responses to ES in the postmenopausal women.

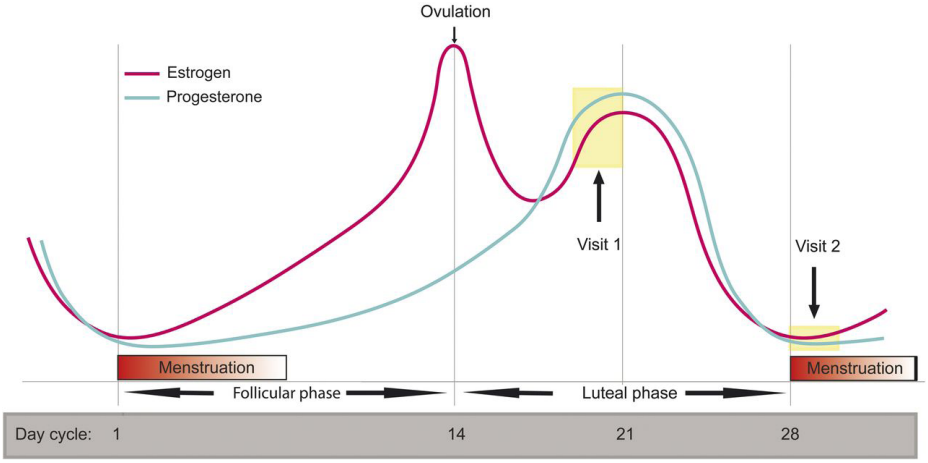

Figure 1. Estrogen and progesterone levels during menstrual cycle and time points of investigations
Yellow highlighted sections: time point of investigations for patients with menstrually related migraine and controls.

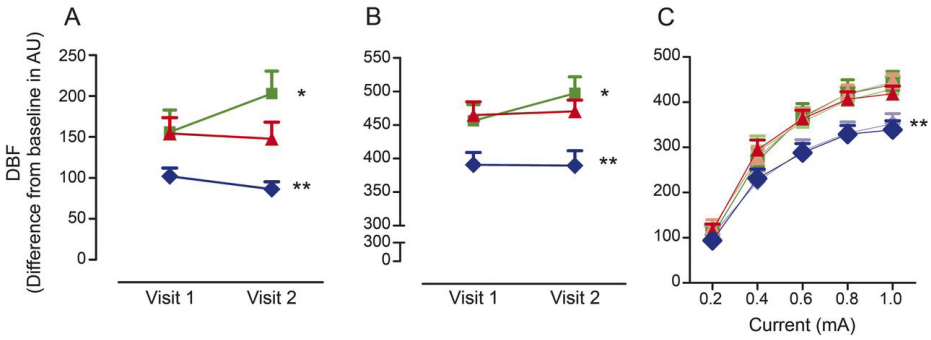

Figure 2. Maximal forehead DBF responses
DBF responses to 0.06 mg/mL capsaicin (A), 6.0 mg/mL capsaicin (B), and electrical stimulation (C). For controls (green square) and patients with MRM (red triangle): visit 1 = days 19–21 of the menstrual cycle and visit 2 = days 1–2 of menstruation. For postmenopausal women (blue diamond): visit 1 and visit 2 planned randomly with 7–10 days in between. (C) Visit 2 controls (light green square), visit 2 patients with menstrually related migraine (light red triangle) and visit 2 postmenopausal women (light blue diamond). *Significant difference in E_{max} between visit 1 and visit 2. **Significant difference in E_{max} during both visits compared to patients with MRM and controls. DBF = dermal blood flow; MRM = menstrually related migraine.

Hormone levels

Serum estradiol levels on day 19-21 of patients with MRM were significantly lower than those of the healthy controls (52±4 pg/ml vs. 75±8 pg/ml respectively), while no differences in estradiol levels between MRM patients and healthy controls (25±5 pg/ml vs. 21±4 pg/ml respectively) were present on day 1-2 of the cycle (Figure 3A). Serum progesterone levels in MRM patients and healthy controls at the two occasions were similar (Figure 3B). Estradiol

and progesterone levels during day 19-21 of the menstrual cycle were significantly higher than during day 1-2. Estradiol and progesterone levels of the postmenopausal women were low, consistent with their non-reproductive life stage.

Peripheral dermal blood flow response to ischemic stimulus

There were no significant differences in PORH responses between visits or between groups (Figure 4). Both age and the maximum PORH response were significant predictors for the E_{max} DBF response to 1 mA electrical stimulation (respectively: Beta:-2.455, 95% CI[-4.206; -0.705] and Beta: 0.813, 95% CI[0.165; 1.461]).

CGRP in saliva

No differences in salivary CGRP levels between visits or between groups were observed (Supplemental figure e-2). There was no correlation between saliva CGRP levels and the time to/since the last migraine attack of the MRM patients. The saliva volume collected from postmenopausal women was significantly lower than from healthy controls and patients with MRM on cycle day 19-21.

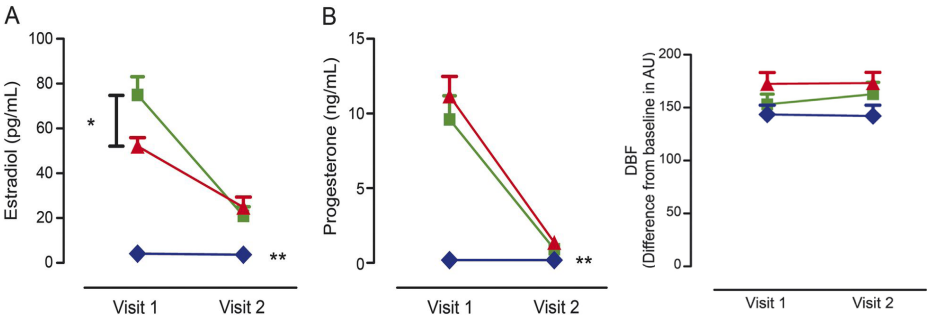

Figure 3. Serum estradiol and progesterone levels

Estradiol levels (A) and progesterone levels (B). For controls (green square) and patients with MRM (red triangle): visit 1 = days 19–21 of the menstrual cycle and visit 2 = days 1–2 of menstruation. For postmenopausal women (blue diamond): visit 1 and visit 2 planned randomly with 7–10 days in between. *Significant difference in estradiol levels between patients with MRM and controls. **Significantly lower levels in postmenopausal women during both visits compared to patients with MRM and controls. MRM = menstrually related migraine

Figure 4. Maximal postocclusive reactive hyperemia responses

For controls (green square) and patients with menstrually related migraine (red triangle): visit 1 = days 19–21 of the menstrual cycle and visit 2 = days 1–2 of menstruation. For postmenopausal women (blue diamond): visit 1 and visit 2 planned randomly with 7–10 days in between.

Discussion

We investigated the association between the menstrual cycling of female sex hormones on trigeminal nerve-mediated vasodilatation in healthy controls and MRM patients.

We clearly demonstrated a DBF response difference to capsaicin over the course of the menstrual cycle in healthy women, indicating that our model is suitable to detect cycle-dependent changes in trigeminovascular reactivity. In contrast, this cyclic DBF response difference was absent in MRM patients. Notably, DBF responses to electrical stimulation were similar throughout the menstrual cycle in both MRM patients and healthy controls. As expected because of their stable low female sex hormone levels, postmenopausal women showed no cyclicity in their DBF responses to either capsaicin or electrical stimulation between visits. Rather, when compared to the healthy controls and MRM patients, they responded significantly lower to both stimuli.

Another surprising finding of our study is the relatively low mean estradiol level detected during day 19-21 of the menstrual cycle of the MRM patients, which seems in agreement with their reduced cyclicity in DBF responses.

In accordance with our hypothesis, DBF responses to capsaicin during day 1-2 of the menstrual cycle were increased in healthy controls. The enhanced DBF response to capsaicin of healthy women around their menstruation is consistent with previously published data, where, in healthy women, sensory and vasomotor responses to intradermal capsaicin injections of the forehead were increased during the menstruation compared to responses during the luteal phase [18]. The higher responses were attributed to either the direct effect of estrogen withdrawal on trigeminal nerve innervated vasculature or to its effect on modulation of serum levels of ionized magnesium. Indeed, estrogen has been suggested to enhance neurogenic vasodilatation, primarily by CGRP, in rats [19, 20]. Since we did not detect cyclic responses to electric stimulation, the higher responses during menstruation in healthy controls may be attributed to differential activity of the TRPV1 channel. Alternatively, release of other neuropeptides in response to electrical stimulation might mask the CGRP-specific component of the DBF response. Finally, the lack of cyclic responses to electrical stimulation could be attributed to the stimulation time and intensity. With electrical stimulation, we applied a brief (1-min) stimulus with a 6-min recovery time. Therefore, this recovery time might have been sufficient for the replenishment of the readily-releasable pool of neuropeptide vesicles at the nerve terminals [21]. In contrast, with capsaicin application there is no recovery phase, but rather a constant stimulation during 40 min. This might lead to not only the exhaustion of the readily-releasable pool of vesicles, but also of the more slowly replenished neuropeptide vesicle reserve pool.

Contrary to our expectations, the DBF responses to capsaicin in MRM patients were unchanged throughout their menstrual cycle. Elevated CGRP levels in jugular blood during migraine attacks have been previously reported [22]. Consequently, we expected MRM patients to have higher DBF responses to capsaicin during day 1-2 of their menstrual cycle. Our results may be explained by activity-dependent transport of neuropeptide in the trigeminal

nerve. In particular, the higher release of CGRP from the dural fibres of the trigeminal nerve during the perimenstrual period might be due to enhanced anterograde transport from the cell soma, where CGRP is synthesized and packaged, to the nerve terminals from where it is synaptically released. Supporting such a potential mechanism is recent evidence from Drosophila that also suggests an activity-dependent mechanism of neuropeptide release [23]. Another possible explanation for the lack of cyclic responses in MRM patients could be an inhibition of TRPV1 channel function induced by the lower decline in estradiol levels in migraine patients as compared to healthy controls. Admittedly, the role of the TRPV1 channel in migraine is still ambiguous [24, 25].

We included salivary measurements of CGRP levels as these reflect the activation state of the trigeminovascular system. Although previous studies have detected elevated CGRP levels in saliva in migraine patients during the premonitory and headache phase of a migraine attack [26, 27], this finding was not replicated in a study with chronic migraine patients [28]. Notably, we observed similar levels of salivary CGRP in MRM patients and healthy controls. These data confirm our DBF measurements, as both the fibres of ophthalmic branch on the forehead (DBF response), as well as the fibres of the mandibular branch of the trigeminal nerve (saliva production), show similar responses in migraine patients independent from the timing in the menstrual cycle or the temporal relationship to a migraine attack. It is important to note that, contrary to the studies mentioned above, we did not collect saliva samples during the premonitory or headache phase of our migraine patients, since we primarily intended to investigate the relation between CGRP and the menstrual phase of healthy controls and migraine patients. Since this limits the conclusions that can be drawn from our salivary measurements, it would be interesting to study ictal salivary CGRP levels in MRM patients in future.

As hypothesized, the DBF responses to capsaicin application and electrical stimulation in postmenopausal women were consistently lower than in menstruating women. To verify whether these findings were caused by general, age-dependent decreases of microcirculatory function, we performed the PORH test. Notably, the PORH response between groups was not significantly different. The relatively low age and good health of the included postmenopausal women may explain the normal PORH responses. Though not significant, the PORH responses of the postmenopausal women tended to be lower than the PORH responses of the premenopausal groups (Figure 4). However, this slight difference in PORH responses is not of such magnitude to plausibly explain the significantly lower DBF responses of the postmenopausal women to capsaicin application and ES, which was confirmed by the regression analysis. Lower DBF responses to capsaicin application and electrical stimulation thus seem to be related to the low estradiol levels after the menopause, which specifically affect the trigeminovascular system and do not induce generalized microcirculatory dysfunction.

Our surprising finding of lower estradiol levels measured in migraine patients during day 19-21 of the menstrual cycle is not due to the mean age of our study groups. In fact, previous studies have reported higher estradiol levels in perimenopausal women [29]. In our study, the MRM patients had a slightly, but not significantly, higher mean age than the healthy

controls (Table 1), despite having lower estradiol levels during the luteal phase (day 19-21 of the cycle). A relationship between low luteal estradiol levels and MRM has never previously been reported. High estradiol levels preceding ovulation have been linked to migraine with, but not to migraine without aura [30]. Our observation is in accordance with previously published data [4], where the ratio between a urinary metabolite of estradiol measured during the luteal phase and during menses in menstrual migraine patients is the same as in our study (estradiol levels MRM patients: day 19-21:day 1-2 = 2:1). To replicate the data observed in our healthy controls, we used data from a previous study at our institution on healthy women with a regular menstrual cycle (n=9) [31]. Indeed, the ratio between serum estradiol during the luteal phase and menses was the same as in our current study (estradiol levels healthy controls: day 19-21:day 1-2 = 4:1). We conceive that the number of subjects in the current study and the replication group is relatively small to make definite statements about the hormone levels in MRM patients. Future studies should investigate these levels in a larger set of MRM patients.

Taken together, our data confirms the pre-existing theory that the premenstrual withdrawal of estradiol influences the trigeminovascular system. Moreover, our study provides evidence for a disturbed systemic as well as trigeminovascular cyclicity in patients with MRM, which may augment their susceptibility to migraine around menstruation.

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