Salivary Inflammatory Markers in Tension Type Headache and Migraine

Salivary Inflammatory Markers in Tension Type Headache and Migraine: A Case-Control Study 1st Department of Neurology, Eginition Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece. Vassilisis Sophias Avenue 72-74, 115 ABSTRACT AIMS: We studied whether headache attacks are associated with changes in CRP, IL-1ß and IL-6 in saliva. We, also investigated whether these markers in tension type headache (TTH) and migraine could be influenced by comorbidities such as depression and anxiety.

METHODS:

This case-control study of 13 migraine, 9 TTH patients that attended our outpatient headache clinic and 15 age matched healthy controls between January to March 2016. We accessed theirs demographic characteristics, headache features, anxiety and depression as measured by the Hamilton Anxiety Rating Scale (HAM-A), and the Beck Depression Inventory (BDI). Salivary IL-6, IL-1ß and CRP were collected in distinct time points as A- headache free period, B – during headache, C- one day after headache attack , and measured by ELISA kits. RESULTS: IL-1ß significantly decreased from time point A to B, while increased from time point B to C in headache groups. Both type of headache had greater IL-1ß levels at time point B as compared with controls. No significant differences were found in time variation of CRP, IL-1ß and IL-6 levels between migraine and TTH ($p>0.05$). CRP was negatively correlated with HAM-A and BDI scores. IL-6 measured at time point A was negatively correlated with BDI scores.

CONCLUSION:

For the first time, it has been showed to exist a similar variation of IL-1ß in both migraine and TTH patients. Migraineurs had elevated IL-1ß levels during attack compared to controls. CRP and IL-6 were correlated with lower symptom scores of anxiety and depression prior or immediately after the headache period in patients groups. Key Words: Tension-type headache (TTH),migraine, inflammation, Interleukin (IL)-1ß, Interleukin IL-6, salivary markers. Introduction Tension-type headache (TTH) and migraine are the most common types of primary headaches, with a high lifetime prevalence of 78% and 18% respectively, and a great overall cost throughout their lifespan [1,2]. Nonetheless, their pathophysiology is still under investigation. Both peripheral and central mechanisms have been implicated. Increased nociception from strained muscles might be the primary cause of both TTH and migraine, possibly favored by a central temporary change in pain control [3]. Inflammation not only generates but also maintains

pain in peripheral structures, while promoting sensitization of central nervous system structures involved in nociception [4].

A high C-reactive protein (CRP) level may be a marker of the proinflammatory state in migraine patients. Activation of brain tissue triggers the release of peptides from the perivascular trigeminal regions, causing inflammation and dilation of extra-parenchymal vessels. Repeated migraine attacks are associated with inflammatory arteriopathy of the cranial vessels [5]. Interleukin (IL)-1ß is also an important mediator of inflammatory response. The induction of cyclo-oxygenase-2 by this cytokine in CNS might contribute to inflammatory pain hypersensitivity [6]. IL-1ß may play a causative role in migraine by activating neuronal and glial cells to release cyclo-oxygenase-2, which in turn could induce neuro-inflammation. Serum levels of IL-1ß were significantly elevated in patients with chronic TTH, which contribute to central sensitization and enhanced general hyperalgesia [7]. Interleukin 6 (IL-6) acts as a pro-inflammatory cytokine that is secreted by T cells and macrophages to stimulate immune response. Both serum and CSF levels of IL-6 were elevated in individuals with the episodic/chronic forms of TTH and migraine [8]. Serum levels of IL-6 were significantly higher in migraine patients during attacks as compared to controls [9]. Both TTH and migraine have been associated with psychiatric comorbidities, such depression and anxiety [10,11]. These disorders have been previously associated with increased expression of IL-6, IL-1ß and CRP [12-14]. No study to date has evaluated whether the increase of these salivary pro-inflammatory cytokines in TTH and migraine could be secondary to psychiatric comorbidities. All the above studies mainly focused on blood and CSF samples [15].

Saliva collection is a simple, non-invasive and cost effective method with evident advantages in the field of psychoneuroendocrinology research [16]. However, no research has been conducted as yet with a view to comparing the salivary levels of CRP, IL-1ß and IL-6 in patients with TTH and migraine versus age matched controls. The primary aim of this study was to investigate whether attacks of migraine and TTH are associated with changes in the concentration of inflammatory markers. The secondary objective was to investigate whether cytokines levels in TTH and migraine could be influenced by psychiatric comorbidities such as depression and anxiety. Materials and Methods.

Study Design and Population This case- control study enrolled 37 subjects (22 migraine, TTH patients and 15 healthy volunteers) that attended an outpatient headache clinic in the Department of Neurology at Aeginition Hospital of Athens between January to March 2016. Subjects of both genders, aged from18 to 60 years old, who suffered from primary headaches (TTH) and migraine, according to the International Classification of Headache Disorders, 3rd edition (beta version) [17], were enrolled.

The patients were given a complete physical and neurological examination. During the

recruitment day, all patients who visited the outpatient clinic were asked to participate in the study after fulfilling the eligibility criteria and providing their informed consent. The main exclusion criteria were (1) abnormal plasma hs-CRP, IL-1ß and IL-6 levels (>10 mg/L) (2) smoking cigarettes > 1 pack/day; (3) current pregnancy, lactation, or hormonal contraceptive use (4) alcohol or substance abuse (5) drug use such as antiplatelet agents, anticoagulants, statins, or hormonal drugs (6) Haedache patients with recent history of a disease with known elevated levels of inflammatory markers (7) Patients under anti-infammatory therapy (8) other primary or secondary headaches (9) major psychiatric disease (10) oral health problems. Healthy control subjects aged between 18 and 50 years were recruited consecutively from hospital staff, laboratory staff and relatives of patients. Inclusion criteria for the control subjects were (1) absence of primary headaches such as migraine, TTH; (2) absence of other neurologic or systemic disease; and (3) presence of a match with migraine patients by age (±2 y), sex, Exclusion criteria for control subjects were the same as for the headaches groups. Initially, the participants were fully informed about the aim of the study and theirs responsibilities and subsequently completed the Hamilton Anxiety [18] (HAM-A), Scale Beck Depression Inventory [19] (BDI) and provided demographic characteristics.

All patients had to keep a headache diary during the four-week run-in period. Every week, until four weeks which were required for the completion of the intervention, participants were contacted, in order to ensure the compliance and the appropriate use of the technique. One month after the end of the study, a follow-up visit has been taken place to ensure the completing of the questionnaires, both in the headache and the control group. Informed consent was obtained from all patients.

The study protocol was approved by the Institutional Review Board (IRB) of Aeginition Hospital of Athens (IRB approval number: 638/5.11.2015) and complied with the 2013 WMA—World Medical Association Declaration of Helsinki [20]. Psychometrics The Hamilton Anxiety Rating Scale (HAM-A) is a psychological questionnaire used by clinicians to rate the severity of a patient's anxiety [18]. Each of the 14 items contains six items of anxious (mood, psychic tension, fears, insomnia, intellectual difficulties, and depressed mood) and eight items of somatic symptoms (muscular, sensory, cardiovascular, respiratory, gastrointestinal, genito-urinary, others autonomic and Behaviour during interview). Each group of symptoms is rated on a scale of zero (not present) to four (severe) with a total score range of 0–56, where 0.05). Significantly greater values for BDI were found for those with TTH as compared with those with migraine (table 4). Correlation coefficients of CRP, IL-1ß and IL-6 levels with scores on HAM-A and BDI are presented in table 5. CRP measured at time point A was negatively correlated with HAM-A, and BDI scores. Additionally, IL-6 measured at time point A was negatively correlated with BDI scores. Discussion The main results of this study were as follows: 1) IL-1ß was found to significantly decrease from time point A to time point B, while a significant increase was recorded from time point B to time point C. 2) All headache sufferers had greater IL-1ß levels at

time point B as compared with controls at time point D. 3) No significant differences were found in time variation of CRP, IL-1ß and IL-6 levels between migraine and TTH 4) CRP was negatively correlated with HAM-A, and BDI scores. 5) IL-6 measured at time point A was negatively correlated with BDI scores. The possibility of measuring these markers in saliva as a stress free method highlights the novelty of this study in the psychobiological basis of headache research.

Most previous studies investigated immune circadian variations using blood samples but not saliva samples. In the few previous studies that investigated salivary inflammatory markers and their circadian patterns, results are contradictory. Sjögren et al. (2006) reported that salivary IL-6 levels in the evening were higher than those in the morning [22]. Out et al. (2012) demonstrated that salivary CRP levels at awakening were higher than those at bedtime and that salivary CRP levels were moderately stable over a period of two years [23]. Izawa et al (2012) showed that salivary IL-6 levels peaked at awakening, gradually declined from morning to noon, and peaked again at midnight, before the participants went to sleep. The salivary CRP levels peaked at awakening, and were lower during the daytime [24]. To date, however, no study has investigated the variability of inflammatory cytokines including IL-6 and CRP over multiple days in headache sufferers.

For the first time, the present study shows a similar variation of salivary IL-1ß in both migraine and TTH. This variation in IL-1ß concentration may reflect feedback inhibition of cytokine concentrations by endogenous cortisol, which is subject to circadian variation. Such a variation may have important implications for future studies in terms of the timing of salivary sampling when measuring for the assessment of primary headaches. The novel feature of the present study is that it demonstrates increased salivary IL-1ß levels in both migraineurs and TTH subjects during the headache period. One possible hypothesis for the ictal increase of pro-inflammatory molecules in TTH is that myofascial pain triggers the release of inflammatory mediators leading to the excitation of peripheral afferent nerves. However, normal levels of inflammatory mediators were found in tender trapezius muscle in patients with chronic TTH [25]. Another interpretation is that the spinoreticulothalamic tract may be activated by peripheral stimuli, stimulating multiple circulating molecules such as corticotropin-releasing hormone, vasopressin and beta-endorphin, which trigger cortisol production that may activate peripheral release of cytokines [26]. This theory is in line with a previous study suggesting that cytokines during headache attacks are involved in the process of pain [15]. Although the results summarized in this section came from blood or CSF samples, our novel findings seems to be similar, but more research is warranted for biological implications of salivary inflammatory mediators in headache sufferers. In the present study, interesting and novel evidence showed that higher levels of CRP and IL- 6 correlated with lower symptom scores of anxiety and depression prior or immediately after the headache attack day. The question as to whether there is a relationship between raised inflammatory markers and depression is more complex

question, and would require longitudinal studies, preferably with a large cohort.

A particular difficulty with longitudinal studies is that of confounding variables, such as biological, psychological and social factors that may contribute to the development of depression and raised levels of inflammatory markers which are impossible to control. There is growing evidence that anxiety and depression can lead to an increased production of pro-inflammatory cytokines. However, in the present study, CRP and IL-6 correlated with lower symptom scores of anxiety and depression in patients groups prior or immediately after the headache period. In other words, anxiety and depression symptoms did not influence the increased levels of IL-6 that were shown to be independently associated with migraine and TTH.

Another strength of this study is the strict control of confounding factors. The measurement protocol was similar for all participants with respect to the circadian rhythm which is related to sleep-wake periods, as previously demonstrated [24,27]. Salivary samples were taken from all participants at 8 a.m. to control for circadian variation in cytokine levels. Moreover, the likelihood of pre-analytic errors was reduced by measuring the transferrin levels for the presence of blood in saliva samples. However, this study is not without its limitations. A cross-sectional study design was used which did not allow us to determine the existence of a causal relationship. Another weakness lies in the limited sample size and uneven gender distribution in the two groups. We experienced problems recruiting sufficient patients and controls, resulting in groups that were smaller than intended. Exclusion of samples due to the presence of blood caused the sample size to decrease further. In other words, the lack of significant findings in the subgroup analyses could be the result of a limited sample size.

Nevertheless, this is the first study to assess salivary inflammatory cytokines in both migraine and TTH. Conclusions To date, this is the first study to show a similar variation of salivary inflammatory cytokines in both migraine and TTH. Migraineurs had elevated IL-1ß levels in saliva compared to controls. Higher levels of CRP and Il-6 correlated with lower symptom scores of anxiety and depression prior or immediately after the headache period. These findings support the role of salivary inflammatory markers in migraine and TTH but larger controlled studies are required before firm conclusions can be drawn. Future studies should adopt a noninvasive sampling method such as saliva collection to overcome the issue of small sample size and increase the number of time points of sampling.