Effect of Ergotamine and its Combination with Vitamin E or Melatonin on Total Antioxidant Status in Migraine Patients

Shahlaa H. Ali [1] , Salim A. Hamadi ** , Ashwaq N. Al- Jaff**

** Department of Clinical Pharmacy ,College of Pharmacy , University of Baghdad, Baghdad , Iraq

Abstract

Free radicals and oxidative damage caused by them have being suggested to be involved in the pathogenesis of migraine. These may result from distorted equilibrium of pro-oxidant/anti-oxidant system that continuously generates and detoxifies oxidants during normal aerobic metabolism. Escape of such system from equilibrium leads to damage of cellular elements with the depletion of cellular stores of anti-oxidants material such as glutathione and vitamin E. Therefore, free radical scavengers (vitamin E or melatonin) seems to be of potential benefit as prophylactic anti-migraine therapy by neutralizing free radicals overproduction and possibly preventing formation of highly toxic intermediates (such as nitric oxide). In addition of being powerful antioxidant, melatonin was shown to possess promising effects in modulating severity, frequency and duration of migraine attacks. For this reason the present study was conducted to investigate the involvement of changed anti-oxidant defense (measured as total antioxidant status "TAS") during migraine attack and the possible modulation of such status by classical anti-migraine therapy (ergotamine), antioxidants (vitamin E and melatonin) and their combination. 23 normal subjects and 21 migraine patients with age range of (17-45) years were enrolled in the study. Patients were diagnosed according to neurologist decision to have migraine with and without aura. Migraine patients were divided into three treatment groups; first group treated with ergotamine alone, second group with ergotamine /vitamin E and third group with ergotamine /melatonin. All groups were advised to take their treatments during attacks. Blood samples were drawn from migraine patients and normal subjects before initiation of therapy and after pain has been relived (from migraine patients only) for the investigation of TAS . The results of the study showed that TAS was significantly lower in migraine patients in comparison to control healthy subjects ($P<0.05$) with a percent reduction ranged from 35.46% to 43.97%. However, there is no significant difference in the level of TAS among migraine patients ($P>0.05$). Treatment with ergotamine raised significantly the level of TAS by 157%. The addition of vitamin E or melatonin greatly raised TAS by 179% and 176% respectively. The addition of vitamin E to ergotamine showed superior effect to that when melatonin was added. The greater reduction in TAS seen in this study among migraine patients in comparison to control healthy subjects suggests the presence of generalized decrease in antioxidant defense elements. Elevation of TAS by all treatments was very clear. In conclusion the decrease in TAS can be implicated in the pathophysiology of migraine and enhancement of antioxidant system can add a beneficial effect for the management of migraine headache with the use of antioxidants (vitamin E or melatonin) with classical anti-migraine drug.

الخلاصـــــــة

تعتبر الجذور الحرة والإجهاد التأكسدي الناجم عنها من النظريات المفترضة حديثاً في سببية داء الشقيقة وهذا الإجهاد التأكسدي قد ينشأ عن عدم التوازن في أنظمة الأكسدة والأنظمة المضادة للأكسدة والتي تنتج جذوراً حرة وتعالج سميتها باستمرار خلال عمليات الأيض الخلوي. إن انعدام السيطرة أو الخلل في عمل هذه الأنظمة يؤدي إلى تلف المواد الخلوية بالإضافة إلى نفاذ المحتوى الخلوي من مضادات الأكسدة الطبيعية مثل الغلوتاثيون وفيتامين هـ. لذا فأن الم,اد القانصة للجذور الحرة مثل فيتامين هـ أو الميلاتونين تبدو ذات فائدة وقائية ضد داء الشقيقة من خلال معالجة الإفراط في إنتاج الجذور الحرة وربما بالتقليل من تكون جذور حرة خطيرة مثل (أوكسيد النتريك). إن الميلاتونين بالإضافة إلى كونه مضاداً فعالاً ضد الأكسدة فانه قد اظهر فعالية موعودة في التقليل من حدة وتردد وفترات الإصابة بألم الشقيقة. وعلى هذا الأساس أجريت الدراسة الحالية لاستكشاف تدخل تغير العوامل الدفاعية ضد الأكسدة (مقاساً بشكل "الحالة الدفاعية الكلية ضد الأكسدة") خلال نوبات صداع الشقيقة ومدى إمكانية تغيير هذه الحالة بالعلاج التقليدي (الارغوتامين)، مضادات الأكسدة (فيتامين هـ والميلاتونين) أو بالاثنين معاً. أُجريت الدراسة على ٢٣ شخص سليم و٢١ مريض بداء الشقيقة وبمعل عمري (١٧-٤٥) سنة. شُخص المرضى كونهم مصابون بداء الشقيقة وفق قرار أخصائي الجملة العصبية. قسم المرضى إلى ثلاث مجاميع علاجية، عولجت المجموعة الأولى بالارغوتامين والثانية بالارغوتامين مع بفيتامين هـ والثالثة بالارغوتامين مع الميلاتونين. ارشد المرضى إلى اخذ العلاج خلال حدوث نوبات الم الشقيقة. أُخذت عينات الدم من مرضى الشقيقة والأصحاء قبل بدء الدراسة وبعد تسكين الم الشقيقة (من مرضى الشقيقة فقط) لقياس الحالة الدفاعية الكلية ضد الأكسدة.

[1] Corresponding author : E-mail : .shahlaa_65@yahoo.com
Received : 26/7/2007
Accepted : 29/12/2007

أظهرت النتائج أن الحالة الدفاعية الكلية ضد الأكسدة منخفضة لدى مرضى الشقيقة بالمقارنة مع الأصحاء وكانت نسبة الانخفاض تتراوح بين ٣٥.٤٦% إلى ٤٣.٩٧%. وعلى أية حال لم يكن هناك فرق منطقي بمستوى الحالة الدفاعية الكلية ضد الأكسدة بين مرضى الشقيقة أنفسهم. إن العلاج بالارغوتامين قد أدى إلى ارتفاع منطقي بمستوى الحالة الدفاعية الكلية ضد الأكسدة بنسبة ١٥٧%. كما وان إضافة فيتامين هـ أو الميلاتونين قد أدى إلى ارتفاع أكبر في الحالة الدفاعية الكلية ضد الأكسدة وبنسبة ١٧٩% و ١٧٦% على التوالي. وعلى العموم فقد اظهر فيتامين هـ تفوقاً في رفع الحالة الدفاعية الكلية ضد الأكسدة عن تلك التي أظهرها الميلاتونين. يظهر الانخفاض الكبير في الحالة الدفاعية الكلية ضد الأكسدة بين مرضى الشقيقة بالمقارنة مع الأصحاء حدوث نقص عام في العناصر الدفاعية ضد الأكسدة كما وان رفع الحالة الدفاعية الكلية ضد الأكسدة كان واضحاً عند جميع المجاميع العلاجية. تستنتج الدراسة بأن انخفاض الحالة الدفاعية الكلية ضد الأكسدة يمكن أن يكون عاملاً في سببية داء الشقيقة وأن تحسينها قد يكون ذا فائدة في علاج صداع الشقيقة باستخدام مضادات الأكسدة مثل فيتامين هـ أو الميلاتونين مضافةً إلى علاجات داء الشقيقة التقليدية.

Introduction

The pathophysiological mechanisms of migraine have been discussed for years to be involving the humoral-vascular and neurogenic theories [1]. The constriction of cortical vessels may explain the aura, while dilation of extracortical (meningeal) vessels may underlay the throbbing headache [2]. Cerebral hypoxia secondary to vasospasm or platelet aggregation may explain impaired vision predisposing migraine headache [3].Causative factors for cerebral vasospasm could be the alteration in ion concentration (low intracellular Mg^{+2}) [4, 5], abnormally released serotonin secondary to platelets aggregation [6], endothelin receptor gene polymorphism [7], impaired mitochondrial oxidative metabolism and altered nitric oxide synthesis and release [8,9,10]. The neurogenic theory is strongly postulated without excluding aspects of the humoral theory [2]. The familial nature of migraine is greatly linked to the hereditary abnormality of monoaminergic transmission [11]. This transmission is vulnerable to sudden changes in internal or external environment to emotional stress, or to overload of afferent systems by excessive glare, smell or other stimuli. Triggering factors, thus could induce a phase of excessive discharge followed by a state of monoamine depletion, hence pain gates would be opened, giving rise to spontaneous pain in the head and neck [12]. Many evidences suggest the involvement of serotonin and norepinephrine to be the monoamines of great interest in the pathophysiology of migraine; the effectiveness of anti-migraine drugs like methysergide , pizotifen (serotonin receptors antagonists) and β-blockers (block the action of norepinephrine) solidify such suggestion [13,14]. Beside that, the level of serotonin metabolities (5-hydroxyindolacetic acid) is highly elevated in plasma from patients during acute migraine [2]. Change in serotonin level can provoke a neurovascular reaction which involve not only constriction or dilation of cerebral and extracerebral blood vessels but also activate nociceptive trigeminovascular system, an effect enforced by releasing vasoactive neuropeptides (substance P, neurokinin A or calcitonin gen-related polypeptide) ended with sterile or neurogenic inflammation [15]. These events were shown to be blocked by sumatriptan or dihydroergotamine [16,17]. Calcium channel blockers on the other hand may diminish vasoconstriction whether produced by humoral agents or by intrinsic monoamine pathways; while non-steroidal anti-inflammatory drugs (NSAIDs) presumably suppress the sterile inflammatory response in vessel walls [18, 19]. Many authors believe that sterile inflammation within the trigeminovascular system is of great importance in the pathophysiology of migraine headache. This postulate the release of the neurotransmitter substance P, vasodilation, increased vessel permeability, edema of cranial blood vessels and sensitization of sensory nerve endings [20]. Biochemical mediators, like nitric oxide and prostaglandins (PGs) may participate in such scenario; where PGE_2 and TXA_2 levels are shown to be elevated in saliva from patients with migraine headache during acute attacks [21]. Furthermore, nitric oxide has the potential to induce oxidative stress by acting as a free radical through the peroxidation pathway [22]. The concept of oxidative stress has been accepted in the increasing association of diseases with advanced age. The plausible explanation for such association is based on the implication of free radicals in the pathogenesis of several disorders like cancer and atherosclerosis [23,24,25].Oxidative stress, however, results from distorted equilibrium of pro-oxidant/anti-oxidant system in intact cell. Such system continuously generates and detoxifies oxidants during normal aerobic metabolism [26]. Outbalancing such equilibrium leads to damage of lipids, proteins, carbohydrates and nucleic acids; these events might deplete cellular stores of anti-oxidants material such as glutathione and vitamin E [27]. Treatment of acute attacks of migraine is achieved with the use of ergotamine. The effectiveness of ergotamine in treatment of migraine attacks was thought to be related to its α-antagonistic activity; in addition the drug is

known to stimulate 5-HTD1 receptors of the cerebrovascular system. However; several limitations may restrict the use of ergotamine for migraine patients, these include: pregnancy; sepsis; hypertension; cerebral, coronary and peripheral vascular diseases; hepatitis; and renal insufficiency. In addition, the drug has many side effects such as abdominal cramps, parasthesia, nausea and tightness of the chest [2]. Melatonin, an endogenous neurohormon, shows a promising effect in relieving migraine headache by means of reducing the severity, frequency and duration of migraine attacks [28]. The mechanism by which melatonin exert such effect has yet been proven, but this agent possessing many pharmacological properties like scavenging free radicals [29], inhibiting nitric oxide synthase [30], regulating neurovascular system [31] and modulating serotonin actions [32]. In the view of the association of various vascular disorders with oxidative stress [33] and because one of the theories of migraine postulate the change in cerebrovascular milieu, the present study was conducted to investigate the involvement of changed anti-oxidant defense (measured as total antioxidant status) during migraine attack and the possible modulation of such status by classical anti-migraine therapy (ergotamine), antioxidants (vitamin E and melatonin) and their combination.

Materials and Methods

Materials:

Ergotamine tartrate (as Cafergot® 1mg tablets, Novartis, Switzerland), Vitamin E (as 400 capsules, Cipla, India), Melatonin (as 3mg tablets, American Nutri-ceutical, USA), Total antioxidant status (TAS) Kit, (Randox Laboratories Ltd, UK).

Patients

Twenty-three normal subjects and 21 migraine patients with age range of (17-45) years were enrolled in this study for three months. For inclusion, patients had to have a long-term history of migraine with and without aura diagnosed according to neurologist decision at specialized neurological centers and were managed under neurologist supervision. Patients who are smokers, alcoholics or those with other apparent disease were excluded. No changes in patients' medications were made during the study and patients were instructed to keep taking their medications. Migraine patients were divided into three treatment groups; first group treated with ergotamine (1mg) alone, second group with ergotamine (1mg)/vitamin E (400mg) and third group with ergotamine (1mg)/melatonin (3mg). All groups were advised to take their treatments during attacks. Blood samples were drawn from migraine patients and normal subjects before initiation of therapy and after pain has been relived (from migraine patients only) for the investigation of TAS using Randox TAS kit. Method of measurement was followed according to the instructions mentioned in Randox TAS kit. Data were expressed as mean ± standard deviation and differences between means were analyzed by Student's *t*-test. P values less than 0.05 were considered significantly different.

Results

Table (1) and figure (1) showed that TAS was significantly lower in migraine patients ($P<0.05$) in comparison to control (normal subjects) with a percent reduction in TAS was ranged from 35.46% to 43.97%. However, there is no significant difference in the level of TAS among migraine patients ($P>0.05$). Table (1) clearly showed that ergotamine rise significantly the level of TAS by 157%. Interestingly, the addition of vitamin E and melatonin greatly raised TAS by 179% and 176% respectively. This accompanied by shortening the time required for pain to alleviate. Ergotamine treated group required (6.28 ± 3.77 hr) for the pain to alleviate; while ergotamine/vitamin E and ergotamine/melatonin required (4.84 ± 3.6 hr) and (2.77 ± 1.4 hr) respectively. However; only melatonin showed significant difference ($P<0.05$) in the time required for the pain to alleviate among other therapies. Although the percent improvement in TAS in patients treated with ergotamine/vitamin E and ergotamine/melatonin appears to be similar, there is a significant difference toward the superiority of vitamin E over melatonin when added to ergotamine. In this regard, it seems that the addition of melatonin did not significantly improve TAS over that produced by ergotamine alone (figure 1).

Table (1): Effect of treatments of migraine patients with ergotamine and its combination with vitamin E and melatonin on serum TAS.

Serum Total Antioxidant Status (mmol/L)				
	Control (n=23)	**Ergotamine (n=7)**	**Ergotamine/ Vit E (n=8)**	**Ergotamine/ Melatonin (n=6)**
Pre-treatment	1.41 ± 0.22 [a]	0.86 ± 0.15 [b]	0.91 ± 0.15 [b]	0.79 ± 0.12 [b]
Post-treatment	---	1.35 ± 0.25 [*a]	1.63 ± 0.09 [*b]	1.39 ± 0.80 [*a]

Data are resented as mean ± SD.n= number of patients.*P<0.05 with respect to pre-treatment value. Non-identical superscripts (a,b) among different groups considered significantly different, P<0.05.

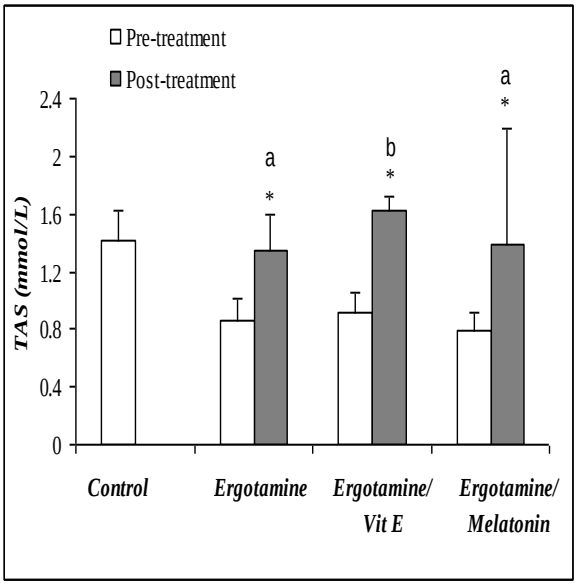

Fig. (1): Serum total antioxidant status in control subjects and migraine patients treated with ergotamine (n=7), Ergotamine/Vitamin E (n=8) and Ergotamine/Melatonin (n=6). Data are presented as mean ± SD. *P<0.05 with respect to pre-treatment value (by paired Student's *t*-test). Non-identical superscripts (a,b) considered significantly different, P<0.05 (by unpaired Student's t-test).

Discussion:

Free radicals in the brain and the implication of oxidative damage caused by them have recently being implicated to playing possible role in the pathogenesis of migraine headache. The greater reduction in TAS seen in this study among migraine patients in comparison to control healthy subjects suggests the presence of generalized decrease in antioxidant defense elements. TAS enables assessment of integrated antioxidant system which encompasses all biological components with antioxidant activity. Reduction in TAS has been implicated in several disease stats such as cancer, ischemic heart diseases and poor nutritional stats [34, 35]. The most convincing evidence for free radical activity comes from nitric oxide, which is a potent vasodilator and is an important biochemical in the trigeminal-vascular peripheral mechanism of migraine headache [36,37]. Infusion of NO donor (glyceryl trinitrate and sodium nitroprusside), immediately induce headache both in healthy subjects and patients suffering from primary headaches with higher intensity in maigiane patients; while slow injection of the nonspecific inhibitor of NO synthase (L-NAM) reduced the spontaneous activity in all neurons [8,38,39]. Koulchitsky and coworkers, suggest that NO can induce activation of central trigeminal neurons and that endogenous release of NO may contribute to the ongoing activity of these neurons. The delayed changes in neuronal activity may include gene expression of pro-nociceptive mediators [46]. In addition, migraine can be induced via a cGMP-dependent mechanism. Kruuse and coworkers showed that sildenafil significantly induce migraine symptoms and propose that triggering mechanisms may reside within the perivascular sensory nerve terminals or the brainstem [41]. NO may interact with other mechanisms for the precipitation of migraine. Strecker and coworkers showed that NO increases meningeal blood flow, an action depends partly on the release and vasodilatory action of calcitonin gene-related peptide (CGRP) from dural afferents; while, prostaglandins show only minimal interaction with NO in this respect [15]. Furthermore, platelet levels of nitric oxide, as well as nitric oxide metabolites such as nitrate/nitrite, are increased in migraine patients and rise further during attacks [9,10]. Therefore, free radical scavengers may provide a potential molecular basis for prophylactic antimigraine therapy by neutralizing nitric oxide overproduction and possibly preventing formation of highly toxic peroxynitrite. Interesting results were observed in this study by observing greater

elevation of TAS by anti-migraine treatments. In deed, the addition of antioxidants (vitamin E or melatonin) to the traditional treatment (ergotamine) greatly potentiate the effect . Although the present study did not concerned with the mechanism of such an elevation in TAS, it seems that the vasoconstriction effect of ergotamine may involved these events. However, the link between effect of ergotamine and the elevation in TAS is the spotlight for further investigation. Vitamin E is powerful antioxidant that scavenges free radicals within the lipid phase of the cell [(42)]. In addition, it have a structural role in stabilizing membranes [(43)], thus nowadays, vitamin E is implicated in the therapy of many diseases [(44, 45)]. Melatonin, beside its known antioxidant activity, it was shown to possess the ability to minimize the intensity, frequency and duration of acute migraine attacks [(28)]. In addition, melatonin has many pharmacological effects, of them is the inhibition of nitric oxide synthase [(30,46)], whether this feature is involved in the relief of migraine headache or in the elevation of TAS this is require further investigation. In our study, melatonin showed no superiority to vitamin E when added to ergotamine with high standard deviation. This may be explained by administration of single rather than maintenance dose of melatonin together with greater fluctuation in plasma melatonin level due to diurnal variation (melatonin plasma level is 10 times higher during night that during the daytime) [(47)]. The age is also an important factor; as we got older the plasma concentration of melatonin decline sharply [(48)]. The present study concluded that the decrease in TAS can be implicated in the pathophysiology of migraine. In addition, enhancement of antioxidant system can add a beneficial effect for the management of migraine headache with the use of antioxidants with classical anti-migraine drug.

Acknowledgments

The authors grateful Dr. Zuhair Abdul Gafoor (neurologist) and Dr. Ibrahem Adham, Dr. May AL-Sabag, and Dr. Haider Fakhry (Clinical Pharmacist) for their kind help in performing this study. This research is abstracted from High Diploma dissertation submitted to clinical pharmacy department .

References

1. Crowell, G.F. and Carlin, L.I.: Neurologic complications of migraine. *Am. Fam. Physicn*. 1982; 26: 139-148.

2. Rang, H.P.; Dale, M.M. and Ritter, J.M.: Clinical conditions in which 5-HT plays a role. In: *Pharmacology* (4th ed.), Churchill Livingstone, London, 1999, pp. 171-172.

3. Marles, R. J.; Kaminski, J.; Arnason, J. T.; Pazos-Sanou, L.; Heptinstall, S.; Fischer, N. H.; Crompton, C. W.; Kindack, D. G. and Awang, D. V. C.: A bioassay for inhibition of serotonin release from bovine platelets. *J. Natural Products.* 1992, 55(8): 1044-1056.

4. McCarty, W.: Magnesium taurate and fish oil for prevention of migraine. *Medical Hypothesis* 1996; 47:461-466.

5. Facchinetti, F.; Sances, G.; Borella, P.; et al.: Magnesium prophylaxis of menstrual migraine: Effects on intracellular magnesium. *Headache* 1991; 31: 298-301.

6. Weber, J.T.; Oconnor, M.F.; Hayataka, K.; Colson, N.; Medora, R.; Russo, E.B. and Parker, K.K.: Activity of parthenolide at 5-HT (2A) receptors. *J. Natural Products* 1997; 60(6): 651-653.

7. Tzourio, C.; El Amrani, M.; Poirier, O.; Nicaud, V.; Bousser, M.-G.; and Alperovitch, A.: Association between migraine and endothelin type A receptor (ETA -231 A/G) gene polymorphism. *Neurology* 2001; 56: 1273-1277.

8. Olesen, J.; Thomsen, L. L.; Lassen, L. H. and Olesen, I. J.: The nitric oxide hypothesis of migraine and other vascular headaches. *Cephalalgia* 1995; 15: 94-100.

9. Shimomura, T.; Murakami, F.; Kotani, K.; Ikawa, S. and Kono, S.: Platelet nitric oxide metabolites in migraine. *Cephalalgia* 1999; 19: 218-222.

10. Stepien, A.; Chalimoniuk, M.; Stepien, A. and Chalimoniuk, M.: Level of nitric oxide-dependent cGMP in patients with migraine. *Cephalalgia* 1998; 18: 631-634.

11. Waeber, C. and Moskowitz, M.A.: Therapeutic implications of central and peripheral neurologic mechanisms in migraine. *Neurology*. 2003; 61(8 Suppl 4): S9-S20.

12. Van den Bergh,V.; Amery, W.K. and Waelkens, J.: Trigger factors in migraine: a study conducted by the Belgian migraine Society Headache 1987; 27:191-196.

13. Silberstein, S.D.: Evidence-based guidelines for migraine headache. *Neurology* 2000; 55: 754-762.

14. Capobianco, D.J.; Cheshire, W.P. and Campbell, J.K.: An overview of the diagnosis and pharmacologic treatment of migraine. *Mayo. Clinic. Proceedings.* 1996; 71(11): 1055-1066.

15. Strecker, T.; Dux, M. and Messlinger, K.: Increase in meningeal blood flow by nitric oxide - interaction with calcitonin gene-related peptide receptor and prostaglandin synthesis inhibition. *Cephalalgia*. 2002; 22(3): 233-41.

16. Akpunonu, B.E.; Mutgi, A.B.; Federman, D.J.; *et al.*: Subcutaneous sumatriptan for treatment of acute migraine in patients admitted to the emergency department: a multicenter study. *Ann. Emerg. Med.* 1995; 25: 464-469.

17. Becker, W.J.; Riess, C.M. and Hoag, J.: Effectiveness of subcutaneous dihydroergotamine by home injection for migraine. Headache 1996; 36: 144-148.

18. Parantainen, J. and Vapaatalo, H.: Prostaglandins. In: *The pathophysiology of migraine.* Prostaglandins: Biology and chemistry of prostaglandins and related eicosanoids. Curtis-Prior, B.P. (Ed.), Churchill Livingstone, Edinburgh, 1988; 386-401.

19. Tfelt-Hansen, P. and McEwen, J.: Nonsteroidal anti-inflammatory drugs in the acute treatment of migraine. In: *The Headaches* (2nd ed.). Olesen, J.; Tfelt-Hansen, P. and Welch, K.M.A. (EDs), Lippincott, Williams and Wilkins, Philadelphia, 2000; 391-397.

20. Moskowitz, M.A.: The neurobiology of vascular head pain. *Ann. Neurol.* 1984; 16: 157-168.

21. Tuca, J.O.; Planas, J.M. and Parellada, P.P.: Increase in PGE2 and TXA2 in the saliva of common migraine patients. Action of calcium channel blockers. *Headache* 1989; 29: 498-501.

22. Bagchi, K. and Puri, S.: Free radicals and antioxidants in health and disease. *East. Medi. Health J.* 1998; 4(2): 350-360.

23. Harman, D.: Role of free radical in aging and disease. *Ann. N.Y. Acad. Sci.* 1992; 673: 126-141.

24. Lea, A.J.: Dietary factors associated with death rates from certain neoplasms in man. *Lancet* ; 1966; 2(7458): 332-333.

25. Halliwell, B. and Gutteridge, J.M.C.: Free radicals in biology and medicine (2nd ed.), Oxford, Clarendon press, 1989.

26. Afanas'ev, I.B.: Mechanism of superoxide-mediated damage relevance to mitochondrial aging. Ann. N.Y. Acad. Sci. 2004; 1019: 343-345.

27. Smythies J.: Recent advances in oxidative stress and antioxidants in medicine. *J. Orthomolecular Medicine* 1998; 13(1): 11-18.

28. Muqdam, M.M.; Salim, A.H. and Ashwaq, N.E.: Clinical evaluation of melatonin alone and in combination with pizotifen in the prophylaxis of migraine. *Iraqi J. Pharm. Sci.* 2007; 16(1): 1-7.

29. Reiter, R.J.; Calvo, J.R.; Karbownik, M.; Qi, W. and Tan, D.X.: Melatonin and its relation to the immune system and inflammation. *Ann. N.Y. Acad. Sci.* 2000; 917: 376-386.

30. Bettahi, I.; Pozo, D.; Osuna, C.; Reiter, R.J.; Acuna-Castroviejo, D. and Guerrero, J.M.: Melatonin reduces nitric oxide synthase activity in rat hypothalamus. *J. Pineal Res.* 1996; 20: 205-210.

31. Savaskan, E.; Olivieri, G.; Brydon, L.; Jockers, R.; Krauchi, K. and Wirz-Justice, A.: Cerebrovascular melatonin MT1-receptor alterations in patients with Alzheimer's disease. Neurosci Lett. 2001; 308: 9-12.

32. Monnet, F.P.: Melatonin modulates [3H] serotonin release in the rat hippocampus: effects of circadian rhythm. *J. Neuroendocrinol.* 2002; 14: 194-199.

33. Langseth, L.: Antioxidant vitamins and disease prevention. *Food Technol*. 1994; 7: 80-84.

34. Zachwieja, J.; Bobkowski, W.; Niklas, A.; *et al.*: Total antioxidant status in children with nephritic syndrome. *POI Merkuriusz lek*. 2000; 8 (46): 216-7.

35. Car, N.; Vucic, M.; Car, A.; *et al.*: Superoxide dismutase and total antioxidant status in patient with diabetic ketoacidosis. *Diabiologia Croatica*. 2000; 29-4:193-196.

36. Silberstein, S.D.; Lipton, R.B. and Goadsby, P.J. (EDs): The pathophysiology of primary headache. In: *Headache in Clinical Practice*. Oxford, England: Isis Medical Media; 1998; 41-58.

37. Srikiatkhachorn, A.; Suwattanasophon, C.; Ruangpattanatawee, U. and Phansuwan-Pujito, P.: 5-HT2A receptor activation and nitric oxide synthesis: A possible mechanism determining migraine attacks. *Headache* 2002; 42: 566-574.

38. Iversen, H.K.; Olesen, J.; and Tfelt-Hansen, P.: Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. *Pain* 1989; 38: 17-24.

39. Tassorelli, C.; Joseph, S.A.; Buzzi, M.G. and Nappi, G.: The effects on the central nervous system of nitroglycerin - putative mechanisms and mediators. *Prog. Neurobiol.* 1999; 57: 607-624.

40. Koulchitsky, S.; Fischer, M.J.M.; De Col, R.; Schlechtweg, P.M. and Messlinger, K.: Biphasic response to nitric oxide of spinal trigeminal neurons with meningeal input in rat – possible implications for the pathophysiology of headaches. *Neurophysiol* 2004; 92: 1320-1328.

41. Kruuse, C.; Thomsen, L.L.; Birk, S. and Olesen, J.: Migraine can be induced by sildenafil without changes in middle

cerebral artery diameter. *Brain* 2003; 126(1): 241-247.

42. Esterbaner, H.; Dieber-Rotheneder, M.; Striegl, G.; *el al.*: Role of vitamin E in preventing the oxidation of low-density lipoprotein. *Am. J. Clin. Nutr.* 1991: 53(suppl 1): 314S-321S.

43. Urano, S.; Inomori, Y.; Sugawara, T.; *et al.*: Vitamin-E inhibition of retinol-induced hemolysis and membrane-stabilizing behavior. *J.Biol. Chem.* 1992; 267: 18365-18370.

44. Swann, I.L. and Kendra, J.R.: Anaemia, vitamin E deficiency and failure to thrive in an infant. Clin. Lab. Haematol. 1998; 20(1): 61-63.

45. Sokol, R.I.: Vitamin E deficiency and neurologic disease. *Ann. Rev. Nutr.* 1988:8:351-73.

46. Leon. J.; Escames, G.; Rodríguez, M.I.; Lopez, L.C.; Tapias, V.; Entrena, A.; Camacho, E.; Carrion, M.D.; Gallo, M.A.; Espinosa, A.; Tan, D.X.; Reiter, R.J. and Acuna-Castroviejo, D.: Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. *J. Neurochem.* 2006; 98(6): 2023-2033.

47. Takimoto, M.; Hamada, A.; Tomoda, A.; Ohdo, S.; Ohmura, T.; Sakato, H.; Kawatani, J.; Jodoi, T.; Nakagawa, H.; Terazono, H.; Koyanagi, S.; Higuchi, S.; Kimura, M.; Tukikawa, H.; Irie, S.; Saito, H. and Miike, T.: Daily expression of clock genes in whole blood cells in healthy subjects and a patient with circadian rhythm sleep disorder. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2005; 289: R1273-R1279.

48. Humbert, W. and Pevet, P.: The decrease of pineal melatonin production with age. Causes and consequences. *Ann. N.Y. Acad. Sci.* 1994; 719: 43-63.