Amitriptyline Effect on Tumor Necrosis Factor-α, Interleukin-1 and Interleukin-6 Serum Level and its Correlation with Pain Severity in Chronic Tension-Type Headache Patients

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Abstract- Introduction : Chronic tension-type headache (CTTH) differs from the episodic form not only in frequency but also with respect to pathophysiology, lack of effect to most treatment strategies, frequent medication overuse, more disability, and higher socioeconomic costs. The purpose of this study is to see the effect of amitriptyline on TNF-α, IL-1, and IL-6 serum levels in CTTH patients and its correlation with pain severity.

Method : This research was done at the Adam Malik Hospital and Bukit Barisan Army Hospital Medan, Indonesia from January 2013 - June 2014 and approved by the Ethics Committee for Health Research School of Medicine in University of Sumatera Utara. The subjects were recruited consecutively from study population. Diagnosis of CTTH was based on the diagnostic criteria as stated in the ICH X. We assessed pain severity by using the Numeric Rating Scale (NRS) for pain. Venous blood was taken to measure serum levels of TNF-α , IL-1, and IL-6. After subjetcs were given amitriptyline 25 mg once daily in the evening for ten days, NRS scores were reassessed and the second measurements of these cytokines serum level were done.

Results : Twenty three subjects, 5 male and 18 female participated in this study. There was a significant difference (p=0.001) between baseline NRS score (4.52 ± 1.78) with NRS score after amitriptyline administration (1.87 ± 1.10). No significant difference (p=0.051) was found between baseline TNF-α (2.15 ± 0.98 pg/dl) with TNF-α level after treatment (1.89 ± 0.86 pg/dl). There was no significant difference between baseline and after 10-day amitriptyline dosage for IL-1 serum level (0.24 ± 0.26 pg/dl vs 0.25 ± 0.22 pg/dl, p=0.954) nor IL-6 serum level (1.84 ± 1.36 pg/dl vs 2.01 ± 1.76 pg/dl, p=0.687). There was a very weak negative (R=-0.178) non significant correlation (p=0.415) between NRS score and TNF-α serum level. In these subjects, we found a very weak negative (R=-0.111) non significant correlation (p=0.615) between NRS score and IL-1 serum level. NRS score and IL-6 serum level had a weak (R=-0.364) non significant negative correlation (p=0.088)
Conclusions : Amitriptyline decreased serum level of TNF-α but not statistically significant.

Amitriptyline had no effect on IL-1 nor IL-6 serum levels. Amitiriptiline lowered pain intensity based on NRS score (p=0.001). NRS score and TNF-α serum level had a very weak and non significant negative correlation. NRS score and IL-1 serum level had a very weak and non significant negative correlation, while NRS score and IL-6 serum level had a weak and non significant negative correlation.

Index Terms- amitriptyline, chronic tension-type headache, IL-1, IL-6, TNF-α

I. Introduction

Tension-type headache (TTH) is the most common form of primary headache.. Chronic tension-type headache (CTTH) differs from the episodic forms not only in frequency but also with respect to pathophysiology, lack of effect to most treatment strategies, more medication overuse, more disability, and higher personal and socioeconomic costs [1]. Globally, the percentages of the adult population with an active headache disorder are 46% headache in general, 11% migraine, 42% TTH and 3% chronic daily headache [2].

A number of studies also have examined the levels of cytokines in the blood of headache patients, generally migraine. Bo *et al*, studied the level of cytokines in cerebrospinal fluid (CSF) in headache patients and found elevated levels of IL-1, TGF-b1 (*transforming growth factor-b1*), and MCP-1 (*monocyte chemoattractant protein-1*) in episodic tension-type headache (ETTH) and migraine compared to controls, and there were significant differences in MCP-1 between cervicogenic headache and migraine without aura [3]. Kocer found an increasing level of IL-6 in patients with ETTH and CTTH compared to controls. Therefore, they believe that IL-6 is involved in the induction of pain or inflammatory mechanisms in TTH [4]. Research by Backonja also found an elevated receptor levels of TNF in CSF and blood, elevated levels of IL-1β in CSF that was associated with pain intensity, whereas IL-10 was inversely correlated with pain symptoms [5]. Serum levels of IL-1β were significantly elevated in CTTH patients relative to healthy controls, while IL-18 levels were significantly elevated in men with CTTH, in a study by Vedova *et.al* [6].

The role of psychological condition in headache has been studied extensively. Chen *et.al* found that anxiety-neurotic and depression correlate with CTTH [7]. Özdemir *et.al* found that patients with migraine and TTH had maladaptive coping responses and more neurotic personality features and these

factors play significant role in the development of headaches and their severity [8]

Antidepressant is one of most commonly used drugs in CTTH management. Amitriptyline was proven better than placebo in many clinical trials [9,10]. Amitriptyline is frequently a first choice for prophylaxis treatment in CTTH despite of it's adverse effects [11]. Amitriptyline is also effective for painful diabetic neuropathy [12]. Tricyclic antidepressants, including amitriptyline, are effective in chronic pain management in systematic review by the WorkSafeBC Evidence-Based Practice Group (EBPG) [13]. In persistent pain in the arms due to overuse, low dose amitriptyline increased the arms function, although did not lower pain intensity significantly [14].

Prior studies have found a positive relationship between the number of cytokines with some types of headache. Unfortunately, most measurements of cytokine levels were performed in the CSF, make it relatively difficult for routine examination in daily practice. The purpose of this study is to measure the serum levels of TNF-α, IL-1, IL-6 in CTTH patients before and after given amitriptyline and its correlation with pain severity.

II. METHODS

This research was done at the Adam Malik Hospital and Bukit Barisan Army Hospital Medan, Indonesia from January 2013 - June 2014 and approved by the Ethics Committee for Health Research School of Medicine in University of Sumatera Utara. The subjects were recruited consecutively from study population. Diagnosis of CTTH was made based on the diagnostic criteria as stated in the ICH X. NRS score were taken from all subjects at baseline as well as blood for TNF-α, IL-1 and IL-6 serum level measurement. Each subject was given amitriptyline 25 mg once daily in the evening to minimize the side effect for 10 consecutive days. The day after the last dosage, all subject were asked to score their pain severity at that time by using NRS. The second blood samples were taken for the second TNF-α, IL-1 and IL-6 serum level measurement. T-paired test with the level of signifcancy $p<0.5$ was performed to analyze differences between NRS score, TNF-α, IL-1 and IL-6 serum level before and after amitriptyline.

III. RESULTS

At the beginning, there were 30 patients with CTTH met the criteria of this study. Seven of them were excluded from the study, 4 subjects because they had never come for blood sampling, and 3 subjects because did not complete the study protocol. Data from 23 subjects who followed the whole procedure were analyzed further. Twenty three CTTH patients participated in this study, 5 men (21.7%) and 18 women (78.3%).

There was a significant difference (p=0.001) between baseline NRS score (4.52 ± 1.78) with NRS score after amitriptyline administration (1.87 ± 1,10). No significant difference (p=0.051) was found between baseline TNF-α (2.15 ± 0.98 pg/dl) with TNF-α level after treatment (1.89 ± 0.86 pg/dl). There is also no significant difference was found between baseline and after 10-day amitriptyline administration for IL-1 serum (0.24 ± 0.26 pg/dl vs 0.25 ± 0.22 pg/dl, p=0.954) and IL-6 serum (1.84 ± 1.36 pg/dl vs 2.01 ± 1.76 pg/dl, p=0.687) (Table 1).

Table 1. NRS score, TNF-α, IL-1 and IL-6 serum level before and after amitriptyline administration

Variable	Amitriptyline		*p* *
	Before (n ; x ± SD)	After (n ; x ± SD)	
NRS	23 ; 4.52 ± 1.78	23 ; 1.87 ± 1.10	0.001 **
TNF-α	23 ; 2.15 ± 0.98 pg/dl	23 ; 1.89 ± 0.86 pg/dl	0.051
IL-1	23 ; 0.24 ± 0.26 pg/dl	23 ; 0.25 ± 0.22 pg/dl	0.954
IL-6	23 ; 1.84 ± 1.36 pg/dl	23 ; 2.01 ± 1.76 pg/dl	0.687

Table 2. Correlation between NRS score and TNF-α, kadar IL-1 and kadar IL-6 serum level after amitriptyline administration

			TNF-α	IL-1	IL-6
NRS amitriptyline administration	after	R	-0.178	- 0.111	- 0.364
		P	0.415	0.615	0.088
		N	23	23	23

After amitriptyline administration, the NRS score showed very weak negative and non-significant correlations with the serum level of TNF- α (R = -0.178 ; p = 0.415), IL-1 (R = -0.111 ; p = 0.615), and IL-6 (R = -0.364 ; p = 0.088) (Table 2).

IV. DISCUSSIONS

At baseline, the mean of the NRS score was 4.52 ± 1.78 and became 1.87 ± 1.10 after amitriptyline administration. There was a significant decrement of the NRS score with p = 0.001. This fact suggests that amitriptyline is effective to lower the pain intensity in CTTH patients. Amitriptyline works by inhibiting the serotonin and norepinefrin re-uptake by pre-synaptic cells (serotonin/noreponefrin re-uptake inhibitor). The effect is strong

on serotonin transporters and moderate on norepinefrin tranporters. Aside from that, amitripytline also works as receptor antagonist toward 5-HT2, 5-HT3, 5-HT6, 5-HT7, α1-adrenergik, H1, H2, H4, and mAch receptors, and receptor agonist toward α1 receptor. Amitriptyline can also block natrium, kalium, and calcium channels [15,16]. Various biological mechanisms of amitriptyline as stated above, have contribute to decrease pain intensity from various medical conditions, including CTTH.

Prior to amitriptyline administration, the mean of TNF-α serum level was 2.15 ± 0.98 pg/dl and became 1.89 ± 0.86 pg/dl after administration. There was a non-significant decrement of the mean of TNF-α serum level (p=0.051). This data suggest that TNF-α serum level had no correlation with decreasing pain intensity after amitriptyline administration in CTTH patients, differs from previous study. A study by Bo *et.al* in 2008, showed significant differences between the CSF level of IL-1ra, TGF-β1 and MCP-1 in TTH and migraine patients when compared to the control group [3]. The non-significant result of TNF-α on this study is in accordance by several previous studies. Tanure *et.al.* found no significant difference in the level of TNF-α, sTNFR1 and sTNFR2 during migraine attack and headache free period [17]. The cytokine was only increased slightly if compared to other severe neurological diseases. This increment was considered as a slight response of cytokine toward headache [16]. A study by Rozen et al found an increment of TNF-α in LCS of NDPH and migraine patients. But the increment was not found in serum [18]. TNF-α is the primary pro-inflammatory cytokine for brain infection diseases. In normal circumstances, the production is little. In infection condition, where there is a strong stimulation by microorganisms, the production will greatly increase so that it can be detected in blood with a quite significant level [19]. The non significant finding in this study maybe due to measurement of TNF-α was performed in the serum where more confounding variables found compare to CSF. The very low serum level of TNF-α found in this study was probably indicated that in CTTH patients, only very small amount of TNF-α produced, in contrast with during brain infection.

There was a contradictory of significance in the result between NRS score and TNF-α serum level, before and after amitriptyline administration. With p = 0.001, it means that amitriptyline effectively reduced pain intensity. On the other side, p = 0.051 after amitriptyline administration, suggest that TNF-α level was not significantly decreased as a result of amitriptyline administration. This fact suggests that pain intensity decrement due to amitriptyline administration, was not through TNF-α decrement mechanism. Regarding pain, there were still many biological mechanisms of amitriptyline, which were still not fully understood [15]. Many in vitro studies regarding effect of TNF-α on CNS has been performed, with still ambiguous conclusion [20].
Before amitriptyline administration, the mean level of IL-1 was 0,24 ± 0,26 and it became 0,25 ± 0,22 after administration (p=0.954). This fact showed that IL-1 serum level did not significantly decrease pain intensity as a result of amitriptyline administration in chronic TTH patients. Together, IL-1 and IL-6 causes trigeminal nociceptor sensitization and play an important role in migraine pathogenesis by reducing sensitivity threshold toward other inflamatory stimulus [21]. As strong mediators for fever, pain, and inflammation, IL-1 and TNF-α function via hypothalamic induction [19, 22]. A research by Bo et al, found an increment of cytokine IL-1, TGF-β1 and MCP-1 level in patient's LCS who had episodic TTH and migraine [3].The non-significant result on IL-1 level in this research was in accordance with previous studies. In normal circumstances, the IL-1 production is very small. In infection condition, where there is a strong stimulation by micro organisms, the production will greatly elevated so that it can be detected in blood with a quite significant level [19]. The small quantity of IL-1 in this study was caused by TTH not being a brain infection.

There was contradictory of significance in the result between NRS score and IL-1 serum level, before and after amitriptyline administration. With p = 0.001, it means that amitriptyline effectively reduced pain intensity. On the other side, p = 0.954 after amitriptyline administration, suggests that IL-1 level was not significantly different as a result of amitriptyline administration. This fact suggests that pain intensity decrement due to amitriptyline administration, was not through IL-1 decrement mechanism. Regarding pain, there were still many biological mechanisms of amitriptyline, which were still not fully understood [15]. The correlation between IL-1 and amitriptyline in reducing pain intensity is still unclear.

Before drug administration, the level of IL-6 = 1,84 ± 1,36 and after administration it became 2,01 ± 1,76 with no significant difference between them (p=0.687). This fact showed that IL-1 serum level did not significantly decrease pain intensity as a result of amitriptyline administration in chronic TTH. Interleukin-6 function as pro and anti inflammation, secreted by T-cell and acts as initial response toward infection and trauma. This substance can penetrate blood-brain barrier and initiates PGE_2in hypothalamus, thus elevating body temperature. Whenever infection is occurred, production of IL-6 will increase [23]. Systemic effect of IL-1 will cause induction of fever, acute phase protein plasma synthesis by liver, and direct or directly stimulate production of IL-6, and production of neutrofil and platelet of bone marrow [21]. In migraineurs, it has been suggested that IL-6 level increase in the attack phase. A study by Yan et al showed that IL-6 strengthen excitability of duramater afferent fiber so that sensitization which contributed toward pathophysiology migraine headache occurred [24].

From statistical analysis there was non-significant difference of IL-6 level, with p = 0.687, after amitriptyline administration. The non-significant result of IL-6 in this study was supported by previous study results. The same as TNF-α and IL-1, IL-6 is very responsive toward infection [23]. Regarding pain in animal experiment, IL-6 can stimulate trigeminal ganglion cell to synthesize COX-2 and PGE2, which will release CGRP that causes pain [25]. Bo et al did not reveal any significant difference in CSF level of several pro inflammatory cytokines in TTH, migraine, and cervicogenic headache [3]. But, IL-6 pain-related detection in those several studies were obtained through LCS, not serum.

There was contradictory of the result between NRS score and IL-6 serum level, before and after amitriptyline administration. With p = 0.001, it means that amitriptyline effectively reduced pain intensity. On the other side, p = 0.687 after amitriptyline administration, suggest that IL-6 level was not significantly different as a result of amitriptyline administration. Regarding pain, there were still many biological mechanisms of amitriptyline, which were still not fully understood [15]. This fact

suggest that pain intensity decrement due to amitriptyline administration, was not through IL-6 decrement mechanism.

V. CONCLUSIONS

In amitriptyline group, there was a reduction of the mean of TNF-α serum level, from 2,15 ± 0,98 pg/ml (before administration) to 1,89 ± 0,86 pg/ml (after administration), but the difference was not significant (p=0.051). As for IL-1 and IL-6 serum level, there was also non-significant difference between before and after administration (p=0.954 and p=0.687 respectively).

In this group , there was statistically significant decrement of pain intensity based on the mean of NRS score (p=0.001), from 4,52 ± 1,78 (before) to 1,87 ± 1,10 (after). After taking amitriptyline, TNF-α serum level had a very weak negative correlation (R=-0.178) and non-significant (p=0.415) with pain intensity. There was a very weak, non-significant negative correlation (R=-0.111; p=0.415) between pain intensity and IL-1 serum level and a weak, non-significant correlation (R=-0.364 ; p=0.088) between pain intensity and IL-6 serum level.

ACKNOWLEDGMENT

Authors acknowledge the immense help receive from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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