Original article

Platelet activating factor and monocyte chemoattractant protein-1 in children with refractory epilepsy

Background: *Epilepsy is an important common and diverse group of symptom complexes characterized by recurrent spontaneous seizures. It is estimated that about 5-10% of all cases of epilepsy eventually become refractory. It has been suggested that inflammation plays a role in epilepsy. In refractory epilepsy, an inflammatory response is produced that leads to rapid release of pro-inflammatory cytokines as platelet activating factor (PAF) and monocyte chemoattractant protein-1 (MCP-1).*
Objective: *The aim of the present study was to evaluate the plasma levels of the monocyte chemoattractant protein-1 (MCP-1) and platelet activating factor (PAF) in children with refractory epilepsy to explore their role in the pathogenesis of refractory epilepsy.*
Methods: *The present study was carried out in Tanta University Hospital, Pediatric Department, Neurology unit. Forty (40) children with idiopathic refractory epilepsy (25 males and 15 females) their age ranging between 4-15 years were included in the study. The control group consisted of thirty healthy children, 20 males and 10 females aged 5 years to 13 years. The serum levels of MCP-1 and PAF were measured for children with refractory epilepsy and the control children.* ***Results:*** *Children with refractory epilepsy had significantly higher serum levels of PAF (P value<0.001) and significantly higher serum level of MCP-1 (P value<0.001) in comparison to the control children. Also there was a significant correlation between the duration of refractory epilepsy and the serum levels of PAF and MCP-1.*
Conclusion: *Higher serum levels of the proinflammatory cytokines PAF and MCP-1 in children with refractory epilepsy suggest that both, PAF and MCP-1, may play a role in the pathogenesis of refractory epilepsy.*

Key words: Platelet activating factor, Monocyte chemoattractant protein-1, Neuroinflammation, Refractory epilepsy.

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INTRODUCTION

Epilepsy represents the most common serious neurological problem affecting children, with an overall incidence approaching 2% for febrile seizures and 1% for idiopathic epilepsy[1,2]. Some authors define intractable epilepsy as failure of two appropriate AEDs, the occurrence of an average of one seizure per month for ≥ 18 months, and no more than a 3-month seizure free during those 18 months[3-5]. Recent findings in experimental models and in the clinical setting highlight the possibility that, inflammatory processes in the brain contribute to the etiopathogenesis of seizures and to the establishment of a chronic epileptic focus[6-8]. In many animal models of epilepsy acute seizures cause glial activation and increased cytokine production. The activated glia and elevated cytokines in turn have been demonstrated to contribute to seizure-related hippocampal pathology such as neuronal death and reactive gliosis[9]. Platelet-activating factor (PAF) is a potent short-lived phospholipid mediator of inflammation that participates in physiologic signaling in the brain. It is a proinflammatory phospholipid that regulates the peripheral cytokines and inflammatory networks[10]. It is also involved in changes to vascular permeability, the oxidative burst, chemotaxis of leukocytes as well as augmentation of arachidonic acid metabolism in phagocytes. In the central nervous system sustained release of PAF is observed during ischemia, encephalitis, meningitis and epilepsy[11]. Another cytokine known as monocyte chemoattractant protein-1 (MCP-1) (also termed a monocyte chemotactic and activating factor (MCAF)[12]), belongs to the family of chemotactic cytokines known as chemokines, was found to be secreted by mononuclear cells and various non leukocytic cells including inflammatory fibroblasts and is synthesized by astrocytes and

microglia within the brain parenchyma. MCP-1 is consistently elevated in the brains in patients with refractory epilepsy[13]. MCP-1 aids in directing leukocytes to specific locales within the brain and spinal cord during central nervous system inflammation and directs the chemotaxis of monocytes and lymphocytes along concentration gradients in vitro. MCP-1 also mediates recruitment of macrophages/microglia and granulocytes during seizure injury[14].

This study aimed to investigate the serum levels the PAF and MCP-1 in children with refractory epilepsy to explore their role in the pathogenesis of refractory epilepsy.

METHODS

The present study was carried out in Tanta University Hospital, Pediatric Department, Neurology unit, during a 12-month period from January 2011 to January 2012. Forty (40) children with idiopathic refractory epilepsy 25 males and 15 females, age range 4-15 years with the mean age of (9.17±3.4 years) were included in the study. Patients with refractory epilepsy had recurrent seizures for more than 18 months with no more than a 3-months seizure free during those 18 months and failure of at least two appropriate AEDs with proper doses, good compliance and within normal therapeutic range. Thorough history of the age of onset, detailed medical history as regard the types of seizures frequency, duration , history of the AEDs given including the dose and the compliance and duration as well as family history of similar conditions, obstetric history of any complication during delivery and past history of trauma were obtained from the parents. Electroencephalography (EEG), Magnetic Resonanace Imaging (MRI) brain, and the serum level of the AEDs were done for the epileptic children. Exclusion criteria including, false refractoriness related to nonepileptic seizures, inadequate AEDs, poor combination of AEDs, subtherapeutic range of AEDs, noncompliance, seizure-precipitating factors and children with any signs of central nervous system or any other infection. Also children with chronic inflammatory diseases, diabetes mellitus, liver or renal impairment, cardiac and pulmonary diseases were excluded from the study. Control group consisted of thirty (30) healthy children 20 males and 10 females, with their age ranging between 5-13 years and their mean age 9.8±2.1 years. The control group had no history of any neurological or systemic diseases or any signs of systemic infections. The control children were matched with the patients as regards to sex and age. All children participated in the study after written informed parental consents had been obtained. The study had been approved by the local ethics committee of the Faculty of Medicine Tanta University. The serum levels of the PAF and MCP-1 were measured for children with refractory epilepsy and the control children.

Methods:

Five ml venous blood samples were collected from each patient in the morning after an overnight fasting. The fasting blood samples were allowed to clot for 30 minutes then centrifuged and the serum obtained were stored at -20°C into clean dry tubes until the time of determinations of the serum platelet activating factor (PAF) and monocyte chemoattractant protein-1 (MCP-1).

Monocyte Chemoattractant Protein-1 (MCP-1)

MCP-1 was measured by enzyme linked immunosorbant assay (ELISA) supplied with the kit (Ray Bio, Catalog n:ELH-MCP-1-0010). Standard and samples were pipetted into the wells and MCP-1 present in the samples was bound to the wells by the immobilized antibody. The wells were washed and a TMB substrate solution was added to the wells and color developed in proportion to the amount of leptin bound .The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm. leptin bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm[15].

Quantitative determination of Human platelet activating factor (PAF)

PAF was measured by immunoassay kit (ELISA) supplied by (EIAab Catalog No: E0526h). Standards and samples were added to the appropriate microtiter plate wells which had been pre-coated with an antibody specific to PAF. The biotin-conjugated polyclonal antibody preparation specific for PAF and Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm[16].

Statistical analysis

Collected data were coded, analyzed and computed using the Statistical Package for Social Sciences (SPSS) version 10. Results were expressed as mean

±SD, and differences between the means of different variables were tested using the independent samples t-test. P <0.05 is considered significantly different. Pearson correlation was done to correlate the duration of refractory seizure and the serum levels of PAF and MCP-1.

RESULTS

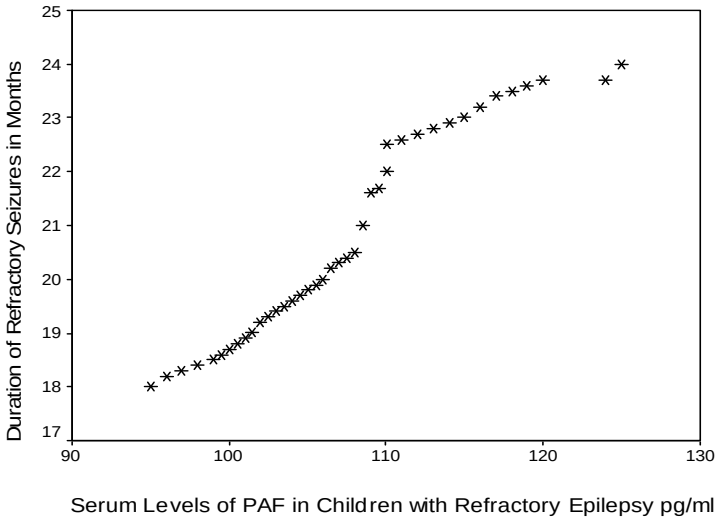
Table (1) shows the characteristics of children with refractory epilepsy as regard the age of onset of the first seizure, the currently used AEDs and the EEG finding in each patient, the frequency of seizures and the duration of refectory seizures. The frequency of seizures was >/= Once /month in 70% of children with refractory epilepsy, >/= Once/week in 25% of children, once/day in 5% of children. The type of refractory seizures are classified as follow, partial seizures were found in 22 children with refractory epilepsy (55%), generalized tonic clonic seizures 6 children (15%), myoclonic seizures 4 children (10%), atonic seizures 6 children (15%), mixed types of seizures 2 children (5%). The AEDs used in children with refractory epilepsy were, valproic acid +carbamazepine in 15% of children with refractory epilepsy , 30% of these children treated with valproic acids +levetiracetam, 20 % treated with lamotrigine +carbamazepine, 15% treated with valproic acid + Topiramate, 10% treated with Valproic acid +Levetiracetam +Carbamazepine, and 10 % treated with Valproic acid +Topiramate+ Clonazepam. Table (2) shows comparison between the mean serum PAF and MCP-1 in children with refractory epilepsy and the control children, the mean serum level of the PAF in children with refractory epilepsy was 107.4±11 pg/ml this value was significantly higher than the mean serum level of the PAF in the control children which was 47.1±6 pg/ml ($p<0.001$). The mean serum level of the MCP-1 in children with refractory epilepsy was 90.4±6 pg/ml, this value was significantly higher than the mean serum level of the MCP-1 in the control children which was 31.5±8 pg/ml ($p<0.001$). Figures (1,2) show significant correlation between the duration of refractory epilepsy and the serum levels of PAF and MCP-1 respectively. Increased duration of refractory epilepsy was associated with increased serum levels of these cytokines ($p<0.001$).

Table 1. Characteristics of children with refractory epilepsy.

Age of the first seizure (years)	
Range	3-9
Mean	6.72±1.8
Duration of refractory epilepsy (months)	
Range	18-24
Mean	20±1.8
Electroencephalography Finding	**Number (percent)**
Generalized epileptogenic activity	20 (50%)
Focal epileptogenic activity	12 (30%)
Multifocal Generalized epileptogenic activity	6 (15%)
No abnormalities	2 (5%)
Frequency of seizures	**Number (percent)**
>/= Once /month	28 (70%)
>/= Once/week	10 (25%)
Once/day	2 (5%)
Currently used AEDs.	**Number (percent)**
Two AEDs	32 (80%)
Three AEDs	8 (20%)

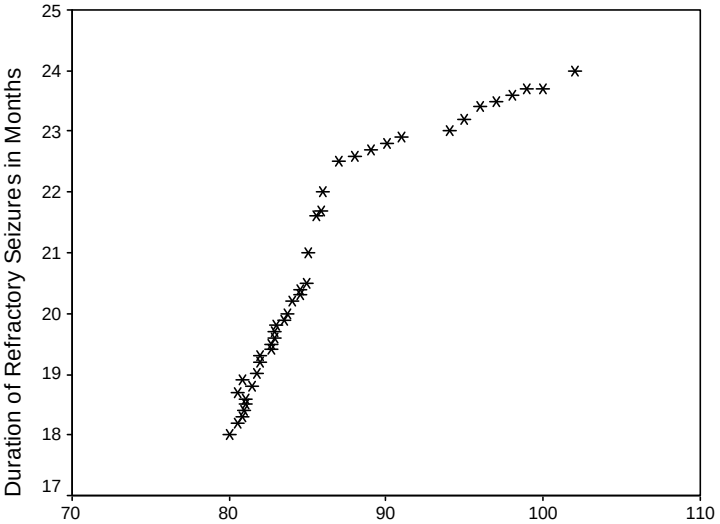
Table 2. Comparison between the mean serum PAF and MCP-1 in children with refractory epilepsy and the control children.

	Children with refractory epilepsy	**Control children**	**P value**
Serum PAF (pg/ml)			
Range	95-125	35-55	<0.001
Mean±SD	107.4±11	47.1±6	
Serum MCP-1 (pg/ml)			
Range	80-102	20-45	<0.001
Mean±SD	90.4±6	31.5±8	



r=0.968 p<0.001

Figure 1. Correlation between the mean serum levels of PAF and the duration of refractory epilepsy.



r=0.929 p<0.001

Figure 2. Correlation between the mean serum levels of MCP-1 and the duration of refractory epilepsy.

DISCUSSION

It has been suggested that inflammation plays a role in epilepsy, inflammation is evoked by pro-inflammatory modulators meant to protect from and heal injuries to the body[17,18]. Pro-inflammatory and anti-inflammatory cytokines, chemokines and prostaglandins are responsible for the production of an early immune response[19]. Rodgers et al.[20] proposed that early inflammation largely increases neuronal excitability and after injury to the brain. An inflammatory response is produced by glial cells that lead to rapid release of pro-inflammatory cytokines. It is suggested that rapid triggering of immune response in the brain can be a precursor to seizures[21,22]. The results of the present study had revealed that, children with refractory epilepsy had significantly higher serum levels of both PAF and MCP-1 than the control children ($p<0.01$). After seizures PAF which is an endogenous proinflammatory agent that mediates neuronal survival and glutamate release was found to accumulate after seizures in the brain and activates intracellular signaling related to inflammation-mediated excitotoxicity and hippocampal hyperexcitability[23]. Although PAF plays a role in the normal functioning of neurons, higher concentrations of PAF have been implicated in the neurotoxicity of epilepsy and contribute to neuronal damage in refractory epilepsy[24]. Increased seizure frequency is associated with increased neuronal and astrocytic injury and with massive glial activation and inflammatory responses in the epileptogenic cortex[25]. Microglial activation, astocytic proliferation and proinflammatory cytokine production may promote seizures, further exacerbate epilepsy and cause subsequent degeneration of neurons, astrocytes and oligodendrocytes[26]. The results of this study agree with pervious results done by Xu Yun et al.[27], who found significantly elevated blood levels of PAF in children with epilepsy 72 hours following seizures in comparison to the control children. On the other hand, MCP-1 is another proinflammatory chemokine that attracts cells involved in the immune/inflammatory response, it lays a significant role in the recruitment of monocytes and lymphocytes to the sites of cellular immune reactions[28]. A previous study done by Wu et al.[29], to investigate the expression of MCP-1 in the brain tissue of patients with intractable epilepsy, had demonstrated that, expression of MCP-1 mRNA was upregulated in the brain tissue of patients with intractable epilepsy compared with that in the control group also expression of MCP-1 protein was significantly increased in patients with intractable epilepsy. Another experimental study done by Manley et al.[30], in which they observed increased expression of MCP-1 in the rat hippocampus after induction of seizures. PAF and MCP-1 have been found to increase in the serum of children with refractory epilepsy with significant correlation to the duration of seizures. Thus, modulation of PAF and MCP-1 overactivity may attenuate seizure susceptibility, hippocampal hyperexcitability, and neuroinflammation, thus may be effective therapeutic strategies to prevent or limit epileptogenesis in children with refractory epilepsy[31].

In conclusion, the results of the present study suggest that plasma PAF and MCP-1 were elevated in children with refractory epilepsy. These results suggest that active neuroinflammation and marked cellular injury may play a common pathogenic role in children with refractory epilepsy. These results may suggest the need for a future novel therapeutic strategy to limit epileptogenesis and cell injury associated with refractory epilepsy.

REFERENCES

1. **Hauser WA.** The natural history of seizures. In: Wyllie E, Gupta A, Lachhwani DK, editors. The treatment of epilepsy, principles and practice. 4th ed. Lippincott, Williams and Wilkins: Philadelphia; 2006. p. 117-24.
2. **Mohanaraj R, Brodie MJ.** Phamacological outcomes in newly diagnosed epilepsy. Epilepsy Behav 2005; 6:382-7.
3. **Schuele SU, Luders HO.** Intractable epilepsy: management and therapeutic alternatives. Lancet Neurol 2008; 7:514-524.
4. **Kwan P, Brodie MJ.** Definition of refractory epilepsy: defining the indefinable? Lancet Neurol 2010; 9:27-9.
5. **Mohanaraj R, Brodie MJ.** Diagnosing refractory epilepsy: response to sequential treatment schedules. Eur J Neurol 2006; 13:277-82.
6. **Choi J, Koh S.** Role of Brain Inflammation in Epileptogenesis. Yonsei Med J 2008; 49: 1-18.
7. **Vezzani A, Granata T.** Brain Inflammation in Epilepsy: experimental and Clinical Evidence. Epilepsia 2005; 46: 1724-43.
8. **Vezziani A, Balossa S, Ravizz T.** The role of cytokines in the pathophysiology of epilepsy. Brain Behavior and Immunity 2008; 22: 797-803.

9. **Vezzani A, Moneta D, Richichi C.** Functional Role of Inflammatory Cytokines and Anti-inflammatory Molecules in Seizures and Epileptogensis. Epilepsia 2002; 43: 30-5.

10. **Musto AE, Samii M.** Platelet-activating factor receptor antagonism targets neuroinflammation in experimental epilepsy. Epilepsia 2011; 52: 551–61.

11. **Steffany B, Jiahua C, Bruce A, et al.** Platelet activating factor receptor expression is associated with neuronal apoptosis in an in vivo model of excitotoxicity. Cell Death and Differentiation 1998; 5:867-75.

12. **Hinojosa AE, Garcia BB, Leza JC, et al.** CCL2/MCP-1 modulation of microglial activation and proliferation. J Neuroinflammation 2011; 88:77.

13. **Xu HJ, Long L, Tang YC.** Kainic acid CCR3, CCR2A and macrophage inflammatory protein (MIP)-1a, monocyte chemotactic protein-1 (MCP-1) in the mouse hippocampus during and after pilocarpine-induced status epilepticus. Neuropathol Appl Neurobiol 2009; 35:496-514.

14. **Baggiolini M, Dewald B, Moser B.** Human chemokines: an update. Ann Rec Immunol 1997;15:675–705

15. **Leonard EJ.** Human monocyte chemoattractant protein-1 (MCP-1). Immunology Today 1990; 11:97-110.

16. **Prescott SM, Zimmerman GA, Stafforini DM.** Platelet-activating factor and related lipid mediators. Annu Rev Biochem 2000; 69: 419-45.

17. **Vezzani A.** Inflammation and Epilepsy. Epilepsy Currents 2005; 5: 1-6.

18. **Billiau AD, Wouters CH, Lgae LG.** Epilepsy and the immune system: is there a link? European Journal of Paeditric Neurology 2005; 9: 29-42.

19. **Mlodzikowska A J, Steinborn B, Zarowski M.** Cytokines, epilepsy and antiepileptic drugs- is there a mutual influence? Pharmacological Reports 2007; 59:129-38.

20. **Rodgers KM, Hutchinson MR, Norhtcutt A.** The cortical innate immune response increases local neuronal excitability leading to seizures. Brain 2009; 132:2478-86.

21. **Ravizza T, Gagliardi B, Noe F, et al.** Innate and adaptive immunity during epilepstogenesis and spontaneous seizures: Evidence from experimental models and human temporal lobe epilepsy. Neurobiology of Disease 2008; 29:142-60.

22. **Aarli J A.** Epilepsy and the Immune System. Arch Neurol 2000; 57: 1689-92.

23. **Aihara M, Ishii S, Kume K, Shimizu T.** Interaction between neurone and microglia mediated by platelet-activating factor. Genes Cells 2000, 5:397-406.

24. **Kanihito N. Yutaka H. Shunro E.** Involvement of platelet-activating factor (PAF) in glutamate neurotoxicity in rat neuronal cultures. Brain Research 2012; 1439:1-108.

25. **Block ML, Hong JS.** Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 2005;76:77–98.

26. **Ravizza T, Rizzi M, Perego C, et al.** Inflammatory response and glia activation in developing rat hippocampus after status epilepticus. Epilepsia 2005; 46:113–7.

27. **Xu Y, Jiang D.** Dynamic Change of Platelet Activating Factor (PAF) in Epilepsy and the Relationship between PAF and EEG. Acta Academiae Medicinae Nanjing 1996; 4.

28. **Jiang Y. Beller DI. Frendl G.** Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocyte. J Immunol 1992; 148:2423-8.

29. **Wu Y, Wang X, Xiao F.** Expression of monocyte chemoattractant protein-1 in brain tissue of patients with intractable epilepsy. Clinical Neuropathology 2008; 27:55-63.

30. **Manley NC. Bentrand AA, Kinney KS.** Characterization of monocyte chemoattractant protein-1 expression following a kainate model of status epilepticus. Brain Res 2007; 28:138-43.

31. **Hulkkonen J, Koskikallio E, Rainesalo S, et al.** The balance of inhibitory and excitatory cytokines is differently regulated in vivo and in vitro among therapy resistant epilepsy patients. Epilepsy Res 2004; 59:199–205.