

Inflammation and endothelium response in epileptic patients: A case-control study

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Abstract

Background: Blood brain barrier (BBB) permeability plays an important role in the brain impairments. The barrier is composed of endothelium cells, due to the presence of tight junctions that connect endothelium cells. The failure of BBB function has triggering chronic or acute seizures through brain inflammation and BBB permeability. Seizure induces vasodilation, BBB leakage and up-regulation of vascular cell adhesion molecules which able to bind integrins blood leukocytes.

Materials and Methods: In this case-control study we included 40 epileptic patients who were sampled during a seizure as a case group and 20 healthy subjects as a healthy control group. Plasma levels of the inflammation and endothelium markers including intercellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM), interleukin 1 beta (IL-1 β) and C-reactive protein (CRP) were measured by enzyme-linked immunosorbent assays (ELISAs).

Results: The ICAM and VCAM concentration in the epileptic patients (135.8 ± 5.35) (52.04 ± 4.24) were significantly higher than healthy control group (110.32 ± 5.04) (23.38 ± 3.01) ($P < 0.005$). IL-1 beta concentration was not significantly different between groups ($P = 0.594$). However, CRP level was significantly up-regulated in epileptic patients ($P < 0.005$).

Conclusion: Epileptic patients have BBB leakage and dysfunction as the up-regulation of the endothelium cytokines showed. The BBB leakage may be the result of the inflammatory impairment.

Key Words: Endothelium, epileptic, Inflammation

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INTRODUCTION

Due to its unique structure, the blood-brain barrier (BBB) permeability plays an important role in the brain impairments. The barrier is composed of endothelium cells, due to the presence of tight junctions that connect endothelium cells.^[1] The

failure of BBB function has triggering chronic or acute seizures through brain inflammation and BBB permeability.^[2] Blood vessels in the brain can respond to the electrical activity due to seizure, causing either transient hyperperfusion in healthy tissue or severe hypoperfusion in tissue at risk of progressive damage.^[3,4] Epilepsy could be resulted of the inflammatory response and the endothelium

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impairments. The inflammatory response includes the secretion of several inflammatory factors from neurons, astrocytes and microglia such as interleukins (ILs) (e.g. IL-1 β) and C-reactive protein.^[5-7] Infiltration of leukocytes through the BBB through the adhesion molecules (e.g., Intercellular Adhesion Molecule 1 [ICAM-1] and Vascular Adhesion Molecule 1 [VCAM-1] might be the reason of endothelium dysfunction and BBB leakage in epileptic patients.^[8]

Brain inflammation and increased level of IL-1 β was found after the seizure has been detected in the post-epileptic animal model.^[9-11] The level of IL-1 β increased during and after induced seizure in rats.^[12] Several human studies has been reported a post-ictal increase in IL-1 β in blood.^[13] However, IL-1 β plasma level after seizures has not yet proved.^[14] In animal induced seizure model an increased level of CRP in blood considered to be brain ischemia, stroke, and vascular events.^[15]

Seizure induces vasodilation, BBB leakage and up-regulation of vascular cell adhesion molecules which able to bind integrins blood leukocytes.^[8] An acute induced seizure associated with vascular leakage which was prevented by inhibiting leukocyte-vascular adhesion, which could linked to vascular damage.^[8] Chronic expression of VCAM after seizure suggests that leukocyte-vascular interactions may continue, contributing to BBB permeability and brain damage.^[16] To the best of our knowledge, there are rare study showed BBB dysfunction including endothelium factors and inflammation response together in epileptic patients versus none epileptic patients. Therefore we aimed to evaluate the serum concentration of endothelium and inflammatory cytokines in epileptic patients versus none epileptic patients.

MATERIALS AND METHODS

This case-control study has been performed in January to March 2012 to evaluate the hypothesis that epileptic patients have BBB dysfunction and leakage. The study was conducted in Ayatollah Kashani and Alzahra hospital, Isfahan, Iran over the 40 epileptic patients as the case group and 20 healthy control people as a control group. All the patients were checked for other diseased, which can effect on the serum level of inflammation cytokines such as a rheumatologic disease, diabetes mellitus (DM) and history of allergy. Epileptic patents were defined as age more than 16 years, idiopathic epilepsy, starting epilepsy at least in the last 6 months. Patients with a disease which can effect on the serum level of inflammation cytokines symptomatic epilepsy, history

of trauma, stroke, metabolic disorder, metabolic syndrome, smoking and drug abuser were excluded from the study.

Blood sampling

This study was approved in local ethic committee of Isfahan University of Medical Sciences and all patients and control signed a consent form.

Blood was sampled at the seizure-free period of epileptic patients. Five milliliters of venous blood was drawn from each participant and immediately centrifuged. Serum samples were then frozen and kept at -70°C.

Plasma samples were thawed and further processed according to manufacturer's instructions. ICAM, VCAM, IL-1 β and CRP concentrations in plasma were quantified by enzyme-linked immunosorbent assay.

Statistics

Data have been analyzed by using SPSS 18 and are presented as mean values \pm standard deviation. Plasma concentrations (ICAM, VCAM, IL-1 β and CRP) between groups were statistically assessed Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's *post hoc* test for variable group sizes. The Pearson Chi-square test or Fisher's exact test was used for comparing the individual variables between groups. $P < 0.05$ was taken as statistically significant.

RESULTS

Participants

Forty epileptic patients had been included as the case group and 20 non-epileptic people as the control group. About 30 patients were excluded because of the reason of their seizures (e.g., Head trauma, drug using, pseudo-epilepsy). Baseline characteristic (sex and age) as Table 1 shows were not significantly different between groups.

Descriptive data

The groups contain 20 (50%) and 12 (60%) male subjects respectively. The means (\pm standard error) of age in each group were 30.48 ± 2.03 and 27.35 ± 3.42 respectively [Table 1].

Main results

Table 2 shows mean levels of serum concentration of ICAM, VCAM, IL-1 β and CRP in each group; ICAM and VCAM serum concentration, as endothelium cytokines, were investigated in epileptic patients and non-epileptic control group. There were significant

Table 1: Baseline characteristics

Variables	Epileptic patients	Nonepileptic subjects	P
Sex (male) (%)	20 (50)	12 (60)	0.473
Age (mean±SE)	30.48±2.03	27.35±3.42	0.409

SE: Standard error

Table 2: The relation between endothelium and inflammatory cytokines in epileptic patients and nonepileptic people

Variables*	ICAM	VCAM	IL-1 β	CRP
Epileptic patients	135.8±5.35	52.04±4.24	15.59±0.75	52.42±7.58
Nonepileptic subjects	110.32±5.04	23.38±3.01	16.27±0.96	28.09±5.95
P	0.004	0.003	0.594	0.040

*Data were mentioned as mean±SE. SE: Standard error, ICAM: Intercellular adhesion molecule, VCAM: Vascular adhesion molecule, IL-1 β : Interleukin-1 beta, CRP: C-reactive protein. P values <0.05 was taken as statistically significant

differences between groups in serum concentration of ICAM ($P = 0.004$) and VCAM ($P = 0.003$). As inflammatory cytokines, IL-1 β and CRP were measured. Serum concentration of CRP were significantly different between groups ($P = 0.04$). However, serum concentration of IL-1 β was not significantly different ($P = 0.594$). Although serum concentration of CRP were significantly different between the two groups ($P = 0.04$), the serum concentration of IL-1 β was not significantly different ($P = 0.594$).

DISCUSSION

The results revealed significant higher serum concentrations of ICAM and VCAM in epileptic patients versus non-epileptic people. Furthermore, two inflammatory cytokines were measured, and there were no significant difference in the level of IL-1 β serum concentration between the epileptics and non-epileptics; however, the level of CRP serum concentration was significantly higher in epileptic patients.

BBB disruption can be triggered by a direct insult to the endothelium or by systemic factors, including activation of circulating leukocytes and release of molecular mediators (ICAM and VCAM) that increases vascular permeability and inflammation (CRP and IL1beta).^[17-19] To the best of our knowledge, there are rare study showed BBB dysfunction including endothelium factors and inflammation response together in epileptic patients versus none epileptic patients.

Vascular mediators

Our results demonstrated that there are higher levels of ICAM and VCAM concentration in epileptic patients which represented the higher permeability of BBB; the up-regulation might show BBB dysfunction in epileptic patients versus non-epileptic people. BBB permeability

plays an important role in the pathogenesis of epilepsy; on the other hand, microvascular permeability has a close relation to endothelium and inflammatory cytokines. Previous studies showed up-regulation of ICAM and VCAM as pro-convulsant agents in the *in-vivo* and *in-vitro* models of provoke seizures in animals and increased infiltration of leukocytes. ICAM and VCAM had a major role in controlling leukocyte traffic into the CNS.^[8,20] Therefore, the infiltration induces BBB leakage and widening the BBB junctions which aggravate the epileptic condition.

Inflammation response

The results showed significant higher serum concentration of CRP in epileptic patients in contrast with non-epileptic people but serum concentration of IL-1 β was not significantly different between these groups. Other study showed IL-1 β had a direct effect on BBB permeability and lowers the seizure threshold and promotes epileptogenesis on-the-spot.^[21] However, IL-1 β up-regulates in the acute phase of the seizure. The current study evaluated the patients in inter-ictal phase; therefore IL-1 β did not significantly different between groups. In contrast the CRP that up-regulated in inter-ictal phase were significantly higher in epileptic patients. CRP participates in brain ischemia, stroke, vascular events and act as a chronic inflammatory cytokine. Previous studies investigated CRP baseline concentration in epileptic patients. They showed that CRP baseline concentration was higher in epileptic patients versus non-epileptic people.^[22,23] Higher baseline CRP levels were also detected in those with older age at diagnosis. In addition, CRP level was higher in elder participants than in youngers in control group.^[24]

Limitation

Small sample size should have been considered as a limitation of this study.

CONCLUSION

The endothelium mediators (ICAM and VCAM) were higher in epileptic patients may show BBB dysfunction in epileptic patients. From the inflammatory cytokines; CRP was higher in epileptic patients in inter-ictal phase and IL-1b had no significant level in epileptic patients in inter-ictal phase.

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Conflicts of interest

There are no conflicts of interest.

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