Circulating Levels of Interleukin-10 and -17 in Patients with Cerebral Sinovenous Thrombosis (CSVT) in Acute and Subacute Stages: A Prospective Case-Control Study

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ABSTRACT

Background: Cerebral sinovenous thrombosis (CSVT) is a neurovascular disorder that occurs when a blood clot develops in a vein near the brain. Evaluating the subsequent changes in inflammatory cytokines can better reveal the underlying pathogeneses. **Objective:** To assess the serum levels of interleukin-10 (an anti-inflammatory cytokine) and IL-17 (a pro-inflammatory cytokine) in patients with aseptic non-vasculitic CSVT. **Methods:** In this prospective case-control study, 31 patients with aseptic non-vasculitic CSVT (admitted in Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran) were enrolled. IL-10 and IL-17 serum levels were measured at diagnosis, before initiation of treatment (acute stage), 3 months later (subacute stage). These cytokines were also measured in samples obtained from 30 gender- and age-matched healthy subjects, which were considered as control values. **Results:** Patients' IL-10 and IL-17 levels were higher in both acute and subacute stages as compared to controls. However, no significant differences existed between the acute stage and control groups for both cytokines. Moreover, subacute levels were significantly higher than their acute and control levels. **Conclusion:** This study demonstrated the alteration of IL-10 and IL-17 levels in aseptic non-vasculitic CSVT. The rise in subacute IL-10 can be explained by the assumption that IL-10 is released as an anti-inflammatory response to subside the effects of IL-17 mediated reactions. More importantly, the immediate sampling in the acute stage did not allow enough time for triggering the immune system to produce such mediators. However, a balance was established between IL-10 and IL-17 in the subacute stage to prevent further tissue damage.

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Keywords: Cerebral sinovenous thrombosis, Interleukin-10, Interleukin-17

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INTRODUCTION

Cerebral sinovenous thrombosis (CSVT) refers to a complete or partial occlusion of cerebral venous sinuses or cortical veins, leading to parenchymal damage or intracranial hypertension (1, 2). CSVT predominantly affects young adults especially women of childbearing age. It is categorized into septic and aseptic types. Aseptic or non-suppurative CSVT can occur due to traumatic, surgical, malignancy, vasculitis, hematologic, thrombophilic, hormonal or idiopathic factors (1, 2). Inflammatory processes may play a pivotal role even in aseptic CSVT. Inflammatory mediators such as tissue necrosis factor (TNF)-α can trigger the production of tissue factor on the monocytes and endothelium, thus inducing the coagulation cascade. Exposure of the membrane surfaces stimulates the factor VIIIa-factor IXa and the factor Xa-factor Va complexes. In addition, inflammatory cytokines enhance the level of fibrinogen, an acute-phase reactant, as well as the level of plasminogen activator inhibitor, which suppresses the fibrinolytic cascade (3).

IL-10, an anti-inflammatory cytokine with immune-suppressing and anticoagulation properties (4), is considered a major clinical predictor of thrombosis (5). IL-10 inhibits human-monocyte-triggered thrombin generation (6), exerting its anticoagulant effects (4). As a source for IL-10 cytokine, regulatory T- and B-cells can suppress inflammation and limit the central nervous system damage induced by infiltrating pro-inflammatory cells (7). IL-17, as a pro-inflammatory T-cell-specific cytokine, mediates its immunological function by inducing pro-inflammatory cytokine, and chemokine secretion by responder cells (8). IL-17 acts on vessels and cardiac cells, leading to inflammation, coagulation, and thrombosis. It enhances adenosine diphosphate-induced platelet activation (9), as well as Von Willebrand factor (10). Moreover, it inhibits anticoagulation by decreasing CD30 and thrombomodulin level (11). Furthermore, by decreasing the mitochondrial transmembrane potential, it induces apoptosis and consequently endothelial damage (10). IL-17 was shown to elevate in the ischemic hemispheres of the human brain compared with the opposite normal hemispheres and peaked ondays 3-5 after brain ischemia (12).This study aimed at evaluating the levels of IL-10 and IL-17 in patients with non-infectious non-vasculitis CSVT in both acute and subacute stages.

MATERIALS AND METHODS

Study Population and Setting. This prospective case-control study was conducted on 31 consecutive patients with a confirmed diagnosis of acute aseptic non-vasculitic CSVT. They were recruited from Namazi and Faghihi hospitals (affiliated with Shiraz University of Medical Sciences, Shiraz, Iran) from 2015 to 2017. These centers are tertiary care medical institutions covering neurologic referrals in Southern Iran. The diagnosis of CSVT was made by a single qualified vascular neurologist based on the American Heart Association/American Stroke Association statement (13). Included patients fulfilled all of the following criteria: presence of relevant neurological findings (intracranial hypertension, focal cerebral deficits, and encephalopathic state), radiological confirmation (computerized tomography (CT) scan, computed tomographic venography (CTV), magnetic resonance imaging (MRI), and magnetic resonance venography (MRV)) (14). Patients with arterial stroke, dissection, reversible cerebral vasoconstriction syndrome, subarachnoid hemorrhage, CNS vasculitis, and other

differential diagnoses of CVST were excluded. Also, any comorbid disease that could involve inflammatory pathways such as infection, neoplasms, vasculitis, and inflammatory bowel disease was excluded. Patients with a family history of autoimmune disorders were excluded as well. Accordingly, etiologies of CVST such as gynecologic causes (pregnancy, post-partum, and oral contraceptive pill (OCP) consumption in women), dehydration such as in fasting (15), mechanical precipitants (trauma and surgery), inherited thrombophilia, acquired thrombophilia such as antiphospholipid syndrome, and hematologic diseases (anemia, polycythemia, thrombocytosis) were included. A total of 30age- and sex-matched healthy controls were selected among volunteer blood donors referring to the Iranian Blood Transfusion Center in Shiraz.

Ethical Considerations: The study protocol was designed in adherence to the Helsinki Declaration of bioethics. Also, it received approval from the Institutional Review Board (IRB) of Shiraz University of Medical Sciences. All participants filled out an informed written consent and the confidentiality of their information was guaranteed.

Data Gathering: Demographic characteristics, clinical manifestations, and laboratory and neuroimaging findings were recorded. Serum levels of IL-10 and IL-17 were measured after diagnosis and before initiation of treatment. This episode was defined as the acute stage. Subsequently, sampling was repeated for the second time after 3 months considered as the subacute stage. In addition, IL-10 and IL-17 serum levels obtained from healthy controls provided baseline reference values. The immunologic assay was performed on a 5cc blood sample (acid washed) in Shiraz Institute for Cancer Research. We used Human IL-10 Platinum ELISA (BMS215/2CE) kit with an assay range of 3.1-200.0 pg/ml and sensitivity of 1.0 pg/ml and Human IL-17 Platinum ELISA (BMS2017) kit with an assay range of 1.6-100.0 pg/ml and sensitivity of 0.5 pg/ml by eBioscience according to the instructions of the manufacturer.

Statistical Analysis: Statistical analysis was performed using Windows SPSS software (Chicago, IL) version 16.0. The Kolmogorov-Smirnov test was used to normalize the data. Based on this test, paired t-test or Wilcoxon test was used to compare the patients' IL-10 and IL-17 serum levels between the two time points (acute and subacute stages). Each of these two episodes was compared with control levels using the independent t-test or Mann Whitney U test. A p-value<0.5 was considered statistically significant.

RESULTS

A total of 31 CSVT patients and 30 age- and gender-matched controls were studied. All patients were closely followed and there were no missing cases due to death or loss of follow-up. The mean age of patients was 35.4 ± 10.11 years ranging from 17 to 54 years. There were 6 (22.6%) male and 24 (77.4%) female patients.

Clinical Findings: The initial manifestation of CSVT comprised of a loss of consciousness (5 patients, 16.1%), headache (30 patients, 96.8%), seizure (18 patients, 58.1%), and motor and sensory dysfunction (2 patients, 6.5%).In compliance with the inclusion criteria, all enrolled patients had at least one site of thrombosis in cerebral venous sinuses or cerebral veins. Regarding medical history, 3 (9.7%) patients were diabetic and 1 (3.3%) had a history of the rheumatologic disorder. Furthermore, the history of abortion and postpartum complications was reported in one and seven female patients, respectively. Also, 14 (45.2%) patients had been taking oral contraceptive pills

(OCP) when CSVT occurred. Through the 3 months' follow-up, none of the patients had optic nerve atrophy or blurred vision. However, 27 (87.1%) still complained of headaches and 1 (3.3%) of seizure and motor and sensory dysfunction. Of all patients, 5 (16.1%) had venous infarction, 11 (35.4%) had a hemorrhagic transformation, and 16 (51.6%) had isolated intracranial hypertension.

Cytokine Levels: The serum level of IL-10 in controls was 0.19±0.98 pg/mL. In patients, IL-10 was 2.01±5.98 pg/mL in the acute stage and 8.8±10.68 pg/mL in the subacute stage. There was no significant difference between control and acute stage levels ($p=0.13$). However, subacute IL-10 was found to have a strongly significant difference in both control and acute levels ($p<0.001$). A similar pattern was observed for IL-17. Control IL-17 was 0.40±1.60 pg/mL, while in patients it was 0.49±2.49 pg/mL in acute stage and 2.07±2.76 pg/mL in subacute stage. Likewise, the difference between control and the acute stage was non-significant ($p=0.88$), whereas subacute IL-17 was significantly higher compared with its control ($p=0.019$) and acute ($p<0.001$) levels.

DISCUSSION

The serum levels of IL-10 and IL-17 were slightly higher in CSVT patients in the acute stage compared to their control levels; however, without any significant differences. Later, a remarkable increase in IL-10 and IL-17 levels in the subacute stage was shown to have a significant difference with both control and acute levels. The relatively increased IL-10 in the subacute injury compared to the acute cases may be explained by the assumption that IL-10 is released as an anti-inflammatory response to subside the effects of Il-17 mediated reactions. More importantly, the increased level of both cytokine in subacute cases compared to acute cases is also explained by the fact that blood sampling wasdone1-2 days immediately after CSVT. Therefore, there was not enough time for triggering the immune system to produce such mediators; however, in subacute cases, a balance between IL-10 and IL-17 may have been established to subside further tissue damage. The precise role and the pattern of change regarding the anti-inflammatory IL-10 is still a matter of debate. Several other studies have shown associations between the markers of inflammation, like IL-10, but the results are conflicting.

In the context of coagulation regulation, IL-10 has been found to down-regulate the synthesis of tissue factor, a potent initiator of the coagulation cascade, which is essential for activating the extrinsic pathway of the coagulation system especially during inflammation (16). Also, IL-10 modulates the fibrinolytic system and inhibits the expression of mRNA for fibrinogen, the secretion of MMPs, and the generation of prothrombin fragment and thrombin-antithrombin complexes (17,18).

An investigation of the pro-coagulant and pro-thrombotic effects of TNFα and IL-17 in endothelial cell (EC) dysfunction demonstrated that IL-17 induced pro-inflammatory genes such as tissue factor alone and in combination with TNFα. Furthermore, IL-17 decreased thrombomodulin and IL-17-treated EC induced strong platelet aggregation and activation, leading to a pro-thrombotic state (11). In vitro studies have confirmed that IL-17 promoted platelet activation is mediated through the MAPK/Erk2 signaling pathway. An analysis of the structure of coronary thrombus in acute myocardial infarction patients has shown that IL-17 promotes thrombus growth and stability (19).

In line with our study, the serum level of IL-10 has been shown to increase after acute brain injury (20). Also, IL-10 has shown an association with a dose- and time-dependent

decrease in inflammation, most significantly at thrombosis initiation (21). In addition, a significant relationship has been reported between IL-10 level and the improvement in the patients' neurologic dysfunction within the first 72 hours after CSVT (22). Inconsistent to our study, patients with idiopathic venous thrombosis reportedly had decreased levels of IL-10 and escalated levels of pro-inflammatory cytokines (23). Furthermore, an assessment of inflammatory cytokines in patients with a history of CSVT revealed no significant differences regarding IL-10 (24). Also, IL-17 has been reported to rise after ischemic brain infarction (25).

Despite the opposing action of IL-10 and IL-17, both had increased levels in the increase in the production of cytokines by activating the source cells, such as the macrophages, monocytes, lymphocytes, endothelial cells, platelets, astrocytes, microglia, and neurons (26). Apparently, increased concentrations of IL-10 moderate the chance of an inflammatory reaction and consequently inhibit the development of the thrombotic cascade (27). Overall, ischemia triggers an immune response, resulting in a change in the level of IL-10 and IL-17 cytokines following CSVT. As mentioned, both cytokines were found to elevate starting from the acute stage, although not yet significantly higher than their control levels. This increase was proved to reach a significantly higher level in the subacute stage. Given the neuroprotective and anticoagulant role of IL-10, the rise in IL-10 can decrease the inflammatory drive of the immune system, resulting in limiting the area of brain infarct and ultimately better patient outcome (28).

As the inflammation occurs, the level of IL-17 is expected to rise. In response, the level of IL-10 increases to suppress the inflammation and limit the damage. As the level of IL-17 is gradually controlled, IL-10 continues to play its protective role for a longer time, which is in the subacute stage.

As to the limitations of this study, the small number of the patients and the extent of severity of stroke in different patients can be considered in future investigations. Moreover, the most important limitation was that only two interleukins were measured. Thrombosis has a complex pathway. In this regard, our study might have yielded more reliable results if other related cytokines were checked. Thus, a better understanding of their interactions could be accomplished.

This novel study demonstrated the alteration of IL-10 and IL-17 levels in aseptic non-vasculitic CSVT. The rise in subacute IL-10 can be explained by the assumption that IL-10 is released as an anti-inflammatory response to subside the effects of IL-17 mediated reactions. More importantly, the immediate sampling in the acute stage did not allow enough time for triggering the immune system to produce such mediators. However, a balance was established between IL-10 and IL-17 in the subacute stage to prevent further tissue damage. The results of this study can help us find effective and new approaches for reducing the mortality and improvingthe quality of life of patients with non-infectious CSVT.

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