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IL-18 AND ADROPIN LEVELS IN PATIENTS WITH ACUTE ISCHEMIC STROKE

AKUT İSKEMİK İNMELİ HASTALARDA IL-18 VE ADROPİN DÜZEYLERİ

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Öz

Amaç

İnmeli hastalarda disfonksiyonel vasküler olaylara yol açan önde gelen faktörlerden bir tanesi olan ateroskleroz; endotelyal disfonksiyon ve vasküler inflamasyonun önemli bir rol oynadığı çok faktörlü ve kompleks bir süreçtir. Biz bu çalışmada endotel disfonksiyonu ve inflamatuar süreçlerle ilişkisi gösterilmiş olan IL-18 ve adropininin akut iskemik inme hastalarındaki serum düzeyleri ile epidemiyolojik, klinik, radyolojik bulgular ve inme şiddeti arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem

Çalışmamıza akut iskemik inme tanısı konulan 61 hasta ve kontrol grubu olarak 30 sağlıklı birey alındı. Hasta grubunda etiyolojik ve klinik olarak inme alt grupları ve inme şiddeti belirlendi. Hasta grubundan ilk 24 saatte, kontrol grubundan herhangi bir zamanda venöz kan örnekleri alınarak serumları ayrıldı ve -80^{0}C'de saklandı. ELISA yöntemi kullanılarak IL-8 ve adropin düzeyleri belirlendi. Hasta ve kontrol gruplarının IL-18 ve adropin düzeyleri ile iskemik inme arasındaki ilişkiler istatistiksel olarak analiz edildi.

Bulgular

Adropin düzeyi hasta grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede düşüktü (sırasıyla 398.01±403.51 ve 509.42±1492.89; p=0.041). Çalışma ve kontrol gruplarının IL-18 düzeyleri benzerdi (sırasıyla 24.87±14.26 ve 21.11±14.93; p=0.112). İnme risk faktörleri, inme alt grupları ve inme şiddeti ile belirlenen IL-18 ve adropin düzeyleri arasında ilişki yoktu.

Sonuç

Bu bulgular, düşük adropin düzeylerinin ateroskleroz göstergesi olarak iskemik inme risk tahmini ölçeklerinde kullanılabileceğini göstermiştir. Akut iskemik inmeli hasta grubu ile kontrol grubu arasında ortalama serum IL-18 düzeyi açısından fark olmaması, IL-18'in iskemiye bağlı inflamasyonda geç dönem bir sitokin olarak rol oynayabileceğini düşündürmüştür.

Anahtar Kelimeler: İskemik inme, Adropin, IL-18, İnflamasyon, Endotel disfonksiyonu

Abstract

Objective

Atherosclerosis, one of the prominent factors causing dysfunctional vascular events in stroke patients, is a multi-factorial and complex process in which endothelial dysfunction and vascular inflammation play significant roles. This study aimed to investigate the relationships between serum levels of IL-18 and adropin, associated with endothelial dysfunction and

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inflammatory processes in acute ischemic stroke patients, with epidemiological, clinical, radiological findings and stroke severity.

Materials and Methods
Sixty-one patients diagnosed with acute ischemic stroke and 30 healthy individuals were included in the study as the patient and control groups. In the patient group, the stroke sub-groups and severity were determined etiologically and clinically. Venous blood samples were obtained within the first 24 hours in the patient group, and at any time in the control group, their serums were separated and stored at -80°C. IL-8 and adropin levels were determined using the ELISA method. The relationships between patient and control groups' IL-18 and adropin levels and ischemic stroke were analyzed statistically.

Results
The adropin level was statistically significantly lower in the patient group than the control group (398.01±403.51 and 509.42±1492.89, respectively; p=0.041). The IL-18 levels of the study and control groups were similar (24.87±14.26 and 21.11±14.93, respectively; p=0.112). There was no relationship between the IL-18 and adropin levels determined with stroke risk factors, stroke sub-groups, and stroke severity.

Conclusion
These results showed that low adropin levels could be used to indicate atherosclerosis in the risk prediction scales of ischemic stroke. The absence of a difference between the patient group with acute ischemic stroke and the control group regarding the first 24-hour mean serum IL-18 level suggested that IL-18 could play a role as a late-stage cytokine in ischemia-related inflammation.

Keywords: Ischemic stroke, adropin, IL-18, inflammation, endothelial dysfunction

Introduction

Stroke is an acute clinical syndrome due to vascular causes and characterized by rapidly developing symptoms and signs of focal neurologic deficit (1). Strokes that develop as a result of decreased cerebral blood flow due to local arterial pathology (mostly atherosclerosis), embolism or hemodynamic reasons and that are pathologically characterized by infarction are called ischemic strokes (2). In addition to many well-known classical risk factors, mechanisms such as free radical formation, lipid peroxidation, excitotoxicity, increased intracellular calcium, and inflammation play a role in the pathophysiology of cerebral ischemia (3). Inflammatory mechanisms play roles in both the stroke development risk and the pathophysiology of cerebral ischemia. In recent years, many inflammatory markers have been defined in ischemic stroke-related studies. Some of these markers have been shown to be helpful to determine the stroke risk, whereas some others were helpful for diagnosis and prognosis (4).

IL-18 is a proinflammatory cytokine considered to play a part in the pathophysiology of acute ischemic stroke like the other proinflammatory cytokines (5, 6). Experimental studies demonstrated that IL-18 was closely linked with atherosclerotic plaque formation and instability (7). Besides, IL-18 is an independent predictor of coronary events in healthy males and a predictor of cardiovascular mortality in patients with coronary artery disease (8). In various studies related to ischemic stroke etiology, it has been hypothesized that the proinflammatory profile due to increased IL-18 level created a pro-thrombotic and pro-atherosclerotic process (9). Even though an increased IL-18 level was not determined in stroke patients, multiple pieces of evidence showed that the IL-18 level could predict stroke development (9, 10, 11, 12).

Atherosclerosis is a principal factor causing cerebrovascular diseases. Atherosclerosis development is a complex process depending on multiple factors such as endothelial dysfunction, vascular inflammation, and thrombus formation (13). Adropin is a newly discovered peptide that plays a role in energy homeostasis and lipid metabolism (14). Adropin, which regulates glucose and fatty acid metabolism, is also associated with endothelial cell function and endothelial nitric oxide synthase (eNOS) bioactivity (15). Adropin can play a protective role by increasing nitric oxide (NO) release through eNOS activation (16). Impairment of endothelial functions brings about the loss of vasomotor control, decreased NO production, formation of a pro-coagulant surface, and increased inflammation. Subsequently, aforementioned events can cause destabilization of atherosclerotic plaques and initiate acute coronary syndromes (17). Another recently conducted study has revealed that decreased serum adropin level would be a coronary

atherosclerosis-related independent determiner and a new predictor (18). Because endothelial dysfunction plays a significant role in atherosclerosis development and progression, besides its favorable metabolic profile, adropin has been predicted to be a new target to limit endothelial dysfunction-related diseases (16). These results have shown adropin would be a novel and convenient determiner for the non-invasive assessment of endothelial functions (19).

The study aimed at examining the relationships between the first 24-hour serum levels of adropin and IL-18, which play significant roles in atherosclerosis and inflammatory processes that have a place in ischemic stroke pathophysiology, with the subtypes and severity of the ischemic stroke.

Materials and Methods

The study included 61 patients admitted between May 2016 and October 2016 to the Neurology Clinic of Medical Faculty Hospital of Atatürk University within the first 24 hours after the onset of their complaints and diagnosed with acute ischemic stroke and 30 age/gender-matched healthy individuals. The approval of the head of the Ethics Committee of Atatürk University Medical Faculty was taken for the study (on April 26th, 2016: 4/29), and ethical principles were observed over the course of the study. Both groups participating in the study gave their consent. Volunteer patients over the age of 18 years and diagnosed with acute ischemic stroke within the first 24 hours were included. Patients with stroke history, brain tumor, or systemic malignancy, severe infection in the last three months, those with autoimmune, rheumatic, hematologic, or immunosuppressive disorders, patients treated with anti-inflammatory drugs during the past six months, those with severe renal or hepatic failure, history of myocardial infarction within the last one year, patients with peripheral arterial disease or deep venous thrombosis, history of major trauma or surgery within the last one year, psychiatric disorders, malnutrition, and intoxication were excluded from the study.

Clinics, Laboratory, and İmaging

Epidemiologic data of the patients in the study, such as age, gender, personal medical, and family histories, were questioned. A detailed history of vascular risk factors (HT, DM, AF, coronary arterial disease (CAD), congestive heart failure (CHF), hyperlipidemia, and smoking was obtained in every patient. Systemic and neurological examinations of all patients were performed. In all patients with no contraindication for MRI, the diffusion MRI was performed within the first 24 hours, following the stroke protocol. Routine hematologic and biochemical tests, complete urinalysis, chest X-ray, electrocardiography, echocardiography, carotid-vertebral artery Doppler ultrasonography, cranial MRI, and MR angiography were carried out in all the study group patients.

Ischemic stroke subtypes in the patient group were determined in accordance with the Bamford classification as the total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), posterior circulation infarct (POCI), lacunar infarct (LACI), and according to the TOAST classification as large- artery atherosclerosis (LAA), cardioembolism (CE), small vessel occlusion (SVO), the stroke of undetermined etiology, and the stroke of other determined etiology. The control group was divided into two sub-groups: those with two or more risk factors (age included) and those with less than two risk factors (age only). In the National Institute of Health Stroke Scale (NIHSS), the NIHSS levels were divided into three groups: an NIHSS score of 0-6 as mild, 7-15 as moderate, and over 16 as severe.

Demographic and clinical data, laboratory and imaging results of all patients were recorded in the forms arranged on an individual basis for every patient.

Blood Collection and Serum Preparation

Approximately five ml of blood was drawn into 10-ml biochemistry tubes with jelly through the antecubital veins of the patients within the first 24 hours following symptom onset, was kept at room temperature for approximately 30 minutes, and then the serum was separated by centrifuging at 4000 rpm for ten minutes. The serum samples were placed in two separate 1.5-ml Eppendorf tubes and stored at -80^{0}C until the analysis day. The IL-18 level was quantitatively measured employing the Human Interleukin 18 (IL-18) ELISA Kit (Cat. No: CK-E10092, China) with the brand name of EASTBIOPHARM. The adropin level was quantitatively measured using the Human Adropin (AD) ELISA Kit (Cat. No: CK-E90267, China) with the brand name of EASTBIOPHARM. The serum IL-18 and adropin concentrations were presented in ng/L.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS)-Windows software, version #17, performed the statistical analysis. The Shapiro Wilks test evaluated the normal distribution of data, and all continuous variables in the study were determined not to have a normal distribution. The numerical variables having a normal distribution were shown as mean±standard deviation, whereas the ones with no normal distribution as median (min-max). The categorical variables were

presented as numbers and percentages. The Mann-Whitney U test determined the factors related to 2-category risk groups (numerical variables with no normal distribution). The Kruskal Wallis test was used to determine the factors related to 3-category risk groups (numerical variables with no normal distribution). The Chi-square and Fisher's Exact Chi-square tests were employed to compare the categorical data. The relationships among the numerical variables were analyzed employing Spearman's correlation analysis. The value for statistical significance was considered as $p<0.05$.

Results

The study included 91 individuals in total involving 61 patients with acute ischemic stroke and 30 controls. The demographic and clinical data obtained from the patient and control groups were presented in the table (Table 1). No statistically significant differences were observed between the patient and control groups regarding age and gender ($p>0.05$). By comparison of the patient group diagnosed with acute ischemic stroke with the control group, a significantly lower mean adropin level was found in the patient group (398.01±403.51 and 509.42±1492.89, respectively; $p=0.041$). No statistically significant difference was established between the mean IL-18 values of the patient and control groups (24.87±14.26 and 21.11±14.93, respectively; $p=0.112$) (Table 1).

When the distributions of risk factors in the stroke subtypes (TOAST) were analyzed, HT was most frequent (87.5%) in the group with small vessel

Table 1 Demographic, clinical and laboratory characteristics in patients and healthy controls.

	Patients (n=61)	Controls (n=30)	p
Age (years, mean±SD)	71.47±11.67	70.00±11.86	0.254
Gender (female)	38 (62.3%)	18 (60%)	0.832
Stroke risk factors			
Hypertension	41 (67.2%)	12 (40%)	
Diabetes	20 (32.8%)	5 (16.7%)	
Atrial fibrillation	21 (34.4%)	2 (6.7%)	
Coronary artery disease	11 (18%)	2 (6.7%)	
Congestive heart failure	13 (21.3%)		
Hyperlipidemia	17 (27.9%)	3 (10%)	
Smoking	17 (27.9%)	2 (6.7%)	
TOAST			
LAA	15 (24.6%)		
CE	15 (24.6%)		
SVO	16 (26.2%)		
UD	11 (18%)		
OD	4 (6.6%)		
Bamford			
TACI	11 (18%)		
PACI	18 (29.5%)		
LACI	22 (32.8%)		
POCI	12 (19.7%)		
NIHSS			
Hafif (0-6)	26 (42.6%)		
Orta (7-15)	18 (29.5%)		
Ağır (>16)	17 (27.9%)		
IL 18 (ng/L)	24.87±14.26	21.11±14.93	0.112
Adropin (ng/L)	398.01±403.51	509.42±1492.89	**0.041**

TOAST: Trial of Org 10172 in Acute Stroke Treatment, LAA: Large-artery atherosclerosis, CE: Cardioembolism, SVO: Small vessel occlusion, UD: undetermined etiology, OD: Other determined etiology, TACI: Total anterior circulation infarcts, PACI: Partial anterior circulation infarcts, LACI: Lacunar infarcts, POCI: Posterior circulation infarcts

occlusion (SVO), which was considered to be statistically significant (p=0.038). AF was most common in the cardioembolism group (66.7%) as expected, being statistically significant (p=0.012). DM was most common in the SVO group; however, there was no correlation (p=0.113). No significant relationships were determined among the other risk factors and the stroke subtypes (Table 2).

The adropin and IL-18 levels were not correlated with age in the patient group (p=0.557, and p=0.649, respectively). The adropin and IL-18 levels of the control group were not correlated with age (p=0.666, and p=0.408, respectively), and not with gender (p>0.05) (Table 3).

Among ischemic stroke sub-groups (according to the TOAST classification), the adropin level was highest in the CE subgroup (536.34±558.09), whereas the lowest in the stroke subgroups of undetermined and other determined etiologies (238.60±64.15 and 299.17±183.25, respectively; p=0.946). The IL-18 level was highest in the SVO subgroup (30.89±17.57), whereas lowest in the CE subgroup (18.95±12.47) (p=0.172) (Table 4).

Table 2 Distribution of risk factors in ischemic stroke subtypes (TOAST).

	LAA	CE	SVO	OD	UD	p
n	15	15	16	4	11	
Hypertension (%)	66.7	46.7	87.5	25	81.8	**0.038**
Diabetes (%)	33.3	13.3	50	0	45.5	0.113
Atrial fibrillation (%)	40	66.7	12.5	0	27.3	**0.012**
Coronary artery disease (%)	13.3	26.7	12.5	0	27.3	0.587
Congestive heart failure (%)	33.3	26.7	18.8	0	9.1	0.452
Hyperlipidemia (%)	40	20	31.3	25	18.2	0.703
Smoking (%)	40	33.3	31.3	25	0	0.224

TOAST: Trial of Org 10172 in Acute Stroke Treatment, LAA: Large-artery atherosclerosis, CE: Cardioembolism, SVO: Small vessel occlusion, UD: undetermined etiology, OD: Other determined etiology

Table 3 Relationship between gender and adropine and IL-18 levels.

		Gender	n	Mean±SD	p
Patients	**Adropin**	Male	23	363.09±270.96	>0.05
		Female	38	419.14±468.15	
	IL 18	Male	23	28.94±18.30	
		Female	38	22.40±10.69	
Control	**Adropin**	Male	12	218.50±73.36	
		Female	18	703.37±1923.25	
	IL 18	Male	12	21.95±12.20	
		Female	18	20.54±16.82	

Table 4 Adropine and IL 18 levels in ischemic stroke subtypes (TOAST).

		n	Mean±SD	p
Adropin	LAA	15	342.19±364.73	0.946
	CE	15	536.34±558.09	
	SVO	16	454.96±424.29	
	UD	11	238.60±64.15	
	OD	4	299.17±183.25	
IL18	LAA	15	24.63±7.47	0.172
	CE	15	18.95±12.47	
	SVO	16	30.89±17.57	
	UD	11	24.34±16.79	
	OD	4	25.30±15.15	

TOAST: Trial of Org 10172 in Acute Stroke Treatment, LAA: Large-artery atherosclerosis, CE: Cardioembolism, SVO: Small vessel occlusion, UD: undetermined etiology, OD: Other determined etiology

Table 5 Adropin and IL 18 levels in healthy controls and controls with risk factors.

	Risk faktörü	n	Ortalama	p
Adropin	<2	13	203.10±97.34	0.170
	≥2	17	743.67±1974.31	
IL18	<2	13	17.80±11.77	0.341
	≥2	17	23.63±16.87	

No statistically significant differences were determined between ischemic stroke risk factors and NIHSS scores and adropin and IL-18 levels in both the patient and control groups.

The control group included 13 individuals (43.3%) with less than two risk factors (no risk factor other than age). Any significant difference concerning the IL-18 and adropin levels was not ascertained between the healthy controls and the controls under risk (having risk factors other than age) (p=0.341, and p=0.170, respectively) (Table 5).

Discussion

The study investigated the relationships of acute ischemic stroke, in which the inflammatory and atherosclerotic processes play significant etiological roles, with IL-18 and adropin. Despite the similar IL-18 levels of the patient and control groups, the serum adropin level of the group with acute ischemic stroke was found to be significantly lower than the control group.

Detailed molecular-level and cellular-level identification of intertwined toxic mechanisms, emerging with sudden interruption of the blood flow and leading to brain cells' irreversible death, is essential for improving diagnostic and therapeutic approaches.

Atherosclerosis, having a significant role in the pathophysiology of ischemic stroke, is a multi-factorial and complex process. Endothelial dysfunction is one of the primary mechanisms in the atherosclerotic

process. The conventional and newly described risk factors cause a chronic injury that leads to impairment of the endothelial vasodilator response. Thus, events such as the vasoconstriction in the endothelium, piling up of inflammatory cells, migration of smooth muscle cells, and enhanced cytokine production lead to atherosclerotic plaque formation. Endothelial dysfunction is not only the first step of the atherosclerotic process that causes plaque formation but also leads the formed plaque to enlarge, crack, triggering the thrombogenic events (20). NO, released from the endothelium, enhances reparative vasculogenesis and acts as an anti-atherosclerotic, anti-inflammatory, and anti-thrombotic factor. Adropin, which modulates eNOS expression, has been considered to play a protective role for the endothelium (16).

The serum adropin level of the patient group with acute ischemic stroke was ascertained to be significantly lower than that of the control group. Few clinical studies were conducted on adropin in patients with acute ischemic stroke. In the conducted studies, the low plasma adropin level reported to be linked with obesity-related insulin resistance, atherogenesis, diabetes, aging, pediatric obstructive apnea, and many other metabolic disorders (16). In their study on diabetic patients, Topuz et al. determined that the group with endothelial dysfunction had lower adropin level (19). In their study analyzing the correlation of serum adropin level with coronary artery disease (CAD) in 356 patients, Zhang et al. determined that the serum adropin level of the CAD group was significantly lower than that of the control group, and adropin was an independent risk factor for CAD (21). Yu et al., in their study on patients with stable angina pectoris and acute myocardial infarction (AMI), reported that the serum adropin level decreased in AMI patients (22). In their study, Wu et al. showed that the adropin level was independently and negatively correlated with the angiographic severity of coronary atherosclerosis, and the serum adropin level could be a new indicator of coronary atherosclerosis (18). In the recent study, Günaydın et al. reported a significantly lower adropin level in ischemic stroke patients compared with the controls and that adropin could be an independent predictor of acute ischemic stroke (23). Our study results supported the studies on both ischemic stroke and CAD, which have similar risk factors and etiologies. Even though adropin is known to reduce the paracellular permeability in brain endothelial cells in ischemic conditions, little is known about adropin's effects on the brain. Adropin has been considered to provide neuroprotection by activating endothelial nitric oxide synthase (eNOS) / NO signal pathway and reducing blood-brain barrier injury (24). Yang et al., in their study, claimed that adropin deficiency in the brain increased neurovascular dysfunction and thus the severity of stroke damage and that over-expression of this peptide reduced the cerebral ischemic damage (25). In our study, low adropin level was found to be related to ischemic stroke. When the studies reporting that low adropin levels could be an indicator of atherosclerosis were considered, it was shown that adropin could be used in ischemic stroke risk prediction scales and could be a novel and useful determiner for the evaluation of endothelial functions. When all these results are considered, it can be suggested that adropin can be used as a biomarker for diagnosing ischemic stroke and is a promising therapeutic agent in the light of more extensive clinical studies that will be performed. Even though adropin seems like a new target to prevent vascular disorders because of its known impacts on metabolic regulation, further studies should be conducted to explain the unique mechanism forming the base for the relationship between adropin and cerebrovascular diseases.

Ischemic stroke causes a severe inflammatory reaction, and cytokines, expressed mainly in the glial cells and neurons, are produced (26). Besides inducing IFN- γ, IL-18 induces the synthesis of cytokines TNF-α and IL-10 that can inhibit the inflammatory process and cause instability of atherosclerotic plaques and thrombosis (27). The studies investigating the relationship between the IL-18 level and ischemic stroke found inconsistent outcomes. In the study, even though the mean serum IL-18 level within the first 24 hours in acute ischemic stroke patients was higher than in the control group, no statistically significant difference was observed between them.

IL-18 is considered to play a part in the pathophysiology of acute ischemic stroke like the other proinflammatory cytokines (6). The relationship between acute ischemic stroke and IL-18 was first shown in a study published by Zaremba et al. in 2003. They studied the IL-18 levels in serum samples obtained within the first 24 hours and determined significantly higher IL-18 levels in the patient group (10). In their study published in 2007, Yuen et al. reported significantly higher IL-18 levels in the patients' venous blood samples obtained at the 48th hour following the acute ischemic stroke onset (27). In their study conducted in 2011, Ormstad et al. determined significantly high IL-18 levels in the group with acute ischemic stroke (28). In their cross-sectional study followed by meta-analysis in 2019, Hao et al. showed that the IL-18 level of the stroke patients was higher than that of the

controls (29). However, unlike these studies, in the studies conducted with animal models, it was shown that the intracerebral IL-18 levels had not increased in the early period (within the first 24 hours) (30, 31). In their study on mice, Wheeler et al. claimed that IL-18 played a minor part in acute ischemic processes, could be induced in the late stage of cerebral infarct, and could change the repair and healing status (31). In their study investigating whether IL-18 was induced after focal ischemia in the rat brain, Jander et al. determined a delayed increase of IL-18 level starting at 48 hours and reaching its maximal level 7-14 days after ischemia (30). In the study conducted by Gürkaş et al., no significant difference was reported between the serum IL-18 levels of ischemic stroke patients within the first 24 hours and the control group, similar to our study (6). After demonstrating the inflammation's role in the pathogenesis of ischemic stroke, numerous studies have been conducted on inflammatory markers such as IL-1β, TNF-α, IL-6, and IL-18. The IL-18-related studies in animal models revealed that IL-18 played a role in the late stage of ischemic cerebral inflammatory response.

On the other hand, while no increase was determined in IL-18 level within the first 24 hours in some studies, other studies reported increases in both the early and late periods. Because IL-18 levels were studied in samples obtained within the first 24 hours (mostly at admission) in our study, the elevation of IL-18 level might not have been determined. Because of the different results of these clinical studies, new studies investigating the IL-18 levels in both the early and late periods on larger sample-sized patient groups are required.

Conclusion

The serum adropin level of the patient group with acute ischemic stroke was significantly lower than the level of the control group; however, the IL-18 levels of the patient and control groups were similar. It can be suggested that adropin can be used as a biomarker for diagnosing ischemic stroke and is a promising therapeutic agent in the light of more extensive clinical studies that will be performed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
The approval of the head of the Ethic Committee of Atatürk University Medical Faculty was taken for the study (on April 26th, 2016: 4/29), and ethical principles were observed during the study.

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

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