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Low serum osteopontin levels in individuals with choroid plexus calcification

[1]Recep Yevgi, [1]Nuray Bilge, [1]Fatma Şimşek, [1]Mustafa Ceylan, [2]Dilcan Kotan, [3]Nazım Kızıldağ

[1]Ataturk University Faculty of Medicine, Department of Neurology, Erzurum; [2]Sakarya University, Faculty of Medicine, Department of Neurology, Sakarya; [3]Erzurum Education and Research Hospital, Department of Neurology, Erzurum, Turkey

Abstract

Background & Objective: The choroid plexus (CP) is a tissue plexus located in the ventricles that produces hormones and nutrients, primarily cerebrospinal fluid (CSF). CP is also an important component of the blood CSF barrier. Osteopontin (OPN) is an extracellular matrix protein that plays a role in various physiological and pathological conditions such as bone reshaping, wound healing, vascular disorders, and inflammatory diseases. It is considered that OPN physiologically regulate bio-mineralization in bone tissue and reduce the growth and accumulation of calcium crystals in epithelial tissues. In our study, the role of OPN in CP calcification was studied. *Methods:* A total of 90 people, without any disease but with CP calcification (45 people) and age and gender matched control without CP calcification (45 people) were studied. Calcified and normal CP tissue was identified in brain computed tomography (CT) images. Serum OPN levels were measured in individuals with and without CP calcification using a human OPN enzyme-linked immunosorbent test kit (ELISA) from morning fasting venous blood samples. *Results:* Serum OPN level was found to be statistically significantly lower in patients with CP calcification than those without CP calcification (p=0.007).
Conclusions: Our results show that OPN may have a significant role in the calcification process of CP.

Keywords: Choroid plexus; calcification; osteopontin

INTRODUCTION

The choroid plexus (CP) is the tissue plexus located in the lateral, third and fourth ventricles and its main function is the production of cerebrospinal fluid (CSF).[1,2] At the same time, epithelial cells of CP form the blood-CSF barrier together with tight junction areas.[3] CP is a source of nutrients and hormones for the CSF and brain, cleans the brain fluids from harmful compounds and waste products.[4,5] CP is highly vulnerable to head injuries, infections, and damage in ischemic conditions.[6] CP degeneration can result in reduced CSF production and hyperthermic brain damage in animals. With aging, CP functions and CSF production reduce.[7] Electron microscopy shows calcium aggregation in subepithelial regions of CP, in the walls of blood vessels and mostly in psammoma bodies.[8] Although CP calcification, which is among the physiological calcifications, does not usually show clinical symptoms, there are studies showing that it is associated with some psychiatric diseases (schizophrenia, depression).[9] Although CP has been reported to be associated with aging and male gender, its etiology is not fully understood.[10]

The levels of extracellular inorganic phosphate (P), calcium (Ca), systemically or locally secreted mineralization inhibitors and extracellular matrix (ECM) suitable for mineralization are important factors in tissue calcification.[11,12] OPN exists as both a component of the extracellular matrix and a soluble cytokine. OPN expression in adults is normally limited to bone, kidney, and epithelium, and it is secreted in body fluids including blood, urine, and milk.[13] Physiologically, OPN is thought to regulate bio-mineralization in bone tissue and reduce the growth and aggregation of calcium crystals in epithelial tissues.[14] It has previously been shown that OPN is a potent inhibitor of ectopic calcification.[15] However, the expression of OPN increases in many tissues under injury and disease conditions and is also associated

Address correspondence to: Dr. Recep Yevgi, Ataturk University, Faculty of Medicine, Department of Neurology, Yakutiye- Erzurum, Turkey 25200. Tel: 90 (442) 344 88 17, Mobile phone:90 (542) 543 55 56, e-mail: recep_yevgi@yahoo.com

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with calcified aggregations found in various pathologies, including atherosclerotic lesions, aortic stenosis, calculary, and tumours.[16]

It is not known whether OPN plays a role in the formation of CP calcification or not. The aim of this study is to research serum OPN levels in patients with and without CP calcification and to determine the relationship between OPN and CP calcification.

METHODS

The study protocol was approved by Atatürk University Ethics Committee (08/37-27.12.2018) and written informed consent was obtained from all subjects.

Among the patients who came to Atatürk University Faculty of Medicine Neurology polyclinic between May 2019-March 2020 and evaluated with brain CT; a total of 90 individuals who met the inclusion and exclusion criteria, 45 individuals with CP calcification and 45 age and gender-matched patients without CP calcification were included in the study.

The inclusion criteria were ages of 18-50 who had normal brain computed tomography (CT), who were examined by the same neurologist in the neurology polyclinic, who did not have any acute or chronic inflammatory diseases and had no chronic diseases. Any pathology detected on brain CT scan and serum calcium (reference range: 8.4-10.6 mg / dL), 25-hydroxy vitamin D (reference range: 30-100 ng / mL), phosphorus (reference range: 2.4-5.1 mg / dL) and parathyroid hormone (reference range: 19.6–74.9 pg / mL) individuals whose levels were abnormal apart from the references were excluded from the study.

After the consent for study was signed, venous blood samples were taken in the morning from individuals who were fasted overnight. Thirty minutes after blood samples were taken, the tubes were centrifuged for 10 minutes at 4000 rpm. Serum samples were stored at -80°C. Serum OPN levels were measured by the enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corp; Catalog number: SEA899Hu) method.

Whole brain CT examinations were performed with a 4-row multi-detector CT scanner (Somatom Spirit, Siemens Healthcare, Forcheim, Germany). CP was localized in the ventricular system on brain CT images. CP density was calculated using multiple regions of interest (ROIs). If Hounsfield Units (HU)> 150 measured at any ROI location for each subject, it was accepted as having CP calcification, and if HU<50, accepted without CP calcification.

Statistical analysis

Study data were analysed by using SPSS 22 (Statistical Package for the Social Sciences) program. Data compliance for normal distribution was evaluated with the Kolmogorov Smirnov test. Chi-Square test was used in the analysis of categorical data between the groups, Student's T test was used for the analysis of numerical data in cases with normal distribution, and the Mann Whitney U test was used for those who did not fit the normal distribution. Spearman's correlation test was used for correlation analysis between the groups. Statistical significance level was accepted as $p < 0.05$.

RESULTS

The study subject consisted of 90 people, 45 with CP calcification (n=45), and 45 age and gender matched people without CP calcification. The demography consisted of 57.8% female, 42.2% male, and the average age of patients with CP calcification was 33.08 ± 9.5 (Table 1). HU values were significantly higher in those with CP calcification than those without CP calcification (Table 1).

Serum OPN level (median=17.64) in patients with CP calcification was lower than those without CP calcification (median=19.36), and this difference was statistically significant (p=0.02) (Table 1).

We examined whether there is a correlation between HU values and OPN levels and age. There was a statistically significant positive correlation between HU values and age in patients with CP calcification (p=0.013) (Table 2). No significant correlation was found between HU values and serum OPN levels in patients with CP calcification (p=0.290) (Table 2).

There was no statistically significant difference in age and Hu values between genders in patients with CP calcification (Table 3).

DISCUSSION

In our study, serum OPN levels were significantly lower in individuals with CP calcification than those without CP calcification. While there was a significant positive correlation between Hu values and age in patients with CP calcification, there was no significant difference in OPN levels and Hu values between genders.

CP, which has multiple functionality with its production, absorption and filtering features, particularly the production of CSF, is an

Table 1: Demographic data, osteopontin levels and HU values of individuals with and without CP calcification

	Have CP calcification n=45	No CP calcification n=45	p
Gender; n (%)			
Female	26 (57.8%)	26 (57.8%)	1*
Male	19 (42.2%)	19 (42.2%)	
Age; year			
Medium±SD;	33.13±9,45	33.04±9.79	0.965**
Median(min-max)	34 (18-49)	34 (18-49)	
HU values			
Medium±SD;	234.20±76.67	36.64±7.27	0.000***
Median(min-max)	219 (150-480)	39 (21-47)	
Osteopontin(ng/ml)			
Medium±SD;	17.32±4.03;	19.70±4.27;	0.023***
Median(min-max)	17.64 (9-26.79)	19.36 (12.46-33.45)	

**Chi-Square **Student T test ***Mann-Whitney U test*

Table 2: Correlation between HU values and age and osteopontin in patients with CP calcification

	Age	Osteopontin
HU values	0.369	0.161
p	**0.013**	0.290

Spearman's correlation test

Table 3: Age, HU values between genders in patients with CP calcification

	Male n=19	Female n=26	p
Age, year			
Medium±SD;	31.79 ±8.92;	34.12 ±9.86;	0.345
Median(min-max)	34 (18-44)	36 (18-49)	
HU values			
Medium±SD;	229.89±65.25;	237±89.90;	0.483
Median(min-max)	236 (168-460)	213.50 (150-480)	

Mann-Whitney U test

intraventricular organ that contains cubic epithelial cells, vascular and connective tissue.[17] CPs produce basic fibroblast growth factor (bFGF)-2, growth factors such as insulin-like growth factor (IGF)-2, and a wide variety of peptides/proteins such as transthyretin, vasopressin, which are secreted into mostly CSF.[7,18,19] In many injuries and diseases, including stroke and traumatic brain injury, CP plays a role in neuroprotection through growth factor release.[19,20] Throughout the human life, CP tends to aggregate physiological calcifications. Daghighi *et al*.[21] estimated the rate of CP calcification at 66.2% with brain CT in 1,569 cases. Physiological calcifications such as CP calcification generally do not show clinical symptoms. However, there are studies showing that there is a relationship between CP calcifications and some psychiatric diseases (schizophrenia, depression).[9] Previous pathological processes such as intraventricular infection, inflammation and bleeding, and chronic calcium and phosphate imbalance can cause early or excessive CP mineralization.[17] With aging, the incidence rate of CP calcification increases.[10,22,23] Modic *et al*.[24] examined CP calcification with approximately 1,000 brain CT and 0.5% in the first decade and in the eighth decade, on the other hand, they detected it with a rate of 86%.

In our study, there was a positive correlation between age and HU values in patients with CP calcification. According to our study, the severity of CP calcification increases with aging. In our study, there was no significant difference in HU values between genders in patients with CP calcification.

OPN is a member of the small integrin-binding ligand, N-linked glycoprotein (SIBLING) protein family that matches with human chromosome 4.[25] After OPN is synthesized, it undergoes post-translational modifications.[26] OPN is a multifunctional protein containing an integrin binding adhesive domain (RGD) and aspartic acid rich calcium binding sites. Besides, OPN can be highly phosphorylated on serine and threonine residues.[27] OPN is presented in the extracellular matrix of mineralized tissues and extracellular fluids at the site of inflammation. It is secreted by many different cell types, including macrophages, lymphocytes, epithelial cells, adipocytes, osteoblasts, osteoclasts, chondrocytes and vascular smooth muscle cells.[28] OPN plays a role in various physiological functions and pathological conditions such as bone reshaping, wound healing, vascular disorders and inflammatory diseases.[29,30] It is thought that OPN physiologically regulate biomineralization in bone tissue and reduce the growth and aggregation of calcium crystals in epithelial tissues.[14] OPN contributes to binding and regulating apatite crystal growth, the calcium-phosphate mineral phase found in bones, teeth, and ectopic calcification sites. Studies support that OPN has an inhibitory role in apatite growth in vitro models.[31-34] Besides regulating many activities, including bone matrix reshaping, tissue calcification, production of various proinflammatory cytokines, OPN can also promote macrophage adhesion, migration, and vascular smooth muscle cell proliferation. Hence, OPN plays many significant roles in calcification, inflammation, and immune response, as well.[28,35] OPN expression increases in many tissues under injury and disease conditions and is closely related to calcified aggregations found in various pathologies, including atherosclerotic lesions, aortic stenosis, calculary, and tumours.[16]

In order to determine the role of OPN in ectopic calcification, an ectopic calcification model was developed in OPN incomplete and deficient mice, and the results showed that OPN is a potent inhibitor of ectopic calcification.[15] In a study to examine the role of OPN in vascular calcification, it was shown that OPN mutant mice crossed with matrix Gla protein (MGP) mutant mice, known to be a potent inhibitor of arterial calcification, mice deficient in MGP alone showed calcification in the arteries after birth. OPN in calcified arteries, the rate of MGP is significantly increased compared to wildtypes, mice deficient in both MGP and OPN have more than two and three times arterial calcification and die earlier than those lacking MGP alone. The cause of death in these animals was found to be vascular rupture followed by bleeding, most likely due to increased calcification. This study demonstrated that OPN has an inhibitory effect on vascular calcification and that OPN has a role as an inducible inhibitor of ectopic calcification in vivo.[36] Due to this effective role in tissue calcification, OPN may be associated with CP calcification. The relationship of fetuin-A levels, which has another calcification inhibitor and inflammatory effects, with CP calcification was examined and serum fetuin-A levels were found to be lower in patients with CP calcification compared to those without CP calcification.[12] Similarly, in our study, serum levels of OPN, which is a calcification inhibitor, were significantly lower in those with CP calcification compared to those without CP calcification. Our findings support the role of OPN in CP calcification.

It is thought that OPN's regulation of pathological calcification is related to several functions.[37] Firstly, it is an effective hydroxyapatite growth inhibitor by binding to crystal surfaces and, by this way blocking further addition of mineral ions to crystal growth sites. The combination of electronegative glutamic and aspartic acid residues, serine / threonine kinase substrate sites, and putative calcium binding motifs give OPN the ability to bind a significant amount of Ca^{+2} (50 moles of calcium to 1 mol of osteopontin).[27] By binding to hydroxyapatite and calcium ions, OPN physically inhibits crystal formation and growth in vivo, previously reported in studies in OPN-deficient mice, showing that the bones of OPN-deficient mice are hypermineralized with increased mineral content and crystal size.[33] Secondly, OPN is a mineral-linked opsonine with cell adhesion and recognition sequences for cell adhesion, migration, and phagocytosis. OPN's inhibition of calcification and the low level of OPN in individuals with CP calcification in our study may be a guide for future studies on prevention or treatment of CP calcification.

The relatively small size of our study group is one of the limitations of our study and should be supported by larger studies.

In conclusion, we showed lower serum OPN

levels in patients with CP calcification compared to those without CP calcification, and we consider that this is related to the role of OPN in calcification inhibition. OPN can be a useful biomarker for CP calcification.

DISCLOSURE

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