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Immunohistochemical Determination of HIF, TSP-1, ADAMTS1, and ADAMTS8 Expressions in the Brains of Alzheimer's Disease Patients: A Preliminary Autopsy Study

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disease that mostly affects the elderly population. Recent studies performed in AD highlight the pathophysiological relevance of disintegrin and metalloproteinase with thrombospondin type 1- like motifs (ADAMTS) genes and their products, namely hypoxia inducible factor-1 (HIF-1) and thrombospondin-1 (TSP-1). Thus, the aim of this study was to describe and identify the distribution, characteristics, and any changes in the expression and immunoreactivity for HIF-1, TSP-1, and ADAMTS1 and 8 in AD brains.

Materials and Methods: Nine patients who were autopsied in the Council of Forensic Medicine, Bursa Morgue Department in 2013, were selected. All patients were sent for autopsy to the Morgue Department within 8 h after death. At the autopsy, tissue samples of the organs were obtained for histopathological examination for determining the cause of death. Among these, two patients were clinically diagnosed with AD.

Results: Immunohistochemical staining was performed, and the staining intensity/extensity was evaluated using a semi-quantitative scoring system. Median distribution (extensity) scores of the immunohistochemical staining were estimated as 2 for HIF-1, 0.67 for TSP-1, 3.11 for ADAMTS1, and 2.78 for ADAMTS8. Intensity scores were estimated as 1.22 for HIF-1, 0.56 for TSP-1, 3 for ADAMTS1, and 2.11 for ADAMTS8.

Conclusion: Our study suggests that ADAMTS1 and ADAMTS8 expressions are not specific for AD. To understand and provide definitive data on all aspects of metalloproteinases, extracellular matrix proteins, and transcriptional factor effects to AD, further studies are needed, where other metalloproteinases and related molecules/enzymes should be studied.

Keywords: Alzheimer's disease, ADAMTS1, HIF-1, ADAMTS8, TSP-1

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that mostly affects the elderly population. The pathologic processes that underlie this disorder are not fully understood, although it is known that oxidative stress and hypoxia play an essential role in neurodegenerative disorders such as AD and frontotemporal dementias (1-3). Hypoxia inducible factor-1 (HIF-1) is a major member of the transcription factor family that is responsible for the induction of genes that facilitate the adaption and survival of cells exposed to hypoxia (4). HIF-1 is a heterodimeric protein composed of a constitutively expressed HIF-1 beta subunit and oxygen-regulated HIF-1 alpha subunit (5). Clinical and experimental studies suggest that HIF-1 is involved in the pathologic processes of AD (6-8). Thrombospondins (TSPs) are extracellular matrix (ECM) proteins and are part of a family of adhesive glycoproteins (9). Thrombospondin-1 (TSP-1) has functions in platelet aggregation, inflammatory response, and angiogenesis regulation during wound repair and tumor growth (10). TSP-1 is predominantly produced by astrocytes and has been implicated in synaptogenesis in the central nervous system (11, 12). A disintegrin-like and metalloproteinase with thrombospondin type-1 motif (ADAMTS) genes were first discovered by Kuno and Matsushima (13) in 1997. ADAMTS1 and 4 may play a role in neurodegenerative disorders such as AD (14, 15).

Herein, we aimed to analyze the immunohistochemical expression of HIF-1, TSP-1, and ADAMTS1 and 8 in the temporal cortex of brains with clinically diagnosed AD and normal adult brains, the latter serving as the control group.

MATERIALS and METHODS

Nine patients who were autopsied in the Council of Forensic Medicine, Bursa Morgue Department, Bursa, Turkey, in 2013, were selected. All patients were sent for autopsy to our institution within 8 h of their death. Seven patients were males and two were females. The youngest patient was 25 years old, and the oldest was 87 years old. Tissue samples were taken for histopathological examination of the organs for determining the cause of death. Two patients were clinically diagnosed with AD, and 7 control patients belonging to different age groups [the age groups in years and the respective number of patients were as follows: 25–35 (n=1), 35–45 (n=1), 45–55 (n=1), 55–65 (n=1), 65–75 (n=1),

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75–85 (n=1), and >85 (n=1)] were analyzed for their ages, causes of death, and cerebral macroscopic and microscopic findings (Table 1).

Tissue samples were taken from the temporal brain regions in each patient and were fixed in 10% formaldehyde and embedded in paraffin wax. Sections of 3–4 µm thickness were cut and stained with hematoxylin and eosin. Ethical permissions for this study were taken from the local ethical committee (Bursa) and ethical committee of the Council of Forensic Medicine (İstanbul).

Immunohistochemistry: As mentioned above, following formaldehyde fixation and paraffin embedding, 5-µm thick sections were taken on APTES (3-Aminopropyl- triethoxysilane)-coated slides. The sections were deparaffinized in xylene and rehydrated through graded alcohols. Rehydration was followed by incubation with 2% hydrogen peroxide and methanol for 5 min to prevent intrinsic peroxidase activity. After the samples were washed three times using phosphate-buffered saline (PBS, pH: 7.4), they were warmed in a microwave oven in 0.1 nM sodium citrate for 10 min, and the antigen retrieval procedure was completed. Later on, incubation and blocking of the non-immune serum at room temperature for 20 min was performed. The sections were taken on polylysine lams and immunohistochemistry with the anti-HIF-1, anti-TSP-1, and anti-ADAMTS1 and 8 antibodies were performed. The procedures were performed according to the protocols recommended for anti-HIF-1, anti-TSP-1, and anti-human ADAMTS1 and 8 antibodies (Abcam, United States). After being deparaffinized at 65°C in a heat chamber and rehydrated, sections were subjected to epitope retrieval in 10× EDTA (ethylenediamine tetra acetic acid) buffer (pH 8.0) in 110 °C for 30 min. Subsequently, the sections were exposed to 3% H_2O_2 for 20 min to bleach endogenous peroxidases, followed by rinsing three times in PBS for 10 min. The sections were respectively incubated with a rabbit anti-TSP-1 antibody, anti-HIF-1 antibody, and anti-human ADAMTS 1 and 8 (all 1:250 in BSA) for 1 h at 37°C, washed three times in PBS, and incubated in a biotinylated goat secondary anti-mouse polyclonal antibody (Abcam) for 15 min at 37°C. The omission procedure of primary antibodies was used to examine the specificity of the antibodies. Following washing in PBS, the tissues were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB chromogen, Abcam) and counterstained with hematoxylin. Finally, the sections were dehydrated in graded ethanol, immersed in xylene, and coverslipped. All the images were examined using a 200× objective (microscope Olympus B 53×). Regarding the exten-

Table 1. Descriptive characteristics of the cases (age, gender, cause of death, cerebral macroscopic, and microscopic findings), staining extensity/intensity scores of HIF-1, TSP-1, ADAMTS1 and 8

Cases	Age	Sex	Cause of death	Histopathology findings in the brain	Macroscopic findings in the brain	HIF-1		TSP-1		ADAMTS1		ADAMTS8	
						EXT	INT	EXT	INT	EXT	INT	EXT	INT
AD Case 1	85	M	Brain hemorrhage due to head trauma	Subarachnoid and subcortical hemorrhages	Subarachnoid and subcortical hemorrhages	2	2	2	1	4	4	3	2
AD Case 2	80	M	Coronary artery disease	Cerebral infarction and subcortical hemorrhage	Atherosclerotic changes in the cerebral arteries (Willis polygon)	3	2	1	1	0	0	0	0
Case 3	25	M	Brain hemorrhage due to head trauma	Subcortical hemorrhage	Subarachnoid and subcortical hemorrhages	0	0	1	1	3	3	3	2
Case 4	41	M	Other internal causes	Congestion	Normal	1	1	0	0	3	3	3	2
Case 5	53	M	Coronary artery disease	Congestion	Atherosclerotic changes in the cerebral arteries (Willis polygon)	2	2	0	0	3	3	2	2
Case 6	56	F	Brain hemorrhage due to head trauma	Subarachnoid hemorrhage	Subarachnoid hemorrhage	2	1	0	0	3	3	3	2
Case 7	66	M	Heart failure	Normal	Normal	2	1	1	1	4	3	3	2
Case 8	76	M	Heart failure	Congestion	Normal	3	1	0	0	4	4	4	3
Case 9	87	F	Aortic Dissection	Normal	Normal	3	1	1	1	4	4	4	4

ADAMTS1: thrombospondin type 1- like motifs; AD: Alzheimer's disease; EXT: extensity; INT: intensity

sity of antibody expression, we determined the staining score for each section and classified it as follows: 0, if it showed no staining; 1, for occasional staining with most fields negative; 2, focally abundant staining with most fields having no staining; 3, focally abundant staining with most fields showing positive staining; or 4, prominent staining throughout the section. The staining intensity was recorded using a semi-quantitative scoring system: 0: absent, 1: weak staining, 2: accumulations with greater staining intensity, 3: strong and dark staining, 4: very strong and the darkest observed staining.

RESULTS

All the specimens were stained by immunohistochemical methods, and the staining scores were determined. ADAMTS1 and 8 were distinctly higher compared to HIF-1 and TSP-1. ADAMTS1 and 8 immunoreactivities were consistently demonstrated in the control group. The extensity of ADAMTS1 staining was very strong and ADAMTS8 staining was strong in one patient (one of the AD patients). ADAMTS1 and 8 expressions were not detected in case 2. Furthermore, the extensities of ADAMTS 1 and 8 staining were detected to be directly proportional to age.

Descriptive characteristics of the patients and the extensity and intensity of the immunohistochemical staining patterns are summarized in the table. The median age of the seven male and two female patients was 63.2 years (min. 25, and max. 87 years). Causes of death of the patients included other causes of internal causes (n=1), aortic dissection (n=1), coronary artery disease (n=2), brain hemorrhage due to head trauma (n=3), and heart failure (n=2). Median distribution (extensity) scores of the immunohistochemical staining were estimated as 2 for HIF-1, 0.67 for TSP-1 (Figures 1, 2), 3.11 for ADAMTS1, and 2.78 for ADAMTS8 (as shown in Figure 3 and 4). Intensity scores were also estimated as 1.22 for HIF-1, 0.56 for TSP-1, 3 for ADAMTS1, and 2.11 for ADAMTS8.

DISCUSSION

Hypoperfusion/hypoxia is among the underlying factors thought to play an important role in AD pathogenesis (1-3). HIF-1 plays

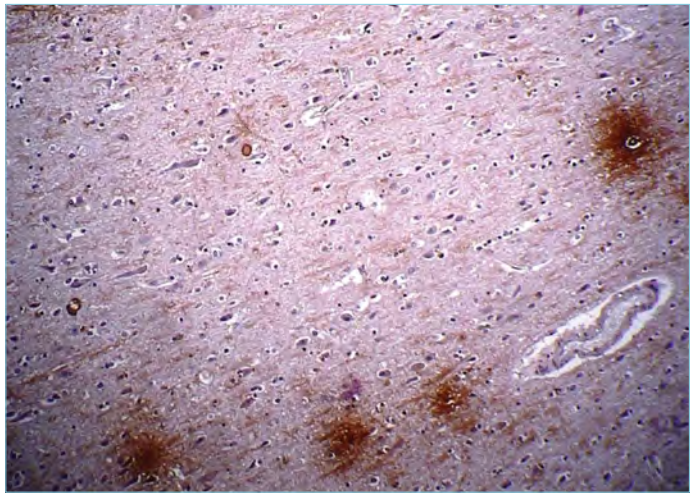

Figure 1. ADAMTS8 immunostaining in postmortem brain tissue. It is shown strongly staining in focal fields (original magnification, ×200). 150x125 mm (300x300 DPI)

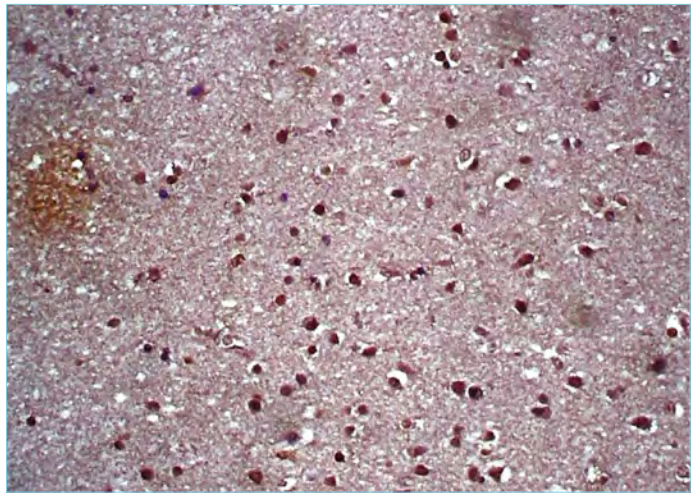

Figure 3. TSP-1 immunostaining in postmortem brain tissue. It is shown focal staining in capillary endothelium (original magnification, ×200). 150x127mm (300x300 DPI)

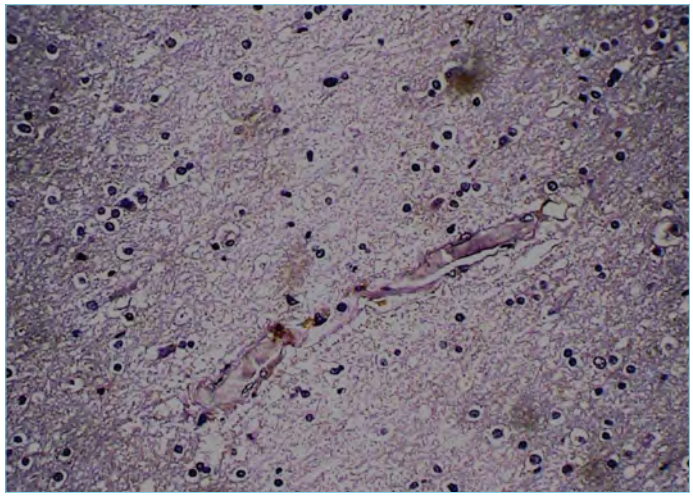

Figure 2. HIF-1 immunostaining in postmortem brain tissue. It is shown weak staining in focal fields (original magnification, ×200). 625x468 mm (72x72 DPI)

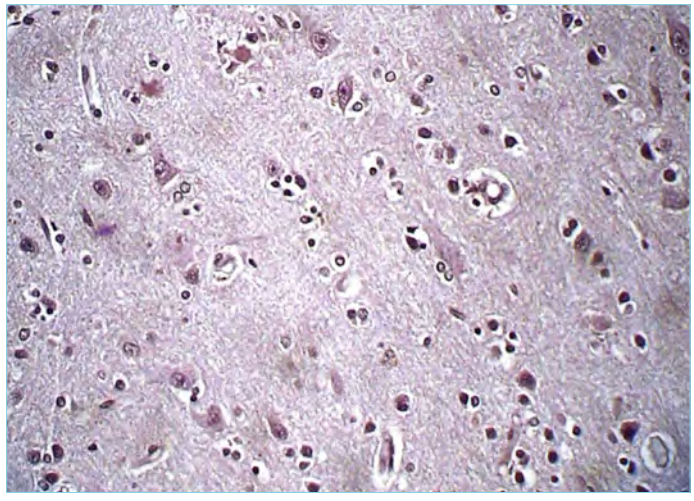

Figure 4. ADAMTS1 immunostaining in post-mortem brain tissue. It is shown diffuse staining (original magnification, ×200). 150x119 mm (300x300 DPI)

key roles in cellular physiology and the pathophysiology response to hypoxia (16). Several target genes that are transactivated by HIF-1 have been identified, including those encoding erythropoietin, glucose transporters, glycolytic enzymes, and vascular endothelial growth factor to adaption to tissue hypoxia (16, 17). HIF-1 activation promotes a cellular response to hypoxia for cell survival (17, 18), and HIF-1 activity can prevent neuron death and ameliorate these symptoms of neurodegenerative disorders such as AD (18). Grammas et al. (19) reported that HIF-1α is elevated in brain blood vessels in Alzheimer's patients compared to in control groups. Grammas et al. (6) reported in another study that brain sections from AD and control mice demonstrated that HIF-1α, angiopoietin-2, matrix metalloproteinase 2, and caspase 3 are elevated and Bcl-xL decreased in the microvasculature of AD mice. Luo et al. (7) reported in their study brain endothelial cell cultures originated from isolated rat brain microvessels and exposed to hypoxia for various periods of time. They observed a time-dependent increase in the accumulation of HIF-1α protein in the brain microvascular endothelial cells exposed to hypoxia (7). In our present study, we observed an HIF-1 increase in the postmortem brain samples from Alzheimer's patients. In addition, HIF-1 expression was observed in the control groups, apart from case 3. The HIF-1 increases in Alzheimer's patients may be explained as a response to exposed hypoxia; such observations have already been described in similar studies (6, 7, 19). Increasing HIF-1 expression was shown to be dependent on the age in the control groups. We believe that brain cells' sensitivity to hypoxia may increase when these are exposed to such conditions, resulting from environmental factors or self-existing diseases (heart failure, atherosclerotic changes in Willis polygon, and coronary artery disease). In addition, HIF-1 expression was not detected in case 3. This can be explained that cause of death in young patients may be traumatic death without hypoxic exposure.

Thrombospondins are ECM proteins and are part of a family of adhesive glycoproteins (9). TSP-1 has functions in platelet aggregation, calcium binding, cell attachment, neurite outgrowth, cell–cell interactions, inflammatory response, and angiogenesis regulation (10). TSP-1 is predominantly produced by astrocytes and has been implicated in synaptogenesis in the central nervous system (11, 12). Buée et al. (20) observed that TSP is found in the normal human brain. In all their examined AD patients, neuronal staining was markedly decreased and microvascular staining appeared to be weaker in AD than control patients. They believe that TSP is a neuronal marker of early neuronal degeneration in AD. In our study, we observed TSP-1 expression in Alzheimer's patients but not in some control patients. In case 2 of AD, the microscopic examination showed cerebral infarction findings and a weaker staining for TSP-than in case 1.

Thrombospondin type 1- like motifs appear to be responsible for the cleavage of proteoglycans in the brain. ADAMTS families of metalloproteinases are important contributors of proteolysis in central nervous system disorders (21-23). The results of a study presented by Reid et al. (24) suggested that ADAMTS9 expression is modulated in response to cerebral ischemia. Miguel et al. (15) reported in their study that ADAMTS1 expression increases in the brains of patients with neurodegeneration, such as AD. In this study, ADAMTS1 and 8 expressions were observed in all patients, except in case 2. In this study, no association was observed between AD and ADAMTS1 and 8.

CONCLUSION

According to our results, ADAMTS1 and ADAMTS8 expressions are not specific for AD. Thus, HIF-1, TSP-1, ADAMTS1, and ADAMTS8 do not have the potential to act as key molecules in AD. To understand and provide definitive data on all aspects of metalloproteinase, ECM proteins, and transcriptional factor effects to AD, further studies are needed. In these prospective studies, other metalloproteinases and related molecules/enzymes should be analyzed.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of local ethical committee (Bursa) and Council of Forensic Medicine (İstanbul).

Informed Consent: Informed consent was obtained from patients who participated in this study.

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Authors' Contributions: Conceived and designed the experiments or case: NTI, RF, KD. Performed the experiments or case: BE, MSG, MNU, BA. Analyzed the data: BE, FE, SA. Wrote the paper: RF, MSG, MNU. All authors have read and approved the final manuscript.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Ogunshola OO, Antoniou X. Contribution of hypoxia to Alzheimer's disease: is HIF-1alpha a mediator of neurodegeneration? Cell Mol Life Sci 2009; 66(22): 3555-63. [CrossRef]
2. Peers C, Dallas ML, Boycott HE, Scragg JL, Pearson HA, Boyle JP. Hypoxia and neurodegeneration. Ann NY Acad Sci 2009; 1177: 169-77. [CrossRef]
3. Zhang X, Le W. Pathological role of hypoxia in Alzheimer's disease. Exp Neurol 2010; 223(2): 299-303. [CrossRef]
4. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995; 92(12): 5510-4. [CrossRef]
5. Kuschel A, Simon P, Tug S. Functional regulation of HIF-1α under normoxia- is there more than post-translational regulation? J Cell Physiol 2012; 227(2): 514-24. [CrossRef]
6. Grammas P, Tripathy D, Sanchez A, Yin X, Luo J. Brain microvasculature and hypoxia-related proteins in Alzheimer's disease. Int J Clin Exp Pathol 2011; 4(6): 616-27.
7. Luo J, Martinez J, Yin X, Sanchez A, Tripathy D, Grammas P. Hypoxia induces angiogenic factors in brain microvascular endothelial cells. Microvasc Res 2012; 83(2): 138-45. [CrossRef]
8. Lonati E, Brambilla A, Milani C, Masserini M, Palestini P, Bulbarelli A. Pin1, a new player in the fate of HIF-1α degradation: an hypothetical mechanism inside vascular damage as Alzheimer's disease risk factor. Front Cell Neurosci 2014; 8: 1. [CrossRef]
9. Frazier WA. Thrombospondins. Curr Opin Cell Biol 1991; 3: 792-9. [CrossRef]
10. Adams JC, Lawler J. The thrombospondins. Int J Biochem Cell Biol 2004; 36(6): 961-8. [CrossRef]
11. Liauw J, Hoang S, Choi M, Eroglu C, Choi M, Sun GH, et al. Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. J Cereb Blood Flow Metab 2008; 28(10): 1722-32. [CrossRef]

12. Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 2005; 120(3): 421-33. [CrossRef]
13. Kuno K, Matsushima K. ADAMTS-1 protein anchors at the extracellular matrix through the thrombospondin type I motifs and its spacing region. J Biol Chem 1998; 273(22): 13912-7. [CrossRef]
14. Satoh K, Suzuki N, Yokota H. ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs) is transcriptionally induced in beta-amyloid treated rat astrocytes. Neurosci Lett 2000; 289(3): 177-80. [CrossRef]
15. Miguel RF, Pollak A, Lubec G. Metalloproteinase ADAMTS-1 but not ADAMTS-5 is manifold overexpressed in neurodegenerative disorders as Down syndrome, Alzheimer's and Pick's disease. Brain Res Mol Brain Res 2005; 133(1): 1-5. [CrossRef]
16. Semenza GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 1999; 15: 551-78. [CrossRef]
17. Wang XJ, Si LB. Advances on hypoxia inducible factor-1. Chin Med J (Engl) 2013; 126(18): 3567-71.
18. Zhang Z, Yan J, Chang Y, ShiDu Yan S, Shi H. Hypoxia inducible factor-1 as a target for neurodegenerative diseases. Curr Med Chem 2011; 18(28): 4335-43. [CrossRef]
19. Grammas P, Samany PG, Thirumangalakudi L. Thrombin and inflammatory proteins are elevated in Alzheimer's disease microvessels: implications for disease pathogenesis. J Alzheimers Dis 9(1): 51-8.
20. Buée L, Hof PR, Roberts DD, Delacourte A, Morrison JH, Fillit HM. Immunohistochemical identification of thrombospondin in normal human brain and in Alzheimer's disease. Am J Pathol 1992; 141(4): 783-8.
21. Cross AK, Haddock G, Stock CJ, Allan S, Surr J, Bunning RA, et al. ADAMTS-1 and -4 are up-regulated following transient middle cerebral artery occlusion in the rat and their expression is modulated by TNF in cultured astrocytes. Brain Res 2006; b1088(1): 19-30.
22. Haddock G, Cross AK, Plumb J, Surr J, Buttle DJ, Bunning RA, et al. Expression of ADAMTS-1, -4, -5 and TIMP-3 in normal and multiple sclerosis CNS white matter. Mult Scler 2006; 12: 386-96. [CrossRef]
23. Plumb J, Cross AK, Surr J, Haddock G, Smith T, Bunning RA, et al. ADAM-17 and TIMP3 protein and mRNA expression in spinal cord white matter of rats with acute experimental autoimmune encephalomyelitis. J Neuroimmunol 2005; 164(1-2): 1-9. [CrossRef]
24. Reid MJ, Cross AK, Haddock G, Allan SM, Stock CJ, Woodroofe MN, et al. ADAMTS-9 expression is up-regulated following transient middle cerebral artery occlusion (tMCAo) in the rat. Neurosci Lett 2009; 452(3): 252-7. [CrossRef]