ARAŞTIRMA MAKALESİ / Research Article

Evaluation of Cognitive Functions in Hypertensive Patients and Its Relationship with Serum Midkine Levels

Hipertansif Hastalarda Kognitif Fonksiyonların Değerlendirilmesi ve Serum Midkine Düzeyleriyle İlişkisi

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Abstract

Objective: Hypertension (HT) is one of the risk factors associated with dementia. Our aim was to research cognitive functions in patients with HT and its relationship to midkine levels in serum.

Material and Methods: This study examined 45 patients who were over 60 years of age, had at least five years of education, and had an essential HT diagnosis. We also had 30 healthy control subjects. The Mini Mental State Examination (MMSE) was applied to the patients. Scores of 24 and lower from the MMSE indicated a cognitive disorder. In relation serum levels of midkine were also evaluated.

Results: The MMSE scores of the HT patients were compared to the control group and were significantly lower ($p<0.01$). Midkine levels in the HT patients (25.10 ±8.16 ng/mL) compared to the control group (19.59±7.53 ng/mL) and were significantly higher ($p< 0.01$). Midkine levels were also higher in HT patients with cognitive impairment compared to HT patients without any cognitive impairment ($p<0.05$). A significant negative correlation was observed between midkine levels and MMSE scores, ($r = 0.558$, $p<0.01$).

Conclusion: This study has demonstrated an important relationship between increased serum midkine levels and cognitive decline in HT patients. **(Sakarya Med J 2017, 7(1):33-38)**

Keywords: Hypertension, Cognitive decline, Midkine, Mini Mental State Examination

Özet

Amaç: Hipertansiyon (HT) demans ile ilişkili risk faktörlerinden biridir. Çalışmanın amacı, HT'lu hastalarda kognitif fonksiyonlar ve serum midkine düzeyleri ile ilişkisini araştırmaktır.

Gereç ve Yöntem: Bu çalışmada, 60 yaşın üstünde, en az beş yıllık eğitim alan ve HT tanısı almış 45 hasta ve 30 sağlıklı kontrol incelendi. Hastalara Mini Mental Durum Değerlendirme (MMDD) testi uygulanmıştır. MMDD skoru 24 ve daha düşük olanlar kognitif bozukluk olarak tanımlandı. Bu gruplarda serum midkine seviyeleri değerlendirildi.

Bulgular: HT'lu hastalarının MMDD skorları kontrol grubuyla karşılaştırıldı ve anlamlı olarak düşük bulundu ($p <0.01$). HT'lu hastalarda midkine düzeyleri (25.10 ± 8.16 ng/mL) kontrol grubuyla (19.59 ± 7.53 ng/mL) kıyaslandığında belirgin olarak yüksekti ($p <0.01$). HT hasta grubunda kognitif bozukluğu olanlar ve olmayanlar arasında Midkine düzeyleri karşılaştıldı. Kognitif bozukluğu olan HT hastalarında Midkine belirgin olarak daha yüksekti ($p <0.05$). Ayrıca Midkine düzeyleri ile MMDD skorları arasında anlamlı bir negatif korelasyon gözlendi ($r = 0.558$, $p <0.01$).

Sonuç: Bu çalışma, HT'lu hastalarda artan serum midkine düzeyleri ile kognitif fonksiyon azalması arasında önemli bir ilişki olduğunu göstermiştir. **(Sakarya Tıp Dergisi 2017, 7(1):33-38)**.

Anahtar Kelimeler: Hipertansiyon, Kognitif Bozulma, Midkine, Mini Mental Durum Değerlendirme

Elevated arterial pressure is interrelated with progressive pathological changes leading to many different cardiovascular and central nervous system related complications such as stroke, vascular dementia, and possibly Alzheimer's disease (AD).[1-3] Cognitive decline may be led by uncontrolled hypertension.[4] Cognitive impairment is considered as a form of dementia. The role of hypertension in determining the loss of cognitive function is not entirely defined.[5,6] It is well known that hypertension is interrelated with increased cerebral vascular resistance with diffuse lesions and multiple lacunar infarcts in the white matter (especially in the subcortical region) are histopathologically detectable and visible by magnetic resonance imaging.[7,8]

Midkine (MK), a heparin-binding growth factor with a molecular weight of 13 kDa, has various biological functions such as the growth of fibroblasts, survival of embryonic neurons, and migration of inflammatory cells; which are elicited through extracellular signal-regulated kinase (ERK) and AKT activation.[9-11] MK, a secreted neurotrophic factor, is normally conceived in brain in the mid-gestation period and promotes both neuronal survival and outgrowth.[12-14] It is present at low levels in the healthy adult brain.15 In addition, MK protein levels are increased around sites of ischemic damage.[16-18] Moreover the levels of serum midkine levels were found to be increased in AD patients.[19]

Consequently, the objectives of this study were to investigate the effect of hypertension on cognitive impairment and the role of midkine in this process.

Materials and Methods

Patients

This study was a cross-sectional study and included total 75 participants as 30 normotensive and 45 hypertensive subjects. Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg, current treatment with antihypertensive medication, or a self-reported diagnosis of hypertension. Inclusion criteria consisted of advanced age (≥60 years) suffering from primary hypertension. Exclusion criteria included secondary hypertension, diabetes, cancer, recent acute infection, severe cardiac, liver or kidney dysfunction, cerebrovascular disease, severe Parkinson's disease, depression or anxiety, hypothyroidism, intracranial space-occupying, alcohol dependence, cognitive dysfunction after traumatic brain injury, alcohol and drug abuse. All the participants underwent a standardized clinical assessment, which included a medical history, and physical and neurological examination together with the Mini Mental State Examination (MMSE) tests. Reliability and validity of revised Turkish version of MMSE was made and normal cognitive function was defined as MMSE test score>24.20 Participants were assigned either to the hypertension with cognitive impairment group (case group, n=25) or to the hypertension with normal cognitive function group (controlHT group, n=20). All patients were under antihypertensive medication treatment. Informed consent was obtained from all participants prior to the study. This clinical investigation was approved by local ethics committee.

Blood collection

Following an overnight fast, blood samples were collected from a vein in the antecubital fossa without venous occlusion and the samples were immediately centrifuged. Serum was separated and then stored at -80 °C until biochemical analysis.

Methodology

Glucose, urea and creatinine levels were measured by assay kit from Cobas, Roche Diagnostics. Serum midkine levels were measured by Biovendor ELISA Kit, based on the competition principle and microtiter plate (MTPL) separation. Inter-assay and intra-assay CV% were <9% and <10% respectively (BioVendor – Laboratorní Medicína a.s.; Brno, Czech Republic).

Statistical analysis

All datum were analyzed with the use of the Statistical Package for the Social Sciences for Windows software (Version 18.0 SPSS, Chicago, IL). Datum were presented as mean and SD (+/-) or percentage (%). The differences between groups were identified by using unpaired t-tests for parametric data and Mann–Whitney U test for nonparametric data. Correlations between the variables were evaluated with the use of Pearson's correlation coefficient. Statistical significance was defined as $p<0.05$.

Results

In the hypertension parameters, SBP (p<0.001) and DBP (p<0.001) were significantly higher in the hypertension patients compared to the control group. MMSE were found to be significantly lower (p<0.01); and MK levels were significantly higher in hypertension patients when compared to the control group (p<0.01) (Table 1).

Table 1: Clinical assessments and laboratory findings in hypertension and and control group

	Control	Hypertension Group
N	30	45
M Age (years)	51.20±6.80	53.80±7.70
BMI (kg/m2)	26.10±3.10	28.20±3.80 *
SBP (mmHg)	120 (90-130)	150 (125-200) ***
DBP (mmHg)	80 (60-90)	100 (95-150) ***
Hypertension Time (Years)	-	6.6 (4-32)
MMSE Score	27 (21-30)	24 (18-30) **
Midkine (ng/ml)	19.59±7.53	25.10±8.16 **

BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MMSE, Mini–Mental State Examination.
*p<0.05, **p<0.01, ***p<0.001;

We also examined the change of midkine and MMSE scores according to the duration of hypertension. Hypertensive patients were classified according to the time of first diagnosis of hypertension: 0-5 years (n=15), 5-10 years (n=12), and longer than 10 years (n=18). In the group of patients with >10 years long hypertension, midkine levels were found to be significantly higher than the <5 years hypertension patients (p<0.05). In contrast, MMSE of the hypertension patients for>10 years were significantly lower than hypertension patients for <5 years (p<0.05) (Table 2).

Table 2: Clinical assessments and laboratory findings according with in hypertension time groups

	Hypertension Time		
	<5 years (n=15)	5-10 years(n=12)	> 10 years (n=18)
Age (years)	50.78±6.99	58.22±6.54	54.27±7.56
MMSE Score	25 (17-28)	24 (18-30)	23 (15-27) a*
Midkine (ng/ml)	22.99±7.25	24.33±6.86	29.62±9.65 a*

*p<0.05; a; < 5 years vs > 10 years

When hypertension patients with MMSE score>24 were compared to those of score below 24, midkine level was significantly lower (p<0.05) (Table 3). Significant negative correlation was observed between midkine levels and MMSE (r= -0.558, p<0.01). MMSE also showed a significant negative correlation with DBP (r=-0.396, p<0.01).

Table 3: Clinical assessments and laboratory findings in MMSE groups

Parameters	MMSE score <24	MMSE score ≥24
N	25	20
Age (years)	55.24±7.38	52.10±7.56
Midkine (pg/ml)	27.95±8.28	22.55±6.84 *

*p<0.05

Discussion

The relationship between hypertension and cognitive function is complex and not fully comprehended. Hypertension-related cognitive decline is a consequence of interplay between functional blood flow reorganisation and vascular damage in brain. Focal and regional dissociations in blood flow and function lead to lesions affecting both grey and white matter, manifesting as complete and incomplete microinfarcts, haemorrhages and white matter hyperintensities.[7,8,21] It is the summation of vascular and degenerative lesions which may contribute to the early expression of still subclinical Alzheimer's disease reaching the dementia threshold earlier.[22] In the present study, we found that MMSE score of patients with hypertension were significantly lower than those of healthy controls. In the >10 years hypertension group, MMSE scores were significantly lower than <5 years hypertension patients. There have been various studies analysing the relationship between hypertension and cognitive function. Although there are few studies reported that there is not a relation between MMSE score and hypertension, majority of the studies on the topic indicated that there is a prominently negative relationship between MMSE score and hypertension.[6,23] In our results, a negative correlation between MMSE and DBP confirms the findings of the majority. Nevertheless the existence of these contradictory results could be associated to the existence of different mechanisms that affect the cognitive performance.

According to literature, one of the mechanisms affecting the cognitive performance could be processed through the MK metabolisms. MK has been found to accumulate in senile plaques in the brain of patients with Alzheimer's disease.[24] Also, MK inhibits cytotoxicity of amyloid beta-peptide and its fibril formation. It is generally produced as a response to tissue damage such as ischemic injury in the brain and can promote the repair of damaged tissue by enhancing the survival of injured cells.[18,25] Furthermore, MK is known to promote the migration of microglias, which operate in the clearance of amyloid beta-peptide plaques.[26] Thus, MK is likely to counteract the deposition of amyloid beta-peptide plaques by both directly binding to amyloid beta-peptide and promoting the migration of microglias.[27,28] We found that the serum MK levels of the patients with hypertension were significantly higher than those of the healthy control patients. Furthermore, serum MK levels are determined to be significantly higher in the patients with hypertension who have cognitive decline. Additionally a negative correlation between MK and MMSE was observed. Salama et al. have reported similar findings to ours; serum MK levels have been observed to be high in Alzheimer disease patients.[19] However, they could not have found any correlation between MK and MMSE suggesting different patophysiological roles of midkine in AD.[19] MK has been defined as a double-edged sword, according to beneficial and harmful outcomes in the tissue. It can be speculated that elevated MK levels in the sera and brains of patients with AD may be produced to prevent A beta peptide-induced cell death in AD. However, high levels of midkine in our hypertensive patients might be a reason or result of mild inflammation due to atherosclerotic complications of hypertension. This speculation may also explain existence of negative correlation between MK serum levels and cognitive impairment based on different way from the pathophysiological mechanism in AD.

Recent research supports an important role of the renin-angiotensin system in both ageing of brain and in dementia progression. Angiotensin is active in the nucleus tractus solitarius and the dorsolateral ventral medulla-BP regulatory area.[21] Interestingly; MK is correlated with hypertension, since it is a regulator of the renin-angiotensin system.[28,29] Upon 5/6 nephrectomy, midkine is secreted in the lung, and induces the production of angiotensin-converting enzyme. Hypertension is induced after the nephrectomy in wild-type mice but not significantly in MK-deficient mice.[28] Therefore this shows that one of the mechanisms which mediate MK increase in patients with hypertension is renin-angiotensin system. Because of MK being a detected mediator in brain, it has been thought that angiotensin induces an increase MK in brain. In our study, midkine levels in the >10 years hypertension patients group were found to be significantly higher than <5 years hypertension patients. Similar to our findings Malyszko et al. states a positive correlation between MK and SBP.[30]

In summary of our study a significant relationship between MK rise in the patients with hypertension and cognitive decrease was observed. MK levels showed a negative correlation with MMSE in hypertension patients. These results support that increased MK levels are related with cognitive decline in hypertensive patients. Through our findings we could also evaluate hypertension as a cause of cognitive impairment.

However this study has some limitations and could be supported with further research with respect to the following points primarily. The study was cross-sectional. The cross-sectional design does not permit to draw any conclusion on a causal relationship between elevated midkine levels and incidence of cognitive impairement in hypertension. Our sample size was relatively small, additionally the blood pressure levels were obtained in a single occasion and that limited information was used throughout. The use of multiple blood pressure measurements would improve accuracy and precision with respect to the data relevance of the study. Furthermore, MMSE has several disadvantages including but not limited to the insensitivity to mild or isolated cognitive deficits.[31] It is necessary to confirm the findings in study with a larger sample size and with the suggested edits to the measurement frequency.

In conclusion serum MK levels were significantly higher in hypertension patients compared to the control group and MK levels were significantly higher in hypertension patients with cognitive decline compared to those without cognitive decline. Furthermore MK levels were negatively correlated with MMSE in hypertension subjects. Thus, these results suggest that increased MK levels co-

uld be related to cognitive decline in hypertension patients. This should be supported with further studies. Our results emphasize the need for further research on the role of MK levels in the etiology and progression of cognitive decline in hypertension patients.

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References

1. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age specific relevance of usual blood pressure to vascular mortality. A metaanalysis of individual data for an million adult in 61 prospective studies. Lancet 2002; 360: 1903–913.
2. Ruitenberg A, Skoog I, Ott A, Aevarsson O, Witteman JC, Lernfelt B et al. Blood pressure and risk of dementia: result from the Rotterdam study and the Gothenberg H-70 study. Dement Geriatric Cogn Disord 2001; 12: 33–9.
3. Kivipelto M, Solomon A, Ahtiluoto S, Ngandu T, Lehtisalo J, Antikainen R et al.. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): study design and progress. Alzheimers Dement. 2013; 9:657-65.
4. Jawed RA. Midlife hypertension is associated with impaired cognition and dementia later. Clev Clin J Med 2002; 69: 664–69.
5. Farmer ME, White LR, Abbott RD, Kittner SJ, Kaplan E, Wolz MM et al. Blood pressure and cognitive performance. The Framingham Study Am J Epidemiol 1987; 126(6): 1103-114.
6. Duron E, Hanon O. Hypertension, cognitive decline and dementia. Arch Cardiovasc Dis 2008;101(3): 181-89.
7. Scheinberg P. Dementia due to vascular disease a multifactorial disorder. Stroke 1988;19(10): 1291-299.
8. Dozono K, Ishii N, Nishihara Y, Horie A. An autopsy study of the incidence of lacunes in relation to age, hypertension, and arteriosclerosis. Stroke1991; 22(8): 993-96.
9. Muramatsu H, Muramatsu T. Purification of recombinant midkine and examination of its biological activities: functional comparison of new heparin binding factors. Biochem Biophys Res Commun 1991; 177: 652–58.
10. Muramatsu T. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. J Biochem 2002; 132: 359–71.
11. Takada T, Toriyama K, Muramatsu H, Song XJ, Torii S,Muramatsu T. Midkine, a retinoicacid inducible heparin-binding cytokine in inflammatory responses: chemotacticactivity to neutrophils and association with inflammatory synovitis. J Biochem 1997; 122: 453–58.
12. Kikuchi S, Muramatsu H, Muramatsu T, Kim SU. Midkine, a novel neurotrophic factor, promotes survival of mesencephalic neurons in culture. Neurosci Lett 1993; 160: 9–12.
13. Matsuzawa MI, Muramatsu T, Yamamori T, Knoll W, Yano R. Novel neuronal effects of midkine on embryonic cerebellar neurons examined using a defined culture system. Cell Mol Neurobiol 1999;19(2): 209-21.
14. Owada K, Sanjo N, Kobayashi T, Mizusawa H, Muramatsu H, Muramatsu T et al. Midkine inhibits caspase-dependent apoptosis via the activation of mitogen-activated protein kinase and phosphatidyl inositol-3-kinase in cultured neurons. J Neurochem 1999;73(5): 2084-92.
15. Kato S, Shinozawa T, Takikawa M, Kato M, Hirano A, Awaya A et al. Midkine, a new neurotrophic factor, is present in glial cytoplasmic inclusions of multiple system atrophy brains. Acta Neuropathol 2000;100(5):481-89.
16. Wada M, Kamata M, Aizu Y, Morita T, Hu J, Oyanagi K. Alteration of midkine expression in the ischemic brain of humans. J NeurolSci 2002; 200:67–73.
17. Wang S, Yoshida Y, Goto M, Moritoyo T, Tsutsui J, Izumo S et al. Midkine exists in astrocytes in the early stage of cerebral infarction. Brain Res Dev Brain Res 1998; 106: 205–9.
18. Yoshida Y, Goto M, Tsutsui J, Ozawa M, Sato E, Osame M et al. Midkine is present in the early stage of cerebral infarct. Brain Res Dev Brain Res1995; 85: 25–30.
19. Salama RH, Muramatsu H, Shimizu E, Hashimoto K, Ohgake S, Watanabe H et al. Increased midkine levels in sera from patients with Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29(4):611-16.
20. Keskinoglu P, Ucku R, Yener G, Yaka E, Kurt P, Tunca Z. Reliability and validity of revised Turkish version of Mini Mental State Examination (rMMSE-T) in community-dwelling educated and uneducated elderly. Int J Geriatr Psychiatry 2009;24(11):1242-250.
21. G secki D, Kwarciany M, Nyka W, Narkiewicz K. Hypertension, brain damage and cognitive decline. Curr Hypertens Rep 2013; 15(6): 547–58.
22. Pasquier F, Leys D. Why are stroke patients prone to develop dementia? J Neurol 1997; 244:135–42.
23. Birns J, Kalra L. Cognitive function and hypertension. J Hum Hypertens 2009;23(2):86-96.
24. Yasuhara O, Muramatsu H, Kim SU, Muramatsu T, Maruta H, McGeer PL. Midkine, a novel neurotrophic factor, is present in senile plaques of Alzheimer disease. Biochem Biophys Res Commun 1993; 192: 246–51.
25. Takenaka H, Horiba M, Ishiguro H, Sumida A, Hojo M, Usui A et al. Midkine prevents ventricular remodeling and improves long-term survival after myocardial infarction. Am J Physiol Heart Circ Physiol 2009;296: 462–69.
26. Muramatsu H, Yokoi K, Chen L, Ichihara-Tanaka K, Kimura T, Muramatsu T. Midkine as a factor to counteract the deposition of amyloid -peptide plaques: in vitro analysis and examination in knockout mice. Int Arch Med 2011;4:1.
27. Muramatsu T. Midkine: a promising molecule for drug development to treat diseases of the central nervous system. Curr Pharm Des 2011;17(5):410-23.
28. Hobo A, Yuzawa Y, Kosugi T.The growth factor midkine regulates the renin-angiotensin system in mice. J Clin Invest 2009; 119: 1616–625.
29. Ezquerra L, Herradon G, Nguyen T, Silos-Santiago I, Deuel TF. Midkine, a newly discovered regulator of the renin-angiotensin pathway in mouse aorta: significance of the pleiotrophin/midkine developmental gene family in angiotensin II signaling. Biochem Biophys Res Commun 2005; 333:636–43.
30. Malyszko J, Bachorzewska-Gajewska H, Koc-Zorawska E, Malyszko JS, Kobus G, Dobrzycki S. Midkine: a novel and early biomarker of contrast-induced acute kidney injury in patients undergoing percutaneous coronary interventions. Biomed Res Int. 2015:879509. doi: 10.1155/2015/879509.
31. Diniz BS, Yassuda MS, Nunes PV, Radanovic M, Forlenza OV. Mini-mental State Examination performance in mildcognitive impairment subtypes. Int Psychogeriatr 2007;19: 647–56.