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CORRELATION BETWEEN SERUM ACTIVITY OF LIVER ENZYMES AND COGNITIVE IMPAIRMENT IN PATIENTS WITH ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA

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Background: Recent research attempts to bring metabolic changes that evolve with aging into the context of neurodegenerative diseases, including dementia. Peripheral levels of liver enzymes may therefore play a central role in explaining the metabolic dysfunctions in Alzheimer's disease (AD) and vascular dementia (VD).

Aim: The aim of this study was to determine the correlation between serum activity of creatine kinase and liver enzymes (ALT, AST, LDH, GGT and ALP) and cognitive impairment in AD and VD. Methods: The subjects were classified as AD patients (n=30), VD patients (n=30) and controls (n=30). Further on, cognitive functions of all subjects were assessed using the Montreal - Cognitive Assessment (MoCA) test.

Results: The obtained results showed significantly lower ALT activity in AD and VD patients compared to controls. The MoCA scores were found to be in positive correlation with serum CK and GGT activity in AD patients. Further research is required to elucidate causal pathways underlying the association between serum liver parameters and the pathophysiology of dementia.

Keywords: Alzheimer's disease, liver enzymes, creatine kinase, cognitive impairment, vascular dementia

INTRODUCTION

Current research firmly proposes a dynamic, multifactorial etiology of dementia. However, cognitive deterioration is thought to be primarily caused by inflammation and oxidative stress processes [1]. Alzheimer's disease (AD), the most common form of dementia, is thus associated with neurodegeneration resulting from astrogliosis, microglial cell proliferation, amyloid plaques, and neurofibrillary tangles [2]. Considering the direct link between aging and the incidence of dementia, in recent years research has been more comprehensive in terms of including the biochemical changes that evolve with years and influence the course of AD. Cerebrovascular changes, disturbed energy metabolism, insulin resistance, high cholesterol levels have been identified as some of the risk factors leading to cognitive impairment [3]. This evidence highlights the importance of liver as one of the centers of metabolic activity and its possible role in the pathophysiology of dementia. Bassendine et al. [4] presented evidence suggesting that the liver is the origin of brain Aβ deposits and that it is involved in peripheral clearance of circulating Aβ in the blood. The authors conclude that dysfunction of peripheral Aβ clearance may contribute to the development of AD, given that the efflux of Aβ to peripheral blood accounts for 50% of total brain Aβ clearance [5]. Therefore, hepatic functionality should be considered when Aβ balance is addressed. Until today, there are rather a few studies bridging peripheral biomarkers of hepatic functioning with central biomarkers related to dementia. Compared to the transaminases where findings point to decreased alanin-aminotransferase (ALT) activity in AD patients, gamma-glutamyltransferase (GGT) has been more extensively investigated and the majority of results confirmed a linear relationship between GGT with the risk of developing AD and vascular dementia (VD), independent of other potential cofounders [6-8]. Even though alkaline phosphatase (ALP) influences developmental plasticity and activity-dependent cortical functions, reports associating the serum ALP activity to dementia are only scarce. The same is valid for creatine kinase (CK) and lactate dehydrogenase (LDH). Hense the aim of this study was to determine correlation between serum activity of liver enzymes and cognitive impairment in patients with AD and VD.

MATERIALS AND METHODS

Study population

A total of 90 female subjects aged ≥ 65 were enrolled in this controlled, cross-sectional study. The subjects were classified as AD patients (n=30), VD patients (n=30) and controls (n=30). The patients were recruited

from a specialized unit at the Health-Care Hospice for persons with disabilities in Sarajevo, Bosnia and Herzegovina. The study was conducted under the approval of the local research Ethics Committee, protocol number 0305-28838. All procedures on human subjects were performed in the accordance with Helsinki Declaration of 2013. Informed consent was obtained from subjects and caregivers upon careful explanation of the study procedure.

Sample collection and measurements

Baseline demographic and health factors

Prior to the sample collection, medical history was obtained from all subjects and laboratory and clinical examinations were performed. In addition, blood pressure was measured and the body mass index (BMI: kg/m2) calculated for all participants. The study excluded subjects with a history of chronic inflammatory disease (asthma and rheumatoid arthritis), hepatic or renal insufficiency and cancer.

Clinical diagnosis and cognitive function evaluation

Subjects diagnosed with AD and VD met the NINCDS-ADRDA [9] and NINDS-AIREN criteria [10], respectively. The Hachinski ischemic score (HIS) was used to distinguish between AD and VD patients. The HIS scale is as follows: score 0-4 implies Alzheimer's dementia, 4-7 implies mixed dementia and a score ≥ 7 implies vascular dementia [11]. The AD patients had a score ≤ 4, while the VD patients had a score ≥ 7. For cognitive assessment, all participants underwent the Montreal Cognitive Assessment (MoCA) [12]. All subjects in the AD and VD groups had a score ≤ 20, while the control group (CG) subjects had a score between 27 and 30.

Blood sample collection

Peripheral blood samples were collected after overnight fast from all participants of the study. Venous blood was drawn from the median cubital vein, collected in serum vacutainers, allowed to coagulate and centrifuged (5 min, 2000 g). The serum samples were stored and frozen at -80 °C until assayed.

Measurement of serum enzyme activity

The measurements were performed at the Clinical Centre of University of Sarajevo, Laboratory for clinical chemistry and biochemistry. Serum activity of creatine kinase and liver enzymes were measured by the Dimension RXL analyzer (Dade Behring). The results were expressed as U/L.

Statistical analysis

All statistical calculations were performed with the SPSS 16 software (version 16.0, SPSS Inc, Chicago, Illinois, USA). The distribution of variables was tested by the Shapiro-Wilk test. Values with normal distribution were expressed as mean ± standard deviation, while those without normal distribution were shown as median and interquartile range. Additionally, since variables were not normally distributed, a comparison between the groups was performed by Kruskal–Wallis test followed by Mann-Whitney U-test. Additionally, correlations were assessed by Spearman's test. Statistical significance was set at $p < 0.05$.

RESULTS

The lowest ALT activity was in the group of AD patients [6,00 U/L (4,00-10,25 U/L)]. The ALT activity in AD patients was significantly lower compared to both VD patients [9.00 UI/L (6.75-14.25)] (p=0.049) and controls [11.00 UI/L (7.5-18.75)] (p=0.002). The activity of all other enzymes did not differ significantly among the investigated groups (Table 2).

Table 1. Serum enzyme activity of AD and VD patients and the control subjects

Variables	AD group (n=30)	VD group (n=30)	CG (n=30)
AST	15.00 U/L (12.00-17.25)	14.0 UI/L (12.00-16.00)	14.00 U/L (12.75-19.25)
ALT	6.00 U/L (4.00-10.25) *	9.00 UI/L (6.75-14.25) #	11.00 UI/L (7.5-18.75)
ALP	102.5 U/L (70.75-131.25)	76.0 UI/L (67.75-100.25)	84.0 UI/L (63.0-116.25)
LDH	157.5 U/L (140.75-180.5)	181.5 UI/L (143.5-209.5)	177.5 UI/L (159.75-189.5)
GGT	16.5 U/L (12.0-21.5)	19.0 UI/L (13.75-25.25)	19.5 UI/L (15.0-27.25)
CK	26.5 U/L (19.5-68.0)	30.5 U/L (13.75-61.25)	53.5 U/L (35.75-72.5)

**Significant difference between AD and CG p=0.002*
#Significant difference between AD and VD p=0.049

The MoCA score was significantly lower in dementia patents compared to controls (p<0,001) (Table 2). The results describing the correlation between enzyme activity and MoCA scores are presented in Table 3.

Table 2. Cognitive assessment scores in dementia patients and control subjects.

Variables	AD group (n=30)	VD group (n=30)	CG (n=30)	p
MoCA Montreal - Cognitive Assessment (score)	9.00 (7.00-12.75)	12.5 (7.00-19.25)	28.00 (27.00-29.00)	<0.001

MoCA scores were in positive correlation with the activity of CK (Rho= 0.388; p<0.05) in patients with AD. The same is true for GGT (Rho= 0.408; p<0.05) (Table 3). The correlation of all remaining enzymes and cognitive assessment scores were not significant in all of the investigated groups.

Table 3. Correlation between enzymes' activities and MoCA scores

Variables	Montreal - Cognitive Assessment (score)		
	CG (n=30)	AD group (n=30)	VD group (n=30)
AST (U/L)	Rho= 0.121	Rho= 0.320	Rho= 0.243
ALT (U/L)	Rho= 0.161	Rho= 0.130	Rho= 0.022
CK (U/L)	Rho= 0.051	Rho= 0.388 *	Rho= 0.219
LDH (U/L)	Rho= -0.021	Rho= 0.215	Rho= -0.086
GGT (U/L)	Rho= 0.099	Rho= 0.408 *	Rho= 0.113
ALP (U/L)	Rho= -0.176	Rho= 0.129	Rho= 0.285

**p<0.05*

DISCUSSION

With aging, major organ systems begin to change in size, functionality and morphology. The liver has a central role in metabolism being responsible for protein synthesis, detoxication, lipid metabolism and secretion of acute phase proteins in response to inflammation [13]. In the elderly population impaired liver functions are common and some of the consequently altered biochemical parameters have been shown to be associated with the susceptibility to developing dementia. Circulating levels of GGT, AST and ALT are routinely used in clinical practice as biomarkers of hepatic or biliary disease. Recent research has placed hepatic enzymes in the context of cognitive impairment, with a large focus on GGT. Many authors have reported a link between increased serum GGT activity and dementia [6-8, 13-15]. The results of two studies regarding the type of dementia are in agreement. One of the studies reported the link between GGT and dementia to be more apparent in those diagnosed with VD [15], while according to the other study [6] the increased risk of developing VD according to GGT concentration was greater compared to AD risk. These results are not surprising, given the substantial implication of GGT in vascular and cardiometabolic diseases [16]. Elevated serum GGT activity might contribute to the development of dementia via its pro-inflammatory and pro-oxidant properties [17]. Being co-localized with oxLDL and CD68+ foam cells, the catalytically active GGT triggers pro-oxidative reactions within the atherosclerotic plaque, impacting its progression and vulnerability [17, 18], which is of particular importance to vascular health that is clearly compromised in VD. Further on, GGT is responsible for the catabolism of the antioxidant glutathione, whereby reactive oxygen species (ROS) are generated, contributing to neurodegenerative diseases such as dementia [19]. Kamada et al. [20] have investigated the effects of plasma transaminase activity on memory functions in healthy subjects. Transaminases play a central role in amino acid metabolism and in the case of ALT and AST, the end product of the reactions they catalyse - glutamate. Not only do the peripheral levels or glutamate positively correlate with glutamate levels in the cerebrospinal fluid (CSF) but also influence the cognitive functions. One of the mechanisms by which glutamate affects memory function is through the induction of long-term potentiation of synaptic strength [21]. The study was limited to healthy subjects and did not assess other metabolic factors but the authors reported that both plasma transaminases and glutamate levels were negatively correlated with memory functions. A more comprehensive study that included AD patients was conducted by Nho et al. [22]. Decreased levels of ALT and elevated AST to ALT ratio that were observed in patients with AD were associated with poor cognition and reduced brain glucose metabolism. Decreased activity of ALT was associated with disturbed energy metabolism, greater amyloid-β deposition and structural atrophy. Vasantharekha et al. [23] who aimed to investigate the interrelationship between MMSE scores and liver enzymes also reported a decline in serum ALT activity in AD patients compared with the adults, old controls and patients with mild cognitive impairment. To the best of our knowledge there are no studies describing the role and alterations in LDH activity related to dementia. The few studies that evaluated ALP from the same perspective presented conflicting findings. Nho et al. [22] observed poor cognition that was associated to higher ALP activity. On the other hand, Vasantharekha et al. [23] reported a decline in serum ALP activity in old men with AD compared with the adults, and in old women with mild cognitive impairment (MCI) and AD compared with the adults and old controls. In addition, a positive correlation was found between MMSE scores and ALP activity in patients with AD that might be indicative of central neuron loss. The activity of CK is critical for CNS function as ATP production regulates the cerebral metabolic rate. A loss of CK-BB activity therefore implicates a perturbed cellular energy state, insufficient energy supply in neurons and impaired neurotransmission processes. Any disturbance of this enzyme may exasperate the AD disease process [24]. Despite the large evidence suggesting an important role of GGT in the development dementia, the serum values in both groups of dementia patients of this study did not significantly differ from control subjects. Our primary finding was that there were minimal effects and no strong connections between serum levels of GGT, cognitive improvement levels and rates in the age-based model. Our findings suggest that GGT serum levels are not only related to those cognitive realms but rather are all-embracing and influence cognitive output and transition in general.

There are obvious limitations that must be considered. Some are related to the restricted sample size, but the major concern relates to the fact that we lack information on GGT levels earlier in life. That prevents us from drawing firm conclusions regarding causality.

CONCLUSIONS

1. This cross-sectional study has revealed that the serum concentration of ALT was significantly lower in female AD and VD patients compared to the control group.
2. The assessment of cognition evaluated by MoCA scores was in positive correlation with serum CK and GGT activity in patients with AD.
3. Current literature evidence suggests that changes in hepatic enzymes activity reflect the pathophysiology of dementia, however some of the results are conflicting and the mechanistic explanations require further, larger studies excluding confounding factors.

REFERENCES

1. Gackowski D, Rozalski R, Siomek A, Dziaman T, Nicpon K, Klimarczyk M, Araszkiewicz A, Olinski R. Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. J Neurol Sci. 2008 Mar 15;266(1-2):57-62. doi: 10.1016/j.jns.2007.08.041. Epub 2007 Sep 20. PMID: 17888453.
2. Brici D, Götz J, Nisbet RM. A Novel Antibody Targeting Tau Phosphorylated at Serine 235 Detects Neurofibrillary Tangles. J Alzheimers Dis. 2018;61(3):899-905. doi: 10.3233/JAD-170610. PMID: 29332046.
3. Ulusu NN. Glucose-6-phosphate dehydrogenase deficiency and Alzheimer's disease: Partners in crime? The hypothesis. Med Hypotheses. 2015 Aug;85(2):219-23. doi: 10.1016/j.mehy.2015.05.006. Epub 2015 May 11. PMID: 26004559.
4. Bassendine MF, Taylor-Robinson SD, Fertleman M, Khan M, Neely D. Is Alzheimer's Disease a Liver Disease of the Brain? J Alzheimers Dis. 2020;75(1):1-14. doi: 10.3233/JAD-190848. PMID: 32250293; PMCID: PMC7306895.
5. Roberts KF, Elbert DL, Kasten TP, Patterson BW, Sigurdson WC, Connors RE, Ovod V, Munsell LY, Mawuenyega KG, Miller-Thomas MM, Moran CJ, Cross DT 3rd, Derdeyn CP, Bateman RJ. Amyloid-β efflux from the central nervous system into the plasma. Ann Neurol. 2014 Dec;76(6):837-44. doi: 10.1002/ana.24270. Epub 2014 Oct 24. PMID: 25205593; PMCID: PMC4355962.
6. Han E, Lee JY, Han KD, Cho H, Kim KJ, Lee BW, Kang ES, Cha BS, Younossi ZM, Lee YH. Gamma glutamyltransferase and risk of dementia in prediabetes and diabetes. Sci Rep. 2020 Apr 22;10(1):6800. doi: 10.1038/s41598-020-63803-0. PMID: 32322005; PMCID: PMC7176727.
7. Hong SH, Han K, Park S, Kim SM, Kim NH, Choi KM, Baik SH, Park YG, Yoo HJ. Gamma-Glutamyl Transferase Variability and Risk of Dementia in Diabetes Mellitus: A Nationwide Population-Based Study. J Clin Endocrinol Metab. 2020 Mar 1;105(3):dgaa019. doi: 10.1210/clinem/dgaa019. PMID: 31955208.
8. Kunutsor SK, Laukkanen JA. Gamma glutamyltransferase and risk of future dementia in middle-aged to older Finnish men: A new prospective cohort study. Alzheimers Dement. 2016 Sep;12(9):931-941. doi: 10.1016/j.jalz.2016.03.003. Epub 2016 Apr 18. PMID: 27103259.
9. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984 Jul;34(7):939-44. doi: 10.1212/wnl.34.7.939. PMID: 6610841.
10. Román GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology. 1993 Feb;43(2):250-60. doi: 10.1212/wnl.43.2.250. PMID: 8094895.
11. Loncarević N, Mehmedika-Sulić E, Alajbegović A, Kucukalić A. Uloga neurologa u dijagnostici i terapiji azheimerove bolesti [The neurologist role in diagnostics and therapy of the Alzheimer's disease]. Med Arh. 2005;59(2):106-9. Bosnian. PMID: 15875473.
12. Montreal Cognitive Assessment. URL: https://www.mocatest.org/ (Accessed: February, 2020)
13. Kunutsor SK. Gamma-glutamyltransferase-friend or foe within? Liver Int. 2016 Dec;36(12):1723-1734. doi: 10.1111/liv.13221. Epub 2016 Aug 31. PMID: 27512925.
14. Yavuz BB, Yavuz B, Halil M, Cankurtaran M, Ulger Z, Cankurtaran ES, Aytemir K, Ariogul S. Serum elevated gamma glutamyltransferase levels may be a marker for oxidative stress in Alzheimer's disease. Int Psychogeriatr. 2008 Aug;20(4):815-23. doi: 10.1017/S1041610208006790. Epub 2008 Apr 17. PMID: 18416873.
15. Praetorius Björk M, Johansson B. Gamma-Glutamyltransferase (GGT) as a biomarker of cognitive decline at the end of life: contrasting age and time to death trajectories. Int Psychogeriatr. 2018 Jul;30(7):981-990. doi: 10.1017/S1041610217002393. Epub 2017 Nov 7. PMID: 29108523.

16. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: a meta-analysis of prospective cohort studies. Atherosclerosis. 2014 Sep;236(1):7-17. doi: 10.1016/j.atherosclerosis.2014.06.006. Epub 2014 Jun 23. PMID: 24998934.

17. Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation. 2005 Oct 4;112(14):2078-80. doi: 10.1161/CIRCULATIONAHA.105.571919. PMID: 16203922.

18. Paolicchi A, Emdin M, Ghliozeni E, Ciancia E, Passino C, Popoff G, Pompella A. Images in cardiovascular medicine. Human atherosclerotic plaques contain gamma-glutamyl transpeptidase enzyme activity. Circulation. 2004 Mar 23;109(11):1440. doi: 10.1161/01.CIR.0000120558.41356.E6. PMID: 15037540.

19. Perry G, Nunomura A, Hirai K, Zhu X, Pérez M, Avila J, Castellani RJ, Atwood CS, Aliev G, Sayre LM, Takeda A, Smith MA. Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radic Biol Med. 2002 Dec 1;33(11):1475-9. doi: 10.1016/s0891-5849(02)01113-9. PMID: 12446204.

20. Kamada Y, Hashimoto R, Yamamori H, Yasuda Y, Takehara T, Fujita Y, Hashimoto K, Miyoshi E. Impact of plasma transaminase levels on the peripheral blood glutamate levels and memory functions in healthy subjects. BBA Clin. 2016 Feb 23;5:101-7. doi: 10.1016/j.bbacli.2016.02.004. PMID: 27051595; PMCID: PMC4802405.

21. Lisman J, Yasuda R, Raghavachari S. Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci. 2012 Feb 15;13(3):169-82. doi: 10.1038/nrn3192. PMID: 22334212; PMCID: PMC4050655.

22. Nho K, Kueider-Paisley A, Ahmad S, MahmoudianDehkordi S, Arnold M, Risacher SL, Louie G, Blach C, Baillie R, Han X, Kastenmüller G, Trojanowski JQ, Shaw LM, Weiner MW, Doraiswamy PM, van Duijn C, Saykin AJ, Kaddurah-Daouk R; Alzheimer's Disease Neuroimaging Initiative and the Alzheimer Disease Metabolomics Consortium. Association of Altered Liver Enzymes With Alzheimer Disease Diagnosis, Cognition, Neuroimaging Measures, and Cerebrospinal Fluid Biomarkers. JAMA Netw Open. 2019 Jul 3;2(7):e197978. doi: 10.1001/jamanetworkopen.2019.7978. PMID: 31365104; PMCID: PMC6669786.

23. Vasantharekha R, Priyanka HP, Swarnalingam T, Srinivasan AV, ThyagaRajan S. Interrelationship between Mini-Mental State Examination scores and biochemical parameters in patients with mild cognitive impairment and Alzheimer's disease. Geriatr Gerontol Int. 2017 Oct;17(10):1737-1745. doi: 10.1111/ggi.12957. Epub 2016 Dec 6. PMID: 27921357.

24. Bürklen TS, Schlattner U, Homayouni R, Gough K, Rak M, Szeghalmi A, Wallimann T. The creatine kinase/creatine connection to Alzheimer's disease: CK-inactivation, APP-CK complexes and focal creatine deposits. J Biomed Biotechnol. 2006;2006(3):35936. doi: 10.1155/JBB/2006/35936. PMID: 17047305; PMCID: PMC1510941.

25. Brayne C, Calloway P. Serum creatine kinase BB isoenzyme levels in an epidemiological study of dementia and cognitive impairment. Acta Psychiatr Scand. 1990 Jan;81(1):6-8. doi: 10.1111/j.1600-0447.1990.tb06440.x. PMID: 2330830.