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Klotho gene and protein expression status in blood of Alzheimer's patients treated with blood pressure or lipid controlling drugs

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Abstract

Background and Objective: Alzheimer's disease (AD) as the most common neurodegenerative disorder. Klotho is an anti-aging protein with important roles in neurodegenerative disorders. This study was done to evaluate the expression of klotho gene and protein in the plasma of AD patients treated with blood pressure control drugs (inhibitors of angiotensin-converting enzyme (ACE)) or blood lipids control drug (simvastatin).

Materials and Methods: Target population was selected from people with AD who visited the neurology clinic of Firouzgar hospital. The tested groups included the control group, Alzheimer's group, Alzheimer's group treated with blood pressure control drugs, and Alzheimer's patients group treated with blood lipid control drug. Expression of klotho gene and protein in the plasma of studied groups was determined using real-time PCR and ELISA techniques and the individual's cognitive disorders were also evaluated using Mini-Mental State Exam (MMSE) and Clinical Dementia Rating (CDR) tests.

Results: The results obtained in this study showed that in addition to the significant difference in cognitive indices between the control groups and three groups of Alzheimer's patients, the level of klotho gene and protein expression was also lower in three groups of Alzheimer's patients compared to healthy group. However, there was no significant difference ($p>0.05$) between the Alzheimer's group and the two Alzheimer's groups treated with blood pressure or blood lipid control drugs.

Conclusion: Drugs controlling blood pressure or blood lipids in Alzheimer's patients possibly have no significant effect on the level of klotho protein. Obviously, more studies are needed in this field.

Keywords: Alzheimer's disease, Klotho, Angiotensin-converting enzyme, Blood lipids, Simvastatin

1. Introduction

ALzheimer's disease (AD) is pathologically described by the presence of amyloid β (Aβ)-containing plaques and tau neurofibrillary tangles. AD is regarded as a genetic and sporadic neurodegenerative disorder that is associated with cognitive impairment in its prototypical picture and with non-amnestic cognitive decline in its less common forms. Although AD is considered as a common cause of cognitive deficits observed in late-life, but its clinical impact is affected by other neurodegenerative and cerebrovascular accidents. AD is a brain disorder that results from a complex interaction of synaptic homeostasis, aggregated species, and modified products of Aβ and tau. Therapeutic options are still searching to find targets within this framework to significantly alter clinical course of patients with AD

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(1, 2).
Dementia is defined by overall cognitive impairment with an acquired decline in cognitive and emotional functions besides behavioral disturbances which severely interferes with daily life. It is regarded as one of the most common clinical syndromes which is observed in old age individuals (3). It has a significant effect on performing daily life activities, leading to great suffering, care dependence, and a decrease in life quality (4-6). Several studies have indicated that klotho overexpression can be of benefit in neurodegenerative conditions (7, 8). The role of klotho is through decreasing neuronal oxidative stress, exerting neuroprotection, and even neutralizing neurodegeneration (9). Klotho is involved in calcium transport in the central nervous system (CNS) through the choroid plexus and this fact explains the strong association between Klotho concentration and risk of cognitive decline (9, 10).
Klotho is a membrane-bound and anti-aging protein that acts as a hormone targeting different tissue cells (11). There exist two forms of klotho in humans, one form as a full-length membrane form linked to fibroblast growth factor receptors (FGFRs) and with an active role in phosphate and calcium homeostasis, and another soluble circulating form which is found in some bodily fluids such as blood, urine, and cerebrospinal fluid (CSF) and which regulates ion channels and transporters and growth factors signaling (12). Klotho gene is highly expressed in kidneys, where it regulates renal function, choroid plexus, and parathyroid glands (9, 13). Klotho pathophysiologically plays an important function in calcium and phosphate metabolism, remyelination process, cognitive functions, and even inflammatory events (11, 14). In human beings, serum concentration of this protein (klotho) falls with age. Klotho exhibits anti-aging properties with important roles in cognitive processes. In this respect, klotho is associated with changes in synaptic structures in the hippocampus and cortex, leading to slower rate of cognitive deficits (15-17).
Hypertension is one of the most important and modifiable risk factors in association with development of AD and targeting hypertension has a considerable effect on lowering AD incidence in the elderly. Hypertension in elderly life is associated with cerebral blood flow dysfunction, neuronal disturbances, and substantial decline in cognitive performance (18). Hyperlipidemia causes disorders such as cardiovascular diseases, metabolic syndrome, and even AD. Accordingly, administration of drugs designed specifically for targeting hypertension and hyperlipidemia are strongly recommended to lower risk of risk factors linked to AD pathogenesis (19, 20).
This research study was undertaken to evaluate the expression of klotho gene and protein in the plasma of AD patients treated with blood pressure control drugs (inhibitors of angiotensin-converting enzyme (ACE)) or blood lipids control drug (simvastatin).

2. Materials and Methods

This study was a clinical trial study (case-control design) related to Iranian Alzheimer's disease Registry of data on AD patients from outpatient neurology clinics and hospitals in Tehran
city (Iran) that was approved by the Medical Research Ethics Committee (R.IUMS-REC.1396.32623) and informed consent was obtained from the patients participating in the study. Data were collected from these centers between September 2018 and December 2021. Subjects were entered into this investigation if they were diagnosed with AD by a neurologist and/or psychiatrist based on cognitive tests and clinical examination. The target population of this study was selected from people with AD and with a minimum age of 65 and a maximum age of 80 who visited the neurology clinic of Firouzgar Hospital. The selection of these patients was based on medical record information. The groups of this study included the control group, Alzheimer's patients group , Alzheimer's patients group treated with blood pressure control drugs (ACE inhibitors), and Alzheimer's patients group treated with blood lipid control drug (simvastatin). In the control group, there were people who did not have any signs of neurodegenerative diseases and were in the age range of 65 to 80 years. The expression of the klotho gene and protein in the plasma of the studied groups was determined using real-time PCR and ELISA techniques and the individual's cognitive disorders were also evaluated using Mini-Mental State Exam (MMSE) and Clinical Dementia Rating (CDR) tests.
MMSE questionnaire was used to assess cognitive status. This questionnaire is usually used as a practical test to assess cognitive functions. This questionnaire, prepared by Folstein et al in 1975, consists of categories of orientation to time, orientation to place, repetition and registration, attention and calculation, recall, repetition, naming, comprehension, reading, writing, and so like (21). It is a practical and useful method to grade cognitive ability of affected patients by the clinician. The maximum total score is 30; a score of 27-30, 20-26, 10-19, and less than 10 is regarded as normal, mild, moderate, and severe AD, respectively. This questionnaire used in our study has been previously standardized (22).
CDR test is a validated instrument in clinical studies for assessment of AD spectrum, which evaluates three domains of cognition (memory, orientation, and judgment/problem solving) and three domains of function (community affairs, home/hobbies, and personal care) using structured interviews. The scores for the six domains (ranging from 0 to 3) tested can be summed (CDR Sum of Boxes or CDR-SB) (23)
Inclusion criteria for this study were male or female cases, age between 65 and 80 years, and normal or corrected normal ability to see and hear. Exclusion

criteria for this study included depression, any other cause of dementia for AD patients (vascular dementia), Any symptoms of disease or abnormalities sufficient to cause memory impairment other than AD (eg. normal pressure hydrocephalus, progressive supranuclear palsy), stroke history, major structural abnormalities on MRI (e.g. infarction, intracerebral malformation), history of seizures, epilepsy, a serious infectious disease affecting the brain in the past, and serious head injury. For control group, the criteria were normal MRI/CT and cognitively normal based on history and psychometric tests and no history of neurodegeneration. In addition, case and control groups were matched regarding age and gender.

Peripheral blood samples were collected (5 ml) from each patient into EDTA-containing tubes. RNA Isolation and cDNA Synthesis was then done. Quantitative Real-Time RT-PCR was done to assess klotho gene expression using Step One Plus real-time PCR system (Applied Biosystems, USA) and SYBR Green PCR mastermix kit (Applied Biosystems, USA). GAPDH housekeeping gene expression was also used as a reference for the level of target gene expression. Klotho gene was amplified using forward primer sequence 5′-CACGGCAAGGGTGCGTCCAT-3′ and reverse primer sequence 5′-TCGCGCCCACGAGATGGAGA-3′. GAPDH gene was amplified using forward primer sequence 5′-CTCATGACCACAGTCCATGC-3′ and reverse primer sequence 5′-

TTCAGCTCTGGGATGACCTT-3′. Klotho Elisa kit was also used to measure its plasma level.
For statistical analysis, GraphPad Prism software version 7 and one-way ANOVA and Mann-Whitney tests were used with $p<0.05$ as significant.

3. Results

Measurement of serum level of creatinine (Fig. 1A) and BUN (Fig. 1B) in different groups indicated that administration of SAC at a dose of 100 mg/kg to the control animals is not associated with significant and marked changes of BUN and creatinine ($p>0.05$). In addition, CCL4 group had higher levels of creatinine ($p<0.01$) and BUN ($p<0.001$) at a significant level versus the control group. Such significant increase was also observed at a lower level in CCL4 group treated with SACA at a dose of 25 mg/kg for creatinine ($p<0.05$) and BUN ($p<0.001$) as compared to the CCL4 group. In contrast, CCL4 group receiving SAC at a dose of 100 mg/kg had lower level of creatinine ($p>0.05$) and BUN ($p<0.05$) versus the CCL4 group.

Table 1: Demographic data of used cases in different groups

	Alzheimer's patients treated for hypertension (Alz+ACEi) (n=10)	Alzheimer's patients treated for hyperlipidemia (Alz+Simvas) (n=12)	Alzheimer's patients without hypertension and hyperlipidemia (Alz.) (n=18)	Control (Normal) (n=12)	P value
Age	65-80	65-80	65-80	65-80	
Sex	women 30%	women 49%	women 52%	women 57%	
	men 70%	men 51%	men 48%	men 43%	
Mean MMSE score	14.11±6.7*	18±2.4*	16.23±4.7*	29.3±2.3	*P<0.05
Clinical dementia rating (CDR)	1.25±0.22*	1.43±0.8*	1.21±0.56*	0.23±0.1	*P<0.05

Measurement of plasma level of klotho in different groups including control group and three groups of Alzheimer's patients without hypertension and hyperlipidemia, treated for hyperlipidemia (simvastatin), or treated for hypertension (ACE inhibitors) employing sandwich ELISA method indicated no significant differences amongst the studied group. However, plasma level of klotho showed non-significant reduction in our three Alzheimer's groups in comparison with the control groups (Figure 1).

Measurement of gene expression level of klotho in different groups including control group and three groups of Alzheimer's patients without hypertension and hyperlipidemia, treated for hyperlipidemia (simvastatin), or treated for hypertension (ACE inhibitors) employing RT-PCR method showed significant and robust reduction in our three Alzheimer's groups in comparison with the control groups ($p<0.01$). However, there was no significant differences between the three AD groups regarding peripheral gene expression of klotho (Figure 2).

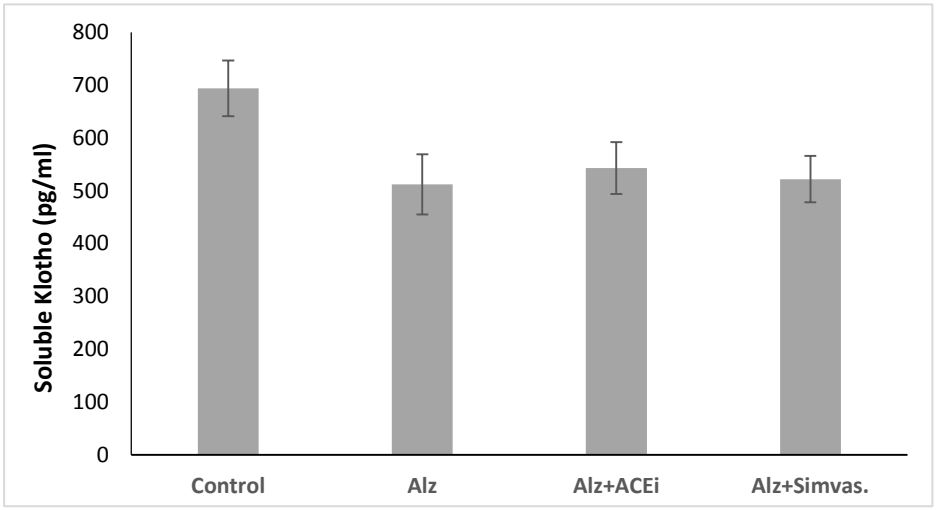

Fig. 1. Plasma level of soluble klotho in diffferent groups

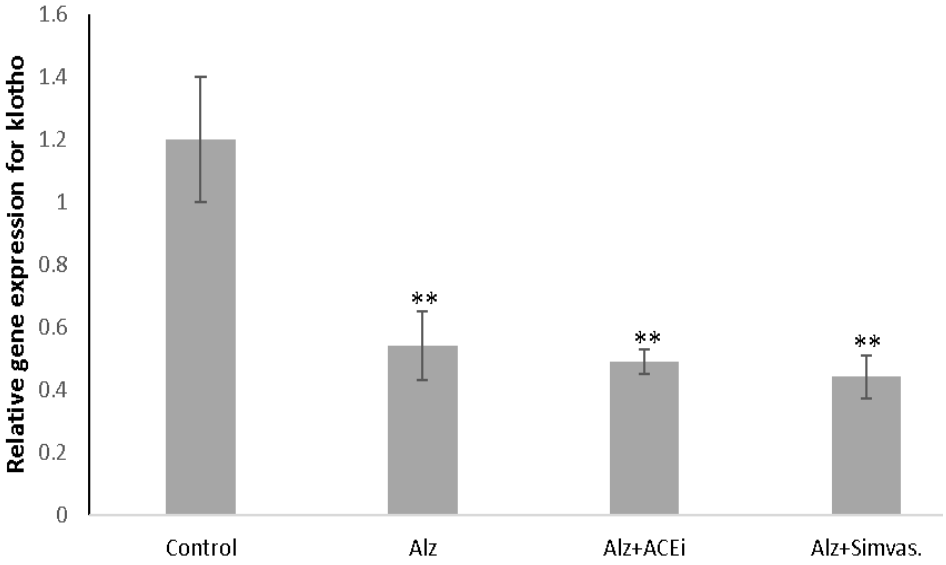

Fig. 2: Gene expression level of klotho in different groups

** p<0.01 (as compared to the control group)

4. Discussion

Alzheimer's disease (AD) is a common neurodegenerative disorder and as the most frequent causative factor of dementia in the world. According to World Health Organization (WHO), prevalence of AD is increasing. It has been estimated that over 50 million people are globally affected with AD and senile dementia (24, 25). AD was initially related to memory deficit, but with disease progression, AD patients develop cognitive impairment, impulsive or unpredictable behavioral difficulties and unusual personality changes (26, 27). Pathologically, AD is typified by neuroinflammation, amyloid-beta (Aβ) plaque, and deposition of neurofibrillary tangle (NFT) which are associated with progressive cognitive deficit of varying degrees and neurodegeneration (28). Over the past years, amyloid beta cascade and tau hypotheses have been the two most known hypotheses for explaining AD pathogenesis process (29-31). Aside from these past hypotheses, researchers are also working on novel theories to explain AD etiology and its pathogenesis (28, 32). Some of these new theories include gamma oscillations, lysosomal deficit, and calcium dysregulation which may be leading to presentation of safe and effective therapy for AD (26, 33).

New research evidence indicate that Klotho protein may be regarded as an appropriate anti-AD target. Klotho is known as a protein encoded by Klotho gene that is highly expressed in the kidney and brain choroid plexus (11, 34). Past studies demonstrated that klotho may be involved in regulating oxidative stress,

ER and Golgi apparatus stresses, apoptosis and even autophagy (11, 34). Klotho is also involved in various ageing-related pathologies cardiovascular disease, kidney disease, and neurodegenerative disorders (35). Klotho expression is reduced in ageing brains besides the brains from patients in the early stages of AD (32, 36, 37). Studies have shown that Klotho overexpression in the brain can prevent AD-related pathology and cognitive deficit and even reverse neuronal damage process. Klotho can alleviate amyloid beta accumulation through regulating different processes (32).

Treatment of neurons with klotho as a neuroprotective factor can protect against neuronal damage in relation to toxic effects of amyloid beta and glutamate and this may be beneficial in retarding pathogenesis rate of AD (38). Thus, enhancing klotho levels at the early phase of AD can be postulated as a therapeutic solution to slow down neuronal deterioration and to lessen the outcome of AD in elderly patients (38). In our study, plasma klotho level (non-significantly) and its gene expression (significantly) was lower in AD patients when compared to control (normal) individuals irrespective of their gender and this clearly suggest possible important role of klotho in pathogenesis of AD and this finding was in correlation of past studies irrespective of tested samples in humans and animals (17, 32, 39, 40). In this study, there was no significant difference between the Alzheimer's group and the two Alzheimer's groups treated with blood pressure or blood lipid control drugs. Although soluble klotho is a circulating factor with protective potential against the development of hypertension (41), however, its level did not change following using antihypertensive drugs, such as ACE inhibitors, as was used in this study.

It seems that according to the role of klotho protein in the aging process, this protein can play an important role in the occurrence of Alzheimer's disease. Drugs controlling blood pressure or blood lipids in Alzheimer's patients possibly have no significant effect on the level of this protein in plasma. Obviously, more studies are needed in this field.

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Ethical considerations

This research study was approved by Ethics Committee of Iran University of Medical Sciences (Tehran, Iran) (IR.IUMS.FMD.REC.1399.294).

References

1. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. Nature Reviews Disease Primers 2021;7(1):33.
2. Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & Dementia 2014;10(6):844-52.
3. Geldmacher DS, Whitehouse PJ. Evaluation of dementia. New England Journal of Medicine 1996;335(5):330-6.
4. Dimities M, Xristina F, Theodora-Elesa K, Maria C. The Impact of Exercising in the Quality of Life of People with Dementia-Alzheimer's Disease. Advances in Experimental Medicine and Biology 2021;1339: 309-15.
5. Iso-Markku P, Kujala UM, Knittle K, Polet J, Vuoksimaa E, Waller K. Physical activity as a protective factor for dementia and Alzheimer's disease: systematic review, meta-analysis and quality assessment of cohort and case-control studies. British Journal of Sports Medicine 2022;56(12):701-9.
6. Li J, Guo Y, Yang K, Zhang G, Huang K, Zhou J, et al. Rehabilitation effects of game therapy in people living with dementia: A systematic review and meta-analysis: Three formats of game therapy for Alzheimer's patients. Worldviews on Evidence-Based Nursing 2023;20(4):361-76.
7. Kuang X, Chen YS, Wang LF, Li YJ, Liu K, Zhang MX, et al. Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. Neurobiology of Aging 2014;35(1):169-78.
8. Sedighi M, Baluchnejadmojarad T, Fallah S, Moradi N, Afshin-Majd S, Roghani M. The Association Between Circulating Klotho and Dipeptidyl Peptidase-4 Activity and Inflammatory Cytokines in Elderly Patients With Alzheimer Disease. Basic and Clinical Neuroscience 2020;11(3):349-57.
9. Ananya FN, Ahammed MR, Lahori S, Parikh C, Lawrence JA, Sulachni F, et al. Neuroprotective Role of Klotho on Dementia. Cureus 2023;15(6):e40043.
10. Abraham CR, Mullen PC, Tucker-Zhou T, Chen CD, Zeldich E. Klotho Is a Neuroprotective and Cognition-Enhancing Protein. Vitamins and Hormones 2016;101:215-38.
11. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological Role of Anti-aging Protein Klotho. Journal of Lifestyle Medicine 2015;5(1):1-6.
12. Dalton GD, Xie J, An SW, Huang CL. New Insights into the Mechanism of Action of Soluble Klotho. Frontiers in Endocrinology 2017;8:323.

13. Brombo G, Bonetti F, Ortolani B, Morieri ML, Bosi C, Passaro A, et al. Lower Plasma Klotho Concentrations Are Associated with Vascular Dementia but Not Late-Onset Alzheimer's Disease. Gerontology 2018;64(5):414-21.

14. Torbus-Paluszczak M, Bartman W, Adamczyk-Sowa M. Klotho protein in neurodegenerative disorders. Neurological Sciences 2018;39(10):1677-82.

15. Castner SA, Gupta S, Wang D, Moreno AJ, Park C, Chen C, et al. Longevity factor klotho enhances cognition in aged nonhuman primates. Nature Aging 2023;3(8):931-7.

16. Sanz B, Arrieta H, Rezola-Pardo C, Fernández-Atutxa A, Garin-Balerdi J, Arizaga N, et al. Low serum klotho concentration is associated with worse cognition, psychological components of frailty, dependence, and falls in nursing home residents. Scientific Reports 2021;11(1):9098.

17. Zhao Y, Zeng CY, Li XH, Yang TT, Kuang X, Du JR. Klotho overexpression improves amyloid-β clearance and cognition in the APP/PS1 mouse model of Alzheimer's disease. Aging Cell 2020;19(10):e13239.

18. Yao Q, Jiang K, Lin F, Zhu T, Khan NH, Jiang E. Pathophysiological Association of Alzheimer's Disease and Hypertension: A Clinical Concern for Elderly Population. Clinical Interventions in Aging 2023;18:713-28.

19. Rai S, Bhatia V, Bhatnagar S. Drug repurposing for hyperlipidemia associated disorders: An integrative network biology and machine learning approach. Computational Biology and Chemistry 2021;92:107505.

20. Fan YC, Hsu JL, Tung HY, Chou CC, Bai CH. Increased dementia risk predominantly in diabetes mellitus rather than in hypertension or hyperlipidemia: a population-based cohort study. Alzheimer's Research & Therapy 2017;9(1):7.

21. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatric Research 1975;12(3):189-98.

22. Seyedian M, Falah M, Nourouzian M, Nejat S, Delavar A, Ghasemzadeh H. Validity of the Farsi version of mini-mental state examination. 2008.

23. Behzad M, Zirak N, Madani GH, Baidoo L, Rezaei A, Karbasi S, et al. CSF-Targeted Proteomics Indicate Amyloid-Beta Ratios in Patients with Alzheimer's Dementia Spectrum. International Journal of Alzheimer's Disease 2023;2023:5336273.

24. Bolós M, Perea JR, Avila J. Alzheimer's disease as an inflammatory disease. Biomolecular Concepts 2017;8(1):37-43.

25. Gaitán JM, Asthana S, Carlsson CM, Engelman CD, Johnson SC, Sager MA, et al. Circulating Klotho Is Higher in Cerebrospinal Fluid than Serum and Elevated Among KLOTHO Heterozygotes in a Cohort with Risk for Alzheimer's Disease. Journal of Alzheimer's Disease 2022;90(4):1557-69.

26. Bondi MW, Edmonds EC, Salmon DP. Alzheimer's Disease: Past, Present, and Future. Journal of the International Neuropsychological Society 2017;23(9-10):818-31.

27. Suzuki K, Iwata A, Iwatsubo T. The past, present, and future of disease-modifying therapies for Alzheimer's disease. Proceedings of the Japan Academy,Ser. B, Physical and Biological Sciences 2017;93(10):757-71.

28. Kocahan S, Doğan Z. Mechanisms of Alzheimer's Disease Pathogenesis and Prevention: The Brain, Neural Pathology, N-methyl-D-aspartate Receptors, Tau Protein and Other Risk Factors. Clinical Psychopharmacology and Neuroscience 2017;15(1):1-8.

29. Cummings JL, Osse AML, Kinney JW. Alzheimer's Disease: Novel Targets and Investigational Drugs for Disease Modification. Drugs 2023, in press..

30. Dave BP, Shah YB, Maheshwari KG, Mansuri KA, Prajapati BS, Postwala HI, et al. Pathophysiological Aspects and Therapeutic Armamentarium of Alzheimer's Disease: Recent Trends and Future Development. Cellular and Molecular Neurobiology 2023, in press.

31. Singh YP, Kumar N, Chauhan BS, Garg P. Carbamate as a potential anti-Alzheimer's pharmacophore: A review. Drug Development Research 2023, in press.

32. Fung TY, Iyaswamy A, Sreenivasmurthy SG, Krishnamoorthi S, Guan XJ, Zhu Z, et al. Klotho an Autophagy Stimulator as a Potential Therapeutic Target for Alzheimer's Disease: A Review. Biomedicines 2022;10(3).

33. Fan L, Mao C, Hu X, Zhang S, Yang Z, Hu Z, et al. New Insights Into the Pathogenesis of Alzheimer's Disease. Frontiers in Neurology 2019;10:1312.

34. Mytych J, Sołek P, Będzińska A, Rusinek K, Warzybok A, Tabęcka-Łonczyńska A, et al. Towards Age-Related Anti-Inflammatory Therapy: Klotho Suppresses Activation of ER and Golgi Stress Response in Senescent Monocytes. Cells 2020;9(2).

35. Prud'homme GJ, Kurt M, Wang Q. Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations. Frontiers in Aging 2022;3:931331.

36. Li D, Jing D, Liu Z, Chen Y, Huang F, Behnisch T. Enhanced Expression of Secreted α-Klotho in the Hippocampus Alters Nesting Behavior and Memory Formation in Mice. Frontiers in Cellular Neuroscience 2019;13:133.

37. Lin Y, Kuro-o M, Sun Z. Genetic deficiency of anti-aging gene klotho exacerbates early nephropathy in STZ-induced diabetes in male mice. Endocrinology 2013;154(10):3855-63.

38. Paroni G, Panza F, De Cosmo S, Greco A, Seripa D, Mazzoccoli G. Klotho at the Edge of Alzheimer's Disease and Senile Depression. Molecular Neurobiology 2019;56(3):1908-20.

39. Semba RD, Moghekar AR, Hu J, Sun K, Turner R, Ferrucci L, et al. Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. Neuroscience Letters 2014;558:37-40.

40. Zhu Y, Prata L, Gerdes EOW, Netto JME, Pirtskhalava T, Giorgadze N, et al. Orally-active, clinically-translatable senolytics restore α-Klotho in mice and humans. EBioMedicine 2022;77:103912.

41. Drew DA, Katz R, Kritchevsky S, Ix JH, Shlipak MG, Newman AB, et al. Soluble Klotho and Incident Hypertension. Clinical Journal of the American Society of Nephrology 2021;16(10):1502-11.