Article

Study The Role Of Functional Proteins In Sera Of Patients With Alzheimers Disease

* Ayat Mahmood Yahya and Rasha Zuhair Jasim

*Department of Scientific Affairs /Ibn Sina University of Medical and Pharmaceutical Sciences /

Baghdad / Iraq

**Department of Chemistry /College of Education for pure Science Ibn Al-Haitham /University of Baghdad / Baghdad / Iraq

rasha.z.j@ihcoedu.uobaghdad.edu.iq

Abstract:

The aim of the current study is to expose novel mechanisms and concepts regarding amyloid beta , albumin and hemoglobin in sera of Iraqi Alzheimer`s disease (AD) patients , and also to examine the biochemical action of memantine on those parameters. . Sixty (60) diagnosed AD patients participated the present study and classified into four (4) groups: G_3 (52-78) included (15) newly diagnosed males / without treatment , G_4 (64-90) years included (15) newly diagnosed females / without treatment , G_5 (60-85) years involved 15) males under treatment with memantine and G_6 (65-73) years involved (15) females also under treatment with memantine. Memantine used as 10 mg /one dose daily. Patients were compared to healthy subjects as two control groups: G_1 included (15) males /(52-80) years and G_2 involved (15) females / (64-82) years. Results of the present study have revealed that Aβ level was significantly increased in sera of G_3 and G_4 compared to G_1 and G_2 respectively, while it was significantly decreased in sera of G_5 and G_6 compared to G_3 and G_4 respectively, non significant difference was reported between (G_5 and G_1) and between (G_4 and G_3) , however the difference between G_6 and G_2 was significant. Albumin level was highly significant increased in G_3 and G_4 compared to G_1 and G_2 respectively while it was highly significant decreased in G_5 and G_6 compared to G_3 and G_4 respectively, the difference

between (G_5 and G_1), (G_6 and G_2) and between (G_3 and G_4)was significant. Hemoglobin level was highly significant decreased in G_3 and G_4 compared to G_1 and G_2 respectively while it was significantly increased in G_5 compared to G_3 and highly significant in G_6 compared to G_4 , the difference between (G_5 and G_1) and between (G_6 and G_2) was non significant but it was highly significant between (G_3 and G_4). The present study submits unique and definite mechanisms regarding Aβ, hemoglobin and albumin (before and after treatment with memantine) and exposes novel concepts to the clinical biochemistry in the term of the studied biochemical parameters. The present study also gives a hint to the difference between male and female AD patients regarding levels of the biochemical parameters before and after treatment.

Keywords: Alzheimer disease, Memantine , Amyloid beta peptide

Introduction:

Alzheimer disease (AD) , one of the most prevaalent neurodegenerative diseases [1,2] is featured by the presence of extracellular amyloid beta peptide (Aβ) in the brain [3] In accordance with amyloid hypothesis , accumulation of (Aβ) in the brain is the major biochemical feature of AD [1] regarding dementia , AD is regarded as the most prevalent type of dementia [1] and [4]. Memantine is an uncompetitive N-methyl-D aspartate (NMDA) receptor. Biochemically , it acts as inhibitor to N-methyl-D-aspartate [5] N-methyl-D-aspartate receptor is a ligand of glutamate , which is the pivotal neurotransmitter in human brain. Practically, amyloid beta peptide (Aβ) is cleaved from the more large precursor (amyloid precursor protein) (APP) which regarded as an integral membrane protein expressed in different tissues mainly in the synapses of neurons and play an essential role in the pathogenesis of AD. According to chemical structure , amyloid precursor protein consists of a single membrane spanning domain , along extracellular glycated N-terminus and shorter cytoplasmic C-terminus , APP is cut by β-secretase and ɣ-secretase producing a 37 - 49 amino acid residue peptide (amyloid beta) with (4 kDa) which is the major and crucial component of amyloid plaques deposited in brains of AD [6,7]. In accordance with amyloid hypothesis , accumulation of Aβ in the brain is the major cause of AD. Indeed, Aβ hypothesis is still the dominant theoretical perspective in AD. Remarkably , Aβ is intrinsically defected protein , but how it is transformed into the highly organized disordered fibrils is still unclear [1]. Hence , abnormal peptide aggregation is a biochemical feature of protein conformational disorders , the presence of aggregated amyloid β (Aβ) in the from of senile plaques and

neurofibrillary tangles of microtubule binding protein is the histopathological diagnostic tool of Alzheimer`s disease [8]. In this context , misfolding of accumulated extracellular amyloid beta in sensile plaques conjugated with the intracellular deposition of misfolded tau protein in neurofibrillary tangles result in memory loss. Definitely , accumulation of amyloid beta in the brain is regarded as an early toxic event in the pathogenesis of AD which constitutes the most prevalent type of dementia [6] The amyloid beta precursor protein gene is old and highly conserved.[7] Aβ has a great role in cardiovascular diseases , the reason may be due to the fact that Aβ promotes the expression of pro-inflammatory mediators , specifically IL-1β , Regarding cardiomyocytes , Aβ decreases cell viability [9]. Albumin is the most prevalent circulating protein in human blood, it constitutes half of the total protein amount in blood of healthy human with level (3-5 g/dL) [10]. Human serum albumin (HSA) is exclusively produced by human liver hepatocytes . HSA is composed of one N-terminus, one C-terminus and also three homologous domains , each domain has two helical subdomains [4]. It regarded as a negative charged protein with 66.5 kDa and characterized by high stability and high solubility. According to polypeptide sequence , it has a single polypeptide sequence consists of 585 amino acids with a relative prevalence of charged amino acids aspartic acid , glutamine , cysine and arginine, in addition to one tryptophan and 35 cysteine residues , 34 of those 35 cysteine residues constitute 17 disulfide bonds which control the biochemical structure of albumin [11] Hemoglobin (Hb) is a globular hematoprotein (hematoproteins are a group of specialized proteins containg heme as a tightly bound prosthetic group) . [12] The major function of Hb is transition oxygen O_2 from the lungs to the tissues and returning carbon dioxide to the lungs. Hemoglobin accompanied by myoglobin ensures a supply of oxygen for oxygen metabolism . [13] where the heme group serves to reversible binding of oxygen . [12] Indeed , Hb is a tetrameric molecule composed of four polypeptide subunits : two alpha (α1 and α2) and two beta (β1 and β2) , the alpha and beta subunits are formed of 7 and 8 helices respectively. Heme group consists of a ferous ion located in a center of porphyrin and cooridinated by four nitrogen atoms of porphyrin ring . [14] A cyclic tetrapyrrole ring within porphyrin structure is linked by α- methylene bridge . [13] Anemia is defined as a lower levels of Hb which being lower than its normal range , anemia constitutes a primary global health detect , most cases of anemia are due to iron deficiency .[15]

Materials and Methods

Patients' selection

Sixty (60) AD patients were enrolled in the current study, each one attended the nervous and psychological division of either Ibn Rushud training hospital /Baghdad or Al-Yarmook teaching hospital / Baghdad. They were diagnosed by both C.T scan and MRI as AD patients with moderate or severe cases. Indeed, those patients were categorized into four (4) groups: G_3 (52-78) years included (15) newly diagnosed male AD patient /with no treatment , G_4 (64-90) years included (15) newly diagnosed female AD patients without treatment, G_5 (60-85) years involved (15) male AD patient under treatment with memantine and G_6 (65-73) years involved (15) female patient also under treatment with memantine. In accordance with G_5 and G_6 , 10 mg film-coated tablets of memantine / one dose daily were used. The duration of treatment with memantine was between (2-12) months for both G_5 and G_6. Definitely, all females enrolled in the current study were menopausal. AD patients were compared to healthy persons within the range of age matched with patients , they did not suffer from any chronic diseases and regarded as control groups: G_1 were composed of (15) males (52-80) years and G_2 were composed of (15) females (64-82) years.

Blood sampling

Five millilters (5mL) of venous blood were collected from each subject participated in the present study. Blood samples collection was performed from November 20th 2019 to February 26th 2020. Each sample placed into plain tubes until being coagulated. Serum was separated from blood cells by centrifugation at 4000 r.p.m. for 3 min, subsequently serum was divided into small portions and kept frozen (-20℃) until analysis to be used in the different biochemical measurements for all patients and control groups. Biochemical measurements were performed in the private center of research and scientific development / Baghdad and other private laboratories.

Biochemical determinations

Amyloid Beta Peptide (Aβ) the microliter plate which provided in this kit and pre-coated with antibody, standard, samples and HRP coupled antibody were put to wells. After incubation and washing to release the uncombined enzyme, chromogen solution A and B were added . The liquid color will convert into blue. About the effect of acid, the color ultimately becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The level of Amyloid Beta Peptide (Aβ) in the samples is then

determined by comparing the O.D. of the samples to the standard curve. Regarding albumin , The reaction between albumin from serum and the bromocresol-green produces a change in colour that is proportional to the albumin concentration. The reaction between albumin from serum and the bromocresol-green makes a change in colour that is proportional to the albumin concentration. Wells are mixed and after 5 minutes incubation the optical density (O.D) was measured . The color is stable up to 30 minutes.

Results and Discussion :

Table (1): Aβ levels (ng/dL) in serum specimens of all groups.

Group	**Mean± S.D**	**Group**	**Mean± S.D**
G_1	39.62 ± 7.31	G_2	30.83 ± 5.26
G_3	63.74 ± 29.63	G_4	48.74 ± 15.5
G_5	45.84 ± 22.89	G_6	38.12 ± 11.92
p G_3/G_1 : S (0.0014)		p G_4/G_2 : S (0.008)	
p G_5/G_3 : S (0.04)		p G_6/G_4 : S(0.0233)	
p G_5/G_1 : N.S (0.266)		p G_6/G_2: S (0.0196)	
		p G_4/G_3:N.S (0.581)	

G_1 : healthy control group (male subjects)

G_2 : healthy control group (female subjects)

G_3 : Alzheimer`s male subjects (newly diagnosed /without treatment)

G_4 : Alzheimer`s female subjects (newly diagnosed /without treatment)

G_5 : Alzheimer`s male subjects (under treatment of memantine)

G_6 : Alzheimer`s female subjects (under treatment of memantine)

S : significantly difference (P< 0.05)

H.S : highly significant difference (P < 0.001)

N.S : non significant difference (P ≥ 0.05)

S.D : standard deviation

As shown in table (1), Aβ level was significantly increased in sera of G_3 (Alzheimer`s male newly diagnosed ) and $G_4$ (Alzheimer`s female newly diagnosed) compared to G_1 (healthy male subjects) and G_2 (healthy female subjects) respectively. Definetly , production and accumulation of amyloid beta peptide in the brain constitutes the pivotal aspect of Alzheimer amyloid hypothesis [9]. Moreover, Alzheimer`s patients brain is microscopically characterized by extracellular amyloid plaques. [16] Additionally , higher levels of Aβ in blood plasma is associated with AD but the mechanism was not clear. [17] As recently reported , the dysregulation in immune system in AD may trigger the inflammatory responses by promoting Aβ production. [18] Importantly, protein misfolding constitutes a major pathogenic cause in a number of chronic diseases involving AD. [19] In view of folded and misfolded proteins , protein folding is a biochemical process in which a polypeptide chain aquires its native and functional three dimentional structure, this folding ensures all required interactions between amino acid atoms in the native conformation [20] On the other hand, protein misfolding is a common event in living cells [21] all misfoldied proteins are characterized by disoriented domains without a definite secondary structure , subsequently becoming dysfunctional. Hence rich beta sheets are mostly available as a secondary structure. Interestingly , in young and healthy subjects the misfolded protein is controlled by protein quality control (PQC) systems. [21] In aging cells and cells of subjects influenced by environmental , physiological and oxidative factors, the misfold proteins load may overcome PQC capacity [21,22] Besides protein misfolding , taking into account dysregulation of Aβ clearance due to turning glial cells (microglia and astrocytes) into inflammatory state, the result is restricted Aβ clearance from the body and subsequently higher levels of Amyloid beta in AD. [23] Remarkably , most previous and recent studies focused on Aβ deposition in brain not body fluids although mentioned by [17,24] that higher amyloid beta levels reported in blood of AD . The present study highlights amyloid beta levels in human sera of AD by a detailed mechanism based on protein misfolding and inflammatory responses of glial cells . Moreover , amyloid beta level is affected by age . [24] Also , the biochemical pathogenic changes regarding AD begins before a period of time (may reach decades prior to clinical features become clear) , that is why G_1 and G_2

subjects detected reasonable levels of Aβ. Anyway , normal aging people must be highlighted to take into account the early changes as soon as possible. In contrast , Aβ level was significantly decreased in G_5 and G_6 compared to G_3 and G_4 respectively . Hence, relationship between intracellular Ca^{2+} and N-methyl-D-aspartate (NMDARs) must be highlighted. A recent study has reported that synaptic dysfunction mostly caused by perturbed synaptic Ca^{2+} handing as a response to over activation of a type of glutamate receptors or N-methyl-D-aspartate receptors (NMDARs). Glutamate (acidic amino acid) constitutes the major excitatory neurotransmitter in the brain functioning at ionotropic and metabotropic glutamate receptors . Importantly , uncontrolled glutamate levels in the brain causes toxicity to neurons, damage to endothelial function and disturpt blood brain barrier integrity [16] Memantine selectively blocks the over activation of NMDARs [25], the result is reducing the excitotoxity in the CNS by memantine via its non competitive antagonism action of N-methyl-D-aspartate NMDA receptors . Regarding Aβ when immune responses shifted into balance, microglia and macrophages stimulate the target cells to eliminate amyloid beta and stop neuronal damaged accompanied by neuronal inflammation [26] this is why amyloid beta level was decreased after treatment with memantine. The present study submits a key mechanism based on immune biochemical responses for amyloid beta level in sera of AD patients before and after treatment with memantine. The non significantly difference between groups G_5 and G_1 reflecting the pivtal role of memantine (Aβ level after treatment approximately reach the range of healthy male subjects). However , the difference between G_6 and G_2 was significant despite the immune modulation action of memantine. The reason mosty due to estrogen deficiency after menopause, estrogen deficiency promotes inflammation. [27].The non significantly difference between G_3 and G_4 indicated that amyloid beta in untreated patients not influenced by genders.

Table (2) : Albumin levels (g/dL) in serum specimens of all groups.

Group	**Mean ± S.D**	**Group**	**Mean ± S.D**
G_1	3.96 ± 0.62	G_2	4.62 ± 0.88
G_3	12.01 ± 4.14	G_4	15.71 ± 4.96
G_5	6.76 ± 4.7	G_6	7.41 ± 3.85
p G_3/G_1 : H.S ($1.58\ E^{-10}$)		p G_4/G_2 : H.S ($1.12\ E^{-11}$)	

p G_5/G_3 : H.S (0.00079)	p G_6/G_4 : H.S (1×10^{-5})
p G_5/G_1 : S (0.014)	p G_6/G_2 : S (0.0038)
	p G_4/G_3 : S (0.017)

Results of table (2) revealed that Albumin level was highly significant increased in serum of G_3 and G_4 (AD patients) compared to G_1 and G_2 (control subjects) respectively . Unexpectly , these results disagree with the previous study [28] which revealed that albumin biosynthesis is down-regulated during acute phase responses (i.e. inflammatory responses) caused by serum albumin is a key negative acute phase protein .The recent study [29] also disagrees with the present results , suggested that higher levels of Aβ peptide is associated with lower levels of albumin . However , albumin status in inflammatory diseases is controversial , another recent study has reported that distrubances in inflammatory responses caused by immunological disorders is associated with abnormal levels of acute phase proteins in blood , brain and cerebrospinal fluid of Alzheimer`s patients . [18] Abnormal levels of acute phase proteins as mentioned may give a hint for higher levels of albumin in Alzheimer patients compared to healthy subjects. Under normal physiological conditions , more than 95% of Aβ peptide are bound with albumin in both blood and CSF , this binding remarkably prevents Aβ peptide from being aggregated or increased . Interestingly , in AD albumin is subjected to conformational changes that impairs albumin-Aβ peptide binding due to albumin became misfolded , the result is higher levels of both albumin and Aβ peptide in blood and CSF , additionally misfolded albumin has toxic effects . [30] This is why albumin level was increased in sera of Alzheimer patients regardless its negative acute phase responses. Accordingly , many neurodegenerative diseases including AD are characterized by accumulation of misfolded proteins that impair neuronal connectivity and adversely affect plasticity . [31]. Conversely , albumin level was highly significant decreased in sera of G_5 and G_6 compared to G_3 and G_4. in other words albumin level was decreased after treatment with memantine. Anyway , the goal of AD treatment is decreasing Aβ peptide production , preventing proteins from being misfolded , and removing or neutralizing toxic aggregated or misfolded forms of these proteins . [32] Importantly , a recent study dealt with bovine serum albumin has revealed that memantine has a stabilizing effect on albumin by interaction with albumin near tryptophan residue , subsequently memantine causes conformational changes around tryptophan residue in albumin . Moreover memantine increases ∝-helical content which promotes albumin

stability . [33] the result is shifting of albumin level towards the balance . In this context , misfolded and aggregated proteins don't have a defined secondary structure . [34] Overall, the present study submits a unique detailed explanation regarding albumin level in Alzheimer`s patients before and after treatment with memantine . Nevertheless, the difference between G_5 and G_1 , and also between G_6 and G_2 was significant . Although albumin levels were shifted towards the balance under treatment with memantine but not reached normal status . Eventually , the significant increase of albumin in G_4 (female patients without treatment) compared to G_3 (male patients without treatment) may due to the role of estrogen deficiency in postmenopausal women on albumin misfolding .[27]

Table (3) : Hemoglobin levels (g/dL) in serum specimen of all groups.

Group	Mean ± S.D	Group	Mean ± S.D
G_1	13.63 ± 1.03	G_2	11.13 ± 0.88
G_3	11.57 ± 1.72	G_4	7.40 ± 1.23
G_5	12.85 ± 1.81	G_6	10.87 ± 1.04
p G_3/G_1 :H.S (4.56×10^{-5})		p G_4/G_2 : H.S ($2.12E^{-13}$)	
p G_5/G_3 :S (0.027)		p G_6/G_4 : H.S ($9.37E^{-12}$)	
p G_5/G_1 :N.S (0.10)		p G_6/G_2 : N.S (0.40)	
		p G_4/G_3 : H.S ($9.6\ E^{-11}$)	

The present study has suggested that hemoglobin level was highly significant decreased in of G_3 and G_4 compared to G_1 and G_2 respectively , as shown in table (3) Alzheimer`s patients may have a disorder in hemoglobin biosynthesis but the etiology is unknown [35] . Accordingly , anemia is an independent risk factor for dementia and the risk will be increased with severe anemia . [36] . Nevertheless , the previous study [38] has a controversial suggestion regarding hemoglobin level that may be elevated or depressed in AD . Both inflammation and perturbed brain iron homeostasis in AD may have a role in decreased hemoglobin level [35] . In view of anemia in AD , a concept called anemia of inflammation (AI) must be highlighted , AI(also called anemia conjugated with chronic disease) is the most prevalent anemia to date (after anemia caused by iron drop) , this type of anemia is the chronically and commonly frequent in patients with diseases associated with immune activation [38]. Interestingly , AI resulted

in iron retention . The most accepted mechanism suggested for iron retention implied that during inflammation , pro-inflammatory cytokines and hepcidin (the major controller of iron homeostasis) stop intestinal iron absorption and lead to iron storage in reticuloendothelial cells . On the other hand , systemic immune activation alters iron trafficking and causes iron storage in macrophages and reduced dietary iron absorption . In this regard , iron retention in macrophage must be highlighted because re-cycling of iron from erythrocytes by macrophages approximately constitutes more than 90% of the daily required iron for hemoglobin biosynthesis and erythropoiesis [39] . Abnormal iron content surprisingly contributes in production of amyloid beta in high levels , as mentioned by [40] , abnormal iron content causes a loss function of the major enzymes required iron as a cofactor , and the result is production of toxic oxidizing compound and amyloid beta with increased level . In normal cases, iron blocks the influx of Ca^{2+} in the cation channel (NMDARs) . In contrast , iron retention enhances NMDARs mediated excitotoxicity via promoting Ca^{2+} release and also calcium-induced cell damage . The present study gives novel mechanism regarding lower hemoglobin levels in AD based on (AI) or anemia of chronic diseases and also highlights the role of iron retention caused by AI.[41] Remarkably , hemoglobin level was significantly elevated in G_5 compared to G_3 and highly significant in G_6 compared to G_4 , reflecting the reactive role of memantine in trying to return hemoglobin levels towards the balance by suppressing the inflammatory status. Memantine may return the balance of iron because it reduces excitotoxicity in the CNS via its non competitive antagonism action of NMDA glutamate receptors [42]. Moreover , memantine supports the release of neurotrophic factors from astroglia which is critical for survival effect [19].The non significant difference between G_5 and G_1 and also between G_6 and G_2 indicating the potential therapeutic action of memantine via shifting hemoglobin towards the balance . The highly significant decrease in G_4 compared to G_3 regarding hemoglobin gives a hint for the role of inflammation associated menopausal women. [43]

Conclusions

The present study provides a definite clarification for higher levels of Aβ in AD patients by a detailed mechanism based on both protein misfolding and inflammatory responses of glial cells. Remarkably.. the biochemical action of memantine on Aβ level is more potent on males than females.On

the other hand , Aβ level in AD patients with no treatment is minimally influenced by the sex. Also, the present study submits a unique detailed concepts regarding albumin level in AD patients before and after treatment with memantine. Interestingly, The biochemical action of memantine on albumin is approximately the same in both males and females patients, albumin level in AD patients with no treatment is influenced in the term of sex. This study gives a comprehensive mechanism regarding lower levels of hemoglobin in AD patients based on anemia of chronic diseases and also highlights the role of iron retention caused by anemia of chronic diseases. Finally, the biochemical action of memantine on hemoglobin level is approximately the same in both males and females patients.

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Ethical Approval

The study protocol reviewed and approved by University of Baghdad and International center of research and development. All clinical trials were conducted according to the Helsinki Ethical Principles.

Conflict of Interest

We indicate that there are no conflicts of interest.

References

[1] Balupuri A, Choi K, & Kang N. [2020]: Aggregation mechanism of Alzheimer's Amyloid β- peptide mediated by α- Strand I α- Sheet structure . International Journal of molecular Sciences. 21(3), pp. 1-13.

[2] Adewole K, and Ishola A. [2021]: BACE1 and cholinesterase inhibitory activities of compounds from cajanus cajan and citrus reticulate: an in silico study. In silico Pharmacology. 9(1),pp.1-17.

[3] Folch J, Busquets O, Ettcheto M, Sa̅nchez-López E, Castro-Torres R, Verdaguerm E, Garcia M, Olloquequi J, Casadesus G, Beas-Zarate C, Pelegri C, Vilaplana J, Auladell C, & Camins A, [2018]: Memantine for The Treatment of Dementia : AReview on its Current and Future Applications . Journal of Alzheimer`s Disease. 62(3), pp. 1223-1240.

[4] Wang J, Gu B, Masters C, & Wang Y, [2017]: A systemic view of Alzheimer disease- insights from amyloid-β metabolism beyond the brain. Nature reviews neurology. 13(10), pp. 612-623.

[5] Ayudhya Th , Rheingold A, & Dingra N. [2019]: Crystal structure of memantine-carboxyborane. Acta Crystallographica Section E: Crystallographic Communications. 75(5), pp. 543-546.

[6] Chen L, Tan Y, & Cao P, [2017]: Original Article Relationship of thyroid stimulating hormone with sex hormones in patients with polycystic ovary syndrome: a single-center, retrospective observational study . Int J Clin Exp Pathol . 10(5), pp. 5989-5993.

[7] William G , & Indra N , [2013]: Origins of amyloid-β. BMC genomics . 14(2013), pp. 290.

[8] Nirmalraj P, List J, Battacharya Sh, Howe G, Xu L, Thompson D, & Mayer M, [2020]: Complete aggregation pathway of amyloid β (1-40) and (1-42) resolved on atomically clean interface . Science advances research article . 6(15), pp. 1-11.

[9] 9. Stakos D, Stamatelopoulos K, Bampatsias D, Saches M, Zormpas E, Vlachogiannis N, Tual-Chalot S, & Stellos K, [2020]: The Alzheimer`s Disease Amyloid-Beta Hypothesis in Cardiovascular Aging and Disease . Journal of the American college of cardiology . 75(8), pp. 953-967.

[10] Moman R, O`Neill C, Ledder R, & McBain A, [2020]: Mitigation of the toxic effects of periodontal pathogens by candidate probiotics in oral Keratinocytes, and in an invertebrate model. Frontiers in Microbiology. 11(999), pp. 1-12.

[11] Carvalho J , & Machado M,[2018] : New insights about albumin and liver disease. Annals of Hepatology. 17(4), pp.547-560.

[12] Harvey R, & Ferrier D, [2011]: Lippincotts illustrated reviews : biochemistry. Library of congress Cataloging -in – Publication Data , Fifth edition , Chapter 3 / Pages 25-28 , Chapter 18 / Pages 227 , 228 and 234.

[13] Beneder D, Mays P, & Botham K. [2006]: Harpers illustrated Biochemistry. Lange Medical Books / Mc Graw-Hill / Medical Publishing Division , twenty seven edition., pp.41- 44.

[14] Ahmed M , Ghatage M, & Safo M. [2020]: Hemoglobin : Structure , function and Allostery. HHS Public Access. 94, pp.345-382.

[15] Milman N, [2011]: Anemia-Still a major health problem. Article in Annals of Hematology. 90(4), pp. 369-377.

[16] Liu J, & Li L, [2019]: Targeting autophagy for the treatment of Alzheimer`s disease: challenges and opportunities. Frontiers in molecular neuroscience. 12(203), pp. 1-9.

[17] Luchsinger J, Tang M, Miller J, Green R, Mehta P, & Mayeux R, [2007]: Relation of plasma Homocysteine to plasma Amyloid Beta levels. neurochemical Research 32(4-5), pp. 715-781.

[18] Domingues C, Silva O, & Henriques A, [2017]: Impact of cytokines and chemokines on Alzheimer`s Disease Neuropathological Hallmarks. Current Alzheimer research. 14(8), pp. 870-882.

[19] Olubiyi O, & Strodel B, [2012]: Structures of the Amyloid β-peptides $A\beta_{1-40}$ and $A\beta_{1-40}$ As influenced by pH and a D-peptide. The Journal of physical Chemistry .116(10), pp. 3280-3291

[20] 20. Yadav K, Yadav A, Vashistha P, Pandey V, & Dwivedi U, [2019]: Protein misfolding Disease and Therapeutic Approaches. Article in current protein and peptide science. 20(12), pp. 1226-1245.

[21] 21. Gregersen N, Bross P, Vang S, & Christensen J, [2006]: Protein misfolding and Human Disease . Article in Annual Review of Genomics and Human Genetics. 7, pp. 103-124.

[22] 22. Herczenik E, & Gebbink M, [2008]: Molecular and Cellular Aspects of Protein misfolding and disease . Article in the FASEB Journal 22(7), pp. 1-19.

[23] 23. Zheng C, Zhou X, & Wang J, [2016]: The dual roles of cytokines in Alzheimer`s disease : Update on Interlukins , TNF-α , TGF-β and IFN-γ. Translational Neurodegeneration. 5(1), pp. 2-15.

[24] 24. Zecca Ch, Tortelli R, Panza F, Arcuti S, Piccininni M, Capozzo R, Barulli M, Barone R, Cardinali R, Abbrescia D, Seripa D, Bresci V, & Logroscino G, [2018]: Plasma β-amyloid $_{1-42}$ refernce values in cognltively normal subjects . Journal of the neurological sciences. 391, pp. 120-126.

[25] 25. . Cheng Q, Fang L, Feng D, Tang S, Yue S, Huang Y, Han J, Lan J, Liu W, Gao L, & Luo Z, [2019]: Memantine ameliorates pulmonary inflammation in a mice model of GOPD induced by cigarette smoke combined with LPS . Biomedicine & pharmacotherapy. 109, pp. 2005-2013.

[26] 26. Gate D, Rezai-Zadeh K, Jodry D, Rentsendovj A, & Town T, [2010]: Macrophages in Alzheimer`s disease : The blood-borne identity . Journal of Neural Transmission "Springer". 117(8), pp. 961-970.

[27] 27. Straub R, [2007]: The complex role of Estrogens in Inflammation .Article in Endocrine Reviews .28(5), pp. 521-574.

[28] 28. Tothova C, Nagy O, & Kovac G, [2014]: Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. Veterinarni Medicina. 59(4), pp. 163-180.

[29] 29. Kim J, Byun M, Lee J, Yi D, Jeon S, Sohn B, Lee J, Shin S, Kim Y, Kang K, Sohn Ch, & Lee D, [2020]: Serum albumin and beta-Amyloid deposition in the human brain . Neurology. 95(7), pp. e815-e826.

[30] 30. Tsao F, Barnes J, Amessoudji A, Li Z, & Meyer K, [2020]: Aging-Related and gender specific Albumin Misfolding in Alzheimer`s Disease. Journal of Alzheimer`s Disease Reports. 4(1), pp. 67-77.

[31] 31. Nakamura T, & Lipton S, [2009]: Cell death : protein misfolding and neurodegenerative disease. Apoptosis. 14(4), pp. 455-468.

[32] 32. Yiannopoulou K, & Papageorgiou S, [2020]: Current and future treatments in Alzheimer Disease : An update. Journal of central Nervous system Disease. 12(11795773520907397), pp. 1-12.

[33] 33. Ameen F, Siddiqui Sh , Jahan I , Nayeem Sh , Rehman S. & Tabish M. [2020]: A detailed insight into the interaction of memantine with bovine serum albumin. A spectroscopic and computational approach. Journal of Molecular Liquids. 303(112671), pp. 1-12.

[34] 34. Bulck M, Sierra-Magro A, Alarcon-Gil J, Perez-Castillo A, & Morales-Garcia J,[2019]: Novel Approaches for the treatment of

Alzheimer`s and parkinson`s disease .international Journal of molecular sciences,20(3), pp. 1-36.

[35] 35. Faux N, Rembach A, Wiley J, Ellis K, Ames D, Fowler C, Martins R, Pertile K, Rumble R, Trounson B, Masters C, & Bush A, [2014]: An anemia of Alzheimer`s disease . Molecular psychiatry. 19(11), pp. 1227-1234.

[36] 36. Jeong S, Shin D, Lee J, Hyeon J, Lee J, & Kim S, [2017]: Anemia is associated with incidence of dementia : a national health Screening study in korea . involving and Therapy. 9(94), pp. 3-8.

[37] 37. Shah R, Buchma A , Wilson R , Leurgans & Bennett D. [2011]: Hemoglobin level in older persons and incident Alzheimers disease. ANN Enterprises Unautharized Reproduction. 77, pp. 219-226.

[38] 38. Wiciński M, Liczner G, Cadelski K, Kolnerzak T, Nowaczewska M, & Malinowski B, [2020]: Anemia of Chronic Diseases: Wider Diagnostics-Better Treatment?. Nutrients. 12(6), pp. 1-17.

[39] 39. Weiss G, Ganz T, & Goodnough L, [2019]: Anemia of inflammation . Article in Blood. 133(1), pp. 40-50.

[40] 40. Wang T, Xu Sh, Fan Y, Li L, & Guo Ch, [2019]: Iron pathophysiology in Alzheimer`s Disease . Chapter 5 in Advances in Experimental medicine and Biology.1173, pp. 67-104.

[41] 41. Munoz P , Humeres A , Elgueta C , Kirkwood A , Hidalo C & Nunez T. [2011] : Iron mediates N-Methyl-D- aspartate Receptor- dependent stimulation of calcium-induced pathways and hippocampal synaptic plasticity. Journal of biological chemistry. 286(15): , pp. 13382-13392.

[42] 42. Hasanagic S, & Serdarevic F, [2020]: Potential role of Memantine in The prevention and treatment of COVID-19 : its antagonism of nicotinic acetylcholine receptors (nAChR) and beyond . EUROPEAN respiratory Journal. 56(2), pp. 1-6.

[43] 43. Malutan A, Dan M, Nicolae C, & Carmen M, [2014]: Proinflammatory and anti-infammatory cytokine changes related to menopause . Article in menopausal review. 13(3), pp. 162-168.