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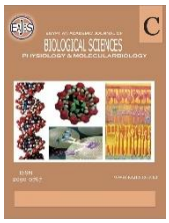

Alpha 1- Anti-chymotrypsin and Other Inflammatory Factors As Biomarkers for Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease characterized by brain function disturbance. It is one of the disorders that affects old age group patients. However, the middle age group could be affected too. Till now, the etiology behind this disease is still obscured, although some researchers attributed it to a genetic factor. The pathology of AD reveals neuronal inflammation and degeneration following amyloid accumulation.

The aim of this research is to study the relationship between AD & the level of some proteins like alpha1- anti chymotrypsin, high sensitive C-reactive protein and 1 interlukin-6 in the blood of participants. Patients, included in this study, are 130 one complaining of AD, they were divided into two groups according to their age. while 80 normal people are involved in this study as a control group. The serum of all patients and normal subjects were taken for detection of the level of alpha-1- anti chymotrypsin, high sensitive C-reactive protein and interlukin- 6.

INTRODUCTION

Alzheimer disease (AD), although is a neurodegenerative disease, is considered as a type of dementia. it is characterized by disturbance in the function of the brain like behavior changes, uncontrolled thinking and loss of recent memory. The temporal lobe is the most part of the brain that is affected by AD (Kim JH, *et al.*, 2018).

Aging is considered to be the greatest risk factor in the etiology of AD since people aged above 65 years are more liable to have this disease (Kim JH, et al.,2018). However, the younger age group of people (below 65 years) are not immune from affection by AD. In this case, the disease is called early-onset Alzheimer disease (Reitz C, Mayeux R, *et al.*, 2014).
Although the etiology of AD is not very well known, many researches elicited rare genes responsible for this disease and is responsible for affecting young age group (30-50 years). This type of disease is called the familial type of AD(Cai Z, Xiao M, *et al*., 2016). The pathological features of AD are characterized by inflammatory process affecting neurons and are usually followed by neuronal degeneration. This inflammatory process is usually associated with amyloid B peptide overproduction and accumulation in the brain tissue. This accumulated amyloid forms amyloid plaque and could be appeared in the extracellular compartment leading to neurofibril formation that affects neuronal function leading to cellular death later on (Allen HB, *et al.*,2016).

This amyloid plaque showed a marked correlation with many proteins (Heneka MT, Carson MJ, *et al.*,2015). Alpha -1- anti chymotrypsin (Alpha1ACT), which is known to be a serine protease inhibitor, is one of these proteins that has the ability to affect B-amyloid formation. Biochemical analysis elicited a strong relationship between the progress and severity of AD and the level of this protein in the blood and CSF (Heneka MT, Carson MJ, *et al* .,2015).

Interlukin-6 (IL-6) is a cytokine that has an essential role in the immune system. Besides, it has a critical effect in the physiology of the nervous system including neurogenesis (Nevrol ZH, Psikhiatr IMS, *et al.,* 2013). Androsova LV *et al*.(2013) detected an increase in the level of IL-6 in the brain of patients with AD. This indicates that disturbance of the brain's immune system could play an important effect in the pathogenesis of AD (Androsova LV, Mikhaîlova NM, *et al .,* 2013). In AD, IL-6 synthesis is stimulated by B-amyloid peptide(Nevrol ZH, Psikhiatr IMS, *et al.,* 2013) (Androsova LV, Mikhaîlova NM, *et al .,* 2013) Another protein has an effect in the pathogenesis of AD, is C-reactive protein (C.R.P). It is a very well-known protein synthesized in the liver whenever and wherever the body exposes to a recent injury, inflammation, or acute infection. Many researchers reported that inflammations especially neuro-inflammation have a critical role in the cognitive function of the brain in AD and other types of dementia like vascular dementia(O'Brynat SE, Johnson L, *et al.,* 2016) (Gong C, Wei D, *et al.,* 2015). Accordingly, C.R.P, which is stimulated by acute inflammation, could have a role in the pathogenesis of dementia (including AD) (Gong C, Wei D, *et al.,* 2015) (Akiyama H, Barger S, *et al.,* 2000).

How inflammatory process affects AD is still not very well understood. However, many researchers attributed it to the effect of systemic IL-1B, IL-6 & CRP, since all these proteins responded to inflammation & to the presence of amyloid.

This research will study the effects of Alph1ACT, IL-6 & CRP in the pathogenesis of AD through comparing the level of these proteins in the serum of old and young patients and control normal subjects.

MATERIALS AND METHODS

Patients were collected from the neurological outpatient clinic of the medical city, Baghdad Teaching Hospital, from September 2021 till the end of March 2022. All the patients were diagnosed as having AD by a neurological specialist. In the current study, 130 patients were included, while 80 normal subjects were chosen as a control group. The patients and controls were divided according to their age & gender as in the following table (Table 1).

Table 1: division of subjects involved in this study according to age and gender.

	Late AD (above 65 years)	**Early AD (below 65 years)**	**Old normal subject**	**Young normal subject (below 65 years)**
No. of participants	100	30	40	40
No. of male	38	18	20	20
No. of female	62	12	20	20
age of male (mean -+ SD)	74.2 +- 1.3	51.1+-1.2	73.3+-1.2	48.4+-1.3
Age of female (mean -+ SD)	73.3+- 1.2	52.1 +-1.4	73.1+-1.3	53.4+- 1.2

All the patients had been asked about family history of AD, or whether they were exposed to previous trauma (head injury, emotional or psychological one) the results obtained from the questionnaire had been summarized in Table 2.

Table 2: grouping of patients with AD (late and early onset) according to their age, family history and exposure to trauma.

	Late AD	Early AD
No. of participants depending on age	100	30
No. of participants having a family history of AD	55	16
No. of participants having trauma	27	7
No. of participants who have nothing abnormal (i.e. no family history or trauma)	18	7

From all participants (control & patient), 5ml of venous blood had been aspirated by using disposable syringes after 12 hours of fasting. The blood obtained was kept in a gelatinous tubes (without anticoagulant) & centrifuged at 3000 RPM for 10 min. The serum was aspirated & kept in deep freeze till analysis. Sandwich –Eliza technique had been used to measure the following parameters :

Alpha1-ACT , hs-CRP , interlukin-6

The kits used in this technique were related to sunlong biotech, China.

The normal values of each parameters are :

- Alpha1-ACT is 0.6-406 ngm/ml
- hs-CRP is 0.3-10 ngm/ml

IL-6 is 2-80 ngm/ ml.

RESULTS AND DISCUSSION

One of the results, obtained from this study, was that female patients above the age of 65 years were more vulnerable to AD than males. While below the age of 65 years, male is more likely to be affected by AD than female. This finding could be attributed to the protective effect of mitochondria against the toxicity of B-amyloid in the cells of females. Besides the apoptotic signals released from male cells are more than that released from female. However, Jose V. and Ana L. (2010) noticed that this protective mechanism of female cells had been reduced with aging. They were attributed this to the +ve estrogenic effect on female cells' mitochondria to protect their cells against the toxic effect of B- amyloid and in consequence this protection had been reduced or lost with aging.

Another explanation for this result (i.e. females affected more than males with AD above the age of 65 years)is that the life span of females is longer than males. Women are more likely to have depression than men with aging. Since depression is one of the major risk factors in dementia, so female is more likely to have AD than male with aging. This explanation is parallel to that of Amyl L *et al.* (2011) & Rebecca A (2018).

Aging is considered to be an essential factor in the pathogenesis of AD. Table(2) demonstrates a wide difference between old patients (above 65 years) affected by AD and patients younger than 65 years old with AD. This finding coincides with other studies which reported that the prevalence of AD in older people is double that of the younger group (Alzheimer's Association, *et al.*,2014). The most logical explanation is an increase incidence of atherosclerotic changes within the whole body in general and within the brain specifically. Another important factor for this obvious increasing in the incidence of AD among old people is the mutation in apolipoprotein E (APOE). that could be occurred due to environmental circumstances or injury (Finkelstein DI, Adlard PA, *et al* ., 2014)(Lalli MA, Bettcher BM, *et al* ., 2015).

APOE is a gene mainly manufactured in the liver and is responsible for many functions, one of them is the transportation of lipids, enzymes, etc…, within the brain tissue (Johsson T, Atwal JK, *et al.*, 2012). Damage of apolipoprotein E is considered to be one of the essential risk factors for AD in old age group people (Ethika Tyagi, Tina Fiorelli, *et al.*, 2013).

Regarding the biomarkers, the results in the patients and control group are summarized in table (3).

Table (3): levels of biomarkers in the serum of AD patients (late and early groups) and in the serum of normal subjects (old and age group) with their significance.

	Late AD	**Early AD**	**Old control**	**Young control**	**P-Value**	**S**
α1antichymotrypsin (ng/ml)	72.2±12	56.8 ± 13.9	15.5±1	5.2±.8	P<0.001	HS
Interlukin-6(ng/L)	92.27±16.77	72.27 ± 6.33	32.7 ± 3.2	12.7 ± 1.2	P<0.001	HS
hs-C-reactive protein(ng/ml)	11.2 ± 1.2	7.4 ± 1.7	4.2 ± 1.7	2.4 ± 0.77	P<0.001	HS

From the data illustrated in Table (3), it is clear that the 3 biomarkers studied in this research were increased with aging (in both patient group and control group). However, if a comparison had been done between these 3 biomarkers in patients with AD versus these in normal subjects, obviously the mean value of these markers showed a highly significant increase in patients with AD than those of normal subjects. Regarding the significant increase in the level of alpha1ACT, the explanation is attributed to the pathology of AD. One of the most characteristic pathological features of AD is the formation of B- amyloid plaque, and with the progression of the disease, neurofibril will be formed (Ethika Tyagi, Tina Fiorelli, *et al.*, 2013). This will be revealed as an inflammation and degeneration of neurons. Alpha 1 ACT is responsible for B-polymarization of such amyloid plaque and so disaggregation of such protein. Therefore its level in plasma and C.S.F of AD patients will be high and its level reflects the severity of the disease. The disaggregation of amyloid plaque and neuronal degeneration will stimulate the immune system in the C.N.S. These include disturbance in the function of microglia and astrocyte cells. This will stimulate the secretion of IL-6, therefore table (3) showed a highly significant increase in the level of IL-6 in the serum of AD patients in the elderly group (16) when compared with the level of IL-6 in the serum of old aged healthy people(Jefferson W, Kinney Shane M, *et al* .,2018).

Table(3) also revealed highly significant increase in the level of C.R.P. This result could be attributed to the fact that the formation of amyloid plaque and neuronal degeneration will stimulate the neuronal immune system and alter the activity of microglia and astrocyte cells. This will release IL-6 which, in turn, stimulates pro-inflammatory cytokines including C.R.P (Wilson W.Tam, Melvyn W, *et al.*,2018)(Marìa Erta, Albert Quintana, *et al.*,2012).

The biochemical markers, that were used in this study, revealed different correlations with AD, regarding pathogenesis and severity of the disease. Table 4 shows the variability between these biomarkers on AD.

Table 4: The most effective limitation factor variable in Alzheimer's disease.

Clinical variable	**R^2**	**P-Value**	**S**
α 1-antichymotrypsin(ng/ml)	98.8 %	P<0.01	(HS)
ILukin-6(ng/L)	72.3 %	P<0.01	(HS)
hs-C-Reactive protein (ng/ml)	39.5 %	P<0.01	(HS)

Although all these factors revealed highly significant changes in AD, yet, Table (4) revealed that alpha 1- anti chymotrypsin factor is the most factor affected in AD patients and followed by 1Lukin-6 then by hs-c-reactive protein. This highlights that

ALPHA-1 ACT is the most important factor in the pathogenesis of the disease and responsible for the changes that occur in the other two biomarkers. Besides the level of ALPHA-1 ACT in the blood of AD patients is a more sensitive test to determine the severity of the disease(Ethika Tyagi, Tina Fiorelli, et al., 2013).

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