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Interaction between apolipoprotein E genotyping, serum inflammatory biomarkers and cognitive functions in Egyptian elderly

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Abstract

There is evidence consistent with the hypothesis that genetic, inflammatory and immune mechanisms are involved in the pathogenesis of AD. The aim of this study is to assess the relationship between Apolipoprotein E (Apo E), serum levels of inflammatory markers, and cognitive functions among elderly patients with Alzheimer's disease (AD) and Mild cognitive impairment (MCI) compared to elderly with normal cognitive function. 88 participants (≥60 years) from Ain Shams University Hospital were enrolled. They were divided into 3 groups: Group A (32 elderly patients with AD), Group B (32 elderly patients with MCI) and Group C (24 controls with normal cognitive function). All participants were subjected to comprehensive geriatric assessment, Apo E genotyping, measurement of C-reactive protein (CRP) and Alpha-1-antichymotrypsin (ACT), by PCR-RFLP, ELIZA and semi-quantitative method respectively. The most common variant of Apo E gene was E3/E3 being more frequent in healthy control group (HC) than the other two groups and the least common variant was E4/E4 detected only in the AD group. ApoE4 allele was associated with 40.6% of AD patients (where 31.4% were heterozygous and 8.6 % homozygous) and 17.1% of MCI patients, whereas ApoE2 was more prevalent in the control group (P<0.05). A significant difference was observed when Mini mental status Examination (MMSE) score in different Apo E alleles was compared (P<0.01). The highest score was associated with (E2/E3) allele whereas, the lowest score was associated with (E4/E4) allele. Regarding inflammatory markers; CRP levels showed a statistically significant difference between the 3 groups and were higher in the AD group than the other 2 groups. (P<0.01). There was no statistically significant difference between the 3 groups as regard ACT level (P>0.05). Carriers of at least one E4 allele showed great risk to develop AD when combined with high CRP serum levels (OR = 36; CI: 11.4-113.7; P< 0.01). In conclusion, Apo E together with CRP may be a useful tool to predict Alzheimer's disease.

Keywords: Alzheimer's disease, Mild cognitive impairment, CRP, alpha1 antichymotrypsin, APOE gene, Elderly

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that slowly destroys memory and thinking skills, resulting in behavioral changes. It is estimated that nearly 36 million are affected globally with numbers reaching 115 million by 2050. In Egypt, the dementia prevalence ranges from 2.01% to 5.07%.[1,2]

Mild cognitive impairment (MCI) is widely considered as the intermediate stage between intact cognition and pathological cognitive ageing. Several genetic, environmental, and physiological factors, including inflammations and metabolic influences, are involved in the pathogenesis of AD.[3]

Apolipoprotein E (Apo E) plays a critical role in the metabolism of lipoproteins and redistribution of cholesterol, and has long been studied in relation to atherosclerosis and cardiovascular disease. In the brain, Apo E is primarily produced by astrocytes, and plays a critical role in cholesterol transfer to neurons for the maintenance of cell membranes and synapses, and for their repair after injury.[4] There are three major isoforms of Apo E: E2, E3 and E4, E3 being the major isoform expressed in humans. Apolipoprotein E4 (ApoE4) is the strongest genetic risk factor for late onset Alzheimer's Disease while E2 is associated with increased longevity and a decreased risk of AD.[5] Mounting evidence suggests that ApoE4 contributes to AD pathogenesis through multiple pathways including facilitated amyloid-β deposition, synaptic dysfunction, neuroinflammation, and cerebrovascular defects.[6] ApoE gene and apolipoprotein E (Apo E), have been proposed as a promising target for therapy and drug development against AD.[7,8]

Recent studies demonstrated that inflammation may play a critical role in the neurodegenerative cascades of AD and MCI. Several markers such as Tumour Necrosis Factor α (TNF-α), interleukin-6 (IL-6), Alpha-1-antichymotrypsin (ACT) and C reactive protein (CRP) have been implicated in detecting disease severity and progression.[9]

C-reactive protein, is a hepatically derived acute phase reactant which has a crucial role in the human immune system and has been widely used as a sensitive marker for systemic inflammation.[10] Studies of brain tissues from patients with AD consistently show evidence of inflammation, as indicated by the presence of activated microglia, activated complement factors, cytokines, and other inflammatory proteins. CRP has been also detected in the senile plaques and neurofibrillary tangles of patients with Alzheimer's disease.[11,12]

Alpha-1-antichymotrypsin (ACT), is a member of the serine protease inhibitor (serpin) family. ACT has been involved in the pathogenesis of AD, since elevated levels of ACT are found in the brain, serum and cerebrospinal fluid (CSF) of AD patients. High levels of ACT in plasma were associated with cognitive decline in elderly subjects suggesting that ACT may serve as a biomarker for early diagnosis of the disease.[13,14]

The aim of this work is to study the interaction between ApoE genotypes, the levels of inflammatory markers [C-reactive protein (CRP), alpha 1-antichymotrypsin (ACT)] and cognitive functions among elderly patients with AD and Mild Cognitive Impairment (MCI) compared to elderly with normal cognitive function, in a trial to explore their clinical utility in predicting Alzheimer's disease.

Subjects and Methods

Subjects

This study was conducted on 88 elderly participants operationally defined as 60 years and more who were recruited from the in patients wards and out patients clinics of Ain Shams University Hospitals. Elderly subjects were divided into 3 groups:

Alzheimer's Disease Group

Included 32 elderly patients with Alzheimer's disease, with a mean age of 66±4.5, diagnosed according to NINCDS-ADRDA criteria (National Institute of Neurological and Communicable Disease and Stroke / AD and Related Disorders Association criteria).[15]

Mild Cognitive Impairment Group

Included 32 elderly patients with Mild Cognitive Impairment (MCI), with a mean age of 64.3± 4.0 years, diagnosed by Petersen criteria.[16]

Healthy Control Group

Included 24 ages and sex matched cognitively normal elderly participants serving as healthy control group (HC), with a mean age of 65.4± 4.6 years.

Subjects who had symptoms or signs of intercurrent infectious conditions, recent myocardial infarction, cerebrovascular stroke or other inflammatory conditions (in the past four weeks) were excluded from the study. Also, patients with depression, marked hearing or visual impairment were not included. An informed consent was obtained from all subjects before enrollment in the study.

All participants were subjected to:

-Comprehensive geriatric assessment including: Detailed history and physical examination. Cognitive assessment was done using: Mini mental status examination (MMSE) for assessment of cognitive function the Arabic version.[17]

-The Arabic version of Montreal Cognitive Assessment (MOCA) was used for diagnosis of MCI.[18, 19]

-Assessments of participants function was done using the Instrumental Activities of Daily Living (IADL); and the Activities of Daily Living (ADL); to detect impairment in activities of daily living.[20,21]

-Screening for depression was done by Arabic version of Geriatric Depression Scale (GDS).[22]

-Laboratory investigations including: Apo E genotyping, CRP assay and alpha-1-antichymotrypsin (ACT) assay

Samples

Five milliliters of venous blood were drawn and equally divided into 2 tubes, an EDTA tube for Apo E genotyping and a non-EDTA tube to obtain serum for CRP & ACT determination.

Methods

CRP assay

It was determined by a semi quantitative method using reagent provided from AVITEX CRP Latex serology test (Omega Diagnostics Ltd 2005). The AVITEX CRP latex particles are coated with antibodies to human CRP. When the latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 minutes, with a detection limit of 6mg/L. Positive results are obtained at a CRP serum concentration above 6mg/L.[23,24]

Alpha-1-antichymotrypsin (ACT)

Assays were carried out by a sandwich enzyme-linked immunosorbent assay (ELISA) technique using reagents provided by EIAab Science Co. Ltd. (A1710 Guangguguoji, East Lake Hi-Tech Development zone, China). Antibody specific for ACT has been pre-coated onto a microplate. Five standards and patients' samples were pipetted into the wells and ACT present was bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated polyclonal antibody preparation specific for ACT was added to each microplate well and incubated. Following a wash step, a substrate solution was added to the wells A color develops in proportion to the amount of ACT bound in the initial step. The color development was stopped and the intensity of the color was measured. To deduce the concentration of ACT in serum samples, a standard curve using logarithmic scale was constructed by plotting the absorbance of each standard on the y-axis against the concentration on the x-axis. The best fit curve was drawn through the points on the graph.[25]

Apo E genotyping

The human apolipoprotein E gene, located on chromosome 19, is polymorphic at two single nucleotides (rs429358 and rs7412) resulting in three known isoforms of ApoE. E3 (Cys112, Arg158) is considered the wild form; a T → C point mutation produces ApoE4 (Arg112, Arg158) while C → T mutation gives ApoE2 (Cys112, Cys158).

ApoE genotyping was performed by polymerase chain reaction followed by restriction fragment length polymorphism analysis using AFLIII and HaeII enzymes, which are specific for the ApoE112 (T/C) and the ApoE158 (C/T) alleles, respectively.[26]

DNA Extraction from Whole Blood: The DNA was extracted from whole blood using genomic DNA purification kits (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer instruction. In this technique, DNA in the sample is liberated using proteinase K (PK) solution and lysis buffer. Released DNA is bound exclusively and specifically to the biospin membrane in presence of binding buffer under appropriate

salt ion and pH conditions. Denatured protein and other contaminants are removed with several washing procedures. The DNA is then eluted from the membrane with elution buffer.

Conventional PCR (cPCR): The DNA extracted from whole blood was subjected to conventional PCR assay to yield a 218-bp DNA fragment that spans both ApoE polymorphic sites using the following primers; F 5'-TCCAAGGAGCTGCAGGCGGCGCA as the forward primer and R5'-GCCCCGGCCTGGTACACTGCCA as reverse primer. Primers were supplied by (Invitrogen, 5791Van Allen Way, Carlsbad, CA, USA)

For each PCR reaction, the following reagents were used : 12.5 µL of Taq Green PCR Master Mix (Dreamtaq Green PCR Thermo Fisher Scientific Waltham,USA), 1 µL forward primer, 1 µL reverse primer, 0.5 nuclease free water and 10 µL DNA template. Amplification was performed using an automated thermal cycler (Biometra T3000, USA). The program used was as follows: step one initial denaturation: 3 minutes 95°C; step two denaturation: 30 seconds 95°C; step three annealing: 30 seconds 57°C; step four extension: 60 seconds 72°C; from step two till step four; number of cycles were 35; and, finally, 5 minutes 72°C**.** Negative control was performed by using the same reaction mixture without DNA.[27]

Amplified DNA was digested using two restriction enzymes, AflIII and HaeII, simultaneously with 2.5 units of AflIII and 5 units of HaeII (Thermo -scientific, 168 Third Avenue, Waltham, MA, USA) for 6 h at 37 °C. The AFLIII cuts the ApoE112 'T' allele, but does not cut the 'C' allele, whereas HaeII cuts the ApoE158 'C' allele, but does not cut the 'T' allele. The resulting fragments were separated on a 2% agarose gel with a 50-bp marker (Biomed; DM0903), and the bands were visualized by ethidium bromide staining. According to restriction map of Apo E amplified product, simultaneous digestion of the 218-bp amplified product produces the following band sizes; 145-bp and 73 bp for E3, 168-bp and 50 bp for E2, 195bp and 23 bp for E4 as shown in Figure1.[26]

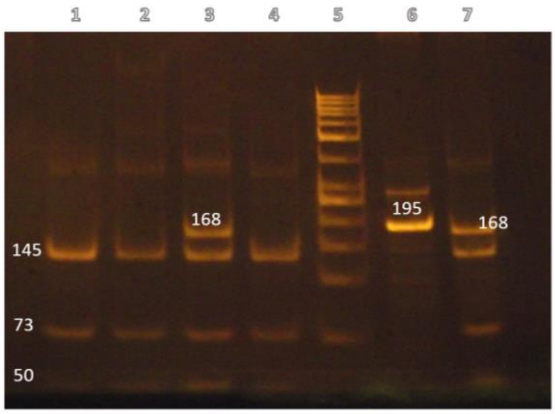

Figure 1. PCR-RFLP analysis of ApoE gene polymorphism. Lanes 1,2,4 show E3/E3 (145, 73bp) Lane 3,7 show E2/E3 (50, 73,145,195bp) lane 6 shows E4/E4 (195,23 bp).Lane5 is 50bp DNA ladder.

Statistical analysis

Statistical analysis was carried out on personal computer using IBM SPSS statistics (V. 25.0, IBM Corp., USA, 2018-2019). Parametric data were expressed as mean and standard deviation ($\bar{X}$ ± SD) while non-parametric data were expressed as median and interquartile range (IQR). Comparative statistics was done by Kruskal-Wallis test in case of non-parametric data whereas ANOVA test was used in case of parametric data. Chi-square test was used for comparison between independent groups as regards the categorized data. Correlation analysis was performed by Spearman's rank correlation (rs). *P* values<0.05 were considered significant. Relative Risk Assessments were calculated as absolute figures and as a standard error of estimate (95%CI). Odds ratio (OR) was used to measure the association between the studied variables and the risk for AD. Finally, Regression analysis was used to search for a panel of independent parameters that can predict AD.

Results

Descriptive and comparative analysis of the studied parameters in healthy control group, mild cognitive impairment group and Alzheimer's disease group is demonstrated in (Table 1).

In our study, the most common variant of Apo E gene in the three studied groups was E3/E3 being higher in HC group and the least common was E4/E4 detected only in AD group. Regarding the distribution of Apo E alleles

among the studied groups: ApoE4 allele was associated with 40.6% of AD patients (where 31.4% were heterozygous and 8.6 % homozygous) and 17.1% of patients with MCI, whereas ApoE2 was more prevalent in the control group (*P*<0.05) (Table 1).

Regarding inflammatory markers; CRP levels showed a highly statistically significant difference between the 3 studied groups using the Chi-square test being higher in AD group than the other 2 groups. (*P*<0.01). On the contrary, by using Kruskal-Wallis test, there was no statistically significant difference between the 3 groups as regards ACT level (*P*>0.05) (Table1).

Table 1. Descriptive and comparative analysis of different studied parameters among healthy control group, mild cognitive impairment group and Alzheimer's Disease group.

	HC (n = 24)	MCI (n = 32)	AD (n = 32)	*P*-value
Age	65.4±4.6	64.3± 4.0	66±4.5	NS
Gender				
Male (%)	47.6%	45.0%	50.0%	NS
Female (%)	52.4%	55.0%	50.0%	
Educational level				
Illiterate	37.1%	37.1%	41.4%	
Primary school	40.0%	40.0%	38.3%	NS
High-school	22.9%	22.9%	20.3%	
ACT (µmol/L) median (IQR)	1.1(1.1-1.5)	1.4(1.1-2.6)	1.1(1.0-1.5)	NS
CRP (mg/L)				
<6	57.2%	54.3%	34.3%	
6-12	37.1%	31.4%	17.1%	<0.01*
12-48	5.7%	14.3%	22.9%	
96	0	0	25.7%	
ApoE alleles				
E3/E3	82.9%	80.0%	60.0%	
E4/E4	0	0	8.6%	<0.01*
E2/E3	17.1%	2.9%	0	
E3/E4	0	17.1%	31.4%	

HC: healthy control subjects. MCI: mild cognitive impairment. AD: Alzheimer disease
ACT: Alpha 1-antichymotrypsin. * Chi-square test; # Kruskal-Wallis test ; *P* >0.05 is not significant (NS).

When comparing MMSE score in different Apo E alleles among the 3 studied groups using ANOVA test, a highly statistically significant difference was observed (*P*<0.01). The highest score was associated with (E2/E3) allele (mean of 28 ± 1.6), while the lowest score was associated with (E4/E4) allele (mean of 10 ± 2). However no statistical significant difference was found when comparing MMSE scores in different Apo E alleles among AD group neither MCI group (*P*>0.05) (Table 2).

Table 2. Comparative analysis between MMSE scores in ApoE gene subtypes using ANOVA test.

Group	ApoE	No.	MMSE Score Mean±SD	*P*-value
All participants	E3/E3	65	23±7.5	0.001
	E4/E4	3	10±2	
	E2/E3	5	28±1.6	
	E3/E4	15	17±8.1	
	Total	88	22±8.1	

Table 2. Continued.

Group	ApoE	No.	MMSE Score Mean±SD	*P*-value
AD	E3/E3	19	14±5.9	NS
	E4/E4	3	10±2	
	E3/E4	10	12±6.4	
	Total	32	13±5.8	
MCI	E3/E3	26	25±1.8	NS
	E2/E3	1	26	
	E3/E4	5	25±1.3	
	Total	32	25±1.6	

P >0.05 is not significant (NS).

Correlation study between CRP levels and cognitive assessment showed that there was a highly statistically significant negative correlation between CRP levels and MMSE scores in AD group (rs = -0.54, *P*<0.01) while in MCI group, there was no statistically significant correlation (rs = -0.18, *P*>0.05).

Similarly, there was a highly statistically significant negative correlation between ACT levels and MMSE scores in AD group whereas no statistically significant correlation was observed in MCI group (rs= - 0.53,- 0.08; *P*<0.01, *P*>0.05; respectively) (Table 3).

Table 3. Spearman's Rank correlation test between MMSE, ACT and CRP.

	AD		MCI		HC	
	rs	*P*-value	rs	*P*-value	rs	*P*-value
MMSE/CRP	-0.535	0.002	-0.182	NS	-0.088	NS
MMSE/ACT	-0.528	0.002	-0.084	NS	-0.047	NS

rs: Spearman's correlation coefficient *P* > 0.05 : not significant difference (NS)
AD: Alzheimer disease MCI: mild cognitive impairment. HC: healthy control subjects

The association between CRP levels as well as Apo E 4 allele with AD was evaluated using odds ratio (OR). It was observed that high serum CRP levels could be considered as a positive risk of AD compared with non- AD elderly participants (OR= 2.41; 95% confidence interval [CI]: 1.0- 5.6; *P*= 0.038). Similarly, Apo E (E4 allele) had been associated with increased risk of AD (OR= 4.89; 95% CI: 1.6-14.7; *P*<0.01).

Positive CRP among AD was 2.41 times as that among those non-AD. Combination of both CRP+E3/E3 confers no risk or non-sig. risk, While between CRP+E3/E4 the risk increased from 4.89 to 36 indicating that combining both serum CRP and Apo E gene with at least one E4 allele could be a promising predictor for AD (OR = 36; 95% CI: 11.4-113.7; *P*< 0.01) (Table 4).

Table 4. Calculated Odds Ratios of CRP and ApoE genotype for AD compared with non- AD elderly participants.

Parameter	Odds Ratio	95% CI	Risk
CRP positive (>6 mg/L)	2.41	1.039-5.599	Pos Sig Risk
Apo E3/E4	4.89	1.628 - 14.68	Pos Sig Risk
Apo E3/E3+CRP	1.77	0.531- 5.89	Non-Risk
Apo E3/E4+CRP	36	11.40 - 113.7	Pos Sig Risk

Regression analysis is usually used to search for a panel (independent parameters) that can predict the target parameter (dependent variable). The results of stepwise multi-regression analysis using the 3 studied parameters (Module 1) compared to the combination of Apo E and CRP (Module2) shows that both APOE and CRP together can be used as sensitive discriminators for prediction of AD (F-ratio = 6.63, *P*<0.01) (Table 5).

Table 5. Two modules of logistic multi regression analysis used to identify the most sensitive predictors of AD among the studied parameters.

	Module 1		Module 2	
	Regression coefficient	*P*-value	Regression coefficient	*P*-value
ACT	0.004	NS		
Apo E	-0.047	NS	-0.047	NS
CRP	-0.165	0.005	-0.171	0.002
F- ratio	4.398		6.63	
P-value	0.007		0.002	

P >0.05 is not significant (NS).

Discussion

There is evidence consistent with the hypothesis that genetic, inflammatory and immune mechanisms are involved in the pathogenesis of AD. In this study we have investigated whether the Apo E genotype and the levels of inflammatory associated proteins (CRP & ACT) in serum can reflect the progressive cognitive decline.

In the current study, the most common variant of Apo E gene in the three studied groups was E3/E3, being higher in HC group and the least common was E4/E4 detected only in AD group.

Regarding the distribution of Apo E alleles among the studied groups: ApoE4 allele was more prevalent in AD group (40.6%) followed by MCI (17.1%) whereas ApoE2 was more prevalent in control group (*P*<0.05).

This is in accordance with previous studies[28,29] who reported that the average allele frequency of APOE E4 in cognitively healthy individuals across African American, white, Hispanic and Japanese populations is 9–20%; the variation being attributed to ethnic differences; while it was dramatically increased to ~40% among patients with AD.

In the study of Mattson *et al*, the prevalence of APOE E4 was 66% in individuals with biomarker-confirmed AD-type dementia further highlighting the strong association of APOE E4 with the risk of AD.[30]

According to our results, MMSE scores showed a highly statistically significant difference in APOE gene subtypes among the three studied groups. The lowest scores were associated with APOE (E4/E4) genotype and the highest scores were associated with (E2/E3). However no statistically significant difference was found when comparing MMSE scores in different Apo E alleles carriers among AD group neither MCI group (*P*>0.05) meaning that different Apo E subtypes are not related to severity of cognitive impairment.

In contrast to the well-established effects of the E4 allele on the onset age of AD, with E4 conferring between a 3- (heterozygous) to 15-fold (homozygous) increase in risk of AD, it is controversial whether it affects the speed of cognitive decline after the symptomatic onset. Several studies have suggested the accelerating effects of the E4 allele on the progression rate of AD.[31,32,33] In sharp contrast, other studies have suggested that the E4 carriage does not affect the rate of symptomatic progression in AD, or even may slow it down.[34, 35,36]

In this context, a recent meta-analysis published in 2020 reported that APOE E4 did not affect the progression rate of cognitive decline in early AD regardless of region of patient recruitment and the number of E 4 alleles. The authors highlighted that the effect of E4 was variable in different disease stages.[37]

Longitudinal studies on a diverse population with biomarker-confirmed AD pathology will be needed to elucidate the long-term effects of the APOE E4 allele on the prognosis of AD.

Regarding ACT, there was no statistically significant difference in serum ACT levels between the 3 groups. However, a significant negative correlation between ACT level and MMSE scores in AD group was reported

In accordance with the current study, a longitudinal study in which ACT were determined in paired serum and CSF samples, reported that no significant differences were

found between AD subjects and controls in the mean levels of ACT.[38]

On the other hand, in the study of Porcellini et al. ACT levels were higher in AD patients than in cognitive impairment with no dementia (CIND) or controls.[39] Also, Ethika *et al*. reported elevated levels of ACT in the brain, serum and cerebrospinal fluid (CSF) of AD patients, and high levels of ACT in plasma were associated with cognitive decline in elderly subjects.[14]

The second inflammatory marker measured in our study was CRP. It was significantly higher in AD group than MCI or control groups. In addition, there was highly statistically significant negative correlation between CRP level and MMSE scores in AD group

In accordance with our results, previous studies indicated that Chronic low-grade inflammation is present in patients with cognitive impairment & peripheral CRP concentrations were negatively correlated with MMSE scores in AD patients.[40,41]

Our results are also consistent with the results of two Meta-analysis published in 2019 about the role of peripheral cytokines and chemokines in AD and MCI.[42,43] They revealed consistently elevated concentrations of inflammatory bio-markers in AD patients, whereas no consistent results were obtained for elevated concentrations of cytokines or chemokines in MCI patients. These results highlighted that systemic inflammation might be a biomarker for AD diagnostics, whereas it might be a later event during AD disease progression.

On the contrary Yarchoan et al. found that subjects with AD had significantly lower levels of plasma CRP than subjects with MCI and normal aging. Also it showed that there was no significant association between plasma CRP and cognitive decline as assessed by MMSE score.[44] Similarly, in the study of Guo *et al,* serum levels of CRP were decreased in AD patients.[45] Moreover; in a Korean study, Jang *et a*l reported that CRP levels were not significantly different among AD, MCI, and NC groups and did not show a significant correlation with the overall cognitive function of the subjects and the severity of the disease stage.[46]

These conflicting findings of studies may be explained by the presence of the blood brain barrier which could be responsible for the mismatch between the circulating levels of inflammatory markers and the intracerebral inflammatory responses. Moreover, different bio-marker levels at different stages of AD & sample differences (plasma or serum, methods for sample handling and difference in detection methods) may have given rise to heterogenicity between studies. Heterogenous results might also derive from participants' different ethnic groups.

The association between CRP levels as well as Apo E 4 allele - and AD was evaluated using odds ratio (OR). It was observed that high serum CRP levels could be considered as a positive risk of AD compared with non- AD elderly participants (OR= 2.41; 95% confidence interval [CI]: 1.0- 5.6; *P*= 0.038). Similarly, Apo E (E4 allele) had been associated with increased risk of AD (OR= 4.89; 95% CI: 1.6-14.7; *P*<0.01). Interestingly, it was found that combining both serum CRP and Apo E gene with at least one E4 allele increased the positive risk (OR = 36; 95% CI: 11.4-113.7; *P*< 0.01). Our results are in accordance with Tao et al, who reported that ApoE4 coupled with chronic low-grade inflammation, defined as a CRP level of 8 mg/L or higher, was associated with an increased risk of AD, (hazard ratio, 6.63; 95% CI, 1.80-24.50; *P* =0 .005), as well as an increased risk of earlier disease onset compared with ApoE4 carriers without chronic inflammation (hazard ratio, 3.52; 95% CI, 1.27-9.75; *P* = 0.009).[47]

Finally, stepwise multiple regression analysis was done showing that the combined use of Apo E and CRP can be useful for prediction of AD (F-ratio = 6.63, *P*<0.01).

In conclusion, combining both serum CRP and Apo E gene with at least one E4 allele could be a promising predictor for AD. Clinical follow-up and treatment of chronic systemic inflammation based on genetic risk in Apo E 4 carriers could be effective for the prevention and early intervention of AD.

Author Contributions

MSA, SAH and HMS conceived of the study and participated in its design and coordination. EEA performed the geriatric and cognitive assessment and was responsible for sample collection and original draft preparation, OKZ carried out the molecular genetic studies. WES and AAS carried out the laboratory work-up of the study, performed the statistical analysis and contributed to the

interpretation of results and writing of the manuscript. All authors read, revised, and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study was approved by the Ethics Committee of Faculty of Medicine at Ain Shams University.

Informed consent

A written informed consent was obtained from each patient.

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