Gene–Environment Interaction in Alzheimer's Disease

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Abstract

Objectives: The aim was to examine the gene environment (GxE) interaction with reference to APO E genotypes, serum lipids and organochlorine pesticides (OCPs) as one of the factors in the etiology of Alzheimer's disease (AD). **Methods:** A case control study was used to examine, APOE *HhaI* polymorphism by polymerase chain reaction (PCR)/PCRrestriction fragment length polymorphism method, serum lipids by autoanalyser and OCPs by gas chromatography (GC). **Results:** APOE ε4 allele frequency was significantly high (p=0.000, OR=5.73, CI=2.68-12.50) in AD as compared to controls. The serum cholesterol, β- hexachlorocyclohexane and dieldrin are risk factors for AD independent of the APOE ε4 risk allele, recording an odds ratio of 1.16, 11.38 and 10.45 respectively. **Conclusion:** GxE interactions exist with APOE ε4 allele status that need to be considered for the study design and analysis of such data in future studies of AD.

Keywords

Alzheimer's disease, gene–environment interaction, organochlorine pesticides, APOE polymorphism, β-HCH, dieldrin

Introduction

Alzheimer's disease (AD) is the most common and progressive neurological disease that results in the irreversible loss of neurons, particularly in the cortex and hippocampus.[1] The clinical hallmarks are progressive impairment in memory, judgement, decision making, orientation to physical surroundings, and language. Pathologically, AD is characterized by deposition of amyloid-β protein (Aβ) and neurofibrillary tangles (tau protein) in the brain. The etiology of AD has not yet been resolved, it could result from multifactorial etiopathogenesis. Alzheimer's disease has been linked to at least 5 chromosomes. However, only a portion of patients with AD account for known genetic association.[2]

The apolipoprotein E gene (*APOE*) polymorphism has been recognized as a risk factor for late onset of AD, the frequency of the APOE ε4 allele being increased in patients with AD as compared to control participants.[3,4] Apolipoprotein E (apoE) plays an important role in the metabolism of cholesterol and triglycerides (TGs) by binding to receptors on the liver and help in clearance of chylomicrons and very-low-density lipoproteins (VLDLs) from the bloodstream.[5] It has been shown that increased serum cholesterol levels affect the Aβ production and are associated with risk of AD.[6] Although *APOE* genotyping increases the specificity of the clinical diagnosis of AD but this association is not absolute. It has been suggested that AD could result from a multifactorial process involving both a genetic predisposition and an exposure to environmental factors.[7,8]

The gene–environment interaction (G × E) research shows potential for the psychiatry and neuroscience, although till date only a few studies have been investigated in AD.[9] In fact, evidence for G × E in AD has recently been started to build up. For example, an association between the presence of APOE ε4 allele and consumption of alcohol and smoking has been shown to increase the risk of AD.[10,11] These epidemiological studies indicate that it makes sense to focus on the future clinical AD studies on measuring both genes and environment and analyzing possible interactions. We are looking for G × E interaction with reference to presence or absence of the ε4 allele as one of the risk factors for AD.

Pesticides are environmental chemicals used extensively throughout the world including India. In India, 40% of all

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pesticides used belong to the organochlorine class of chemicals.[12] Organochlorine pesticides (OCPs) are chemically stable, strongly lipophilic as well as very slow in degradation.[12] These compounds are concentrated up in the food chain and can be detected in the diet including drinking water. Thus, the combination of their persistence in the environment and their potential to neurotoxicity suggest that the risk of AD may be associated with OCPs. This study has been designed to examine the G × E interaction with reference to *APOE* genotypes and OCPs as one of the factors in the etiology of AD.

Materials and Methods

Study Population and Design

In this case–control study, patients with AD and controls belonged to the state of Delhi and other surrounding states of North India. They were examined by neurologist in the outpatients department of neurology, Institute of Human Behaviour and Allied Science, Delhi, during February 2010 to January 2012. The sample size was calculated (α = 0.05, β = 0.20, and power = 0.80) by using PS software, version 3.0.14. The study objectives were explained to 70 patients diagnosed with AD and 75 control participants. Patients who were 50 to 85 years old with complaints of memory or other cognitive impairment were subjected to define by the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association[13] for AD and a magnetic resonance imaging/computed tomography/positron emission tomography brain scan supporting the clinical diagnosis of AD were eligible. Additional inclusion criteria were a score of <23 on the Mini-Mental State Examination and a Clinical Dementia Rating score of ≥0.5. Control group comprised of age- and sex-matched healthy volunteers who came to the hospital for routine health checkup.

Participants were excluded in both case and control groups if there was no consent for participation in the study, history of cerebral stroke, epilepsy, a history of head trauma, and other concomitant disease potentially associated with dementia, chronic intake of drugs affecting cognitive processes, moderate-to-severe depressive episode, and familial history of any kind of cognitive/behavioral abnormality. Nutritional deficiency, metabolic abnormalities, and infections in the central nervous system were ruled out.

The study protocol and the informed consent form were reviewed and approved by institutional ethics committee of the institute. Before any study-specific procedures were performed, written consent was obtained from each participant.

APOE Genotyping

Genomic DNA was isolated from peripheral blood by salting-out method[14] and amplified for the region of the fourth exon of *APOE* gene, which encodes amino acid residues, 112 and 158. The specific primers 5′-ACAGAATTCGCCCCGGCCTGG-TACAC-3′ and 5′-TAAGCTTGGCACGGCTGTCCAAGGA-

3′ were used for amplification as described by Pandey et al.[15] Polymerase chain reaction (PCR) assay was performed on Biorad iQ5 cycler, USA, in a 20 μL volume of 1× high-fidelity master mix (Biorad, United States) containing high-fidelity buffer, 0.04 U/μl DNA polymerase, 1.5 mol/L MgCl$_2$, 200 μmol/L dNTPs. 7.5% dimethyl sulfoxide, 10 ng of genomic DNA, and 0.5 μmol/L of each primer were used in final concentration. The PCR protocol consisted of a 1-minute hold at 98°C followed by 35 cycles of 98°C (30 seconds), 65°C (30 seconds) and 72°C (30 seconds), and final extention at 72°C for 3 minutes. The PCR product of 244 bp was digested by 10 units of *Hha*1. The digested product was run on 12% polyacrylamide gel followed by the ethidium bromide staining. Allelic sizes were compared with known size molecular weight marker. Approximately 10% samples were repeated for quality control.

Estimation of OCP Levels

Blood samples were collected using standard venepuncture techniques and stored at −80°C until analysis. The OCPs α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, aldrin, dieldrin, endosulfan, *pp*′-dichlorodiphenyldichloroethylene (*pp*′-DDE), *op*′-DDE, *pp*′-dichlorodiphenyltrichloroethane (*pp*′-DDT), *op*′-DDT, *pp*′-dichlorodiphenyldichloroethane (*pp*′-DDD), and *op*′-DDD were assayed. The compounds selected for assay were based on previous findings[16] that indicated the pesticides most commonly found in human samples and based on the compounds assayed by the Environmental Protection Agency. Samples were analyzed on Perkin Elmer gas chromatograph equipped with ^{63}Ni electron capture detector under standard operating procedure.[16]

The OCPs were extracted from blood according to the method described by Bush et al.[17] Quantitative analysis of OCPs in each sample was analyzed by comparing the peak area with those obtained from a chromatogram of a mixed organochlorine standard (Supelco, Sigma-Aldrich, United States) of known concentration. The detection limit of the detector was <0.05 pg per chloroethylene with nitrogen as a carrier gas. The detection limit of the method was 4 pg/mL for each OCP. For quality control process, 5 blood samples in triplicate were spiked with a mixed standard of OCPs at 5 and 25 ng/mL. The average recoveries of prepared samples exceeded 95%. The case and control samples were run in the same analytical batches and moreover, a quality check sample was always run with each set of samples for pesticide analysis to maintain accuracy.

Estimation of Lipids

Serum lipids (cholesterol, TGs, high-density lipoprotein [HDL], and LDL) were estimated by commercially available kit manufactured by Centronic GmbH, Germany, on autoanalyzer (Random Access Discrete Autoanalyser, XL-300 from Transasia, Mumbai, India).
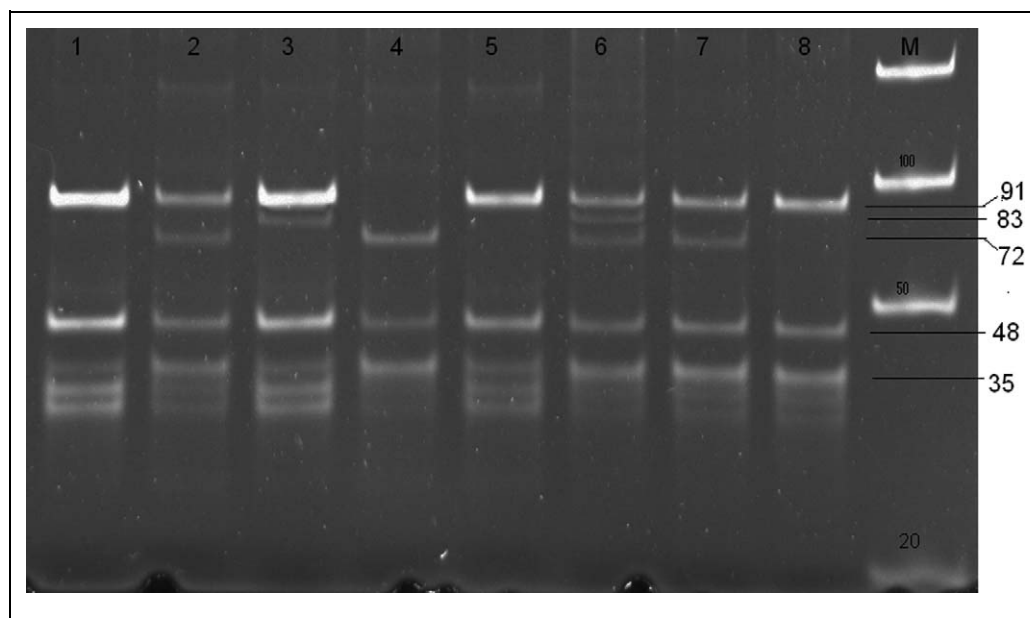
Figure 1. Picture of polyacrylamide gel showing ApoE polymorphism ε3ε genotype in lanes 2 and 7. ε3ε2 genotype in lane 4 and ε4ε2 genotype in lane 6. Molecular weight marker in lane M.

Statistical Analysis

The statistical analysis was carried out by SPSS version 14.0. Mean $\pm$ standard deviations were calculated to describe the quantitative data, whereas percentages were calculated to describe qualitative data. The χ^2 test was applied for the analysis of genotypic and allelic distribution. Odds ratio (OR) and their 95% confidential intervals (CIs) were calculated by 2×2 contingency tables. Independent sample t test was used for comparing mean of normally distributed continuous variables. Mann-Whitney U test was used for comparing continuous variables that did not follow normal distribution. Logistic regression analysis was done with serum cholesterol, HDL cholesterol, LDL cholesterol, β-HCH, dieldrin, and pp'-DDE as predictor variables for AD versus controls status.

Results

No significant difference was found between cases and controls in terms of demographic characteristics. Cases and controls were homogenous in terms of sex, habitat, dietary habit, smoking habit, and alcohol intake habits (data not shown). Restriction analysis of the 244 bp expected PCR product yielded the specific combination of fragments which is shown in Figure 1. The fragment sizes 38, 18, and 16 bp were common in all genotypes except 38 bp other 2 were too small to detect. The allele frequencies and prevalence of *APOE* genotypes results are shown in Table 1. The APOE ε4 allele frequency was significantly high ($P = .000$, OR = 5.73, CI = 2.68-12.50) in AD as compared to controls. Among the *APOE* genotypes, ε3ε4 showed a strong significant association ($P = .000$, OR = 9.56, CI = 3.81-24.55) with the patients with AD.

The independent sample t test results (Table 2) showed that in all participants the mean levels of serum cholesterol and LDL cholesterol were significantly higher ($P < .001$ in both) and HDL cholesterol levels were significantly lower ($P < .01$) in patients with AD as compared to the controls. In participants with ε3ε3 genotypes, the mean level of serum cholesterol was significantly higher ($P < .001$) and HDL cholesterol level was significantly lower ($P < .05$) in patients with AD as compared to the controls. The mean level of serum cholesterol and LDL cholesterol were significantly higher ($P < .01$ and $P < .05$, respectively) in patients with AD as compared to control participants which have ε3ε4 genotypes. No significant difference was found in serum lipid levels in participant with other genotypes (ε3ε2, ε4ε4, and ε4ε2).

Mann-Whitney U test (Table 3) revealed a significant difference in β-HCH levels ($U = 1237.00$, $W = 4087.00$, $z = -6.29$, $P = .000$), dieldrin levels ($U = 1449.00$, $W = 4299.00$, $z = -5.81$, $P = .000$), and pp'-DDE levels ($U = 2062.00$, $W = 4912.00$, $z = -2.69$, $P = .007$) in all participants of cases and controls. These 3 OCP levels β-HCH ($U = 193.00$, $W = 1733.00$, $z = -5.74$, $P = .000$), dieldrin ($U = 360.00$, $W = 1900.00$, $z = -4.23$, $P = .000$), and pp'-DDE ($U = 449.50$, $W = 1989.50$, $z = -2.53$, $P = .012$) were also significant in participants which have ε3ε3 genotypes, whereas participants which have ε3ε4 genotype, only the mean level of dieldrin was found to be significantly higher in patients with AD as compared to controls ($U = 85.00$, $W = 140.00$, $z = -3.03$, $P = .002$). The OCP levels were not significant in other *APOE* genotypes (ε3ε2, ε4ε4, and ε4ε2). The OCPs, op'-DDE, op'-DDT, pp'-DDD, and op'-DDD were not detected in both the groups.

Direct logistic regression (Table 4) was performed to assess the impact of a number of factors on the likelihood of

Table 1. The APOE Allele and Genotype Frequencies, P Value, OR, and 95% CI for Alzheimer's Disease (AD) and Control Populations[a]

Alleles/Genotypes	Controls (n = 75)	AD (n = 70)	P Value	OR	CI
ε3	129 (0.860)	90 (0.643)			
ε4	11 (0.073)	44 (0.314)[b]	.000	5.73	2.68-12.50
ε2	10 (0.066)	6 (0.043)	NS		
ε3ε3	55 (0.733)	23 (0.328)			
ε3ε4	10 (0.133)	40 (0.571)[b]	.000	9.56	3.81-24.55
ε3ε2	9 (0.120)	4 (0.057)	NS		
ε4ε4	–	1 (0.014)	NS		
ε4ε2	1 (0.013)	2 (0.028)	NS		
ε2ε2	–	–	–		

Abbreviations: CI, confidence interval; NS, not significant; OR, odds ratio; -, not present.
[a]Figures within parentheses indicate the frequency.
[b] $P < .001$.

developing AD. The full model containing all predictors was not statistically significant. Only serum cholesterol ($P < .05$), β-HCH ($P = .01$), and dieldrin ($P < .05$) levels were statistically significant.

Discussion

This is one of the first studies in which the role of *APOE* genotypes along with OCP levels was seen in patients with AD and healthy participants. No differences were found between demographical profiles such as age, sex, habitat, dietary habit, smoking, and alcoholic status. The human glycoprotein (apoE), one of the least abundant plasma apoproteins, is coded by APOE. The *APOE* gene is polymorphic and is located on chromosome 19q13 with 3 common allele, ε2, ε3, and ε4 (coding for 3 isoforms E2, E3, and E4) which produce 3 homozygous (ε2ε2, ε3ε3, and ε4ε4) and 3 heterozygous (ε2ε3, ε3ε4, and ε4ε2) phenotypes in the human population.[18] These isoforms differ by single amino acid substitutions (missense mutation) at codons 112 and/or 158. The ε3 allele, considered to be the parental allele, has cystine at codon 112 and arginine at codon 158.[19] The ε2 allele has cystine at both positions, while the ε4 allele contains arginine at both positions.[19] The *APOE* ε3 allele and homozygous ε3ε3 genotype were most prevalent in controls, whereas APOE ε4 allele and heterozygous ε3ε4 genotype in AD.

In the present study, 31.4% of the patients with AD and 7.3% of controls carried at least 1 ε4 allele and 4.3% of patients with AD and 6.6% of controls carried ε2 allele, respectively. The patients with AD are 5.7 times more susceptible when having APOE ε4 allele. Among the genotypes, ε3ε4 shows a strong significant association with AD (9.56 times elevation). The present findings regarding the APOE ε4 allele frequency is in the corroboration with other studies of Indian origin.[20,21] Allele frequencies of the APOE polymorphism have been reported to vary among different populations.[20,21] A possible explanation for this might be due to genetic drift by founder effect and/or due to the different prevalence rates of AD and/or the selection bias of samples.

Earlier studies indicated that the higher serum cholesterol and LDL cholesterol levels were associated with the risk of AD.[22,23] The present study showed that the mean levels of serum cholesterol and LDL cholesterol were significantly higher, whereas HDL cholesterol levels were significantly lower in patients with AD as compared to the controls. Experimental studies suggest that serum cholesterol and LDL cholesterol levels are associated with increased deposition of Aβ 1-42 in AD.[24,25] Huaqi Xiong et al,[24] explained the detailed mechanism by which higher cholesterol levels affects this process but the detailed mechanism is still unclear by which higher LDL cholesterol affects this process, whether they increase AD risk directly or through indirect mechanisms in which their metabolites can enter the brain and promote the accumulation of Aβ. Higher levels of HDL cholesterol were associated with a decreased risk of AD as compared with lower HDL cholesterol levels.[26] Amyloid β binds to HDL, maintaining its solubility in cerebrospinal fluid and plasma. This HDL–Aβ interaction prevents the deposition of Aβ in the brain.[27]

We have also observed the influence of *APOE* genotypes on serum lipid levels. Apolipoprotein E is involved in the transportation of cholesterol from peripheral tissues to the liver for catabolism. Overexpression of ε4 allele increases serum cholesterol and LDL cholesterol levels[28] and it may be involved in the development of cognitive impairment and may also promote extracellular aggregation of Aβ peptide in the aging brain,[29] which is the main pathological feature of AD. The APOE has been shown to bind directly to the Aβ peptide and influence its fibrillogenesis and clearance. However, ε4 allele is less effective than the ε3 and ε2 alleles to mediate neuronal repair, remodeling, and protection.[30,31]

The present study showed that the mean levels of serum cholesterol and LDL cholesterol in ε3ε4 genotypes were significantly higher in patients with AD as compared to controls. The ε3ε4 genotype codes E4 isoform of apoE. The binding capacity of E4 isoforms to the VLDL receptor is high[32] as a result of which serum cholesterol and LDL cholesterol levels were elevated. In wild type/common ε3ε3 genotypes, serum cholesterol levels were significantly higher. However, HDL cholesterol levels were significantly lower in patients with AD as compared to controls which follow the observations of previous studies.[26,27]

Table 2. Distribution of Serum Lipid Levels According to the *APOE* Genotypes in Patients With Alzheimer's Disease (AD) and Control Participants[a]

Variables (ng/mL)	All Participant		ε3ε3		ε3ε4		ε3ε2		ε4ε4		ε4ε2	
	Control (n = 75)	AD (n = 70)	Control (n = 55)	AD (n = 23)	Control (n = 10)	AD (n = 40)	Control (n = 09)	AD (n = 04)	Control (n = 00)	AD (n = 01)	Control (n = 01)	AD (n = 02)
Cholesterol	122.03 ± 30.37	169.89 ± 38.84[b]	114.45 ± 24.67	134.48 ± 23.97[b]	161.70 ± 26.17	190.60 ± 24.68[c]	121.33 ± 34.73	145.0 ± 19.69	–	300.0	147.0	147.50 ± 10.61
TG	158.19 ± 53.02	157.96 ± 76.81	150.36 ± 50.41	162.17 ± 90.05	176.40 ± 29.61	167.05 ± 69.58	165.56 ± 53.25	118.0 ± 40.03	–	109.0	340.0	101.50 ± 21.92
HDL	46.29 ± 9.09	41.54 ± 8.40[c]	46.75 ± 8.93	41.35 ± 7.04[d]	46.12 ± 5.97	41.38 ± 9.56	44.78 ± 13.10	44.50 ± 4.36	–	36.0	37.0	44.0 ± 8.48
LDL	71.47 ± 24.61	106.13 ± 45.63[b]	62.07 ± 16.33	55.52 ± 13.86	113.80 ± 11.83	137.80 ± 27.92[d]	79.22 ± 26.06	74.50 ± 4.43	–	176.0	100.0	83.0 ± 1.41

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; -, not detected.
[a] The values are presented as mean ± SD.
[b] $P < .001$.
[c] $P < .01$.
[d] $P < .05$.

Table 3. Distribution of Organochlorine Pesticides (OCPs) According to the *APOE* Genotypes in Alzheimer's Disease (AD) and Control Participants[a]

Variables (ng/mL)	All Participants		ε3ε3		ε3ε4		ε3ε2		ε4ε4		ε4ε2	
	Control (n = 75)	AD (n = 70)	Control (n = 55)	AD (n = 23)	Control (n = 10)	AD (n = 40)	Control (n = 09)	AD (n = 04)	Control (n = 00)	AD (n = 01)	Control (n = 01)	AD (n = 02)
α-HCH	0.56 ± 0.27 (4.0)	0.25 ± 0.95 (8.57)	0.08 ± 0.32 (5.45)	0.10 ± 0.50 (4.35)	–	0.30 ± 1.08 (10.0)	–	–	–	3.40	–	–
β-HCH	0.25 ± 0.59 (17.33)	4.16 ± 4.61[b] (60.0)	0.27 ± 0.64 (16.36)	5.85 ± 5.75[b] (73.91)	0.28 ± 0.49 (30.0)	3.07 ± 3.75 (50.0)	0.11 ± 0.33 (11.11)	2.84 ± 3.42 (50.0)	–	7.12	–	7.66 ± 1.78
γ-HCH	0.07 ± 0.35 (5.33)	0.32 ± 1.35 (10.0)	0.04 ± 0.22 (3.36)	0.51 ± 2.14 (8.69)	0.04 ± 0.13 (10.0)	0.20 ± 0.69 (10.0)	0.29 ± 0.89 (11.11)	–	–	2.61	–	–
Dieldrin	0.16 ± 0.61 (9.33)	4.82 ± 6.65[b] (50.0)	0.12 ± 0.44 (9.09)	3.70 ± 4.94[b] (47.82)	–	6.27 ± 7.59[c] (57.5)	0.15 ± 0.46 (11.11)	0.30 ± 0.6 (25.0)	–	–	4.02	–
Aldrin	0.09 ± 0.04 (5.33)	0.22 ± 0.81 (8.57)	0.12 ± 0.47 (7.27)	0.37 ± 1.21 (8.69)	–	0.07 ± 0.33 (5.0)	–	0.30 ± 0.60 (25.0)	–	2.80	–	–
α-Endosulfan	0.07 ± 0.30 (6.66)	0.26 ± 0.85 (10.0)	0.06 ± 0.23 (7.27)	0.19 ± 0.64 (8.69)	–	0.25 ± 0.81 (10.0)	0.22 ± 0.66 (11.11)	1.05 ± 2.10 (25.0)	–	0.33	–	–
β-Endosulfan	0.05 ± 0.27 (4.0)	0.10 ± 0.50 (4.28)	0.06 ± 0.32 (5.45)	–	–	0.11 ± 0.49 (5.0)	–	0.70 ± 1.40 (25.0)	–	–	–	–
pp′-DDT	0.14 ± 0.64 (6.66)	0.55 ± 1.86 (10.0)	0.17 ± 0.73 (7.27)	0.30 ± 1.44 (4.35)	0.14 ± 0.44 (10.0)	0.67 ± 2.22 (12.5)	–	1.20 ± 2.40 (25.0)	–	–	–	–
pp′-DDE	0.82 ± 1.93 (22.66)	2.52 ± 5.69[d] (41.42)	0.66 ± 1.44 (21.82)	4.38 ± 9.15[d] (43.48)	0.91 ± 2.11 (30.0)	1.90 ± 2.55 (47.5)	1.78 ± 3.78 (22.22)	–	–	–	–	–

Abbreviations: DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCH, hexachlorocyclohexane; -, not detected.
[a] The values are presented as mean ± SD, figures within parentheses indicate percentage of sample in which OCP was detected.
[b] $P < .001$.
[c] $P < .01$.
[d] $P < .05$.

Table 4. Risk Factors for Alzheimer's Disease

		B	SE	Wald	df	Sig	OR	95.0% CI for OR	
								Lower	Upper
Step 1[a]	Cholesterol	0.145	0.067	4.724	1	0.030	1.157	1.014	1.319
	HDL	0.015	0.082	0.033	1	0.856	1.015	0.864	1.192
	LDL	−0.075	0.057	1.696	1	0.193	0.928	0.829	1.038
	β-HCH	2.432	0.942	6.669	1	0.010	11.380	1.797	72.058
	Dieldrin	2.347	1.093	4.613	1	0.032	10.454	1.228	89.012
	pp′-DDE	0.189	0.274	0.479	1	0.489	1.209	0.707	2.067

Abbreviations: CI, confidence interval; df, degrees of freedom; HCH, hexachlorocyclohexane; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; pp′-DDE, pp′-dichlorodiphenyldichloroethylene; SE, standard error; Sig, significance.
[a] Variable(s) entered on step 1: cholesterol, HDL, LDL, β-HCH, dieldrin, pp′-DDE.

Studies have shown an association of ε4 allele with AD, which proved that it is a susceptible gene for AD.[20,21] The present study indicates that ε4 allele is not absolutely contributing to AD genotypes as we have found the presence of ε4 allele in healthy participants, whereas it was absent in diagnosed patients with AD (n = 27). The *APOE* genotyping might improve the specificity of the clinical diagnosis of AD. However, the presence of the ε4 allele alone is neither necessary nor sufficient for the risk of AD. Other factors may participate independently or in concert with APOE to determine the overall risk of AD. The G × E research shows potential for the etiology of several neurological diseases. The OCPs are persistent in the environment and their potential to neurotoxicity suggests that the risk of AD may be associated with OCPs.

In the present study, the mean levels of β-HCH, dieldrin, and pp′-DDE were found to be higher in patients with AD as compared to the controls. It has been previously suggested that OCP produce free radicals which lead to oxidative stress.[33] The brain having high lipid content and poor antioxidant defenses makes the brain an ideal target for free radical attack. One theory on the pathogenesis of AD postulates that neurodegeneration is the result of oxidative stress and damage to vulnerable cerebral tissues.[34] Richardson et al,[35] indicated that β-HCH levels were not associated with AD, whereas pp′-DDE levels were associated with AD but this observation was based on only 20 patients with AD and 43 controls and done in different cohorts. It has also been noticed that most of the OCPs that are banned in the developed countries are still being used extensively in the developing nations throughout the world including India. The higher levels of pp′-DDE were also found as per the observation of previous studies.[36,37] Dieldrin is a major metabolite of aldrin and metabolized at a much slower rate to hydrophilic metabolites due to extremely apolar nature. The present study indicates that elevated dieldrin levels are associated with AD. Experimental studies (in vitro and in vivo) using mammalian system suggest that dieldrin induces oxidative stress and apoptosis.[38] These cellular responses are common during the progression and manifestation of human neurological diseases, such as AD and Parkinson's disease. However, the dieldrin-mediated neurotoxicity mechanisms are not fully characterized.

In this study, the intergenotypic OCP levels were also screened. Of the total number of patient with AD, 32.85% patients had common/wild type of ε3ε3 genotypes, and in these patients the β-HCH, dieldrin, and pp′-DDE were found to be significantly higher as compared to the controls. However, 57.14% of patients with AD had ε3ε4 genotype and only dieldrin level was significantly higher. It has also been suggested that the antioxidant property among APOE alleles is followed as ε2 > ε3 > ε4.[34] This study shows the role of β-HCH, dieldrin, and pp′-DDE with the presence of AD in those with the absence of ε4 allele. Interpretation cannot be made for the rest of the genotypes because of less sample size which is the major limitation of this study.

Alzheimer's disease is influenced not only by genetic factors but also by environmental components. The study indicates a potential association between OCPs and AD varied by APOE ε4 allele status. The OCPs showed a positive association with AD in participants without an APOE ε4 allele. The direct logistic regression result shows that beside ε4 allele, higher levels of total cholesterol, β-HCH, and dieldrin were also associated with increased risk of AD, recording an OR of 1.16, 11.38, and 10.45, respectively. This indicates that for an additional 1 unit increment of total cholesterol, β-HCH, and dieldrin levels, chances of developing AD in a population will be 11.16, 11.38, and 10.45, respectively. β-Hexachlorocyclohexane and/or dieldrin were not detected in 3 patients diagnosed with AD which were not carrying ε4 allele which may suggest the multifactorial etiopathology nature of AD.

In conclusion, the results of the study suggest that besides the APOE ε4 allele, the presence of β-HCH and dieldrin in serum are important determinants of the presence of AD. Among lipids, higher total cholesterol levels significantly increased the odds of the AD presence. Multifactorial genetic and environmental determinants of the AD should be studied in more detail by prospective studies with a larger sample size to further understand the mechanisms involved.

Declaration of Conflicting Interests

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

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