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Synergistic Epistasis of Paraoxonase 1 (rs662 and rs85460) and Apolipoprotein E4 Genes in Pathogenesis of Alzheimer's Disease and Vascular Dementia

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Abstract

Genetic polymorphism and epistasis play a role in etiopathogenesis of Alzheimer's disease (AD) and vascular dementia (VaD). In this case-control study, a total of 241 patients were included in the study to see the effect of paraoxonase 1 (PON1; rs662 and rs85460) and apolipoprotein E (ApoE) genes in altering the odds of having AD and VaD along with serum PON and lipid profile. The presence of at least 1 variant allele of rs662, but not rs85460, increased the risk of having AD by 1.8-fold (95% confidence interval [CI]: 0.97-3.40) and VaD by 3.09-fold (95% CI: 1.4-6.9). The interaction between PON1 genes (rs662 and rs85460) and ApoE genes showed synergistic epistasis in altering the odds of significantly having both AD and VaD. On the other hand, low serum level of high-density lipoprotein and low level of serum PON activity were found associated significantly ($P \leq .001$ in both cases) only in patients with VaD as compared to healthy control.

Keywords

Alzheimer's disease, vascular dementia, paraoxonase, synergistic epistasis, apolipoprotein, lipid profile

Introduction

Dementia is one of the major causes of disability in elderly people. The number of such cases has been increasing remarkably because of increasing life expectancy of humanity. World Alzheimer's report (2009) estimates that more than half of these patients with dementia worldwide live in low- and middle-income countries; the number is expected to rise up to 71% by 2050. Because of population, the disease burden will be enormous for India. Two most common types of dementia, Alzheimer's disease (AD) and vascular dementia (VaD), have several possible pathophysiological causes and mechanisms that contribute to a common pathway of neuronal loss.

Genetic polymorphism is supposed to cause variation in the gene product, the biochemical parameters that could be measured in serum. At a deeper and broader level, gene–gene interaction or epistasis plays a role in causation of the disease. Sporadic AD is presumed to be the result of interactions of many genes and environmental factors, with each gene only having a small effect on the disease. Epistasis has been found to play an important role in the pathogenesis of AD in the context of amyloid β metabolism, lipid metabolism, oxidative stress, and inflammatory cytokines.[1]

In this study, we assessed whether polymorphism in paraoxonase 1 (PON1; rs662 and rs85460) gene has any predictive effect on the pathogenesis of AD and VaD. We also estimated serum level of lipids and PON, which are influenced by PON1 genes. At deeper level, we looked for gene–gene interaction

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between PON1 genes and apoliporotein E (ApoE4; from our previously published data) genes in the studied group of patients.

Paraoxonase 1 not only has antioxidant activity but also has antiatherogenic function.[2] It is synthesized in the liver and transported in plasma with high-density lipoprotein (HDL). It prevents oxidation of low-density lipoprotein (LDL). Its serum concentration is influenced by inflammatory changes. The LDL is responsible for the transport of triglycerides (TGs) and cholesterol from the blood and tissues to the liver and is highly susceptible to oxidation.[3] The LDL in oxidized form has been shown to promote smooth muscle cell proliferation, platelet adhesion, and foam cell formation.[4-6] All these phenomena are operative in the process of early atherogenesis. That serum lipid-related known cardiological risk factors also contribute to the development of AD has been reported long back in 1999 by Kalman et al.[7] The demonstration that susceptibility of LDL to oxidation is increased in cerebrospinal fluid and in plasma of patients with AD, in parallel with reduced levels of antioxidants, further indicates a crucial role for LDL oxidation in AD.[8] Therefore, in the present study, we took up the measurement of the whole spectrum of lipid parameters along with PON in serum.

As mentioned earlier, PON1 serum levels and activity are genetically determined and are strongly influenced by common single-nucleotide polymorphism in the coding region. This fact prompted us to look into the study on genetic polymorphism of PON1 (L55M: rs854560 and R192Q: rs622) in patients with AD and VaD along with their connection, if any, with serum PON and lipid profiles.

We also examined gene–gene interaction of PON1 polymorphism (rs854560 and rs622) with our previously published data on polymorphism of ApoE gene in patients with AD and VaD in terms of alteration in odds ratio (OR).[9]

Methods

In this case–control study, patients with AD (n = 75) and VaD (n = 46) were recruited following strict inclusion and exclusion criteria described in our previously published article[9] at the cognitive disorder clinic at All India Institutes of Medical Sciences (AIIMS), New Delhi. Clinical and computed tomography/magnetic resonance imaging criteria were critically examined to avoid any clinical contamination between patients with AD and VaD. Healthy controls (HCs; n = 120) of comparable gender and age (approximate ± 3 years) were recruited mostly from genetically unrelated relatives of the patients and their attendants after exclusion of cognitive impairment by the Mini-Mental State Examination. Both patients and HCs were part of our previously published study.[9] All the patients are of Indian origin. None of the patients included in the study was under treatment with any drug that can alter the lipid profile level. Institution ethics clearance and informed consent were obtained from all study patients. The cognitive states of the patients were fair enough for signing the informed consent. The blood sample was collected after an overnight fasting, and further processing was done as indicated subsequently.

DNA Extraction

Genomic DNA was extracted from the EDTA blood by the salting-out method.[10] Extracted DNA was further used for genotyping.

Detection of rs622 Genotypes

The rs622 genotypes were determined by polymerase chain reaction (PCR)-restricted fragment length polymorphism (RFLP) according to previously published protocols with contingent modification. The gene was amplified using sense primer 5′-TAT TGT TGC TGT GGG ACC TGA G 3′ and antisense primer 5′-CAC GCT AAA CCC AAA TAC ATC TC 3′. After digestion of amplicon (99 bp) with restriction enzyme *BspPI* (NEB: Massachusetts, USA), variant allele produced 2 fragments (66 bp + 33 bp), while wild type remained undigested.[11,12]

Detection of rs854560 Genotypes

The rs854560 genotypes were determined by PCR-RFLP according to previously published protocols with contingent modification. The gene was amplified using sense primer 5′-GAA GAG TGA TGT ATA GCC CCA G-3′ and antisense primer 5′-TTT AAT CCA GAG CTA ATG AAA GCC-3′. Amplified product was digested with RE *NlaIII* (NEB). After digestion, wild allele remained undigested, while variant allele produced 2 fragments (110 bp + 60 bp).[11,12]

Lipid Assay

Total serum cholesterol, TGs, and HDL were estimated using kits from RANDOX Labs Ltd (Antrim, United Kingdom) in a semi-auto analyzer. Low-density lipoprotein was calculated using Friedewal's formula[13]:

$$LDL = TC - HDL - TG/5.0 \text{ (mg/dL)}.$$

Paraoxonase Assay

Paraoxonase activity in serum was determined by highly sensitive, homogeneous fluorometric assay (excitation/emission maxima ~360/450 nm) for the organophosphatase activity of PON (Invitrogen: Carlsbad, CA, USA). The method is based on the hydrolysis of a fluorogenic organophosphate analog and is 10-fold more sensitive than the colorimetric assay.

Statistical Analysis

Statistical analysis was carried out using state 11.0 (Stata Corp, College Station, Texas). Data were presented as numbers (%) or mean $\pm$ standard deviation (SD)/median (minimum-maximum) as appropriate. Continuous variables were compared among the 3 groups using analysis of variance/Kruskal-Wallis test followed by post hoc analysis (Bonferroni correction). Categorical variables were compared among the groups using chi-square test/Fisher exact test. The association

Table 1. Allele and Genotype distribution of PON1 (rs662 and rs854560) Genes Among AD, VaD, and HC Groups.

Polymorphisms	AD (n = 75)	VaD (n = 46)	HC (n = 120)	*P* value	OR (95% CI)
rs662					
Alleles					
Q (wild)	100 (66.7%)	49 (53.2%)	173 (72.1%)	.26[a]	1.29 (0.81-2.05)[a]
R (variant)	50 (33.3%)	43 (46.8%)	67 (27.9%)	.001[b]	2.27 (1.33-3.83)[b]
Genotypes					
QQ (wild)	32 (42.7%)	14 (30.4%)	69 (57.5%)		
QR (heterozygous)	36 (48.0%)	21 (45.7%)	35 (29.2%)	.044[a]	1.82 (0.97-3.40)[a]
RR (variant)	7 (9.3%)	11 (23.9%)	16 (13.3%)	.002[b]	3.09 (1.42-6.91)[b]
rs854560					
Alleles					
L (wild)	121 (80.7%)	72 (78.2%)	192 (80.0%)	.87[a]	0.96 (055-1.65)[a]
M (variant)	29 (19.3%)	20 (21.8%)	48 (20.0%)	.72[b]	1.11 (0.58-2.06)[b]
Genotypes					
LL (wild)	50 (66.7%)	31 (67.3%)	76 (63.4%)		
LM (heterozygous)	21 (28.0%)	10 (21.8%)	40 (33.3%)	.64[a]	0.86 (0.45-1.65)[a]
MM (variant)	4 (5.3%)	5 (10.9%)	4 (3.3%)	.62[b]	0.83 (0.38-1.80)[b]

Abbreviations: AD, Alzheimer's disease; CI, confidence interval; OR, odds ratio; PON1, paraoxonase 1; VaD, vascular dementia; HC, healthy control.
[a] *P* value for AD versus HC.
[b] *P* value for VaD versus HC.

between polymorphism and AD/VaD and HC groups was assessed using logistic regression analysis. The results were reported as OR (95% confidence interval [CI]). The *P* value less than .05 were considered as statistically significant.

Results

In this case–control study, a total of 241 patients were genotyped for 2 polymorphisms of PON1 gene (rs662 and rs85460) in patients with AD (n = 75) and VaD (n = 46) and in HCs (n = 120). Of all the cases with AD, 68.0% were male and for VaD it was 74.0%. When compared to HCs (62.5%), there was no statistically significant ($P = .43$, $P = .15$) difference. In the patients studied, the following mean age with SD was found: AD (66.2 $\pm$ 9.2 years), VaD (66.1 $\pm$ 8.8 years), and HC (63.8 $\pm$ 8.2 years). There was no statistically significant difference in the age ($P = .1$). The frequency of the PON1 genotype (rs854560) was consistent with the Hardy-Weinberg principle ($\chi^2 = 1.68$, $P = .19$) in our sample, but the frequency of the PON1 genotype (rs622) was not consistent with the Hard-Weinberg principle ($\chi^2 = 4.68$, $P = .03$). This variation could be a change due to mutation, population migration, or nonrandom distribution of sample.

Allele Frequency

The frequency of rs622 variant (R) allele was observed highest in patients with VaD (46.8%) when compared to patients with AD (27.9%) and HCs (33.3%; Table 1). The frequency difference between VaD and HC was statistically significant ($P = .001$) but not between AD and HC ($P = .26$). The frequency of variant (M) allele of rs85460 was almost same in all 3 groups, patients with AD (19.3%), patients with VaD (21.8%), and in controls (20.0%). The correlation between prevalence of the 2 PON variant alleles with age was calculated, and no significant association was observed.

Gene Frequency

Presence of at least 1 variant allele (QR/RR) in genotype of rs662 showed significant difference (Table 1) in frequency of both the groups (AD vs HC: $P = .044$ and VaD vs HC: $P = .002$), and it increased the risk of having AD by 1.8-fold (95% CI: 0.97-3.40) and VaD by 3.09-fold (95% CI: 1.4-6.9). However, variant (RR) genotype of patient groups (AD: 7.3% and VaD: 23.9%) did not show any statistically significant difference in frequency when compared to the control group (13.3%). In the analysis of genotype of rs854560, the presence of at least 1 variant allele (LM/MM) did not show any significant difference when compared to controls. However, a low frequency (5.4%) of variant (MM) homozygous genotype was observed in all 3 study patients; in controls (3.3%), in patients with AD (5.3%), and in patients with VaD (10.9%), respectively.

In this study, no such case was found either in patients with AD and in patients with VaD who had homozygous variant genotype of rs622 and rs854560. Only 2 cases of AD were found where homozygous variant genotype of rs622 was present along with a heterozygous variant genotype of rs85460.

Gene–Gene Interaction

To find out the epistatic interaction in altering the odds of having disease (AD and VaD), the allelic/genotypic frequency of PON1 (rs662 and rs8545260) gene was analyzed in combination with the ApoE polymorphism data reported earlier by our group, which is as follows: the frequency of genotype ε3ε4 was higher in patients with AD (38.7%) and VaD (26.1%) than in

Table 2. Relationship Between Combination of PON1 rs854560 (LM + MM) and ApoE ε4 Genotypes and the Outcome of Having AD and VaD.[a]

rs854560 (LM+MM)	ApoE ε4	AD (n = 75)	VaD (n = 46)	HC (n = 120)	OR (95%CI)	*P* value
Absent	Absent	27 (36.0%)	24 (52.2%)	64 (53.4%)	1 (reference)	-
Absent	Present	23 (30.7%)	7 (15.2%)	12 (10.0%)	4.5 (1.9-10.4)[b]	<.001[b]
					1.5 (0.5-4.4)[c]	.4[c]
Present	Absent	13 (17.3%)	8 (17.4%)	40 (33.3%)	0.7 (0.3-1.6)[b]	0.5[b]
					0.5 (0.2-1.3)[c]	0.1[c]
Present	Present	12 (16.0%)	7 (15.2%)	4 (3.3%)	7.1 (2.1-24.0)[b]	0.002[b]
					4.6 (1.2-17.3)[c]	0.02[c]

Abbreviations: AD, Alzheimer's disease; VaD, vascular dementia; HC, healthy control; CI, confidence interval; OR, odds ratio; PON1, paraoxonase 1; ApoE, apolipoprotein E.
[a] Odds Ratio of having AD and VaD against HC.
[b] *P* values and OR of AD versus HC.
[c] *P* values and OR of VaD versus HC.

Table 3. Relationship Between Combination of PON1 rs662 (QR + RR) and ApoE ε4 Genotypes and the Outcome of Having AD and VaD.[a]

rs662 (QR+RR)	ApoE ε4	AD (n = 75)	VaD (n = 46)	HC (n = 120)	OR (95%CI)	*P* value
Absent	Absent	15 (20.0%)	8 (17.4%)	63 (52.5%)	1 (reference)	-
Absent	Present	17 (22.7%)	6 (13.0%)	6 (5.0%)	11.9 (4.0-35.3)[b]	<0.001[b]
					7.8 (2.0-30.4)[c]	0.003[c]
Present	Absent	25 (33.3%)	24 (52.2%)	41 (34.2%)	2.5 (1.2-5.4)[b]	0.014[b]
					4.6 (1.8-11.2)[c]	0.001[c]
Present	Present	18 (24.0%)	8 (17.4%)	10 (8.3%)	7.5 (2.9-19.6)[b]	<0.001[b]
					6.3 (1.9-20.6)[c]	0.002[c]

Abbreviations: AD, Alzheimer's disease, VaD, vascular dementia; HC, healthy control; CI, confidence interval; OR, odds ratio; PON1, paraoxonase 1; ApoE, apolipoprotein E.
[a] Odds Ratio of having AD and VaD against HC.
[b] *P* values and OR of AD versus HC.
[c] *P* values and OR of VaD versus HC.

controls (12.5%). Genotype ε4ε4 was more frequent in patients with AD (8.0%) and VaD (4.3%) while totally absent in controls.[9]

Interaction between rs85460 and ApoE ε4 gene. Presence of wild-type genotype of both polymorphisms (rs85460 and ApoE ε3) was taken as reference for multinomial logistic regression (Table 2). It was observed that singular presence of ApoE ε4 increased the odds of having AD by 4.5 times ($P \leq .001$, 95% CI: 1.9-10.4) and VaD by 1.5 times ($P = .4$, 95% CI: 0.5-4.4), while the singular presence of rs85460 variant allele in genotype (LM/MM) did not alter the odds of having AD and VaD. However, their presence in combination with ApoE ε4 increased the odds of having AD to 7.1 times ($P = .002$, 95% CI: 2.1-24.0) and VaD to 4.6 times ($P = .02$, 95% CI: 1.2-17.3; data shown in Table 3). The result exemplifies synergistic epistasis.

Interaction between rs622 and ApoE ε4 gene. For the analysis of interaction between ApoE ε4 and rs622 (Table 3), the presence of wild genotype was taken as reference. Singular presence of ApoE ε4 increased the OR of having AD by 11.9-fold ($P \leq .001$, 95% CI: 4.0-35.3) and VaD by 7.8-fold ($P = .003$, 95% CI: 2.0-30.36). Singular presence of rs622 (QR/RR) also increased the odds of having AD by 2.5-fold ($P = .014$, 95% CI: 1.2-5.4) and VaD by 4.6-fold ($P \leq .001$, 95% CI: 1.8-11.24). Presence of variant genotype of both polymorphisms together increased the odds of having AD and VaD by 7.5-fold ($P \leq .001$, 95% CI: 2.9-19.6) and 6.3-fold ($P = .002$, 95% CI: 1.9-20.6), respectively.

Serum Chemistry

Low level of serum HDL and PON showed significant difference in their mean value in patients with VaD when compared to HCs (HDL: $P = 0.001$ and PON: $P \leq .001$). This difference was not observed in patients with AD (Table 4).

Serum TGs, total cholesterol (TC), and LDL did not show any significant difference between diseased (AD and VaD) and HC groups. In analysis of interrelation of different lipid parameters, it was found that TC showed significant positive correlation with TG ($r = .1$ and $P = .001$) and LDL ($r = .84$ and $P \leq .001$), whereas LDL showed significant negative correlation ($r = -.29$ and $P = .001$) with HDL (data not shown). Correlation analysis between PON activity and HDL cholesterol has been done only in case of HCs, and no significant correlation has been observed.

Table 4. Serum Levels of Lipid (mg/dL) and Paraoxonase (U/mL) in Patients With AD, VaD, and HC.[a]

Groups	AD (n = 75)	VaD (n = 46)	HC (n = 120)	*P* value
Lipid level				
TG	129.0 ± 43.7	124.7 ± 35.6	122.6 ± 31.3	0.71[b], 1.0[c]
TC	170.7 ± 31.7	168.7 ± 30.5	167.6 ± 22.8	1.0[b], 1.0[c]
HDL	40.5 ± 9.5	37.2 ± 6.1	42.4 ± 8.3	0.36[b], 0.001[c]
LDL	104.4 ± 28.8	106.6 ± 27.1	100.6 ± 21.8	0.92[b], 0.5[c]
Paraoxonase	4.3 (1.1-9.2)	2.8 (0.1-9.5)	5.5 (0.1-14.9)	0.19[b], < 0.001[c]

Abbreviations: AD, Alzheimer's disease; VaD, vascular dementia; HC, healthy control; SD, standard deviation; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a] Data presented for Lipid level: mean ± SD and for paraoxonase: median (range).
[b] *P* value for AD versus HC.
[c] *P* value for VaD versus HC.

Correlation of Genetic Polymorphism With Serum Level of PON and Lipids

Correlation between genetic polymorphism and biochemical levels were analyzed by categorization of the HCs on the basis of presence or absence of polymorphism against the respective parameters. Homozygous wild-type genotypes QQ of rs662 were associated with a high level of serum PON (median [range] = 5.7[0.96-14.9]) when compared to heterozygous QR (median [range] = 5.4[0.54-10.1]) and variant homozygous RR (median [range] = 5.2[0.13-8.9]), but the difference was not statistically significant. Similarly, in case of rs85460, variant homozygous genotype MM was associated with high level of serum PON (median (range) = 6.8 [3.0-9.4]) when compared to heterozygous LM (median [range] = 5.1[0.54-14.9]) and wild homozygous LL (median [range] = 5.5[0.13-10.3]), but the difference was not statistically significant. No association was observed between PON1 (rs662 and rs85460) gene polymorphism and serum level of lipids (TG, TC, HDL, and LDL) in the HC group.

Discussion

Frequency of rs622 alleles varies among different ethnic populations. The variant allele frequency in present study (27.9%) shows similarity with those reported from Italy (27.0%), Korea (30.0%), Japan (33.0%), and India (29.0%), while high frequency of this allele has been reported in studies conducted in China (65.0%) and Taiwan (64.0%).[14-19] Varying results have been reported for the PON1 rs662 polymorphism in patients with AD and VaD. Studies from Italy, China, Japan, and Poland suggested that rs662 polymorphism is not associated with AD.[20-23] There are other studies which observed that the variant allele of rs662 had a protective role for the development of AD.[24,25] In this study, we observed a significant association of rs622 polymorphism which increased risk of having AD (by 1.8-fold). Our result is in agreement with a previously published report from France, which studied 27 cases of VaD and 45 cases of AD and found rs622 to be a reliable marker to distinguish patients with AD from patients withVaD and HCs.[26] The present study however has also shown the association of rs622 polymorphism with the frequency of VaD, increasing the OR by 3.09. Another study from French population showed influence of rs622 polymorphism on non-AD dementia.[27] Unlike other geographical regions why rs622 gene polymorphism does not alter OR of having VaD in French population cannot be easily explained. Lifestyle difference and difference in nutritional contents and habits of the patient might have the answer. Migration of different ethnic population with intermixing of genes could be another factor that merits to be looked into.

The frequency of variant homozygous genotype (MM) was found very low (HC = 3.3%) in the present study, while studies from Taiwan, Mexico, and Thailand found that the rs85460 genotype was rare.[19,28,29] No significant difference in rs85460 frequency was observed between patients with AD and VaD and HCs. The result concurs with a study from Polish population in late-onset AD, while another study from Canada shows that the Met allele of the rs85460 was associated with an increased risk of developing AD.[23,30] One of the study from France as quoted earlier has also not shown any association of rs85460 gene polymorphism with AD and VaD.[26]

The study by Janka et al[31] is based on 53 patients with AD, 55 patients with VaD, and 51 HCs. Also, the study by Kalman et al[7] is on 25 patients with AD and 15 HCs. In the present study, it was possible to recruit 75 patients with AD, 46 patients with VaD, and 120 HCs. Even then the sample size is not large enough to draw any robust conclusion from our study.

Epistasis is gene–gene interaction where presence or absence of 1 gene influences the function of another gene. Both AD and VaD are complex diseases, and multiple genes are implicated in their pathogenesis. If suspected candidate genes are examined individually, it may reflect a small effect on the disease. Thus, for better understanding of gene function in the involvement of complex disease, epistasis is more relevant. Epistasis has been demonstrated in disease such as diabetes, rheumatoid arthritis, and stroke.[32-34] In the present study, we found this interaction in cases of both AD and VaD. Although presence of (LM or MM) genotype of rs85460 did not alter any risk in the patient groups, singular presence of ApoE ε4 genotype increased the odds of having AD by 4.5-fold but did not alter odds for VaD in a significant way; however, the presence of both rs85460 and

ApoE ε4 alleles together increased the odds significantly in the diseased groups, 4.6-fold for AD and 7.1-fold for VaD. Most remarkable part of this observation is the role of ApoE ε4 which usually does not affect odds of having VaD, here increasing the odds of having VaD by 7.1-fold in association with rs85460 which by singular presence has no effect on frequency of VaD (Table 2). This example of epistasis and synergism, to our knowledge, has not been reported earlier. It strengthens the theory of multiple gene interaction in AD and VaD. From 2 available studies on gene–gene interaction between PON1 and ApoE ε4 in patients with AD in caucasians and African Americans population, no evidence of interaction between ApoE ε4 and PON1 (rs622 and rs85460) had been observed.[35] Another study in the Italian population regarding rs705381 failed to detect any interaction with ApoE ε4 allele status in AD.[36]

Further, in the case of combined presence of rs622 (QR or RR) genotype and ApoE ε4 allele, OR of having AD did alter significantly but not to the expected summated level or more. On the contrary, this appeared slightly lower in magnitude when compared to singular presence of ApoE ε4 allele. Then, the argument flows, does this combination have a protective role? Since the number of controls and patients was small, it restrains us from drawing an affirmative conclusion on a definitive synergism or protectionism in this interaction. The mechanism and the reason of such synergism of ApoE ε4 and PON1 polymorphism are yet to be explored. To make a definite conclusion, a study with larger sample size is warranted.

The effect of genes is supposed to be reflected in several biochemical parameters, and in this study, this was expected on serum lipid levels of TG, TC, and LDL. A study by Zuliani et al found that HDL-C has been associated with dementia, independent of the factors like age, gender, stroke, ApoE4, and interleukin 6 status.[37] However, their levels were not significantly increased in AD and even in patients with VaD when compared to HCs. In a previous study, Moroney et al[38] had observed high level of LDL-C in dementia with stroke but could not find any association of LDL-C level in the patients with AD. Kalman et al reported that increased HDL-C levels were significantly higher in patients with AD when compared to HCs.[7] However, this study included very low number (AD = 24, HC = 15) of cases and controls. Another study by Janka et al found PON2S allele and ApoE4 alleles have interactive effect on AD and VaD and supported the hypothesis that the cardiovascular factor contributes to development of AD.[31] In the present study, low serum level of HDL was significantly associated in patients with VaD when compared to HC but not in the case of patients with AD. The results are in accordance with the previously published data from the United States.[39] Also, there is a significant difference ($P \leq .001$) in the PON activity observed only in patients with VaD when compared to HC, and the result is in accordance with previous report as published in French population.[40] The study by Paragh et al[41] reported significantly decreased PON activity in both patients with AD and patients with VaD. It is still not clear whether PON activity has any independent role in etiopathogenesis of VaD or the difference in PON activity between VaD and controls may be due to alteration in the components of HDL as suggested by Corrigan et al.[42] As PON is a HDL-associated enzyme, decrease in HDL-associated PON activities in patients with VaD could offset its protective role against lipid peroxidation of LDL. Our study did not show any statistically significant correlation between genetic polymorphism with serum PON or HDL level. In contrast to Kálmán et al's report, we did not find any difference in PON and lipid parameter between patients with AD and HCs.[7]

To conclude, the present study shows statistically significant increased risk of having both AD and VaD because of presence of at least 1 variant allele (QR/RR) of rs662 but not with any variant of rs85460. In addition, the result suggests synergistic epistatic interaction between 2 genes, PON1 (rs662, rs85460) and ApoE, pointing toward involvement of several genes in the etiopathogenesis of both AD and VaD. A significant difference in the serum PON activity and low level of HDL was observed in patients with VaD when compared to HC, whereas TG, TC, and LDL levels were not significantly altered in either patients with AD or VaD . One of the limitations of the study is to draw any robust conclusion from such small number of patients. Since nutrient component in the diet has an influence on serum lipids, one of the other limitations of the study is the lack of recent dietary intake record of the patients and controls. Taking stock of above-mentioned limitations, it is recommended that such study may be conducted on a large sample size collected from different ethnic groups with proper nutritional history to reach a definitive conclusion.

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Declaration of Conflicting Interests

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