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Advances in Applied Science Research, 2017, 8(3):65-75

ISSN : 0976-8610
CODEN (USA): AASRFC

Assessment of Modifiable and Non-Modifiable Risk Factors of Dementia as Early Diagnostic Blood Based Biomarkers in Western Indian Population

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ABSTRACT

Background: *Worldwide prevalence of dementia is growing rapidly. Epidemiology of dementia consists of both the modifiable as well as non-modifiable risk factors and there is a dearth of knowledge about use of these as early diagnostic biomarkers. Hence our study aimed at assessment of these risk factors which can be used as promising biomarkers for early diagnosis of dementia.*

Methods: *The study population consisted of patients with Dementia (n=33), Mild Cognitive Impairment (MCI; n=30) and elderly age-matched Controls (n=30). All the participants were subjected to medical history, psychological, clinical and biochemical evaluations, serum biomarker estimations like vitamin B12, Folic acid and homocysteine and genetic studies which included gene expression and polymorphism of APOE and MTHFR genes.*

Findings and conclusion: *Our study showed significant difference in various biomarkers including modifiable metabolic factors (serum levels of vitamin B12, folic acid and homocysteine) and non-modifiable genetic factors (APOE & MTHFR) across the three study groups. Thus, if validated at early stages of ageing or before start of cognitive decline, together these biomarkers and Psychological assessment will help in early diagnosis of dementia risk.*

Keywords: APOE, MTHFR, Gene expression, Homocysteine, Dementia risk

INTRODUCTION

Aging is associated with cognitive deterioration and many factors are implicated in individual cognitive differences in these aged population. Dementia, an age associated illness with a devastating impact on patients and their families, has become a prominent health issue. The total number of people with dementia worldwide in 2010 is estimated at 35.6 million and is projected to nearly double every 20 years [1]. Prevalence of dementia reported from Indian studies range from 0.6% to 3.5% in rural areas and 0.9% to 4.8% in urban areas [2]. Mortality risk was 2.3 times more for older people with dementia and linearly correlated with the severity of cognitive impairment [3]. Hence, detecting dementia at the earliest possible stage is vital and considerable efforts are being invested in the identification of biomarkers for this purpose.

Epidemiological studies have identified many risk factors for dementia, including age, medical factors (cardiovascular disease, history of depression, head injury), demographic factors, as well as family history and genotype. The use of biological markers offers the potential for more accurate diagnosis of dementia [4,5]. These biomarkers consist of both modifiable as well as non-modifiable risk factors. The modifiable risk factors include biological markers such as serum levels of lipids, lipoproteins, thyroid hormones and nutritional deficiencies of Vitamin B12, Folate, Homocysteine, etc. Historically, antioxidative nutrients and B-vitamins have been evaluated for neuroprotective effects. Vitamin B12 and Folate play vital roles as enzyme cofactors or substrates in the methylation of homocysteine to methionine and in the

synthesis of S-adenosylmethionine (SAM) through One Carbon Metabolism (OCM). As B-vitamins and homocysteine are interlinked and their metabolism is dependent on each other in the methylation cycle, deficiency in the levels of any one of these vitamins leads to dysregulation of methylation cycle and consequently affect homocysteine metabolism. This results in the accumulation of intracellular and serum homocysteine in the body leading to a clinical condition called hyperhomocysteinemia, which is considered as a risk factor for dementia.

Among relevant non-modifiable risk factors are a number of common gene polymorphisms which play a role in the development of dementia, but their effects are complicated and patterns of inheritance vary considerably. Many genes, including apolipoprotein E (APOE), and methylenetetrahydrofolate reductase (MTHFR) are considered putative risk factors for vascular pathology in dementia.

Apolipoprotein E (APOE) gene codes for a protein involved in cholesterol transport and lipid metabolism [6]. APOE gene is polymorphic and the different alleles of APOE include ε2, ε3 and ε4. Literature suggests that the variant APOE-ε4 is a major risk factor for the development of Alzheimer-type dementia (AD) [7,8]. The major effect of APOE isoforms on the risk of developing AD is via its effect on Aβ aggregation and clearance, influencing the onset of Aβ deposition. Other mechanisms include the effects of APOE isoforms on synaptic function, neurotoxicity, tau hyperphosphorylation and neuroinflammation, which may also contribute to the disease process [9,10].

Dysregulation of one-carbon metabolism (OCM) through different mechanisms is also considered as a causative factor for deterioration of cognitive abilities in elderly population. Components of OCM such as low blood levels of folate and vitamin B12 and elevated homocysteine levels were associated with poor cognitive performance in elderly people [11,12]. Another important component of OCM is methylenete trahydrofolate reductase (MTHFR) enzyme, which is a central enzyme forming the substrate needed for the transferring reaction. Furthermore, insufficient one-carbon metabolism has been suggested to have a contributory role in the development of dementia and has found to be significant in AD patient [13]. A polymorphism at position 677 of the MTHFR gene (C677T) produces a thermolabile form of the enzyme with low activity; this mutation results in hyperhomocysteinemia, a known risk factor for cognitive decline [13,14]. Recently different studies correlated MTHFR gene polymorphism with homocysteine and folate levels and it had been observed that for adequate functioning of MTHFR enzyme in both homozygous and heterozygote mutants, folate supplementation is required [14,15]. A large number of studies have provided a broad overview of the prevalence of the 677C>T polymorphism in different human populations, showing that the distribution of frequencies is diverse [16].

Although abundance of literature suggests effect of polymorphism in APOE & MTHFR gene on the risk of dementia, there is a dearth of knowledge about gene expression of both these genes in the field of dementia research. Therefore our study was proposed to analyse gene expression levels as well as the genotype and allelic frequency of both the genes in Western Indian population and their relation to the risk of dementia. We also assessed association of some modifiable risk factors to the risk of dementia.

METHODS

Subjects and samples

In this prospective study, total of 93 study participants aged >50 years were enrolled. The study population was divided into three groups; viz., patients suffering from Dementia (n=33), patients with Mild Cognitive Impairment (MCI) (n=30) and elderly age-matched controls (n=30). The diagnosis of Dementia and MCI cases was made according to the standard Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) diagnostic criterion; according to which dementia is characterized by multiple cognitive deficits, which include memory impairment and at least one of the following: aphasia, apraxia, agnosia or disturbance in executive functioning. Hence, DSM IV criteria were used along with the MMSE score to divide patients into the category of Dementia, Mild Cognitive Impairment and Normal. A score of MMSE below 24 indicated dementia along with subjective complaints of forgetfulness or aphasia or impairment in executive functioning in daily living. MMSE score of >27/30 indicated Normal controls.

Dementia and MCI cases were referred from two hospitals located in Mumbai; Sir H. N. Hospital & Research Centre, Mumbai, India and T.N. Medical College & B.Y.L. Nair Hospital, Mumbai, India. Elderly age matched controls were healthy individuals from the nearby vicinity of Sir H.N. Hospital & Research Centre falling in the same age category.

Ethics statement

The procedures of this study were approved by the Scientific Advisory Committee and the Institutional Ethics

Committee. The study was carried out in accordance with the "Ethical Guidelines for Biomedical Research on Human Participants, 2006" by the Indian Council of Medical Research and the Declaration of Helsinki, 2008, and written informed consent was obtained from all participants involved in this study.

At the time of recruitment, all the participants were subjected to a baseline clinical and neurological examination.

Psychological assessment

Cognitive assessment of study participants was conducted by a Clinical Psychologist. All the participants underwent a comprehensive geriatric assessment, including history, medication history, physical and neurological examination. They were put through questions for assessment of the Mini Mental State Examination (MMSE) and Addenbrook's Cognitive Examination (ACE). In MMSE, functions such as registration, attention, calculation, recall, language (Comprehension, reading, writing and naming), ability to follow simple commands and orientation were examined. However, in ACE along with MMSE other additional questions for remote memory, verbal fluency, naming, language and visuospatial were assessed to test various specific domains of cognition.

Clinical and biochemical evaluations

Anthropometric measurements such as height and weight of each study participant were calculated. BMI was calculated as weight/(height)^2 and expressed in kg/m^2. Two readings of blood pressure (systolic and diastolic) were recorded using mercury sphygmomanometer as recommended by the American Society of Hypertension, and the average was used for analysis.

6 ml of blood was collected by venipuncture after an overnight fasting and serum was separated after centrifugation at 3000 rpm for 10 min. Analysis of blood glucose levels, lipid profile, SGOT, SGPT, VDRL, and Thyroid profile (T3, T4 and TSH) were performed on fresh serum on the same day. Biochemical estimations were performed using a clinical chemistry auto analyser Konelab i20 (Thermo Scientific, Waltham, USA). In haematology, erythrocyte sedimentation rate (ESR) and complete blood count (CBC) were performed using VES-MATIC 20 and Sysmex 2000I haematology analyser respectively. All the measurements except ESR measurement which was done immediately after blood collection were done within 2 h of collection.

Biomarker estimation

4 ml of blood was collected in plain bulb and the separated serum was used for the ELISA assays using commercial ELISA kits as mentioned below [17-19].

- Total Homocysteine (MyBioSource, USA)
- Vitamin B12 (MyBioSource, USA)
- Folic Acid (MyBioSource, USA)
- Holotranscobalamin (IBL International, Germany)

For genetic studies, 3 ml of blood was collected from all the study subjects into tubes containing EDTA.

Gene polymorphism study

Genomic DNA was extracted from peripheral leukocytes using salting out method. All DNA samples were stored at –20°C until further genotyping studies. Genotyping was performed using Polymerase Chain Reaction (PCR) – Restriction Fragment Length Polymorphism (RFLP) method.

APOE genotyping

The APOE gene was amplified using primers described by Wenham et al. [20]. The PCR profile involved initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 60°C for 1 min and 70°C for 2 min, with a final extension at 70°C for 10 min. The amplified product was electrophoresed on a 1% agarose gel. Further, the amplified fragments were digested with the 5 units of HhaI enzyme (New England Biolabs) overnight at 37°C and separated on a 4% agarose gel electrophoresis and visualized under UV spectrophotometer after ethidium bromide staining [20,21].

MTHFR 677 C>T genotyping

PCR amplification of exon 4 of MTHFR gene using primers described by Frosst et. al. [22] resulted in a 198 bp product. PCR Conditions were as follows: Initial denaturation at 94°C for 8 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 1 min and extension at 72°C for 1 min, with final extension at 72°C for 7 min. The amplified PCR product was digested with 5 units of HinfI restriction enzyme at 37°C for 1 h and separated on 3% agarose gel stained with ethidium bromide [22].

Gene expression study

To study mRNA expression of APOE and MTHFR gene, EDTA anticoagulated blood samples were used for extraction

of the Total RNA from whole blood using QIAamp® RNA Blood Mini Kit (Qiagen). RNA was dissolved in DEPC-treated water and the concentration was measured by fluorometry using the Qubit® 2.0 Fluorometer (Invitrogen, USA). cDNA was produced from 1 µg of total RNA by High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems, USA). The mRNA expression was measured by quantitative TaqMan real-time PCR (qPCR) assays in 96 well plates using a StepOnePlus Real-Time PCR system (Applied Biosystems, CA, USA). The assay ID Hs00171168_m1 and Hs01114487_m1 were used to access the APOE and MTHFR mRNA expression respectively. Gene specific TaqMan probes and primers were used. GAPDH gene was used as an endogenous control for both the genes. The relative quantification of mRNA was calculated by the comparative Ct method using the formula $2^{-\Delta\Delta Ct}$.

STATISTICAL ANALYSIS

Statistical evaluation of the results was performed using the statistical software SPSS, version 21.0 (SPSS, Chicago, IL, USA). The allele frequencies and genotype distribution were estimated by gene counting. SNP analyzer software v. 1.0 was used to verify whether the genotypes distribution were in Hardy-Weinberg equilibrium (HWE) using the expectation-maximization algorithm. Differences in non-continuous variables, genotype and allelic distributions were compared by chi-square test. Normality distribution for all continuous variables was tested by Kolmogorov-Smirnov test. Comparison between groups was done using either one-way analysis of variance-ANOVA (if normally distributed) or Kruskal-Wallis test (if not normally distributed) with post-hoc tests. Correlation between two numerical variables was assessed using Spearman's rho correlation coefficient. Student's t-test was used to compare the patient group with the control group. Baseline differences were analysed using Mann-Whitney U-test and independent samples t-tests (continuous data). A p-value <0.05 was accepted as statistically significant.

RESULTS

Assessment of markers of cognitive decline

Table 1 shows significantly lower global MMSE and ACE score in patients with dementia when compared to controls and patients with MCI. Also significant difference was observed between MMSE score of MCI patients and controls (P<0.001).

Table 1: Comparison of global cognitive scores of MMSE and ACE analysis

Parameter	Dementia (n=33) Mean ± S.D	MCI (n=30) Mean ± S.D	Controls (n=30) Mean ± S.D	Overall P value (Post-hoc P value after Bonferroni's correction)
MMSE score (Range: 0-30) (Normal score: ≥ 27)	20.5 ± 6.5	27.3 ± 1.4	29.4 ± 0.7	**<0.001***** a: 0.000 b: 0.000 c: 0.000
ACE score (Range: 0-100) (Normal score ≥ 90)	58.9 ± 20.5	83.9 ± 5.9	90.6 ± 6.0	**<0.001***** a: 0.000 c: 0.000

*p<0.05, ** p<0.01, ***p<0.001; a: Dementia vs. Controls; b: MCI vs. Controls; c: Dementia vs. MCI

Clinical and biochemical evaluations

Clinical profile

As shown from Table 2, all the groups were comparable with respect to the demographic details and anthropometric measurements.

Table 2: Baseline characteristics of study population (mean ± SD)

Parameters	Dementia (n=33)	MCI (n=30)	Controls (n=30)	Overall P value (post hoc P value after Bonferroni's correction)
Age (years)	68.5 ± 6.9	65.4 ± 8.4	66.8 ± 7.7	0.305
Male:Female	20:13	22:7	13:17	-
Weight (kg)	59.01 ± 12.8	63.5 ± 10.3	59.8 ± 14.1	0.375
Height (ft)	5.2 ± 0.4	5.3 ± 0.3	5.2 ± 0.3	0.857
BMI (kg/m²)	10.76 ± 2.06	10.95 ± 1.89	10.15 ± 2.27	0.776
Diastolic blood pressure (mm Hg)	78.8 ± 5.65	78.8 ± 10.2	82.7 ± 6.48	0.09
Systolic blood pressure (mm Hg)	123.93 ± 15.1	129.6 ± 25.8	126.2 ± 15.4	0.830

Biochemical estimations

Table 3 represents all the biochemical estimations undertaken in our study. Total Cholesterol and HDL concentrations were significantly lower in patients with Dementia compared to MCI and elderly age-matched controls (all $p<0.05$). We observed a significant decrease in serum ApoA1 levels in Dementia and MCI patients compared to elderly age-matched controls ($p<0.01$). Further, serum ApoB levels were found to be decreased significantly only in patients with Dementia compared to elderly age-matched controls ($p<0.05$). In thyroid profile, significant decrease in Total T4 serum levels was observed in patients with Dementia compared to elderly matched controls ($p<0.001$).

Note: This part of our study was previously published [23].

Table 3: Biochemical parameters across the Study groups represented as Mean ± SD

Parameters	Dementia (n=33)	MCI (n=30)	Controls (n=30)	Overall P value (post hoc P value after Bonferroni's correction)
Glucose profile				
Fasting blood sugar (mg/dl)	111.4 ± 38.7	110.5 ± 46.2	95.9 ± 21.86	0.18
Post-prandial blood sugar (mg/dl)	152.4 ± 76.7	157.8 ± 84.4	121.6 ± 45.1	0.13
Lipid profile				
Total cholesterol (TC) (mg/dl)	178.4 ± 26.5	178.9 ± 34.8	210.0 ± 63.3	**0.01***a: 0.03 c: 0.02
High-density lipoprotein (HDL) (mg/dl)	49.1 ± 11.7	51.0 ± 14.4	73.0 ± 55.5	**0.01*** a: 0.01
Low-density lipoprotein (LDL) (mg/dl)	108.4 ± 29.7	97.0 ± 31.7	108.1 ± 26.3	0.26
Very low-density lipoprotein (VLDL) (mg/dl)	24.9 ± 2.8	29.7 ± 8.6	20.9 ± 7.47	0.12
Triglycerides (mg/dl)	124.7 ± 79.2	148.5 ± 93.6	104.7 ± 37.3	0.14
Apolipoprotein A1 (g/L)	1.24 ± 0.1	1.29 ± 0.1	1.43 ± 0.2	**<0.001*****a: <0.001 b: 0.003
Apolipoprotein B (g/L)	.05 ± 0.1	1.15 ± 0.18	1.15 ± 0.16	**0.024*** a: 0.04
Apolipoprotein B/AI ratio	0.84 ± 0.05	0.88 ± 0.16	0.81 ± 0.19	0.21
Thyroid profile				
Total T3 (ng/dL)	92.6 ± 18.8	96.1 ± 28.5	97.6 ± 18.0	0.65
Total T4 (µg/dL)	4.29 ± 2.5	6.23 ± 2.5	6.83 ± 1.4	**<0.001*****a: <0.001
Thyroid stimulating hormone (TSH) (µIU/ml)	3.56 ± 1.3	2.74 ± 1.7	3.15 ± 1.14	0.35
Liver function tests				
Serum glutamic oxaloacetic transaminase (SGOT) (U/L)	19.4 ± 8.1	20.2 ± 6.8	22.3 ± 7.8	0.31
Serum glutamic pyruvic transaminase (SGPT) (U/L)	17.1 ± 9.2	17.4 ± 6.9	20.1 ± 9.6	0.35

*p<0.05, ** p<0.01, ***p<0.001; a: Dementia vs. Controls; b: MCI vs. Controls; c: Dementia vs. MCI

Serum biomarker estimation

As represented in Table 4, significantly elevated levels of serum homocysteine were seen in patients with dementia when compared to controls and MCI patients (p<0.001). Similarly, we found significant decrease in serum levels of holotranscobalamin and folic acid in dementia and MCI patients as compared to controls (p<0.001). Though Vitamin B12 concentration was found to be low in dementia group and slightly higher in MCI group as compared to controls, but difference was not statistically significant.

Table 4: Estimation of serum levels of different biomarkers in all the study groups by ELISA

Parameters	Dementia (n=33)	MCI (n=30)	Controls (n=30)	Overall P value (post hoc P value after Bonferroni's correction)
Homocysteine (nmol/ml) (Mean ± S.D)	26.9 ± 3.0	21.07 ± 4.4	12.6 ± 4.0	**<0.001***** a: <0.001 b: <0.001 c: <0.001
Vitamin B12 (pg/ml) (Median, minimum & maximum)	109.7 (87.3, 161.1)	132.3 (90.1, 175.5)	125.4 (85.1, 180.1)	0.7
Holotranscobalamin (pmol/L) (Mean ± S.D)	45.2 ± 20.8	75.2 ± 30.4	89.8 ± 24.7	**<0.001***** a: <0.001 b: <0.001
Folic Acid (ng/ml) (Mean ± S.D)	6.8 ± 1.5	8.3 ± 1.8	9.9 ± 3.7	**<0.001***** a: <0.001 b: 0.04

*p<0.05, ** p<0.01, ***p<0.001; a: Dementia vs. Controls; b: MCI vs. Controls and c: Dementia vs. MCI

Correlation studies

In this study, we observed a moderate positive correlation of markers of cognitive decline (MMSE & ACE score) with serum levels of holotranscobalamin and folic acid (p<0.001). On the other hand, MMSE and ACE score showed a strong negative correlation with serum homocysteine levels. Also, an inverse correlation was seen between serum levels of homocysteine and holotranscobalamin (p<0.001) (Table 5).

Table 5: Association between markers of cognitive decline & serum biomarker concentrations

Parameters		Spearman's correlation coefficient (σ)	P value
MMSE score	**Holotranscobalamin (pmol/L)**	0.4	<0.001
	Homocysteine (nmol/ml)	-0.61	<0.001
	Folic Acid (ng/ml)	0.34	0.001
ACE score	**Holotranscobalamin (pmol/L)**	0.4	<0.001
	Homocysteine (nmol/ml)	-0.62	<0.001
	Folic Acid (ng/ml)	0.31	0.002
Holotranscobalamin (pmol/L)	**Homocysteine (nmol/ml)**	-0.54	<0.001
	Folic Acid (ng/ml)	0.33	0.001

*p<0.05, ** p<0.01, ***p<0.001

Genetic studies

APOE genotyping

Genotype and Allele frequencies observed among the patients are summarized in Table 6. All observed genotype frequencies in our study met the Hardy-Weinberg equilibrium (χ^2 P-value>0.05). In dementia group, the genotype frequencies of E2/E4, E2/E3, E3/E3, E3/E4 and E4/E4 were 6.06%, 12.12%, 12.12%, 42.42% and 21.21%, respectively. Similarly for MCI group, frequencies for six genotypes E2/E2, E2/E4, E2/E3, E3/E3, E3/E4 and E4/E4 were 3.3%, 13.3%, 26.7%, 40%, 10% and 6.7%, respectively. Further among controls we observed four genotype frequencies as follows: E2/E2 (20%), E2/E4 (3.3%), E2/E3 (30%) and E3/E3 (46.7%). Frequency of APOE alleles did differ between the dementia cases and controls (P<0.01): E2, 9.1% vs. 36.7%; E3, 42.4% vs. 61.7%; and E4, 48.5% vs. 1.6%.

Table 6: APOE genotype and allele frequency among the controls and patients

Groups	Dementia (n=33)	MCI (n=30)	Control (n=30)
Genotype frequency	**N (%)**	**N (%)**	**N (%)**
E2/E2	0	1 (3.3)	6 (20)
E2/E4	2 (6.06)	4 (13.3)	1 (3.3)
E2/E3	4 (12.12)	8 (26.7)	9 (30)
E3/E3	4 (12.12)	12 (40)	14 (46.7)
E3/E4	16 (42.42)	3 (10)	0
E4/E4	7 (21.21)	2 (6.7)	0
χ^2 (P-value)	0.02 (0.8)	0.04 (0.8)	0.24 (0.61)
Allele frequency	**N (%)**	**N (%)**	**N (%)**
E2	6 (9.1)	14 (23.3)	22 (36.7)
E3	28 (42.4)	35 (58.3)	37 (61.7)
E4	32 (48.5)***	11 (18.3) **	1 (1.6)

***: Significant difference in the E4 allele frequencies between dementia cases and controls (OR=0.024; 95% CI: 0.003 to 0.183; P<0.0001)
**: Significant difference in the E4 allele frequencies between MCI cases and controls (OR=0.086; 95% CI: 0.0105 to 0.701; P=0.009)

MTHFR genotyping

Observed genotype frequencies in our study population were found to be consistent with Hardy-Weinberg equilibrium (χ^2 P-value>0.05). The frequency of genotypes MTHFR 677CC, 677CT and 677TT in the dementia patients was, respectively, 54.4%, 39.4% and 6.1%. Among the patients with mild cognitive impairment, 70% presented genotype 677CC, 26.7% genotype 677CT and 3.3% genotype 677TT. While in the control group, genotype frequencies of 677CC and 677CT were found to be 83.3% and 16.7%, respectively (Table 7).

Table 7: MTHFR genotype and allele frequency among the controls and patients

Groups	Dementia (n=33)	MCI (n=30)	Control (n=30)
Genotype frequency	**N (%)**	**N (%)**	**N (%)**
CC	18 (54.5)	21 (70)	25 (83.3)
CT	13 (39.4)	8 (26.7)	5(16.7)
TT	2 (6.1)	1 (3.3)	0
χ^2 (P-value)	0.02 (0.8)	0.04 (0.8)	0.24 (0.61)
Allele frequency	**N (%)**	**N (%)**	**N (%)**
C	49 (74)	50 (83)	55 (92)
T	17 (26)*	10 (16.7)	5 (8)

*: Significant difference in the T allele frequencies between dementia cases and controls (OR=3.81; 95% CI: 1.31-11.11; P=0.01)

Gene expression studies

Figures 1A and 1B shows the evaluation for mRNA expression levels of APOE and MTHFR gene represented by their corresponding relative quantification (RQ) values. Expression of MTHFR gene was observed to be significantly decreased in patients of dementia when compared to MCI and controls (p<0.01). Similarly, APOE gene mRNA expression was also found to be decreased in dementia as well as MCI patients when compared to controls (p<0.05).

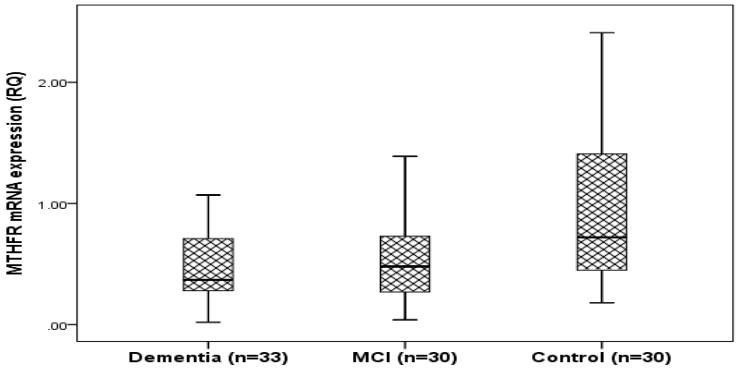

Figure 1A: mRNA expression levels of MTHFR gene across the study groups

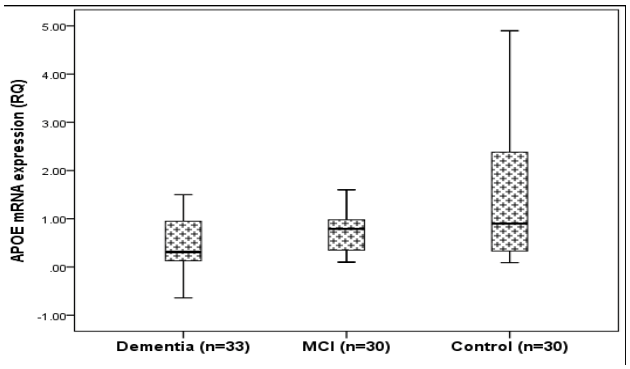

Figure 1B: mRNA expression levels of APOE gene across the study groups

DISCUSSION

Epidemiologies of dementia consist of both modifiable as well as non-modifiable risk factors. The modifiable risk factors include biological markers such as serum levels of lipids, lipoproteins, thyroid hormones and nutritional deficiencies of Vitamin B12, Folate, Homocysteine, etc. whereas non-modifiable risk factors of dementia include certain genes which play a role in the development of dementia by way of different mechanisms [24].

The silent findings of our study are: 1) Significant difference was observed between expression levels of APOE and MTHFR gene in patient and control group. 2) Further gene polymorphism study of APOE ε4 allele and MTHFR C677T revealed that frequency of risk allele was observed to be high in dementia group as compared to control group. 3) Patients in dementia and MCI group had reduced serum levels of vitamin B12 and folic acid, and elevated serum levels of homocysteine when compared to elderly age-matched controls. 4) Further association studies demonstrated a moderate positive correlation of markers of cognitive decline (MMSE and ACE score) with serum levels of holotranscobalamin and folic acid whereas a strong negative correlation with serum homocysteine levels. An inverse correlation between serum homocysteine and holotranscobalamin was also seen.

Changes in gene expression are associated with numerous biological processes, cellular responses and disease states. Literature from aging studies conducted in model organisms such as yeast, worms, and flies have constantly shown that changes in the expression of certain genes have an effect upon longevity. Although similar aging processes are likely to operate across multiple species, it has been much more difficult to identify longevity candidate genes in human studies [22]. There are no recent human studies performed in India or abroad where expression levels of APOE and MTHFR gene has been assessed in relation to dementia risk or cognitive decline related to aging. Hence, according to our knowledge this is a novel and pilot study which evaluates mRNA expression levels of APOE and MTHFR from blood of dementia patients from Indian population to understand if there is any correlation with cognitive impairment and dementia risk.

MTHFR gene codes for an enzyme called methylene tetrahydrofolate reductase which is associated with higher plasma homocysteine, a well-known mediator of neuronal damage and brain atrophy. In the present study, dementia patients showed significantly decreased expression of MTHFR gene as compared to MCI patients and controls. Further gene polymorphism study of MTHFR C677T revealed that the frequency of mutant T allele was significantly high in dementia group compared to controls. Also in the present study significantly elevated levels of serum homocysteine were observed in dementia group. Hence, these findings suggest that lower expression of MTHFR gene and higher frequency of mutant allele may be responsible for reduced activity of MTHFR enzyme thereby affecting folate and methionine metabolism through increased levels of homocysteine. This theory is supported by the study of Rajagopalan et al. in which the MTHFR risk variant has been shown to promote brain atrophy by elevating homocysteine levels in elderly people with MCI [25]. Although, results of genotyping study are in concordance with the results of study by Liu et al. 2010 [26], a study by Pandey et al. demonstrated contradictory results which suggests no true correlation of MTHFR genotypes with the risk of dementia in the elderly [27].

Apolipoprotein E is a plasma protein involved in the transport of cholesterol, which is encoded by a gene on chromosome 19 [28]. Numerous ethnic populations have established APOE genotype as the most important genetic risk factor for susceptibility of AD risk [29]. Various studies have shown that APOE4 allele is linked to accelerated memory decline in ageing and AD. Both post-mortem studies [30] and positron emission tomography imaging studies [31] have demonstrated an increased amyloid plaque load in brains of APOE4 carriers. While most studies have observed

consistent reports about effect of the APOE4 allele on the risk and age of onset for AD, few gene polymorphism studies of Indian origin depict contradictory reports [32]. In our population, gene polymorphism study of APOE revealed that frequency of APOE ε4 allele was observed to be high in dementia group as compared to MCI and control group. These results are in harmony with the results of study by Bales et al. [33].

Further we assessed levels of APOE mRNA expression in all study subjects. APOE gene expression was found to be decreased in dementia as well as MCI groups when compared to controls. This lower APOE gene k expression may cause abnormal cholesterol transport which is considered as one of the contributing factor for cognitive decline. A study by Wisniewski et al. [34] revealed ApoE's role as an accelerator for Aβ formation. Low levels of APOE in the brain correlate with an increased risk of AD. But studies were unable to determine whether or not the cholesterol-carrying function of APOE is involved in AD. Further, we tried to see if there is any relationship between gene polymorphism and gene expression levels of MTHFR and APOE in our study groups. But we could not compare due to small sample size of each study group which made it difficult to subcategorise. A longitudinal study with large sample size will help in understanding and enlightening this theory.

Biomarkers like vitamin B12, folic acid and homocysteine also play role in cognition through different mechanisms. The relation between total homocysteine, its main determinants (vitamin B12 and folate) and dementia risk is still controversial as cross-sectional studies yielded mixed results. In India limited studies have been done on the association of these biomarkers to dementia risk and observed data is inconsistent. In our study, significant decrease in serum levels of holotranscobalamin and folic acid were seen in dementia patients as compared to controls. Though Vitamin B12 concentration was found to be low in dementia group and slightly higher in MCI group as compared to controls, but difference was not statistically significant. Further correlation studies between these biomarkers showed an inverse correlation of serum homocysteine with serum holotranscobalamin levels. Therefore these findings supports the fact that elevated homocysteine levels, a considered risk factor for cognitive decline among elderly population, are dependent on deficiency of Vitamin B12 and folic acid levels and these can be helpful as early diagnostic markers for cognitive decline. Our findings are similar to findings of other studies [35]. We have also included our previously published data which is a part of this project. It highlights the role of biochemical markers as helpers in early diagnosis of dementia risk.

LIMITATIONS

The major limitation of the study was that it was performed on a small sample size and therefore is not representative of complete Indian population. Owing to ethnic and cultural heterogeneity in Indian population, it is imperative to study the ethnic groups collectively. To validate the clinical utility of the current study results, an independent study is required of a greater size, which should include large, longitudinal, population-based cohorts to test the accuracy of this panel of biomarkers. Such a study would be able to address potential slipups of the data reported in this study. This will give more insight into proper understanding of the association lying between modifiable and non-modifiable risk factors and progress of cognitive decline among Indians.

CONCLUSION

In conclusion, present study demonstrated significant difference in various biomarkers including modifiable metabolic factors (serum levels of lipids, vitamin B12, folic acid and homocysteine) and non-modifiable genetic factors (APOE and MTHFR) across the three study groups. It summarizes the evidence in support of gene expression levels as promising non-modifiable risk factor which can aid in the diagnosis of diseased stage and may also represent a novel approach in the genetic studies of dementia. Thus, if validated at early stages of ageing, together these biomarkers and Psychological assessment will help in early diagnosis of dementia risk and in delaying the conversion of MCI to dementia.

FUNDING

Sir H. N. Reliance Foundation Hospital and Research centre.

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