ERYTHROCYTE OMEGA-3-POLYUNSATURATED FATTY ACIDS, SELECTED ANTIOXIDANTS AND CARDIOVASCULAR DISEASE RISK FACTORS IN ELDERLY NIGERIANS WITH DEMENTIA

BY

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A Thesis in the Department of Chemical Pathology

Submitted to the Faculty of Basic Medical Sciences

in Partial Fulfilment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

August, 2012

ABSTRACT

Dyslipidemia, reduced omega-3 and -6 fatty acids and vitamins are risk factors for cerebrovascular disease associated with dementia. Prognosis differs between Alzheimer's disease (AD) and Vascular Dementia (VD) but derangements of the fatty acids and their clinical values for making distinction are unclear. In addition, inadequate antioxidant levels have been associated with hyperhomocystenemia in dementia patients. Therefore, levels of lipids, omega-3 and-6 fatty acids, folate, homocysteine, selenium and vitamin E were evaluated in VD and AD patients.

Forty consenting patients with VD and fifteen with AD were recruited at the University College Hospital, Ibadan. Forty healthy adults served as control group. Anthropometric indices and Blood Pressure (BP) were measured in all subjects. Fasting venous blood sample was obtained and erythrocytes were separated by centrifugation. High Performance Liquid Chromatography (HPLC) was used for analysis of erythrocyte fatty acids: Docosahexanoic Acid (DHA), Eicosapentanoic Acid (EPA), Linolenic Acid (LA) and Arachidonic Acid (AA) as Total Fatty Acids (TFA). Plasma homocysteine was determined using HPLC with fluorescence detection. Plasma folate was determined by Ion Capture Technology using pteroic acid and alkaline phosphatase as signal-generators; vitamin-E by colorimetry; selenium, Total Cholesterol (TC), triglyceride, High Density Lipoproteins-Cholesterol (HDL-C) by spectrophotometry and Low Density Lipoprotein- Cholesterol (LDL-C) was estimated using Friedewald's formula. Data were analysed using ANOVA, Chi Square, Student's t-test and Pearson correlation at $p = 0.05$.

Mean ages for AD (71.1 ±5.0 years), VD (69.0 ±8.2 years) and control (67.5 ±6.8 years) were not significantly different. Mean systolic BP was lower in VD (148.3 ±41.8 mmHg) than AD (156.0 ±36.0 mmHg). Mean BMI, weight and height of the three groups were not significantly different. Mean DHA and EPA in VD (6.7 ±1.9% and 2.4 ±1.7 % of TFA) and AD (5.4 ±2.1% and 3.0 ±1.7% TFA) were lower than in the control (8.9±3.8% and 6.0 ±4.7% TFA) but there were no differences in LA and AA among the three groups. Mean triglyceride and LDL-C were higher in VD (122.7 ±47.3 mg/dL, 101.6 ±28.5 mg/dL) than AD (86.0 ±17.5 mg/dL, 84.7 ±24.6 mg/dL) and control (72.7 ±37.3 mg/dL, 71.2 ±37.1 mg/dL), while

HDL-C was lower in VD (39.8 ±22.4 mg/dL) than AD (46.2 ±21.3 mg/dL) and control (52.2 ±18.9 mg/dL). Mean homocysteine was higher in VD (9.6 ±1.8 µmol/L) and AD (10.0 ±1.7 µmol/L) than control (6.4 ±3.5 µmol/L). Mean selenium and folate were lower in both VD (1.4 ±0.7 µmol/L, 3.5 ±0.7 ng/mL) and AD (1.5 ±0.6 µmol/L, 5.4 ±0.9 ng/mL) than in control (3.1 ±0.5 µmol/L, 9.5 ±2.8 ng/mL). There were negative correlations between homocysteine and folate (r = -0.67), homocysteine and selenium (r = -0.14) but positive correlations between folate and DHA (r = 0.49); homocysteine and TFA (r = 0.36).

Deficiencies of fatty acids, folate and selenium were associated with occurrence of dementia subtypes in Nigerians. Differences in erythrocyte fatty acids levels did not vary distinctly in Vascular dementia and Alzheimer's disease patients. Omega- 3 fatty and folic acids and antioxidant supplements have the potential to reduce vulnerability to neurodegeneration.

Keywords: Senile dementia, polyunsaturated fatty acids, antioxidants.

ACKNOWLEDGEMENTS

I give all glory and honour to Jehovah God almighty, the father of celestial lights and king of eternity for his loving-kindness and protection over me through all my coming and going. I am greatly indebted to my able supervisor and Head of Department, Prof. E. O. Agbedana for his invaluable fatherly attention and constant assistance despite his tight schedule that made this work a success. May Jah bless you and all that is yours, sir.

My immense gratitude goes to my family members for their loving concern and moral support during the course of my study : my husband, Mr Gideon Ayelagbe; son, Eniola Ayomide; parents, Mr and Mrs E. A. Giwa; siblings, Mr and Mrs Gideon Giwa and Dr and Mrs Temidayo Giwa as well as my in-laws. May we continue to share happy moments as a family. I cannot but thank Prof. A. Ogunniyi for his professional assistance and for making his patients available for the study. May God continue to meet you at your point of need, sir. Also Dr Owolabi of Neurology Unit, Department of Medicine, University College Hospital, Ibadan. I appreciate all my lecturers in the Department of Chemical Pathology for their encouragement and useful suggestions.

The thoughtful words and concerns of Prof C.E. Dioka, Drs Oluyemi Akinloye, O. Ojurongbe, J. Onuegbu and Kunle Olowe cannot be overlooked. I quite cherish the companionship and support of my colleagues : Dr Elizabeth Bolajoko, Dr T.O. Adedosu, Mrs Bose Orimadegun and Ijeoma Esiaba, not forgetting the administrative staff in the department for their encouragement.

I am ever grateful for the tremendous assistance of the technical staff at the Central Laboratory, Obafemi Awolowo University, Ile-Ife and IITA, Ibadan in the analysis of most of the biochemical parameters studied. I recognize the untiring efforts of Mrs F. Osasona, Mrs L.Ogundipe, the staff of the Ibadan Dementia Project and most importantly the willingness of the participants in this study.

To all my Christian brothers and sisters and loved ones too numerous to mention who have contributed in one way or the other to make my Ph.D pursuit come to fruition, I say a big thank you. May Jehovah in his infinite mercy be with you all and shower his blessings on all your endeavours

CERTIFICATION

I certify that this work was carried out by Olubunmi Gloria, Ayelagbe in the Department of Chemical Pathology, University of Ibadan.

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VASCULAR DEMENTIA

S/N	CHOL	TG	HDL	AA	LA	DHA	EPA	HCY	FLT	SE	Vit E
1	141.4	90.0	43.3	14.3	9.6	6.2	2.1	9.8	3.7	3.9	2.1
2	151.6	105.1	25.4	13.3	9.8	5.8	2.4	10.0	3.9	0.2	1.0
3	139.1	105.2	52.2	11.2	10.4	5.1	2.1	9.2	3.3	1.1	1.4
4	177.9	122.3	73.1	11.2	10.3	5.1	2.3	8.3	4.9	2.0	0.2
5	120.6	78.8	23.9	14.3	8.9	7.7	1.6	10.2	4.0	1.4	2.2
6	150.7	123.3	13.4	14.3	9.0	7.0	1.3	8.2	3.8	2.3	2.3
7	168.2	164.9	55.2	13.2	11.2	5.2	2.3	10.0	3.3	3.2	2.1
8	132.2	80.8	20.9	12.2	10.9	5.1	2.5	10.6	4.3	0.3	1.9
9	196.4	182.0	17.9	10.2	9.7	6.5	1.4	9.8	3.4	1.2	1.8
10	185.8	139.3	89.6	14.2	9.6	6.4	1.7	8.0	4.6	2.1	1,6
11	176.5	117.4	52.2	15.3	8.5	7.4	1.7	9.7	3.2	1.3	1.7
12	163.6	148.3	20.9	15.3	9.2	7.3	1.0	9.9	3.5	1.4	1.5
13	145.6	74.6	17.9	14.6	10.0	6.1	2.4	9.1	4.0	0.3	0.9
14	138.2	100.8	81.9	14.1	10.1	6.4	2.2	8.2	3.9	0.5	0.7
15	177.0	56.2	52.2	12.6	9.6	7.5	1.5	10.3	3.2	0.3	1.3
16	166.4	200.6	20.9	12.9	9.9	5.4	1.3	8.1	4.8	1.2	1.4
17	212.3	203.1	40.3	11.0	10.8	8.4	2.6	9.9	3.7	2.1	2.0
18	217.8	137.2	12.9	11.2	10.3	6.3	2.9	10.5	3.2	1.5	0.6
19	232.7	206.3	25.4	14.0	8.9	7.1	2.8	9.7	4.2	2.4	1.8
20	202.9	161.8	40.3	10.2	8.2	5.4	2.3	7.9	3.4	3.3	1.7
21	207.5	111.2	89.6	13.9	11.9	5.4	3.1	17.8	4.5	0.4	1.6
22	210.9	80.8	53.7	13.9	11.9	6.8	1.4	9.6	3.1	1.3	1.4
23	193.5	70.6	47.8	12.8	10.9	4.3	3.1	10.4	3.4	2.2	1.2

24	202.1	91.0	19.4	12.9	10.9	6.1	1.3	9.8	2.9	1.4	2.2
25	140.3	92.0	55.2	13.2	11.2	6.9	2.6	8.0	2.4	1.5	1.3
26	150.4	120.2	46.0	13.8	11.8	8.0	0.3	10.0	3.9	0.4	1.4
27	138.7	110.2	90.0	11.2	10.2	6.3	3.3	8.1	2.9	0.6	0.1
28	176.7	210.1	42.4	11.9	10.9	5.9	1.4	9.0	2.4	0.2	2.0
29	131.2	121.4	24.4	9.5	8.4	5.2	2.4	9.8	3.4	0.5	2.4
30	167.7	125.9	15.4	9.2	8.2	5.2	0.7	9.5	2.6	1.1	2.1
31	133.4	220.3	19.9	9.9	9.9	7.8	2.7	9.5	3.7	0.3	1.9
32	186.2	130.0	11.6	9.4	9.4	7.1	1.0	10.0	2.3	2.0	1.7
33	177.8	90.9	14.9	13.4	13.3	7.2	1.4	8.3	2.6	1.4	1.7
34	164.5	130.0	21.4	15.8	14.9	4.8	3.2	9.4	3.9	1.4	1.4
35	146.4	201.0	22.3	10.8	9.8	6.1	0.5	9.8	3.3	1.3	0.9
36	167.2	105.7	24.4	9.4	8.4	4.1	2.3	9.8	4.8	2.3	0.6
37	201.4	122.3	42.3	12.7	11.7	8.7	1.6	10.1	3.7	2.1	1.2
38	210.5	112.7	85.0	9.5	8.5	6.0	3.9	9.3	3.2	3.2	1.3
39	222.6	193.9	45.1	13.3	12.3	6.2	1.8	8.4	4.3	1.2	2.1
40	202.7	128.2	87.5	16.1	10.1`	4.1	3.3	10.2	3.4	1.3	0.6

ALZHEIMER'S DISEASE

S/N	CHOL	TG	HDL	AA	LA	DHA	EPA	HCY	FOL	Se	Vit E
1	131.6	90.3	43.3	13.1	10.1	5.2	3.1	9.8	4.9	1.3	3.1
2	157.9	78.7	25.4	13.1	10.1	4.8	3.4	10.0	4.3	2.2	2.3
3	130.7	74.8	52.2	14.1	11.4	4.1	3.1	9.2	5.8	2.2	1.5
4	148.3	74.8	55.2	12.1	9.3	4.1	3.3	8.3	4.7	1.6	1.3
5	176.4	79.0	52.2	14.4	11.1	6.7	2.6	10.2	4.3	2.4	2.2
6	165.8	89.4	40.3	14.3	11.2	6.0	2.3	8.2	5.3	3.3	2.2

7	156.6	96.9	40.3	13.1	9.0	4.2	3.3	10.0	4.5	0.4	2.0
8	143.6	91.3	53.7	12.2	8.6	4.1	3.5	10.6	5.6	1.3	1.8
9	157.0	79.7	47.8	15.0	12.1	5.5	2.4	9.8	4.2	1.3	1.7
10	146.4	75.9	55.2	14.1	10.0	5.4	2.7	8.0	4.6	2.2	1.5
11	178.8	74.9	46.0	11.2	7.1	6.4	2.7	9.7	5.0	1.4	0.9
12	135.0	80.0	42.4	14.0	10.5	6.3	2.0	9.4	5.4	1.5	0.8
13	157.9	90.4	52.1	13.2	9.4	5.5	3.4	10.1	5.5	0.4	1.3
14	148.3	97.9	55.1	14.3	10.2	5.4	3.2	8.2	5.5	0.6	1.4
15	165.8	96.5	40.2	12.1	8.0	6.5	3.5	10.4	6.0	1.2	2.0

CONTROLS

S/N	CHOL	TG	HDL	AA	LA	DHA	EPA	HCY	FLT	Se	VitE
1	224.0	85.0	58.0	7.2	7.6	8.2	5.1	6.7	7.8	1.2	2.0
2	167.0	92.0	49.0	13.9	7.8	7.8	5.4	3.4	7.9	2.1	2.1
3	181.0	157.0	40.0	11.9	8.4	7.1	5.1	6.8	7.4	3.0	3.0
4	145.0	110.0	43.0	11.1	8.3	7.1	5.3	6.4	7.0	3.3	2.9
5	160.0	88.0	52.2	15.9	6.9	9.7	5.6	6.4	8.9	2.4	2.8
6	166.0	87.0	56.2	15.4	7.0	9.0	1.3	5.1	15.0	4.2	1.9
7	227.0	56.0	50.3	11.9	9.0	7.2	2.3	6.5	11.9	1.3	1.9
8	195.0	190.0	50.2	11.9	8.9	7.1	5.5	8.4	18.0	2.2	1.1
9	135.0	58.0	52.1	13.1	7.7	8.5	4.4	8.1	17.8	3.1	1.2
10	154.0	177.0	46.0	12.9	7.6	8.4	4.7	5.5	6.9	2.3	1.7
11	95.0	83.0	46.0	16.5	6.5	9.4	4.7	14.0	11.0	2.4	3.0
12	140.0	45.0	55.0	16.4	6.5	9.3	4.0	7.0	6.5	1.3	1.6
13	100.0	98.0	56.0	12.2	8.0	8.1	5.4	11.0	14.0	1.5	1.7
14	135.1	74.0	50.0	12.2	8.1	8.4	5.2	5.4	6.8	1.2	2.6

15	105.1	95.0	51.0	14.1	7.7	9.5	4.5	9.1	8.0	2.1	2.7
16	194.0	74.7	55.1	14.0	7.9	7.4	4.3	7.1	6.0	3.0	1.7
17	138.0	74.4	58.0	12.9	8.9	10.4	5.6	9.0	9.5	2.4	1.7
18	153.0	76.7	44.0	12.1	8.8	8.3	5.9	5.8	6.1	3.3	0.7
19	187.0	74.5	52.2	15.8	8.3	9.1	5.8	5.7	7.5	4.2	0.8
20	153.0	62.0	58.0	15.3	6.9	4.4	5.3	10.1	4.4	1.3	1.4
21	140.0	73.0	61.0	15.2	7.0	4.4	6.1	6.7	4.5	2.2	2.6
22	110.2	86.0	50.0	12.2	7.7	5.8	4.4	3.0	7.5	3.1	2.4
23	130.2	56.0	40.3	14.6	7.9	3.3	6.1	6.4	6.0	2.3	2.7
24	125.3	70.6	46.0	13.9	8.8	5.1	4.3	6.0	9.5	2.5	2.6
25	120.4	72.6	43.0	11.0	8.7	6.9	5.6	6.7	6.6	1.3	1.5
26	115.3	76.5	36.9	10.0	6.5	8.0	3.3	5.0	8.0	1.5	0.8
27	110.2	45.0	82.0	15.9	7.0	6.3	6.3	6.0	7.8	0.3	0.7
28	165.0	110.0	57.1	15.4	9.1	5.9	4.4	8.0	7.5	1.2	1.4
29	213.0	52.0	57.0	11.0	8.8	5.2	3.7	8.0	7.6	2.1	1.2
30	179.0	83.0	47.0	10.8	7.3	5.2	5.7	5.1	9.0	1.5	2.3
31	118.0	59.0	50.1	13.1	7.9	7.8	4.0	14.0	15.0	2.4	0.6
32	74.0	104.0	50.1	12.8	6.4	7.1	4.4	11.0	6.9	3.3	0.6
33	125.0	69.0	66.0	16.4	6.0	7.2	6.3	7.0	11.0	0.4	2.2
34	145.0	56.0	48.0	16.4	8.3	4.8	3.5	5.7	6.8	1.3	1.5
35	175.0	68.0	44.8	12.2	8.1	6.1	5.3	9.1	18.0	2.2	1.4
36	164.0	74.0	50.5	12.2	7.6	4.1	4.6	9.0	7.7	1.4	2.0
37	116.0	72.0	50.1	12.0	7.9	10.8	6.9	10.1	7.5	1.5	1.8
38	192.0	72.6	56.2	10.9	8.7	8.1	4.8	7.1	12.0	0.4	1.3
39	98.0	70.5	52.2	10.1	8.3	8.3	6.2	5.4	15.0	0.6	1.2
40	140.0	72.4	54.3	14.9	6.8	6.2	6.1	5.7	9.0	0.2	1.9

CHAPTER ONE

1.1 INTRODUCTION

Dementia is a descriptive term for a collection of symptoms that can be caused by a number of disorders that affect the brain. People with dementia lose their ability to solve problems and maintain emotional control, and they may experience personality changes and behavioural problems such as agitation, delusions and hallucinations. Dementia is diagnosed only if two or more brain functions- such as memory, language skills, perceptions or cognitive skills including reasoning and judgement – are significantly impaired without loss of consciousness (NINDS, 2007).

Alzheimer's disease (AD) is the most common cause of dementia (Heerema, 2012) in elderly individuals with symptoms appearing after age 60 in most people. However, some early-onset forms of the disease, which may appear as early as age 30, has been reported in some subjects in rare cases, this is usually linked to a specific gene defect. AD is characterised by two abnormalities in the brain: amyloid plaques and neurofibrillary tangles (NINDS, 2007).

Vascular dementia is considered the second major cause of dementia after Alzheimer's disease (Heerem,2012) in Western populations. While the role of coincident vascular disease in patients with AD has received attention, the cause of vascular dementia remains unclear (Lentz, 2005). It is thought to be caused by brain damage from cerebrovascular or cardiovascular problems- usually strokes. Many conditions that can cause dementia or dementia-like symptoms include: reactions to medications, metabolic and endocrine disorders (thyroid problems, hypoglycemia, pernicious anemia, disorders of sodium and calcium metabolism), micronutrients deficiencies (thiamine, vitamin B6, vitamin B12), infections (meningitis and encephalitis, untreated syphilis, AIDS, leukemia), brain tumours and anoxia (NINDS, 2007).

In a comparative prevalence study involving two populations of African descent, the findings of Hendrie et al (2001) seem to indicate that both vascular dementia and AD were more prevalent in a typical African-American community in Indianapolis, USA as compared to an African community of Yoruba descent in Ibadan, Nigeria. They also reported a weak association between a molecular variant of ApoE and AD in the Indianapolis community and even a weaker association in the Yoruba community in Ibadan unlike in other populations (Noguchi et al, 1993) where a strong association was observed between ApoE genes and incidence of AD. The results probably suggest that lifestyle and environmental factors like habitual diet may play important roles in the neurological disorder.

In the two communities, the majority of subjects who developed dementing disorder, with higher preponderance in females, developed AD. In the Yoruba community in Ibadan, 1.35% per year of the subjects studied developed dementia including 1.15% per year for those who developed AD (Hendrie et al 2001). Therefore with the ageing population the incidence of dementia may increase rapidly in Nigeria.

Conflicting data show that dyslipoproteinemia, a modifiable risk factor, is associated with a higher risk of dementia. Reduced high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I levels, as well as increased levels of Lp(a) have been reported in VD in some studies (Scacchi et al,1998, Rygllewicz, 2002). Alterations in lipoprotein profile such as hypercholesterolemia and reduced levels of HDL-C are considered risk factors for cardiovascular disease and perhaps for cerebrovascular disease (Postglione and Napoli, 1995). It has equally been suggested that cerebrovascular disease might play a role in vascular dementia (Zuliani et al, 2001). Vascular dementia and AD share common risk factors like age, Apo E4 allele, hypertension and smoking (Pasquier et al, 1999). Moroney et al (1999) postulated that elevated levels of LDL-C were associated with the risk of dementia

in elderly patients suffering from stroke. Elevated LDL-C and serum cholesterol was also reported in AD patients (Kuo et al 1998), and lower HDL-C levels in patients with VD when compared with controls (Kartzman et al, 1989).

It was consequently hypothesized that the association between low HDL-C values and small vessels disease could be due to the role of HDL particles in the removal of excess cholesterol from the brain by interaction with apoE and heparan sulphate proteoglycans in the subendothelial space of cerebral microvessels (Matsuda et al, 1993). It is also known that HDL particles favour endothelium dependent vasorelaxation by inhibiting the action of oxidized LDL particles thereby interfering with induction of endothelial cell adhesion molecules .

Fatty acids are long chain organic acids and form a major composition of membrane lipids. Long chain PUFAs in brain include both omega-3 (e.g DHA, 22:6n-3) and omega-6 (e.g AA, 20:6n-6). PUFAs are prone to free radical attack and hydrogen abstraction leading to oxidative damage (Sinclair et al, 1991). Oxidative damage in the form of lipid peroxidation is known to play a significant role in pathogenesis of neurodegeneration in AD (Merkesbery et al, 2001). Laboratory studies show high level of oxidative damage to DNA in AD, which is associated with lower plasma antioxidant levels. In fact in early AD, it was reported that markers of oxidative damage are elevated by as much as 210% (Keller et al, 2005). Epidemiological studies support antioxidant intake for reduction of AD risk due to oxidative stress (Engelhart et al, 2002; Morris et al, 2003).

Fatty acids with multiple bonds confer increased fluidity to membrane. Each double bond causes a 37^0 angle in the carbon chain, thus resulting in a fatty acid that is not easily compressible. The phospholipids in the brain membrane are enriched in docosahexanoic acid (DHA) which appears to be important for central nervous system function. Zellweger

syndrome and neonatal adrenal leukodystrophy are inherited disorders which present early in life with severe neurologic symptoms and are associated with markedly lower amounts of DHA in plasma and brain when compared to the respective values in healthy controls. It is known that the final step in DHA formation occurs in the peroxisome in the liver via beta oxidation, and patients with Zellweger syndrome lack peroxisomes and do not adequately convert alpha-linolenic acid (ALA) to DHA (Johnson and Schaefer, 2006). DHA is crucial for the development and maintenance of the human central nervous system and its neuronal cells.

High intake of saturated fat and cholesterol and a low intake of polyunsaturated fatty acids (PUFAs) have been related to an increased risk of cardiovascular disease which has been associated with dementia (NINDS, 2007). In contrast, some studies reported that high intake of total cholesterol, saturated and trans fatty acids and low intake of monounsaturated fatty acids (MUFA), PUFA, n-6 PUFA, and n-3 PUFA were not necessarily associated with increased risk of dementia or its subtypes (Engelhart, 2002; Engelhart et al, 2002). An inverse relationship between fish intake and cardiovascular disease mortality and a strong correlation between fish consumption and reduction in sudden death from myocardial infarction was observed in a prospective cohort study by Leys et al (1998). Not only does fish oil reduce triglycerides in the blood and decrease thrombosis, but it also prevents cardiac arrhythmias. Many of the disorders connected with essential fatty acids (EFA) deficiency are also correlated with depression. Prognosis is generally worse when these pathologies are associated with depression (Maes et al, 1994).

Dietary omega-3 fatty acids are involved in prevention of some aspects of cardiovascular disease especially at the level of cerebral vascularization and in some neuropsychiatric disorders, particularly depression as well as in dementia, notably Alzheimer's disease.

Dietary ALA deficiency induces more marked abnormalities in certain cerebral structures such as the frontal cortex and pituitary gland. These selective lesions are accompanied by behavioural disorders more particularly affecting tests of habituation and adaptation to new situations during ageing (Bourre, 2004).

Dyslipoprotenemia (which describes high levels of cholesterol and triacylglycerol, as well as a decrease in PUFA) and hyperhomocystenemia have been identified as independent risk factors for atherosclerosis (de Gomez et al, 2003). Hyperhomocysteinemia is closely linked to plasma folate and pyridoxine concentration. Once formed, homocysteine is either remethylated to methionine which requires vitamin B12, folate and riboflavin as a cofactor, cosubstrate, and prosthetic group respectively, or it undergoes a transsulfuration reaction to form cysteine. The transsulfuration pathway is catalyzed by cystathionine beta synthase which requires pyridoxine as a cofactor. Impairment of homocysteine remethylation has been reported as the predominant cause of high homocysteine levels in different conditions (de Gomez et al, 2003).

The greatest risk factor for the development of neurodegenerative diseases is age, but in addition, hyperhomocysteinemia has been identified as an independent risk factor for these conditions. Seshadri et al (2002) demonstrated that hyperhomocysteinemia is an independent risk factor for the development of AD. The primary symptom of AD is a decline in cognitive function. Earlier reports suggest that there is an inverse relationship between cognitive function and total plasma homocysteine levels.

Hyperhomocysteinemia is also a prominent feature in Parkinson's disease (PD) patients. Plasma homocysteine concentration greater than 14μM double the risk of developing AD. In addition, homocysteine sensitizes neurons to agents linked with the onset of AD such as accumulation of the amyloid β-peptide (Doherty, 2007). Homocysteine does not induce

neurotoxicity in some animal models but enhances the depletion in neuronal viability caused by other neurotoxins. Other mechanisms include homocysteine's excitotoxic actions mediated via the N-methyl-D-aspartate (NMDA) receptor and its actions on the class 1 metabotropic glutamate receptors. The oxidised metabolite of homocysteine, homocysteic acid, is thought to mediate this excitotoxity. Homocysteine metabolites are taken up into cells where they accumulate at relatively high levels. Also homocysteine can increase cellular levels of oxidative stress by a host of mechanism including modulation of the activity of endothelial nitric oxide synthase (Doherty, 2007). Nitric oxide exposure is a condition associated with hyperhomocysteinemia.

Trace elements have been associated with several central nervous system (CNS) degenerative diseases. Neurons and glia are affected by free radicals. Application of superoxide dismutase and other antioxidants may therefore be of value in treating brain injury and edema mediated by free radicals (Chen et al, 1984). Experimental as well as clinical data show that lipophilic antioxidants such as vitamin E are neuroprotective and may help patients suffering from AD (Behl,1999) since oxygen free radicals had been reported as probably causing the damage in AD (Riviere et al, 1998). Increased free radical formation together with reduced antioxidant defense may increase neuronal injury. Low concentration of antioxidants such as alpha-tocopherol may influence the development of post-stroke dementia (Ryglewicz et al, 2002).

Findings indicate that Alzheimer's disease and vascular dementia may have common risk factors and or pathogenesis but the basis for distinction is yet to be clearly defined. Although most of the analytical results of biochemical parameters associated with neurodegenerative disorders are often considered separately, multivariate analysis of laboratory investigations had been shown to differentiate patients from control subjects (Boyd, 1986). A possible interrelationship between omega-3 and -6 fatty acids, nutritional antioxidants and

cardiovascular disease risk factors was assessed in elderly Nigerian men and women with dementia. Also a simple two- and three-dimensional representation was applied to a set of data from patients and control subjects to demonstrate if this would provide useful information that could aid in better diagnostic separation of VD, AD patients and control subjects.

1.2 RATIONALE OF THE STUDY

PUFAs especially omega-3 fatty acids play a crucial part in development and function of the central nervous system. Overlap between cardiovascular disease and dementia has been reported in VD, with omega-3 status emerging as a common factor. Major depression in acute coronary syndrome patients is associated with significantly lower plasma levels of n-3 fatty acids particularly DHA. There has been speculations that n-3 long chain PUFAs specifically EPA and DHA, exert a range of biochemical and physiological effect consistent with cardioprotection (Schmidt et al, 2000). Evidence from observation and controlled trial studies indicate that n-3 long chain fatty acids lower the risk of cardiovascular disease (Hooper et al, 2006), cognitive decline and dementia (Johnson and Schaefer, 2006).

Dyslipoprotenemia and hyperhomocystenemia are important factors associated with onset of atherosclerosis (de Gomez et al, 2003) and neurodegenerative diseases including dementia (Seshadri et al, 1999; Moroney et al, 1999). Elevating circulating plasma concentration of homocysteine has been identified as an independent risk factor for increased risk of cardiovascular disease (Klerk, 2002) and probably AD and VD (Hankey and Eikelboom, 1999; Clarke et al, 1998). Low blood folate and increased homocysteine concentrations are also associated with poor cognitive function (Das, 2008).

Selenium is of potential significance in preventing oxidative stress in AD (Caban-holt et al, 2007). Se also co-operates in the defence against increased total homocysteine levels and has been reported to ameliorate effect of folate deficiency in the elderly (Gonzalez et al, 2004). Selenium and vitamin E play major roles in antioxidant defense (Gonzalez et al, 2004). In addition, status of antioxidant nutrients is often inadequate in the elderly probably because of

age-related changes in function and composition of the human body (Cornelius, 2004) and this can result in hyperhomocystenemia, a feature of omega-3 fatty acids and folate deficiencies. Research on the relationship between erythrocyte fatty acids, plasma homocysteine, folate, selenium and other cardiovascular disease risk factors is yet to be conducted among Nigerians suffering from VD and AD. Moreover, prognosis differs between AD and VD but the use of results from laboratory analysis of biochemical parameters and their clinical values for making distinction are still unclear.

1.3 SIGNIFICANCE OF THE STUDY

It is hoped that the findings in this study will provide reliable scientific data on the erythrocyte fatty acids, plasma lipoproteins, homocysteine, folate, selenium and vitamin E levels in patients suffering from VD and AD. It is also hoped that this study will contribute significantly to the understanding of the etiology of VD and AD and probably come up with new approaches for the diagnosis, management and most importantly discrimination between these two neurodegenerative disorders.

It is equally expected that by comparing data from this study with others from other populations, the role of plasma lipids and non-vascular risk factors such as environment and diet in the aetiology of the neurodegenerative disorders would be ascertained.

1.4 OBJECTIVES OF THE STUDY

The objectives of the present study are to :

- Investigate the mechanisms of altered plasma lipids and erythrocyte fatty acids concentrations in dementia patients

- Compare changes in plasma concentrations of homocysteine, folate, selenium and vitamin E in vascular dementia , Alzheimer's disease patients and controls

- To assess the involvement of omega-3 and -6 fatty acids and other cardiovascular disease risk factors in occurrence of dementia

- To establish whether dietary fatty acids and other related nutritional factors may influence vulnerability in patients with dementia

- To evaluate the possibility of using a three-dimensional representation of biochemical parameters to distinguish Alzheimer's disease, vascular dementia and control subjects

CHAPTER TWO

LITERATURE REVIEW

2.0 LIPIDS AND LIPOPROTEINS

Lipids are non-polar substances that can be utilized by living organisms for energy and cellular integrity (Hyde and Draisey, 1974). Chemically, lipids are either compounds that yield fatty acids on hydrolysis or complex alcohols that can combine with fatty acids to form esters. Some lipids can attach to nonlipid groups like sialic, phosphoryl, amino or sulphate groups and these are commonly referred to as complex lipids (Hyde and Draisey, 1974). The lipids in mammalian tissues include fatty acids, glycerides (mono-, di- and triglycerides), cholesterol, cholesterol esters and glycerophospholipids. Lipids circulate in body fluids as soluble protein complexes known as lipoproteins (Mayne,1994, Basile-Bogia and Abel, 1996). The major lipoprotein classes (chylomicrons, very-low density lipoproteins, low density lipoproteins and high- density lipoproteins) are defined based on the density at which they are isolated by density gradient centrifugation (Brouillete and Anantharamaiah, 1995). Each lipoprotein contains a specific protein known as apolipoprotein which confers unique functions to the lipoprotein class by directing particle assembly, targeting particle interaction with cell surface receptors and activating or inhibiting enzymes involved in lipid metabolism (Brouillete and Anantharamaiah, 1995). Apolipoprotein A, B, C, and E are examples of apolipoproteins found in human lipoproteins.

2.1 TRIGLYCERIDES

These are triesters of the trihydric alcohol (glycerol) and fatty acids (such as stearic, palmitic or oleic acid). Naturally occurring fats usually exist as mixtures of triglycerides (Naito, 1989) but partial glycerides known as mono- and diglycerides are also found in the human body. Monoglycerides are mostly present in intestinal mucosa during absorption of dietary triglyceride. The melting point of triglyceride increases with increased chain length and increased saturation of the fatty acids (Naito, 1989). Triglycerides of both exogenous and endogenous origin are stored in adipose tissue (Mayne, 1994). Nestel et al (1964) estimated the half life of triglyceride to be between 5 to 15 minutes. The rate of triglyceride turnover is, however, faster in skeletal muscle and adipose tissue compared to liver, spleen, heart and other tissues (Fredrickson et al, 1958)

In cross-sectional studies of men with premature coronary heart disease, plasma triglyceride as well as LDL-cholesterol levels were increased (Schaefer et al, 1988).The atherogenicity of triglyceride appears to be controversial as some previous investigators have reported that elevated plasma triglyceride concentration is an independent risk factor for the development of premature coronary heart disease (Garber and Avins, 1998, Steiner, 1993), while others have failed to identify hypertriglyceridemia per se as an independent risk factor (Austin, 1988, Garber and Avins, 1994,) since high-density lipoprotein (HDL) cholesterol is also significantly lowered in such condition.

It has been suggested that significant hypertriglyceridemia may be associated with mood symptoms (Rogers et al, 1989). After lowering of plasma triglyceride values in some depressed patients with severe hypertriglyceridemia (Glueck et al, 1994), significant reductions occurred in the Beck Depression Scores, which correlated positively with the

reduction in the triglyceride value. It was proposed that hypertriglyceridemia could cause increased plasma viscosity and distrupt the normal cerebral perfusion leading to depression (Rogers et al, 1989). Other investigators have not been able to confirm the relationship between high triglyceride levels and psychopathologic symptoms (Maes et al, 1994, Horsten et al, 1997).

2.1.1 CHOLESTEROL

Cholesterol is one of the most essential lipid molecules for mammalian cells (Yokoyama, 1998). It belongs to the family of sterols, which are complex cyclic alcohols based on the cyclopentanophenathrene ring (Hyde and Draisey, 1974). It is a steroid alcohol having a hydroxyl group at carbon 3 which facilitates the formation of esters with long chain fatty acids. Tissue cholesterol is in the free form while in plasma, about 60 – 70% is present as an ester (Glomset, 1968). Esterification of cholesterol occurs in plasma through the selective transfer of fatty acyl group on the C2 position of lecithin to cholesterol by the action of lecithin: cholesterol acyl transferase (LCAT) (Glomset, 1968, Yokoyama, 1998). The half-life of cholesterol has been observed to be between 40 and 60 days, though this may vary from one tissue to another. The turnover of cholesterol is rapid in liver and red blood cells but very slow in adipose tissue, muscle and bone. Cholesterol is a precursor of bile acids (Grundy and Denke, 1990) and is particularly abundant in the nervous tissue (Tichy, 1967), probably suggesting a major role for cholesterol in the normal function of the nervous system.

Low cholesterol values have been shown to be associated with various psychopathologic conditions such as schizophrenia (Sletten et al, 1964) and affective disorders (Glueck et al, 1993), but this does not appear to be a uniform finding. For instance, one study reported that serum cholesterol values were higher in patients with major depression than in controls

(Shizuka and Yambe, 2001) while other studies did not observe any significant changes in cholesterol concentrations in such patients (Lundberg et al, 2001).

The mechanisms linking low cholesterol levels to affective disorders, psychopathologic disorders and suicide tendencies remain unknown and highly speculative (Glueck et al, 1994). Jacobs (1993) stated that anxiety, depression and low total cholesterol are all indicators of poor health in general. Poor health and/or existing medical conditions as well as anxiety and depression are known causes of low plasma total cholesterol (Verdery, 1997).

2.1.2 LIPOPROTEINS

Lipoprotein is a class of macromolecules associated with lipid transport (Biggerstaff and Wooten, 2004). The major lipids in human plasma, cholesterol, cholesteryl ester, triglycerides and phospholipids are carried in the circulation by lipoproteins (Basile-Bogia and Able, 1996). A lipoprotein is composed of an outer coat of proteins known as apolipoproteins, free cholesterol and phospholipids and an inner hydrophobic core of triglycerides and cholesterol esters (Naito, 1989). Specific apolipoproteins control plasma lipoprotein metabolism through their involvement in the transport and redistribution of lipids among various cells and tissues, co-factors for enzymes of lipid metabolism or through their maintenance of the structure of the lipoprotein particles (Mahley et al, 1984).

Lipoproteins share common lipid and apolipoprotein components but apolipoproteins and the amounts of cholesterol, triglyceride and phospholipids vary between lipoprotein particles. Consequently, lipoproteins can be identified based on either particle size, chemical composition, physicochemical and floatation characteristics or electrophoretic mobility. There are four major types of plasma lipoproteins, which are classified based on the density at which they are isolated by ultracentrifugation. They are namely, the chylomicrons, very low density lipoprotein, low-density lipoprotein and high-density lipoprotein.

Chylomicron: Chylomicron contains about 82% triglyceride, 9% cholesterol, 7% phospholipids and 2% protein. It shows electrophoretic mobility at the origin and is isolated at a density of less than 1.006g/ml. Apo A-I, A-II, B, C-I, C-II, C-III and E are the major apoproteins of chylomicron and they make up 2% of the weight of chylomicron (Mayne, 1994). Chylomicrons are the major lipoproteins responsible for the transport of dietary lipids to various tissues. Lipoprotein lipase hydrolyzes triglyceride present in the core of chylomicrons, converting them to remnants (Hussain et al, 1996) and chylomicron remnants are rapidly cleared from plasma by the liver through a process mediated primarily by apolipoprotein E (Cooper, 1997).

Very low-density lipoprotein (VLDL): VLDL contains 50-70% triglyceride, 15-20% phospholipids, 15-20% cholesterol and 7-20% of protein. The ratio of the free cholesterol-to-cholesterol ester is 1:1 by weight (Levy, 1966). VLDL carries mostly endogenous triglyceride and using ultra-centrifugation technique, it is isolated at a density of 1.006g/ml (Skipsi et al, 1967). It has a molecular weight of 5-10 x 10^6 daltons, a diameter that lies between 25-70nm and shows a pre-β mobility on serum electrophoresis (Granda and Scanu, 1966). VLDL contains different proportions of apolipoprotein B, C, E and other apolipoproteins (Mayne, 1994). ApoB-100 is the major protein of VLDL. Apo-B-100 is combined with lipid in the liver by the action of microsomal protein (Schaefer,2002) and approximately 20.4mg apoB-100/kg/day is secreted into VLDL (Welty et al, 1999). VLDL rapidly lose much of its triacylglycerol via lipolysis and about one-half is converted to rich apoB-100 LDL in the fed state (Schaefer,2002).

Low – density Lipoprotein (LDL): Approximately 42-46% of cholesterol and its esters, 9% triglyceride, 23% phospholipids and 21% protein are contained in LDL. The structural integrity of LDL is provided by ApoB (Rudel et al, 1997). LDL has a density of between

1.006 and 1.063 g/ml, β mobility on serum electrophoresis with molecular weight of 2.3 x 10^6 to 1.8 x 10^6 daltons and a mean diameter of approximately 19-23nm (Maten et al, 1972). The major function of LDL is the transport of lipid, mainly cholesterol, from the liver to the peripheral tissues where they are either further utilized or stored (Roheim and Asztalos, 1995). Investigators have identified several LDL subpopulations (Krauss, 1991), but only two major LDL subclasses are usually quantified in terms of their association with coronary heart disease in man. The larger LDL and the smaller LDL often referred to as phenotype A and phenotype B, respectively (Austin et al, 1990). Elevated concentration of LDL (especially phenotype B) is reported to be closely associated with an increased risk of developing premature coronary heart disease in different populations, probably because of its relative slower rate of clearance from circulation as well as increased susceptibility to oxidation in the presence of circulating oxidants like free radicals (Teng et al, 1986, Tribble et al, 1992). This has led to renewed interest in the lowering of LDL-cholesterol level in the prevention of coronary heart disease in different populations.

High Density Lipoprotein (HDL) : HDL contains 55% proteins, 24% phospholipids, 15% cholesterol ester, 2% free cholesterol, 4% glycerides (Scanu and Granda, 1966). It is the smallest of the lipoproteins (9-12nm) and floats at the highest density (1.063-1.21g/ml) (Naito, 1989, Brouillete and Anatharamaiah, 1995). The major phospholipids class in HDL is phosphatidylcholine (lecithin) and high density lipoprotein 3 (HDL_3). High density lipoprotein 2 contains twice as many cholesterol molecules per unit of apolipoprotein as does high density lipoprotein 3 (Rifai et al, 1999) and it has been shown that in vitro HDL_2 is converted back to HDL_3 in the presence of hepatic lipase (Patsch et al, 1984). The apolipoproteins on the HDL particle are Apo A-I and Apo A-II, Apo B and Apo C, but the major ones are Apo A-I and Apo A-II (Scanu, 1969). High-density lipoproteins are considered anti-atherogenic because of their major role in mediating peripheral cholesterol

transport from extrahepatic tissues to the liver for excretion and degradation via a process known as the reverse cholesterol transport mechanism. (Lie et al, 2001). An important step in this reverse cholesterol-transport pathway is the uptake of cellular cholesterol by a specific subclass of small, lipid-poor apolipoprotein A-1 particles termed pre β-HDL (Lie et al, 2001). The two lipid transfer proteins present in human plasma, cholesteryl ester transfer protein (CETP) and phospholipids transfer protein (PLTP), have both been implicated in the formation of pre-β HDL. Circulating HDL concentration is thus important in the aetiology of coronary heart disease and it is well established that there is an inverse relationship between HDL cholesterol concentration and the risk of developing premature coronary heart disease in man. (Schaefer et al, 2002). More detailed blood lipid profiles from the Whitewhall II Study and elsewhere suggest that high-density lipoproteins promotes reverse transport of cholesterol from the arterial wall (Brunner et al, 1993). Maes et al, (1997) have suggested that major depression may be accompanied by an impairment of the reverse cholesterol transport system. Their study revealed a significantly lower serum HDL-C level in subjects with major depression than in normal controls. This supports the findings of a previous report which showed a significant decrease in esterified cholesterol but not in total or free cholesterol levels in depression (Maes et al, 1994).

2.1.3 APOLIPOPROTEIN E (ApoE)

Apo E is a glycoprotein, containing 299 amino acids, with relative molecular mass of 34200Da.There are three major isoforms of ApoE (E2,E3 and E4) that are the products of three allelic forms (e2,e3 and e4) of this single gene located on the long arm of chromosome 19 (Helsalmi, 1998). Apo E alleles (ε) are genetic determinants of the initiation and progression of Alzheimer's disease.The various combinations of these alleles give rise to six different genotypes of which the most common is ApoE E3/3. The three isoforms differ by the interchange of cysteine and arginine residues at positions 112 and 158 of the mature

ApoE. A recent research showed that ApoE polymorphism may have impact on neuronal plasticity and regenerative capacity and degree of cholinergic deficit in AD brains (Poirier et al, 1995). There is evidence for an association of HLA-A2 allele and patients with early or late-onset AD on chrosome 6 , and the gene coding for the LDL receptor-related protein gene (LRP) on chromosome 12 with late onset Alzheimer's disease (Kang et al, 1997).

Apo E proteins influence β -amyloid deposition, cytoskeletal integrity, and efficiency of neuronal repair. The different isoforms of ApoE exert different effects on lipoprotein distribution and amyloid plaque formation in AD (Folin et al, 2004). e2 allele is associated with high ApoE and low total cholesterol and LDL-C, e4 allele is associated with high serum cholesterol and low ApoE concentration and is considered a genetic risk factor for vascular disease. Population studies suggested that e4 allele is somehow indirectly involved in the biological chain of events leading to AD and may thus be considered a susceptibility gene (Folin et al,2004). In AD families containing an APP mutation, an increasing number of e4 alleles decrease the age of onset in affected patients. The e2 allele at the ApoE locus may be protective against AD (Helsalmi, 1998).

ApoE is involved in the mobilisation and redistribution of cholesterol during neuronal growth and after injury, nerve regeneration, immunoregulation and activation of several lipolytic enzymes (Siest et al, 1995). The risk of developing AD seems to be allele dose dependent.Individuals carrying two e4 alleles are at greater risk and have an earlier onset of disease than those with one or no e4 alleles (Poirier et al, 1995). An association of increased e4 allele frequency with coronary artery disease has been reported in a number of studies (Frisoni et al, 1994, Helsalmi, 1998) while another study reported no association between e4 allele frequency and ischemic cerebrovascular disease (Couderc et al, 1993). AD patients carrying an e4 allele often had significantly more severe coronary sclerosis than AD patients without the e4 allele but no association between ApoE genotypes and the extent of

atherosclerosis in cerebral vessels has been reported in humans (Kosunen et al, 1995). It was also suggested that the e4 allele could as well be a genetic marker predisposing to cerebrovascular disease. Variants in *SORL1* may also be involved; they are more common among people with late-onset Alzheimer's disease. These variants may cause the gene to malfunction, possibly resulting in increased production of β -amyloid.

ApoE4 has an elevated affinity for beta-amyloid binding, thereby favouring beta-amyloid deposition in senile plaques. It was hypothesised that ApoE binding to tau protein inhibits the assembly of this protein and hampers neurofibrillary tangle (NFT) formation. The net increase in ApoE serum concentration in AD patients could be related to a higher synthesis or lower degradation of lipoproteins (Helsalmi, 1998).

2.1.4 POLYUNSATURATED FATTY ACIDS:

Fatty acids are long chain organic acids and form a major component of membrane lipids (Sinclair et al, 1991).Polyunsaturated fatty acids (PUFAs) of animal origin can be subdivided into families according to their derivation from specific biosynthetic precursors. In each instance, the families contain from two up to a maximum of six cis- double bonds, separated by single methylene groups, and they have the same terminal structure. Some members of these families include:

Linoleic acid [18:2(n-6)]; arachidonic acid [20:4(n-6)]; α-linolenic acid [18:3(n-3)]; eicosapentaenoic acid [20:5(n-3)]; and docosahexaenoic acid [22:6(n-3)].(William, 1989).

There are two distinct classes of essential fatty acids (EFAs), omega-6 (n-6) and omega-3 (n-3).The n-3 and n-6 EFAs are not interconvertible in humans (Fig. 1). These two classes are distinct and have opposing physiological functions. Arachidonic acid (20:4n-6 or AA) and eicosapentaenoic acid (EPA or 20:5n-3) are precursors of eicosanoids which have pervasive

physiological activities. It is likely that susceptibility to many pathological conditions are due to a diminished ability to elongate and desaturate the 18 carbon chains into 20 and 22 carbon chains (Kris-Etherton et al,2000).

PUFAs play a critical role in determining lipid-protein interactions in synaptic neuronal membranes which affect receptor conformation, ion channels, enzymes, and the movement of compounds into and out of the cell (Salem et al, 1999). PUFAs are prone to free radical attack and hydrogen abstraction, this result in oxidative damage termed lipid peroxidation.This causes a reduction in membrane fluidity and permeability. These changes in lipid bilayer are also thought to interfere with electron transport chain and signal transduction across the cellular membrane (Sinclair et al, 1991). Neuronal membranes contain high concentrations of DHA and AA; both of these EFAs are crucial components of the phospholipid bilayer. Neurotransmitter receptors lie embedded within the matrix of this membrane and their 3-dimensional conformation is dependent on the specific fatty acids that give structure to the membrane (Mitchell et al, 1998).

The n-6 and n-3 fatty acids influence eicosanoid metabolism, gene expression, and intercellular communication (eicosanoids include prostaglandins, cytokines, cytokine mediators, and other components of the immune response). A balance of n-6 and n-3 EFAs is critical for health because they compete with one another for synthetic enzymes and have many opposite metabolic functions via their metabolism to their respective eicosanoids. An uncontrolled n-6 pathway with inadequate opposing n-3 metabolism can create a physiological situation that promotes chronic inflammation, and propagation of cancer, heart disease, stroke, diabetes, arthritis, auto-immunity and impaired neuronal functioning – including mental disorders (Connor, 2000, James et al, 2000). There will likely be an imbalance between n-3 and n-6 based eicosanoids if there is a significant imbalance in

dietary EFA precursors. It had been shown that diets that provide n-6 oils at the expense of n-3 will stimulate production of *pro-inflammatory* prostaglandins, while n-3 stimulate *anti-inflammatory* prostaglandins. The PUFA composition of neuronal membranes is, to a great extent, dependent on dietary intake (James et al, 2000).

The CNS has the second highest concentration of lipids after adipose tissue. These brain lipids contain very high amounts of arachidonic acid (AA) and docosahexaenoic acid (DHA) and these two, which are the major constituents of neural cell membrane phospholipids, belong to the omega-6 and omega-3 PUFA families (Heude et al, 2003).

2.1.5 OMEGA-6 FATTY ACIDS:

The initial step in PUFA biosynthesis is the desaturation of alpha-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6) by the enzyme δ-6-desaturase. PUFA modulate inflammatory response through a number of different mechanisms including modulation of activities of cyclooxygenase and lipoxigenase enzymes. Cyclooxygenase and lipoxigenase are essential for production of eicosanoids and resolvins. Since n-3 and n-6 fatty acids compete for the same metabolic pathway and produce eicosanoids with differing effects, it has been theorized that the balance of the two classes of PUFA may be important in the pathogenesis of inflammatory diseases (Serhan et al, 2008). LA (cis-9, cis-12-octadecadienoic acid)) is the most abundant fatty acid and is found in most animal and plant tissues. It is an essential fatty acid in animal diets, as it cannot be synthesized in animal tissues yet is required for normal growth, reproduction and healthy development. Linoleic acid serves as the precursor of a family of fatty acids that is formed by desaturation and chain elongation,in which the terminal (n-6) structure is retained. Of these, arachidonic acid is particularly important as an essential component of the membrane phospholipids and as a precursor of the prostaglandins (William, 1989).

Studies indicate that a high dietary intake of n-6 fatty acids is characterized by increases in blood viscosity, vasospasm, vasoconstriction and decrease in bleeding time (De Caterina et al,2000). Heude et al (2003) observed that subjects wth high erythrocyte levels of linoleic acid had a 59% increase in the risk of developing cognitive decline and postulated that high circulating levels of n-6 fatty acids tend to 'harden' membrane of brain cells (Heude et al, 2003).

2.1.6 OMEGA-3 FATTY ACIDS:

The enzymes in plant tissue are capable of inserting a double bond in the terminal region of an existing unsaturated fatty acid, and linolenic acid [18:3(n-3)] is the end-point of biosynthesis in most higher plants. When it is absorbed into animal tissue through the diet, it forms the precursor of a family of PUFAs with an (n-3) terminal structure. These fatty acids are essential dietary components, especially in fish (Heude et al, 2003).

α-linolenic acid (18:3n-3)(ALA), eicosapentanoic acid (20:5n-3) (EPA), and docosahexanoic acid (22:6n-3) (DHA) are the 3 major n-3 fatty acids in the diet and also in human plasma and tissue. 20:5(n-3) and 22:6(n-3) fatty acids appear to have special functions in the phospholipids of nervous tissue (William, 1989). Of these, DHA is the most abundant fatty acid in plasma and brain. DHA can be made from ALA by desaturation and elongation in human liver or can be obtained directly in the diet from fish, fish oil, meat or supplement that are rich in DHA. The efficiency of the conversion of ALA to DHA is decreased in premature infants and may decline with aging (Johnson and Schaefer, 2006). N-3 fatty acids, particularly docosahexanoic acid (DHA) are highly concentrated in brain especially in the more active areas including the cerebral cortex, mitochondria, synaptosomes and synaptic vesicles (Morris et al, 2003) and may prevent or delay the progression of dementia. Low dietary intakes and plasma concentrations have been reported to be associated with dementia

and cognitive decline risk (Johnson and Schaefer, 2006). EPA and DHA besides up-regulating the gene concerned with neurogenesis, neurotransmission and connectivity, have been associated with cardiovascular benefit and are found in fish oils especially fatty cold water fish such as herring, mackerel, salmon and tuna (O'Keefe, 2008). Trials showed reductions in cardiovascular events of 19-45% in subjects receiving omega-3 fatty acid supplement containing EPA and DHA (O'Keefe, 2008).

N-3 fatty acids, have anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. Highly unsaturated fatty acids, especially n-3 fatty acids, have anti-atherogenic properties via modulation of endothelial activation. EPA (eicosapentaenoic acid) is anti-inflammatory, lowers triglycerides and raises HDL cholesterol, and reduces the tendency of the blood to thrombosis. DHA (docosahexaenoic acid) is a highly unsaturated fatty acid found in particular abundance in the membranes of mitochondria and neurons where it may assist their function by increasing their fluidity (De Caterina et al, 2000). Physiological functions of the EFAs include the control of inflammation, cardiovascular health, myelin sheath development, allergic reactivity, immune response, hormone modulation, cognition, and behavior. These beneficial effects of n-3 fatty acids have been shown in the secondary prevention of coronary heart disease, hypertension, type 2 diabetes mellitus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and chronic obstructive pulmonary disease (Hu et al, 2002; Connor,2000; Simopoulos, 2000). DHA is reported to be broadly neuroprotective via mechanisms that include formation of neuroprotective DHA metabolites, reduced arachidonic acid metabolites, and increased trophic signal transduction (Cole et al, 2009). DHA limits the production and accumulation of the amyloid beta peptide toxin that worsens AD, and also suppresses several signal transduction pathways induced by Abeta including two major kinases that phosphorylate the microtubule-associated protein tau and promote neurofibrillary tangle pathology (Mitchell et al, 1998).

Cytokines are important biologic mediators with tightly regulated production.Overproduction contributes to pathogenesis of acute and chronic inflammatory,atherosclerotic, and neoplastic diseases. Animal and human studies have shown that production of cytokines can be reduced by long-chain (n-3) PUFA. This, in turn, results in reduction of the severity of certain inflammatory, and atherosclerotic diseases (Meydani, 2001).

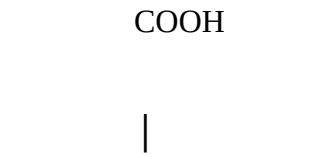
An excess of n-6 EFAs can suppress the synthesis of long-chain n-3 PUFAs such as EPA and DHA. The 18-carbon PUFAs have been replaced by trans-fatty acids in food processing because they are not easily oxidized and do not become rancid. The formation of long-chain PUFAs (LCPUFAs) also depends on adequate amounts of zinc as a cofactor for several desaturase enzymes along the formation pathways. Thus, there are at least three major factors limiting the synthesis of n-3 LCPUFAs: excessive n-6 EFAs, introduced trans-fatty acids, and zinc deficiency. The conversion of 18-carbon n-3 linoleic acid to the 20-carbon EPA is not an efficient process, only about 0.2% is converted (Salem et al, 1999, Pawlosky et al, 2001).

Omega-3 fatty acids may reduce coronary heart disease incidence and mortality by its ability to reduce triacylglycerol levels and platelet aggregability and help prevent abnormal heart rhythms thus protecting people against sudden deaths (Hu et al, 2002). ALA deficiency alters the structure and function of membrane and induces minor cerebral dysfunction, alters the course of brain development, perturbs the composition and physicochemical properties of brain cell membrane, neurones, oligodendrocytes and astrocytes. This leads to physicochemical modifications, induces biochemical and physiological pertubations, and results in neurosensory and behavioural upset (Bourre, 2004). ALA (flaxseed oil) intake was not associated with AD except in the case of people with the ApoE-epsilon 4 allele where a high intake was strongly protective (Morris, 2003). Epidemiological studies have shown that fatty acid consumption and plasma levels, in particular of the n-3 family, are associated with

reduced risk of cardiovascular disease (Albert et al, 2005), diabetes (Hu et al, 2002), depression (Silvers & Scott, 2002), and dementia (Johnson & Schaefer, 2006).

2.1.7 HOMOCYSTEINE METABOLISM

Homocysteine is a metabolic product of methyl-group donation by the amino acid methionine and it is emerging as a risk factor for cardiovascular disease and Alzheimer's disease (Seshadri et al, 2002).



(Structure of homocysteine)

Homocysteine forms part of the methionine and folate cycles; methionine being an essential amino acid that is required for protein synthesis and as a methyl donor for the synthesis of numerous methylated compounds (Pritchard, 2006).

Homocysteine was first linked to neuropathy in 1962, when it was recovered from the urine of mentally retarded children. Methionine and ATP form S-Adenosylmethionine (SAM), the most important methyl group donor of the human body and the only one available to the central nervous system (CNS) (Perna et al, 2003). Methionine synthase (MS), betaine homocysteine methyltransferase and cystathionine β-synthase are directly involved in homocysteine metabolism. Folate is a substrate in MS-mediated reactions. Impaired SAM synthesis may cause impaired neurotransmitter turnover and therefore interfere with cognitive function. Result from a study on possible interaction between homocysteine and nitric oxide

strongly support that homocysteine can induce oxidative stress, to which neurons are extremely sensitive (Perna et al, 2003).

Methionine synthase is a vitamin B12-dependent enzyme which catalyzes the conversion of homocysteine to methionine. In turn, methionine is required in the synthesis of S-adenosyl-methionine, which is a methyl –donor in numerous methylation reactions taking place in the neural membrane. Homocysteine accumulates in folate- and vitamin B12-deficient patients. In that case, homocysteine will be rapidly metabolized to S-adenosylhomocysteine, which is a strong inhibitor of methylation reactions (Ariog^ul et al, 2005).

The majority of homocysteine is bound to protein - principally albumin – through a disulphide bond, or linked to cysteine through a disulphide linkage in vivo. Only around 5% of total homocysteine in blood exists in a free form. Assays for homocysteine measure the total homocysteine – free and complexed (Smith, 2006). Plasma total homocysteine is determined by genetic, lifestyle and nutritional factors. Although folate, vitamins B6 and 12 are major nutritional determinants of plasma total homocysteine, other nutrients such as riboflavin and choline may also be important (Jacques et al, 2001). Several trials assessed the effects of folate, vitamin B6 and vitamin B12 supplementation on homocysteine.It was observed that although high-dose folic acid supplementation reduced fasting levels of homocysteine, vitamins B6 and B12 supplementation had only a minimal effect on homocysteine concentration in the subjects studied (Jacques et al, 2001; Ford et al, 2002) . Homocysteine can influence gene expression in a variety of manners including activation of transcription factors such as the ubiquitous transcription factor, NF-KB. It is well established that NF-KB is activated in synapses in response to excitatory synaptic transmission and therefore may play a pivotal role in processes such as memory. Thus many of the intracellular signal transduction pathway triggered by homocysteine play pivotal roles in normal neuronal

physiology, influencing the ability of neurons to form functional neural networks (Doherty, 2007).

Homocysteine can influence gene expression in a variety of manners including activation of transcription factors such as the ubiquitous transcription factor, NF-KB. It is well established that NF-KB is activated in synapses in response to excitatory synaptic transmission and therefore may play a pivotal role in processes such as memory. Thus many of the intracellular signal transduction pathways activated by homocysteine play pivotal roles in normal neuronal physiology, influencing the ability of neurons to form functional neural networks (Doherty, 2007).

Modifiable lifestyle factors that cause elevated homocysteine include : dietary B vitamin deficiencies : poor diet (folate deficiency), vegetarianism (B12 deficiency), smoking, excessive coffee, low physical activity, low or excessive alcohol intake (Pritchard, 2006). Results from case-control and cohort studies suggest that elevated circulating level of homocysteine are associated with increased risk of cardiovascular disease independent of traditional risk factors such as smoking, blood pressure, serum lipids and obesity (Wald et al, 2002, Klerk et al, 2002). It was observed that there was a higher risk of cardiovascular disease in homozygous carriers of the 677C→T polymorphism on the methylene tetrahydrofolate reductase gene, a genotype that confers naturally higher homocysteine concentration on individuals (Klerk et al, 2002).

Ebesunun et al (2008) in their study on plasma lipoprotein(a), homocysteine, and other cardiovascular risk factors in Nigerians with cardiovascular disease, reported a negative correlation between waist-to hip ratio and plasma total homocysteine concentration in all the patients studied, but a mild hyperhomocysteinemia in only 15% of their patients. They then

postulated that hyperhomocystenemia may still undoubtedly play a role in determining vascular risk factors in Nigerian Africans.

2.1.8 FOLATE METABOLISM AND DEMENTIA

Folate is required for the biochemical conversion of homocysteine to methionine and the subsequent synthesis of S-adenosylmethionine (SAM). S-adenosylhomocysteine, a product of SAM-dependent methylation reactions, subsequently loses its adenosyl group to form homocysteine. Homocysteine can then enter a new cycle of methionine synthesis and methylation, or it can be catabolized through cystathionine synthesis.Elevated plasma homocysteine is one of the primary consequences of folate deficiency (Riggs et al, 1996).

Low blood folate and raised homocysteine concentrations are associated with poor cognitive function. Folic acid supplementation had been reported to improve cognitive function (Das, 2008). Folic acid enhances the plasma concentrations of DHA and EPA. DHA and EPA are of benefit in dementia and AD by up-regulating the gene concerned with neurogenesis. They also form precursors to anti-inflammatory compounds such as lipoxins, resolvins and thus protect neurons from oxidative stress. Folic acid improves endothelial nitric oxide generation and by enhancing plasma levels of DHA and EPA could augment the formation of neuropectin (Das, 2008).The active form of folate is tetrahydrofolate which is essential for the transfer of 1-C units, it is particularly important in purine and pyrimidine metabolism and therefore deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Methotrexate, a cytotoxic analogue of folate, competes with it for metabolism and therefore inhibits DNA synthesis (Mayne, 1996).

Folic acid is important for functioning of the nervous system at all ages. Botez et al (1977) described 16 patients whose impaired intellectual function, confirmed on neuropsychological

testing, was strikingly improved after 6 – 12 months of folic acid therapy. It was observed from another study by Reynolds (2002) that the syndrome of folate responsive dementia and depression, associated with peripheral nerve signs, was commoner in patients attending the geriatrics and psychogeriatric units of the hospital.

In neonates, infants, children, and adolescents, inborn errors of folate transport and metabolism are associated with a variety of overlapping syndromes. These syndromes include developmental delay, cognitive deterioration, motor and gait abnormalities, behavioural or psychiatric symptoms, seizure, signs of demyelination or failure of myelination and vascular changes seen on magnetic resonance imaging. Subacute combined degeneration and peripheral neuropathy may also occur in this group of individuals (Reynolds, 2002).

Folate deficiency is associated with depression and dementia. In elderly people it may be related to ageing, poor diet, malabsorption, drugs, or increased demand. Folic acid has particular effects on mood, cognitive and social function. Impaired folate metabolism may result in a pattern of cognitive dysfunction that resembles ageing. Deficiency of folic acid in the elderly contributes to ageing brain processes, increases the risk of AD and VD and if critically severe, can lead to irreversible dementia (Reynolds, 2002).

2.1.9 ALZHEIMER'S DISEASE (AD)

AD is the most common cause of dementia in people aged 65 years and older. Most cases are sporadic, with late onset (≥ 60 years) and unclear etiology. However, about 5 to 15% are familial; 50% of these cases have an early (presenile) onset (< 60 years) and are typically related to specific genetic mutations. AD usually causes a gradual decline in cognitive abilities, usually during a span of 7-10 years.Nearly all brain functions including memory, movement, language, judgement, behaviour and abstract thinking, are eventually affected.

AD is characterised by two abnormalities in the brain : presence of amyloid plague and neurofibrillary tangle (NFT) (NINDS, 2007). Extensive neuronal damage and loss of synapses are also found in AD brain. Cerebrocortical atrophy is common, use of cerebral glucose is reduced, there is increased brain and CSF concentration of tau protein (which is a component of NFT and β-amyloid) and reduced levels of cholineacetyl transferase and various neurotransmitters (e.g somatostatin) in AD patients (The Merck Manual, 2007).

At least 5 distinct genetic loci, located on chromosomes 1, 12, 14, 19, and 21, influence initiation and progression of Alzheimer's disease. Mutations in genes for the amyloid precursor protein, presenilin I, and presenilin II may lead to autosomal dominant forms of Alzheimer's disease, typically with presenile onset. In affected patients, the processing of amyloid precursor protein is altered, leading to deposition and fibrillar aggregation of β -amyloid. β -amyloid may lead to neuronal death and formation of neurofibrillary tangle and senile plaque, which consist of degenerated axonal or dendritic processes, astrocytes, and glial cells around an amyloid core (Helsalmi, 1998).

Mutations in at least three different genes are responsible for early-onset familial AD (FAD) (Helsalmi, 1998).The amyloid precursor protein (APP) gene is located on chrosome 21 and its mutation is responsible for 2% of all FAD cases. 41 different mutations found in the presenilin-1 gene on chrosome 14 account for 30-50% of presenile AD families. The presenilin-2 gene mutations on chrosome 1 are much rarer causes of early-onset FAD than mutations in the presenilin-1 (Helsalmi, 1998).

The clinical diagnosis of AD is based on criteria defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The criteria is characterized by multiple cognitive deficits which include memory impairment and at least one of aphasia (impaired ability to comprehend or use language), agnosia (impaired ability to identify objects despite intact

sensory function), and apraxia (impaired ability to perform previously learned motor activities despite intact motor function), or disturbance in executive functioning. Social or occupational function is also impaired (APA-DSMMD, 1994). Neuronal degeneration and plastic dendritic remodeling is affected severely in a number of subcortical areas of AD patients carrying the ApoE e4 allele . ApoE4 allele imparts risk of both hyperlipidemia and AD (Folin et al, 2004). AD patients carrying an e4 allele often had significantly more severe coronary sclerosis than AD patients without the e4 allele, but no association between ApoE genotypes and the extent of atherosclerosis in cerebral vessels has been found in humans (Kosunen et al, 1995).

Types of AD include early onset Alzheimer's, late-onset Alzheimer's and familial Alzheimer's disease . Dietary therapy for AD involves appropriate intake of darkly coloured fruits and vegetables, soy, alcohol, folate and vitamin B12 (Kosunen et al, 1995).

The role of dyslipidemia in the development of AD remains unclear. Brain cholesterol alters the degradation of amyloid precursor protein, which contributes to the pathogenesis of AD. However, brain cholesterol is almost entirely synthesized in situ and not transfered from the plasma into the brain because of blood brain barrier (BBB). Evidence suggests that plasma cholesterol levels have no effect on brain or levels of 24S- hydroxylcholesterol which is a degradation product of brain cholesterol (Reitz et al, 2004). Reduced cellular cholesterol levels promote tau phosphorylation in neurons, inhibit dendrite outgrowth and synaptogenesis, and induce neurodegeneration (Reitz et al, 2004). The gene coding for the low-density lipoprotein receptor-related protein gene, which is the ApoE receptor and resides on chromosome 12, may be associated with the expression of late-onset AD (Helsalmi, 1998).

Several studies (Pasquier et al, 1999, Kuo et al, 1998, Moroney et al, 1999) suggested that the risk of developing AD is increased when a patient is exposed to vascular risk factors such as hypertension, diabetes mellitus, peripheral arterial disease, and smoking which usually are associated with cerebrovascular disease and VD. Apo E4 increases the risk of dementia in stroke survivors and is a strong risk factor for the development of cerebral amyloid angiopathy in patients with AD. In elderly individuals, many cases of dementia may be caused by the cumulative effect of cerebrovascular and Alzheimer's pathology (Alagiakrishnan and Masaki, 2000).

2.2.0 VASCULAR DEMENTIA (VD)

Dementia is a syndrome of progressive, global decline in cognition that is severe enough to degrade the individual's well being and social function (Kennedy, 2003) Dementia could be defined as the presence of consistent deficits in short term memory coupled with a decline in other areas of cognitive function. These include:

(a) aphasia –this is characterized by impaired or absent comprehension, production of speech and writing,

(b) apraxia – this describes a difficulty in carrying out familiar purposeful movement. This difficulty is not due to physical limitation such as arthritis

(c) agnosia – associated with impairment of ability to recognize or comprehend the meaning of various sensory stimuli or disturbance of exercise function such as planning, organizing and sequencing (Kennedy,2003).

VD is the second most common cause of dementia, after AD, accounts for up to 20% of all dementias and is caused by brain damage from cerebrovascular and cardiovascular problems – usualy strokes. Most commonly there is blockage of small blood vessels (arteries) deep within the brain (NINDS, 2007). A diagnosis of probable VD is made according to National Institute of Neurological Disorders and Stroke criteria (NINDS, 2007) which included: incidence of cerebrovascular disease (CVD) with or without history of stroke and evidence of relevant CVD by brain imaging, including multiple large-vessel infarcts or a single strategically placed infarct , as well as multiple basal ganglia and white matter lacunas . Other criteria include any combination of the trio of: onset of dementia within three months following a recognized stroke; abrupt deterioration in cognitive functions; or fluctuating, stepwise progression of cognitive deficits (Roman et al, 1993).

Vascular disease produces either focal or diffuse effects on the brain and causes cognitive decline.Focal cerebrovascular disease occurs secondary to thrombotic or embolic vascular occlusions. Hypertension is the major cause of diffuse disease. The 3 major mechanisms of vascular dementia are multiple cortical infarcts, a strategic single infarct and small vessel disease. VD is associated with a higher mortality rate than AD, presumably because of the coexistence of other atherosclerotic diseases (Alagiakrishnan and Masaki, 2000).

VD may result from genetic diseases, endocarditis, or amyloid angiopathy. In many cases, it may co-exist with AD. The incidence of VD increases with advancing age and is similar in men and women. Two of the most common types of VD are multi-infarct dementia (MID) and Binswanger's disease. MID is caused by numerous small strokes in the brain. MID typically includes multiple damaged areas called infarcts, along with extensive lesions in the white matter, or nerve fibres of the brain. A single infarct dementia occurs when a single stroke can damage the brain enough to cause dementia. Dementia is more common when the

stroke takes place on the left side of the brain and when it involves the hippocampus (NINDS, 2007). Patients with Binswanger's disease show signs of abnormal blood pressure, stroke, blood abnormalities or disease of the large blood vessels in the neck.

High blood pressure, high cholesterol, diabetes and heart rhythm problems are risk factors for VD. Symptoms of VD often begin suddenly, frequently after a stroke.Patients may have a history of high blood pressure, vascular disease or heart attacks. Management of vascular dementia is aimed at reducing these risk factors (NINDS, 2007).

The causal role of vascular risk factors in different types of dementia has been stressed during the past decade. The sclerosis of small cerebral arteries and arterioles is considered to be responsible for diffuse periventricular white matter abnormalities, which play an important role in the development of VD (Reitz et al, 2004). High concentration of LDL-C and low levels of HDL-C are known to be independent risk factors for coronary heart disease and carotid artery atherosclerosis, which in turn may lead to cognitive impairment through cerebral hypoperfusion or embolism (Reitz et al, 2004).

Lipid peroxidation may be a major factor in the aging process and intake of hypercholesterolemic diets may lead to microglial activation and 6-amyloid plaque deposition (Moroney et al, 1999). Dietary restriction of foods high in cholesterol can reduce brain vulnerability to acute insults, and may also slow the progression of age-related changes in the brain associated with VD. Cholesterol oxidation in the brain may be particularly relevant to the pathogenesis of those cases of VD with mixed pathology (Moroney et al, 1999). Evidence suggests that decreased levels of antioxidants such as vitamins E, A and C or serum paraoxonase lead to higher susceptibility to oxidative stress and a higher grade of LDL-C oxidation, and lower levels of antioxidants have been found in patients with VD (Reitz et al, 2004).

Noguchi et al (1993) reported that ApoE e4 allele frequency was increased in AD and multi-infarct dementia patients than nondemented controls.On the other hand, another study failed to show any significant association between the frequency of ApoE e4 allele and clinically diagnosed VD (Goate et al, 1991). The variation in e4 allele frequency in VD relative to AD might be due to difficulties in assessing the diagnosis and different ApoE-genotype distribution in the population. AD, VD and mixed AD-VD are responsible for up to 90% of all dementias in different cases. Many studies have reported an association between increased e4 allele frequency and coronary artery disease (Helsalmi, 1998; Frisoni et al, 1994) probably suggesting that e4 allele could be a genetic marker predisposing to cardiovascular disease. In constrast, another study reported no association between e4 allele frequency and ischemic cerebrovascular disease (Couderc et al, 1993).

2.2.1 OXIDATIVE STRESS AND ANTIOXIDANTS

Oxidative stress exists when reactive oxygen species (ROS) generation exceeds availability of antioxidant defences. Under normal physiological conditions, low levels of ROS are produced, predominantly through leakage of electrons from mitochondrial electron transport chain. Normal oxidative metabolism relies on controlled radical reactions involving oxygen. However in all cases, free radical production is tightly controlled. For instance a number of of enzymes utilize free radicals at their active site for catalysis, and superoxide (O_2) production by phagocytes is a pivotal aspect of their bactericidal function (Babior, 1984).

In diseases in which the aetiology is linked with oxidative stress, ROS generation is either excessive, or antioxidant defence levels are compromised such that they cannot deal with the normal ROS load. When free radical production exceeds antioxidant defence system, the

excess radicals react with all classes of biological molecules , including lipids, proteins and nucleic acids (Slater, 1984)

Antioxidants play the roles of:

- scavenging oxygen-derived species, either by using protein catalyst (enzymes), or by direct chemical reaction.
- minimizing the formation of oxygen derived species.
- binding metal ions needed to convert poorly reactive species (such as O_2 and H_2O_2) into free radicals such as OH^-.
- destroying badly damaged target molecules and replace them with new ones (Halliwell, 1992)

Insufficient antioxidant enzyme synthesis may be due to decrease micronutrient availability (such as selenium, manganese, copper and zinc). Hence if the nutritional supply of these minerals is inadequate, enzymatic defences against free radicals may be impaired.

The balance between the production of free radicals and the antioxidant defences in the body has important health implications. If there are too many free radicals produced and too few antioxidants, oxidative stress develops which may cause chronic damage.

2.2.2 VITAMIN E (α-Tocopherol) AND DEMENTIA

Oxidative damage in the form of lipid peroxidation, protein and DNA oxidation and glyco-oxidation are known to play a significant role in the pathogenesis of neurodegeneration in AD (Markesbery et al, 2001). Laboratory studies showed an association between lower plasma antioxidant levels and a high level of oxidative damage to DNA in AD (Markesbery et al, 2001). Epidemiological studies support antioxidant intake as a means of reducing AD risk resulting from oxidative damage (Englehart et al, 2002, Morris et al, 2002). One of such antioxidants is vitamin E. Vitamin E is a family of naturally occurring lipid soluble vitamin

compounds including α, β, γ and δ tocopherol that have been shown to have antioxidant properties. α –tocopherol is the most active and abundant form of the vitamin. Vitamin E reacts with singlet oxγgen, hydroxyl, lipid peroxyl, hydroperoxyl and nitrogen-based radicals to prevent propagation of free radical damage in biological membrane. This is particularly effective with regard to neuroprotection (Caban-holt et al, 2007).

Low levels of antioxidants has been attributed to the consumption of such antioxidants due to high levels of oxidative stress in vivo as well as to insufficient dietary intake (Ryglewicz et al, 2002). The oxidation of LDL to give cholesterol ester and phosphatidyl choline hydroperoxides as major products generate radicals in which alpha-tocopherol acts as a radical scavenger (Gotoh et al,1996). Increased free radical formation together with reduced antioxidant defense may increase neuronal injury. A low concentration of antioxidants such as alpha-tocopherol may influence the development of post-stroke dementia (Ryglewicz et al, 2002). Some workers reported that large doses of antioxidant vitamins C and E might be helpful to people with AD (Van Dyke, 1997).

Pathological oxidation has been proposed to be among the most important factors in the pathogenesis of AD. The brain is especially vulnerable to oxidative stress due to its high oxygen consumption as well as high concentration of easily oxidizable lipids and transition metal ions, capable of producing reactive oxygen species. Brain tissue from AD patients has been reported to have higher levels of oxidized proteins, advanced glycation end products, 4-hydroxylnonenal – derived adducts and products of lipid peroxidation than tissues from non-demented elderly controls (Kontush et al, 2001). Clinical trials found a beneficial effect of α-tocopherol and selegiline by slowing the progression of dementia disease. α –tocopherol is shown to accumulate in brain and reduce peroxidation of brain lipids in animal models. It

also reduces the neurotoxicity of Aβ, a major component of senile plaques in neuronal cell culture (Kontush et al, 2001).

Lipoproteins are identified as new targets for oxidation. Oxidation can impair the normal function of lipoproteins and brain development and oxidized lipoprotein can be toxic to neuronal cells. Lipoproteins in density range of plasma HDL have been found in human CSF. They contain PUFAs, the major substrate for lipid peroxidation, as well as tocopherols, the major lipophilic antioxidant, and can be easily oxidized in vitro. CSF PUFA and antioxidant vitamins were decreased in AD, supporting the concept of oxidation as an important factor in the pathogenesis of AD (Artl et al, 2000). Ryglewicz et al, (2002) found significantly decreased plasma levels of triglycerides and low density lipoprotein in patients with VD compared to AD patients and a similar atherogenic index in both groups. They also reported reduced alpha-tocopherol concentrations in VD patients when compared with the corresponding values in AD patients and controls probably indicating a reduced antioxidant defense in VD patients.

2.2.3 SELENIUM (Se) METABOLISM AND DEMENTIA

Selenium is an essential trace element that is an integral part of many proteins, with catalytic and structural functions. The antioxidant properties of some selenoproteins, such as glutathione peroxidase, may be particularly important in carcinogenesis and heart disease. The content of selenium in food depends on the Se content of the soil where the plants are grown or the animals are raised and this is contributory to the wide variations found in selenium status in different parts of the world (Gonzalez et al, 2004). In animal studies, selenium deficiency is associated with cardiomyopathy and sudden death, as well as reduced T-cell counts and impaired lymphocyte proliferation and responsiveness. In humans,

selenium deficiency has been implicated in the etiology of cardiovascular disease and other conditions in which oxidative stress and inflammation are prominent features (Neve, 1996). However, the therapeutic benefit of selenium administration in the prevention and treatment of cardiovascular diseases remains insufficiently documented. Intervention studies are currently been carried out to assess the benefits of selenium supplements in primary and secondary prevention of atherosclerosis (Alissa et al, 2003).

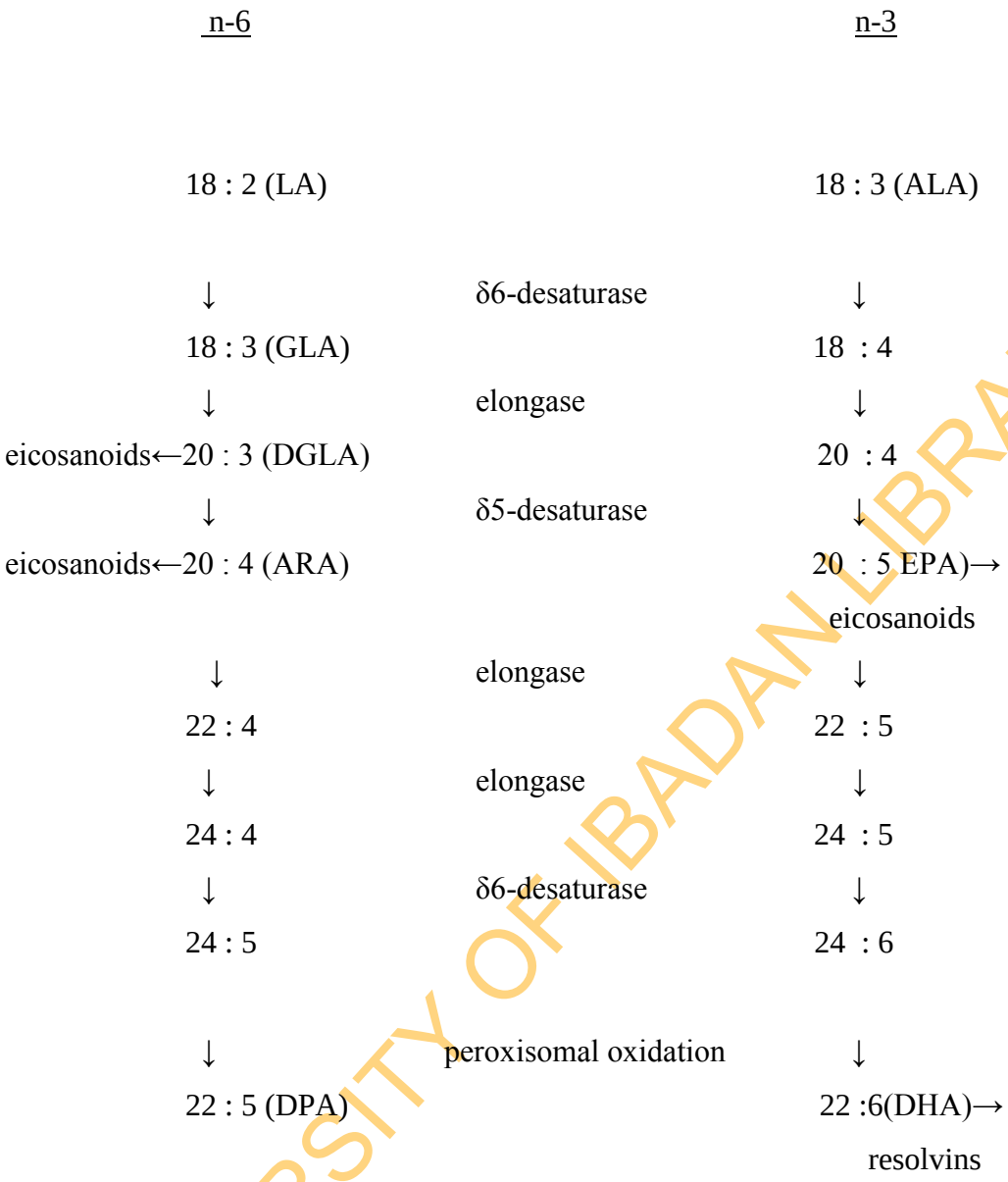
The reactive, damaging effects of intermediates of lipofuscin metabolism are reported to be inactivated by glutathione peroxidase (a Se-containing enzyme), vitamin E and catalase (Clausen, 1984). Lipofuscin is formed by a complex series of reactions which involve reaction of hydrogen peroxide with polyunsaturated fatty acids followed by auto-oxidation, with the formation of organic peroxides, organic free radicals and other reactive intermediates. The final product, lipofuscin, is a polymerisation product of phospholipids, proteins and reactive intermediates. Lipofuscin may have a direct toxic effect on brain cells, also, the peroxides, free radicals and other intermediates are reactive compounds which could damage neuronal DNA as well as membrane structures. It was suggested that vitamin E and Se supplementation may be of benefit in treatment of dementia (Clausen, 1984).

Akbaraly and Hininger, (2007) in their study on association between cognitive decline and plasma selenium, found out that among subjects who had a decline in their plasma Se levels, the greater the decrease in their plasma Se levels, the higher the probability of occurrence of cognitive decline. It was concluded that cognitive decline was associated with decreased plasma selenium over time and they suggested that since brain oxidative stress is a cause of cognitive impairment, Se, which is an antioxidant may protect against cognitive decline.

The mode of action of selenium was thought to involve antioxidant defense mechanisms (Al-Bader A, 1998). Plasma Se concentration less than 1.2umol/L was believed to be that

necessary for full glutathione peroxidase activity (Venn et al, 2003).Changes in glutathione and protein-bound glutathione were highly correlated with changes in plasma selenium levels and were believed to reflect a reduction in oxidation stress. Suboptimal selenium status has been suggested to reduce resistance to infectious diseases and to increase cancer and cardiovascular disease risk (Venn et al, 2003).

In a study on the interactive effect of dietary Se and folate in animal models, Gonzalez et al, (2004), observed that some of the effects of folate deficiency seem to be ameliorated in the animals when they were fed Se supplemented diets and therefore postulated that this effect is probably due to the shunting of the build-up of total homocysteine to glutathione by Se supplementation. They subsequently suggested that Se should be considered as a potential factor to lower total homocysteine. Optimal concentrations of antioxidant vitamins and Se were found to decrease risk of cardiovascular disease in a study on patients with peripheral vascular disease (Mansoor et al, 2000). The antioxidant vitamins and Se protects against peroxidation of lipids and hyperhomocysteinemia. Serum Se is directly dependent on the quantity of Se ingested in the daily diet (Gonzalez et al, 2004).



KEY : ALA : α-linolenic acid ARA : arachidonic acid
DGLA : dihomo-γ-linolenic acid DHA : docosahexanoic acid
DPA : docosapentaenoic acid GLA : γ-linolenic acid
LA : linoleic acid

FIGURE 1 : BIOCHEMICAL PATHWAY FOR THE INTERCONVERSION OF N-6 AND N-3 FATTY ACIDS (Burr, 2000)

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY POPULATION

Ninety-five subjects (58 males, 47 females; mean age: 65.6±6.7 years) were enrolled in this study. These comprised of:

- Forty patients (24 males, 16 females; mean age: 69.0±8.2 years) who were attending the Neurology clinic at the Medical Outpatient Unit of the University College Hospital, Ibadan were diagnosed as suffering from vascular dementia using the National Institute of Neurological Disorders and Stroke (NINDS) criteria which includes incidence of cerebrovascular disease (CVD) with or without history of stroke, periventricular white matter lesions, onset of dementia within 3 months following a recognized stroke and abrupt or fluctuating deterioration in cognitive functions (Roman et al, 1993). Diagnosis by the Consultant Neurologist also included taking a medical history, performing a physical and neurological examination, and administering a neuropsychological battery using Mini-Mental State Examination (MMSE). The maximum score for the MMSE is 30 and a score lower than 23 is indicative of cognitive impairment (Folstein et al, 1975).

- Fifteen patients (7 males, 8 females: mean age: 71.1 ±5.0 years) suffering from Alzheimer's disease were diagnosed using the National Institute of Neurological and Cognitive Disorders (NINCD) and Stroke/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria. The criteria include: dementia established by examination and objective testing, deficits in 2 or more cognitive areas, progressive worsening of memory and other cognitive functions, onset between ages 40 and 90 years and establishment of absence of systemic disorders or other brain diseases (McKhann et al, 1984).

- Forty apparently healthy subjects (27 males, 13 females; mean age: 67.5 ± 6.8 years) who had no report of endocrine diseases, diabetes mellitus, hyperlipidemia, hypertension, cirrhosis of liver or chronic nephritis and had not used any medications or drugs influencing fat metabolism during the last 2 months before commencement of the study were selected as controls.

Only two of the VD patients were less than 60 years old (45 and 53 years respectively). However their data were not included in the study since they do not affect the final results significantly.

Each participant underwent an interview- with the aid of a structured questionaire- of general health and detailed dietary habits using a food frequency questionaire followed by a standard assessment. All the subjects gave their informed consent prior to participation in the study. The study was approved by the University of Ibadan / University College Hospital Ethical Review Committee.

INCLUSION CRITERIA

Subjects that were diagnosed as suffering from VD and AD.

EXCLUSION CRITERIA

Individuals that use psychotropic drugs or consume alcohol regularly

Individuals below 60 years old.

Unwillingness to participate in the study.

3.1.1 BLOOD SAMPLE COLLECTION

SEPARATION OF BLOOD SAMPLES

10ml blood sample was drawn by venipuncture after 10-14 hours fast. 4ml of blood was collected in lithium heparin and 6ml was dispensed into ethylenediaminetetraacetic acid

(EDTA)-containing bottles respectively. Samples were immediately placed on ice before centrifugation. The heparinised blood sample were maintained at -20^{0}C until analysis for homocysteine, folate, vitamin E and selenium.

3.1.2 DETERMINATION OF PACKED CELL VOLUME (PCV)

PCV was determined by centrifugation of EDTA whole blood collected into a capillary tube. PCV is a measure of the relative mass of red cells present in a sample of whole blood.

MATERIALS:

i. plain capillary tube (modulhn A/S. Denmark)
ii. plasticine
iii. micro-hematocrit centrifuge and reader (Hawksley, England)
iv. EDTA whole blood

METHOD:

The blood sample collected in EDTA tube was properly mixed by inversion. A plain capillary tube was then filled to two-thirds its volume with the mixed blood. This was achieved by capillary action. One end of the tube was sealed with plasticine. The capillary tube were placed in the troughs of the micro-hematocrit centrifuge and spun at 1200g for 5 minutes. The volume of packed red cells was measured in a micro-hematoctrit reader and expressed as percentage of the total blood volume.

i.e PCV = volume of packed red blood cells/total volume of blood sample x 100

3.1.3 ISOLATION OF ERYTHROCYTE MEMBRANE

The 6ml blood sample was collected in test tubes containing EDTA solution. To obtain the erythrocytes, whole blood was centrifuged, the plasma was immediately separated, and the packed red blood cells were washed four times at $4^{O}C$ for 10 min, and centrifuged at 16,000g for 15min. The plasma and buffy coat were removed after centrifugation. This procedure was done twice, leaving a substantially hemoglobin-free pellet of erythrocyte membranes, which was resuspended in twice its volume of phosphate buffer saline and stored at $-70^{O}C$ until analysed for fatty acids.

The separated plasma sample was used for lipid analysis.

3.2.0 DETERMINATION OF TOTAL CHOLESTEROL

Plasma total cholesterol estimation was carried out using the enzymatic method as described by Allain et al (1974)

Cholesterol ester hydrolase hydrolyses cholesterol esters to free cholesterol. The free cholesterol produced is oxidised by cholesterol oxidase to cholesten-4-ene-3-one with simultaneous production of hydrogen peroxide which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at wavelength 510nm. The colour intensity is proportional to the cholesterol concentration.

METHOD:

10ul of each sample was added to 1ml of the reaction mixture. Each solution was incubated for 5 minutes at $37^{O}C$ for colour development. The absorbance was read at wavelength 510nm using CE 272 Linear Readout Ultraviolet Spectrophotometer (Cecil, United Kingdom).

A plasma sample for quality control was obtained from Randox Diagnostics (Crumlin, United Kingdom).

Calculation:

$$\text{Concentration of test} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200\text{mg/dl}$$

3.2.1 TRIGLYCERIDE ESTIMATION BY ENZYMATIC METHOD

The enzymatic method of Buccolo and David (1973) was used in the determination of triglycerides in plasma.

PRINCIPLE :

Triglycerides are hydrolyzed by lipases to yield glycerol and fatty acids. The glycerol produced is oxidized to dihydroxyacetone phosphate with the production of hydrogen peroxide, which oxidatively couples with 4-aminophenazone and 4-chlorophenol to produce a chromogen referred to as quinone imine. The reaction is catalyzed by peroxidase. The degree of absorbance of the chromogen is directly proportional to the concentration of triglycerides.

REAGENTS :

To prepare the working reagent, 15ml of the buffer solution was used to dilute the enzyme reagent.

METHOD :

10 ul of each sample was pipetted into a test tube and 1ml of the working reagent added to each tube. The solution was incubated for 5 minutes at 37^{O}C for colour development and the absorbance was read at wavelength 500nm using the CE

272 Linear Read out Ultraviolet Spectrophotometer (Cecil, United Kingdom). Quality control sample was obtained from Randox Diagnostics (Crumlin, United Kingdom). All test samples were analysed in duplicates.

CALCULATION :

$$\text{Concentration of Test} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.2.2 ESTIMATION OF HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

The precipitation method by Assman et al (1983) using polyanion solution was used to isolate and estimate HDL-C.

PRINCIPLE :

The addition of phosphotungstic acid in the presence of magnesium ion precipitates quantitatively LDL, VLDL, and chylomicron fractions from whole plasma, leaving the HDL fraction in the supernate. The cholesterol in the HDL, which remains in the supernatant after centrifugation, is estimated using the enzymatic method of Allain et al (1974).

METHOD :

50ul of the sample was pipetted into a centrifuge tube and 1ml of precipitant was added to the mixture and then left at room temperature for 10 minutes after which the mixture was centrifuged at 4000rpm for 10minutes. The resultant clear supernatant was separated and analyzed for cholesterol using the enzymatic method for cholesterol described above.

3.2.3 LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C) DETERMINATION

This was calculated according to the Friedwald et al's formular (1972)

LDL-Cholesterol (mg/dl) =

$$\text{Total Cholesterol (mg/dl)} - \left[\frac{\text{Triglyceride(mg/dl)}}{5} + \text{HDL-C(mg/dl)}\right]$$

3.2.4 ERYTHROCYTE FATTY ACID DETERMINATION

Fatty acid determination was carried out using the method developed by Pandey et al (2003). Erythrocytes were thawed and 2ml of distilled water was added to hemolyze the erythrocytes. The hemolyzed solution was saponified by refluxing with N/2 alcoholic KOH in a water bath and the fatty acids were extracted in ether. The remaining saponified matter was acidified with 10% HCl and extracted with ether. The ether extract was washed with distilled water till acid free, dried under anhydrous sodium sulphate to obtain free fatty acids. These were then converted to fatty acid methyl esters (FAME) by methylating with methanol sulphuric acid. The mixture was heated in a water bath for 4 hours, cooled to room temperature and then extracted with ether. The ether soluble layer was washed with 1% KOH and then by distilled water till alkali free. The ether was evaporated and FAME were dissolved in benzene and stored at $4^{O}C$ till analysis.

For chromatographic analysis 100µg of FAME, 10ml of Phenacyl bromide solution and 10µl of triethylamine solution were combined and allowed to stand overnight at room temperature. An aliquot of this solution was injected directly into the liquid chromatograph using the Rheodyne Manual Injector. High performance liquid chromatography was carried out using Waters 616/626 LC chromatograph equipped with Water's 501 pump solvent programmer

and Waters UV detector. Absorbance was measured at 300nm. A 90cm x 0.64cm : bondapak C-18 column was used with acetonitrite (8:20v/v) as eluent at a flow rate of 0.50ml/minute. FAMEs were identified by comparison with internal standards. Peak retention times for total fatty acids were identified by injecting known standards.

3.2.5 DETERMINATION OF UREA IN PLASMA

Urea concentration in plasma was determined by the method of Coulombe and Favreau (1963)

Principle

Urea reacts with diacetyl monoxime containing adjacent carbonyl groups when heated to form a pink coloured complex. The reaction which takes place in two stages was carried out at 100^0C in strong acid solution. The diacetyl monoxime decomposes to give hydroxylamine and diacetyl which then condenses with urea to give diazine derivative (a coloured compound). The colour formed is enhanced by thiosemicarbazide and ferric ions. The resulting pink solution is read at 525nm in a spectrophotometer.

Reagents

All reagents were supplied in a ready to use form by Cromatest Laboratory Knickerbocker S.A.E., Barcelona Spain.

1. OXIDANT REAGENT

Phosphoric acid 4mol/l (500ml) containing ferric chloride ions (60mmol/l). Storage temperature; 2^0 - 30^0C

2. Diacetylmonoxime (140mmol/l) and thiosemicarbazide (5mmol/l). Storage temperature; 2^0 - 10^0C.
3. Urea/Glucose Standard

   Urea/Glucose standard contains 100mg glucose and 50mg Urea/dl. Storage temperature: 2^0 - 30^0C.

Procedure

1. Into labeled tubes place reagents, samples and standard as shown below

Reagents	Blank	Standard	Test Samples
Sample	**-**	**-**	**0.02ml**
Oxidant Reagent	**4.0ml**	**4.0ml**	**4.0ml**
DAM-TSC	**1.0ml**	**1.0ml**	**1.0ml**
Standard (50mg/dl)	**-**	**0.02ml**	**-**

2. All tubes were stoppered, mixed and placed in boiling water bath (Fissons Scientific Instrument, England) for 5 minutes.
3. The tubes were removed and placed in cold-water for about 5 minutes
4. Absorbances of the coloured samples and standard were read at 525nm in 21-UVD Spectrophotometer (Milton Roy, Analytical Product Division, Rochester, USA) after the instrument was adjusted to zero with the blank.

Calculations

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 50 = \text{(Concentration of sample) mg/dL}$$

3.2.6 DETERMINATION OF SODIUM AND POTASSIUM IN PLASMA

Sodium and potassium in plasma were determined with flame photometer (CORNING Clinical Flame Photometer 410C).The atoms of sodium and potassium (in the ground state) are dissociated and excited by heat from the flame and emit spectra with sharp bright lines at 589nm for sodium and 768nm for potassium. As the thermally excited atoms return to ground state, light is emitted. Sodium produced a yellow colour while potassium a violet colour. The intensity of each colour is proportional to the amount of each of these elements in the sample. The colour is measured with a photodetector and the result is displayed in mmol/l on a digital read-out device.

Procedure

1. The flame photometer was switched on and the gas supply turned on fully. The pump was turned on and the instrument ignited.
2. The instrument was allowed to stabilize for about 30 minutes while aspirating deionized water.
3. The air pressure was kept constant at 101bs/sq inch (10 psi) and the gas smoothly regulated to obtain discrete cones of flame.
4. The flame photometer was first set to zero with deionized water. The instrument was then standardized with a solution containing 140mmol/l sodium and 5.0mmol/l potassium. The standard was diluted 1:200 with deionized water according to the manufacturer's specification.
5. To determine sodium, the filter (589) was inserted and calibrated at 140mmol/l with the standard. The instrument is now ready for use.

6. The samples for the determination of sodium were diluted 1:100 by adding 9.9ml of diluents (deionized water) to 0.1ml plasma. The containers were then mixed by gentle rotation.
7. The solution was aspirated and the sodium content was then automatically displayed by the digital read-out device.
8. The procedure was repeated for potassium. The instrument was calibrated with potassium standard (5.0mmol/l) and the potassium content of samples was determined after replacing the sodium filter with the potassium filter (768nm).

3.2.7 DETERMINATION OF TOTAL HOMOCYSTEINE IN PLASMA

The total homocysteine concentration was measured by high performance liquid chromatography with fluorescence detection involving derivatization with 7-fluorobenzo-2-axo-1, 3-diazole-4-sulfonate according to the method of Ubbink et al (1991).

The thiol compounds in plasma are reduced with tri-n-butylphosphine, deproteinized with trichloroacetic acid, centrifuged and derivatized with 7-fluorobenzo-2-axo-1,3-diazole-4-sulfonate. The compounds are isocratically separated by C_{18} HPLC and quantified by fluorescence detection. The mobile phase was 0.1mol/l phosphate buffer:acetonitrile (pH 1.5; 94:6 by vol). The retention time was 5 min for homocysteine and 11min for the internal standard (N-acetylcysteine).

3.2.8 DETERMINATION OF PLASMA FOLATE

Plasma folate was determined on an Abbot IMX analyzer (Abbott Laboratories, Abbot Park, IL) with a between-run CV of <8% based on the manufacturer's control samples.

This method is based on ion capture technology. In this technology, a high molecular weight quarternary ammonium compound, Ion Capture solution (Bulk solution 2), is dispensed on

the glass fiber matrix of the matrix cell. This imparts a positive charge to the matrix which enables capture of negatively charged analyte complexes. During the assay, negatively charged polyanion analyte complexes are formed. These complexes are captured through electrostatic interaction with the positively charged glass fiber matrix.

This assay utilizes a soluble affinity reagent composed of folate binding protein (FBP) coupled to monoclonal antibodies, which are in turn covalently coupled to carboxymethylamylase. Negatively charged analyte complexes are then captured through electrostatic interaction with the positively charged fiber matrix.

Folate is quantified by measuring the population of unoccupied FBP sites bound to the matrix using a conjugate of pteroic acid (a folate analog) and alkaline phosphatase as the signal-generating molecule and a substrate,4-methylumbelliferyl phosphate.

Calculation

The AXSYM folate result unit is nmol/L; to convert to ng/mL,

$$\text{ng/mL} = \frac{\text{nmol/L}}{2.265}$$

Normal range = 7.2 to 15.4 ng/mL

3.2.9 DETERMINATION OF PLASMA TOCOPHEROL

(Quaife et al, 1949; Baker and Frank, 1968)

PRINCIPLE:

Tocopherol and carotenes in plasma was first extracted into xylene. Tocopherol reduces ferric ions to ferrous ions which then form a red complex with $\alpha 1 \alpha^1$ dipyridyl. The extinction was read at 460nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520nm.

REAGENTS

1. Absolute ethanol, aldehyde free

2. Xylene

3. $\alpha 1\alpha^1$ – Dipyridyl, 1.20g/l in n-propanol

4. Ferric Chloride solution, 1.20g $FeCl_3 .6H_2O$/l in ethanol kept in a brown bottle.

5. Standard solution of D-L α-tocopherol, 10ng/l in ethanol.

PROCEDURE

1.5ml plasma, standard, and water (blank) were measured into three-stoppered centrifuge tube, respectively. To test and blank was added 1.5ml ethanol and to the standard 1.5ml water. Then 1.5ml xylene was added to all tubes, stoppered, mixed well and centrifuged. 1ml of the xylene layer was transferred into another stoppered tube taking care not to include any ethanol or protein. 1ml of $\alpha 1\alpha^1$ – dipyridyl reagent was added to each tube, stoppered and mixed. 1.5ml of the mixture pipette into colorimeter cuvette and the extinction of test and standard were read against the blank at 460nm. Then in turn beginning with the blank, 0.33ml of ferric chloride solution was added, mixed and after 2min, the test and standard were read against blank at 520nm.

Calculation:

Plasma tocopherol (mg/l) =

$$\frac{\text{Reading of unknown at 520} - \text{Reading at 460nm} \times 0.29}{\text{Reading of standard at 520nm}} \times 10$$

Since the standard contains 10mg/l

3.3.0 DETERMINATION OF PLASMA CHLORIDE (Schales and Schales, 1941)

PRINCIPLE

When mercuric nitrate solution is added to a chloride solution, unionized mercuric chloride is formed. At the end-point, the first excess of mercuric ions gives a bright purple colour with the indicator, diphenylcarbazone.

REAGENTS:

All reagents were supplied in ready –to-use form by Cromatest laboratories, Knickerbocker, S.A.E, Barcelona, Spain.

1. 0.10N mercuric nitrate
2. Diphenylcarbazone indicator (60µmol)
3. 2/3N H_2SO_4
4. Phosphorus – Chloride standard (100mmol/L)

PREPARATION OF WORKING REAGENT:

Indicator solution: Five ml of methanol or 95% ethanol was added to one vial and swirled to dissolve. The original cap was replaced with stopper provided. Reagent is stable for 2 months at 0 - 4^0C.

PROCEDURE

All glassware and test tube were washed with potassium dichromate solution and thoroughly rinsed with distilled water. Into 25ml Elenmeyer flasks were added the following:

FLASKS	UNKNOWN	STANDARD
Distiled water	2.0ml	2.0ml
Sample	0.2ml	-
Standard, 100mmol/L	-	0.2ml
2/3N H_2SO_4	1 drop	1 drop
Indicator solution	2 drops	2 drops

Contents of each flask were titrated with mercuric nitrate from a micro burette calibrated at 0.01ml intervals to a definite violet-blue colour.

CALCULATION

Plasma chloride (mmol/L) = $\frac{\text{Titre of Test}}{\text{Titre of standard}} \times 100$

3.3.1 DETERMINATION OF BICARBONATE (Wotton, 1974)

PRINCIPLE

The sample containing bicarbonate is reacted with dilute solution of hydrochloric acid (0.01M). Carbon dioxide is liberated and the excess hydrochloric acid is titrated with dilute sodium hydroxide (0.01M) in the presence of methyl red as indicator. The end point is indicated when the solution changes from red to yellow.

REAGENTS

1. 0.01M NaOH

This was prepared by dissolving 40g of NaOH in 200ml-distilled water in a beaker (Becton-Dickinson, Dublin, Ireland). When solution was complete, it was transferred to a one litre volumetric flask **(**Becton-Dickinson, Dublin, Ireland) and the volume made up to1 litre, togive a one molar solution of NaOH (stock solution). The working solution (0.01M) was prepared by making a 1 in 100 dilution of the stock solution. This was prepared by diluting 1ml of stock solution to 100ml with distilled water.

2. 0.01M HCL

A concentrated solution of hydrochloric acid (HCL, 12M) was first diluted 1 in 12 to give 1 molar solution. A working solution (0.01M HCL) was made by diluting the stock (1 mol solution) 1 in 100 with distilled water.

3. INDICATOR SOLUTION

Methyl Red 0.1g

Distilled water 100ml

The salt of the indicator was dissolved in about 10ml of distilled water and volume made up to 100ml with distilled water.

4. STANDARDIZATION OF SODIUM HYDROXIDE

To ascertain the concentration of 0.01M NaOH, it was standardized against 0.01M HCL using methyl red as the indicator. Two ml of 0.01M HCL was titrated with 2ml 0.01M NaOH in a beaker. The final volumes of 0.01M NaOH and 0.01M HCL were equal.

PROCEDURE

1. Two ml of distilled water were put into a new, clean, dry universal container (Sterilin Scientific Co., England) and 0.2ml of serum was added and mixed.

2. The content was mixed with 1ml of 0.01M HCL, controls were similarly treated.

3. One drop of methyl red indicator was added to each tube and mixed by swirling.

4. The solution was titrated with 0.01M NaOH until the colour changed from red to yellow.

CALCULATION

mmol/L HCO^-_3 = 1-titre/2

3.3.2 STATISTICAL METHODS

Statistical analyses were performed using SPSS version 14.0. The data are expressed as means ± SD. Gender differences in variables of lifestyle status, food and nutrient consumption, and blood lipids and fatty acid concentrations were compared by χ2-tests and student's t-test. $P<0.05$ was regarded as significant. Group comparisons were carried out using one way analysis of variance (ANOVA). The relationship between variables was determined using Pearson product-moment coefficient of correlation.

CHAPTER FOUR

RESULTS

4.0 PHYSICAL PARAMETERS

Table 1 shows the BP readings, MMSE score and other physical parameters in patients and control subjects. The mean SBP readings and MMSE score were significantly different in VD and AD patients when compared with the respective value in control subjects ($p<0.05$) with increase in SBP and decreased MMSE recorded in the patient group than in controls. None of the subjects were anaemic.

From table 2, there were no significant differences in the mean values of age, BMI and DBP in VD and AD patients but there was a significant increase in SBP in AD compared with the value in VD patients. Similarly, in VD, the mean value for weight was significantly ($p<0.05$) elevated when compared with the value in AD patients. The difference in the mean ages of AD and control subjects was also significant ($t = 2.14$; $p < 0.05$)

Table 1. Physical Characteristics of All Subjects (Mean ± SD)

Physical Parameters	Vascular dementia (n=40)	Alzheimer's disease (n=15)	Control (n=40)	F-value	p
Age (years)	69.0±8.2	71.1±5.0	67.5±6.8	0.76	NS
BMI(kg/m^2	28.4±4.5	26.1±2.6	25.9±2.7	0.35	NS
Weight(kg)	65.7±12.2	55.6±6.1	61.8±13.5	1.48	NS
Height(m)	1.6±0.3	1.5±0.1	1.6±0.3	0.60	NS
SBP(mmHg)	148.3±41.8	156.0±36.0	141.0±24.2	2.58	S*
DBP(mmHg)	85.3±20.9	89.0±18.0	81.0±15.1	1.54	NS
MMSE	12.3±2.2	12.0±3.4	26.1±2.2	3.56	S*
PCV	40.4±13.7	40.8±14.8	41.9±15.4	0.24	NS

NS : not significant

*significant at $p < 0.05$

SBP – systolic bood pressure

DBP – diastolic blood pressure

MMSE – Mini-Mental State Examination

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Table 2. Comparison of Physical Characteristics of VD and AD Patients

Parameters	VD N=40	AD N=15	t-value	p
Age(years)	69.03±8.2	71.06±5.0	0.871	NS
BMI(kg/m^2)	28.5±4.5	26.1±2.6	0.242	NS
Weight(kg)	65.7±12.2	55.6±6.1	2.365	S
Height(m)	1.6±0.3	1.46±0.1	0.612	NS
SBP(mmHg)	148.3±41.8	156.0±36.0	2.587	S
DBP(mmHg)	85.3±20.9	89.0±18.0	1.451	NS
MMSE	12.3±2.2	12.1±3.4	1.782	NS

NS – not significant; S – significant @ $p < 0.05$

SBP – systolic blood pressure

DBP – diastolic blood pressure

MMSE – Mini Mental State Examination

4.1 GENERAL CHARACTERISTICS

Table 3 shows the general characteristics of VD, AD patients and control subjects. A higher percentage of VD patients had either smoked before or were current cigarette smokers (18.4%) compared with either AD (3.1%) or control subjects (6.2%). The percentage consumption of fish in VD and AD patients was similar (24% respectively) and higher than that in control subjects (21%). A larger percentage of AD and VD patients had less than six years of formal education unlike in the control subjects. Although the incidence of cardiovascular disease and stroke was seen in VD but not in AD, about 62.5% of VD and 20% of AD patients were hypertensive.

Table 3: General characteristics of Vascular Dementia, Alzheimer's Disease patients and Controls

Parameters	Vascular Dementia (N=40)	Alzheimer's Disease (N=15)	Control (N=40)
Gender (%) men(women)	60 (40)	46.7(53.3)	68.6(31.4)
Smokers(%)(Current or past)	18.4	3.1	6.2
Alcohol intake (%)*	23	22	18
Fish intake (%)**	24	24	21
Education(%) <6yr (>6yr)	54 (46)	55(45)	43 (57)
Hypertension(%)	62.5	20	-
Cardiovascular disease(%)	25	-	-
Stroke (%)	15	-	-

* ≥ 2drinks/day

**1mg/d = 1.05kg fish servings /week (Morris et al, 2006)

4.2 PLASMA LIPIDS AND LIPOPROTEINS

As shown in table 4, using ANOVA, the mean plasma triglyceride ($p = 0.001$), LDL-C ($p = 0.000$) and HDL-C ($p < 0.05$) concentrations showed significant variations between the dementia subtypes and control subjects. In contrast, there was no significant change in the mean plasma value of total cholesterol in the patient group compared with controls.

Table 5 shows the comparisons of the differences in mean plasma values of total cholesterol, triglyceride, HDL-C and LDL-C in all subjects. The results showed significant increases in mean concentrations of triglyceride and LDL-C ($p < 0.05$ respectively) in VD compared to the corresponding values in AD patients while there was decreased HDL-C concentration in VD than in AD patients ($P<0.05$). There were significant differences recorded in mean plasma triglyceride and LDL-C concentrations in VD patients and control subjects ($p < 0.05$, $p = 0.000$) with the higher value recorded in VD.

When the subjects were divided into two groups based on the body mass index, BMI, 100% of the AD patients had BMI < 30kg. There were significant increases in mean plasma values of total cholesterol, triglyceride, LDL-C and decreased HDL-C in vascular dementia patients with BMI > 30kg/m^2 compared to the value in those with BMI < 30kg/m^2 (Table 6). There were no significant differences in the mean erythrocyte concentrations of total PUFAs, plasma values of homocysteine and folate between VD patients with BMI >30kg/m^2 and those with BMI < 30kg/m^2.

Table 4: Plasma Cholesterol, Triglycerides, HDL-C, and LDL-C Levels in Vascular Dementia, Alzheimer's Disease Patients and Controls (Mean ± SD)

Parameters	Vascular Dementia N=40	Alzheimer's Disease N=15	Control N=40	F-value	P
Total Cholesterol mg/dl	164.9±37.4	153.1±36.2	146.9±35.2	1.28	NS
Triglyceride mg/dl	122.7±50.9	86.0±45.1	72.7 ± 35.3	2.79	S*
HDL-C mg/dl	39.8 ± 22.4	46.2 ±16.8	52.2 ± 16.9	4.41	S*
LDL-C mg/dl	101.6±41.4	84.7 ±28.2	71.2 ± 30.1	4.30	S*

*Significant at P< 0.05

N = number of subjects

HDL-C : high density lipoprotein cholesterol

LDL-C : low density lipoprotein cholesterol

Table 5 : Plasma Cholesterol, Triglycerides, HDL-C, and LDL-C Levels in patients and Controls (Mean ± SD)

Parameters	VD (n = 40) vs AD	VD vs C (n = 40)	AD (n = 15) vs C
Total Cholesterol mg/dl	VD:164.9±37.4 AD:153.1±36.2 p : NS	VD:164.9±37.4 C:146.9±35.2 p : NS	AD:153.1±36.2 C: 146.9±35.2 p : NS
Triglyceride (mg/dl)	VD:122.7±50.9 AD:86.9±45.1 p : S*	VD:122.7±50.9 C:72.7±35.3 p : S*	AD:86.9±45.1 C:72.7±35.3 p : NS
HDL-C (mg/dl)	VD:39.8±22.4 AD:46.2±16.8 p : S*	VD:39.8±22.4 C:52.2±16.9 p : NS	AD:46.2±16.8 C:52.2±16.9 p : NS
LDL-C (mg/dL)	VD:101.6±41.4 AD:84.7±28.2 p : S*	VD:101.6±41.4 C:71.2±30.1 p : S*	AD:84.7±28.2 C:71.2±30.1 p : S*

*significant at $p < 0.05$

Table 6: Biochemical Parameters in Vascular Dementia patients with BMI > 30kg/m^2 and BMI < 30kg/m^2 (Mean ± SD)

Parameters	BMI > 30 (n = 16)	BMI < 30 (n = 24)	t – value	P
Total Cholesterol (mg/dl)	143.9± 12.5	134.8±16.0	2.620	S*
Triglyceride (mg/dl)	136.7 ± 38.3	117.2 ± 38.4	5.700	S*
HDL-C (mg/dl)	34.1 ± 15.3	43.1 ± 19.3	4.176	S*
LDL-C (mg/dl)	72.3 ± 7.5	61.4 ± 14.6	2.670	S*
Total PUFA (%TFA)	28.1 ± 2.7	26.1 ± 2.1	1.257	NS
Homocysteine (umol/l)	8.55± 0.65	9.0 ± 0.54	0.726	NS
Folate (nmol/l)	4.38 ± 1.62	5.48 ± 0.98	1.726	NS

*significant at $p < 0.05$

PUFA – polyunsaturated fatty acids

TFA – total fatty acids

Table 7: Biochemical Parameters in Vascular Dementia and Alzheimer's disease Patients with BMI < 30kg/m^2 (Mean ± SD)

Parameters	VD (n = 24)	AD (n = 15)	t-value	p
Total Cholesterol (mg/dl)	143.9± 12.5	153.1±36.2	2.51	S*
Triglyceride (mg/dl)	136.7 ± 38.3	86.0±45.1	4.60	S*
HDL-C (mg/dl)	34.1 ± 15.3	46.2±16.8	0.52	NS
LDL-C (mg/dl)	72.3 ± 7.5	84.6±28.2	2.78	S*
Total PUFA (%TFA)	28.1 ± 2.7	35.1±3.0	1.26	NS
Homocysteine (umol/l)	8.55± 0.7	10.0±0.4	0.84	NS
Folate (nmol/l)	4.38 ± 1.6	5.37±0.9	0.73	NS

*significant at $p < 0.05$

4.3 ERYTHROCYTE MEMBRANE FATTY ACIDS

As shown in Table 7, there were significant increases in total cholesterol and LDL-C levels and reduced triglycerides in AD patients with BMI < 30kg/m^2 when compared with the corresponding values in VD patients.

Group comparisons (Table 8) using ANOVA showed that the mean percentage contributions of total n-6 PUFAs: AA (20:4n-6) and LA (18:2n-6) to total fatty acid composition and the ratio of n-3: n-6 fatty acids showed minimal but statistically insignificant variations among the dementia subtypes and the control subjects. On the other hand, the mean relative contributions of total n-3 PUFAs (p = 0.002), DHA (22:6n-3) (p = 0.002) and EPA (20:5n-3)(p = 0.001) to the total fatty acid contents in VD and AD were lower than the corresponding values in the control subjects. Pairwise comparisons showed that there were no significant differences in the mean percentage content of erythrocyte fatty acids between AD and VD patients. In VD patients, the mean relative contributions of total n-3 PUFAs, DHA and EPA were decreased when compared with the control subjects and this difference reached level of significance (P = 0.002, 0.000 and 0.001 respectively) . A similar pattern of change was obtained in AD versus the control group (Table 8).

Table 8. Comparison of Erythrocyte Membrane Fatty Acid contents in Vascular Dementia, Alzheimer's disease patients and Control subjects (Mean ± SD)

Parameters (% total fatty acid)	Vascular Dementia N = 40	Alzheimer's Disease N=15	Control N = 40	F – value	p
Total PUFAs	37.0 ±2.9	35.1±3.0	39.2 ±2.7	1.368	NS
AA (20:4n-6)	13.6±2.4	14.0±2.3	14.3 ±3.5	0.153	NS
LA (18:2n-6)	10.1±2.5	10.3±2.4	8.1 ± 3.3	0.146	NS
Total n-3 PUFAs	9.0±2.8[a]	8.8±2.8[b]	15.8±4.9[a,b]	4.312	S
DHA (22:6n-3)	6.3±2.2[c]	5.4±3.1[d]	8.9±3.8[c,d]	3.675	S
EPA (20:5n-3)	2.0±1.6[e]	3.0±1.7[f]	6.0±4.7[e,f]	4.301	S
n-3 : n-6 fatty acids	0.3±1.2	0.3±1.3	0.4 ± 1.3	1.212	NS

aa: p = 0.002; bb: p = 0.000, cc: p = 0.001; dd: p = 0.002; ee: p = 0.001; ff: p = 0.002

ab, cd, ef : NS

4.4 OTHER BIOCHEMICAL PARAMETERS

The concentrations of potassium, chloride, and urea though decreased in VD patients were not significant as compared with the control subjects. A similar pattern was observed in AD patients with a slightly lower mean bicarbonate value than that in control subjects (Table 9).

The mean plasma concentrations of triglyceride and LDL-C for male VD patients were 123.5±58.8 and 85.8 ± 27.0 respectively versus 121.5±35.4 and 82.9 ± 31.2 in the female group (Table 10). The decreased plasma levels of total cholesterol and HDL-C in male VD were also not statistically significant when compared with the corresponding values in female AD patients (Table 11).

Tables 12 and 13 showed the mean percentages of erythrocyte fatty acids content and the plasma concentrations of electrolytes and urea in male and female VD and AD patients respectively. The percentage contributions of arachidonic, linoleic, docosahexanoic and eicosapentanoic acids to erythrocyte total fatty acids content in male VD patients was not significantly different from the values in female VD patients. A similar pattern was shown in the mean plasma values of Na+, K+, Cl-, HCO3- and urea in male and female VD patients. There were also no significant differences in the percentage erythrocyte fatty acids content in male AD patients compared to the female subjects. However, the mean plasma concentrations of K^+ ($p<0.05$) and Cl- ($p<0.05$) were significantly decreased in male AD patients when compared to the values in female AD patients.

From table 14, when patients with VD and AD as well as control subjects were compared according to their mean values for homocysteine, folate, vitamin E and Se, using ANOVA, there was a significant increase in plasma homocysteine and decreases in folate and Se concentrations in VD and AD patients compared to control subjects ($p<0.05$ in all cases) whereas the changes in mean plasma vitamin E concentration among the different groups

were not significant.Table 15 showed the statistical comparisons of homocysteine, folate and selenium in all subjects. Folate concentration increased in AD patients by 1.88ng/ml than in VD patients ($p = 0.000$) (Table 15).

In VD patients, consistent positive correlations were found between homocysteine and total PUFA ($p<0.01$) and folate and DHA ($P<0.01$). On the other hand, the negative correlations between homocysteine and folate ($r= - 0.67$, $p<0.01$) and homocysteine and selenium ($r = -0.14$, $p<0.01$) were significant (Table 16). In AD patients, only the total PUFAs showed a significant association with homocysteine ($r = 0.98$, $p<0.01$). All other parameters were not significantly correlated.There were no associations found between the parameters analyzed in control subjects.

Table 9. Plasma Sodium, Potassium, Chloride, Bicarbonate and Urea in Vascular Dementia, Alzheimer's Disease patients and Controls (Mean ± SD)

Parameters	Vascular Dementia N = 40	Alzheimer's Disease N=15	Control N = 40	F –value	p
Na+(mmol/l)	135.8±3.53	136.8±3.61	134.9±2.41	1.886	NS
K+(mmol/l)	3.6±0.34	3.5±0.33	3.6±0.49	0.587	NS
Cl-(mmol/l)	116.9±0.34	116.8±0.81	119.1±5.94	2.161	NS
HCO^-_3 (mmol/l)	19.4±9.89	21.0±6.69	22.2±2.25	0.790	NS
Urea(mg/dl)	19.7±2.81	17.8±7.67	20.5±2.78	1.549	NS

Table 10. Plasma Total Cholesterol, Triglycerides, HDL-C and LDL-C Levels in Male and Female Vascular Dementia patients (Mean ± SD)

Parameters	Male N=23	Female N=17	t – value	p
Total Cholesterol (mg/dl)	147.7±35.2	161.1±35.3	0.909	NS
Triglyceride (mg/dl)	123.5±58.8	121.5±35.4	0.098	NS
HDL-C (mg/dl)	37.5±25.7	43.2±17.1	0.605	NS
LDL-C(mg/dl)	85.8±27.0	82.9±31.2	0.246	NS

Table 11. Plasma Total Cholesterol, Triglycerides, HDL-C and LDL-C Levels in Male and Female Alzheimer's Disease patients (Mean ± SD)

Parameters	Male N=7	Female N= 8	t – value	p
Total Cholesterol (mg/dl)	120.3±4.63	136.9±4.64	0.818	NS
Triglyceride (mg/dl)	104.7±6.99	105.1±4.65	0.199	NS
HDL-C (mg/dl)	53.1±3.68	52.2±2.82	0.716	NS
LDL-C(mg/dl)	54.9±3.81	71.2±4.23	0.357	NS

Table 12. Erythrocyte Membrane Fatty Acids, plasma Electrolytes and Urea Concentrations in Male and Female Vascular Dementia patients (Mean±SD)

Parameters	Male (n = 24)	Female (n = 16)	t – value	P
AA (20:4n-6)(%TFA)	13.8±1.53	13.7±1.89	0.068	NS
LA (18:2n-6)(%TFA)	9.5±1.69	9.2±1.69	0.430	NS
DHA (22:6n-3)(%TFA)	6.2±1.38	6.8±1.69	0.915	NS
EPA (20:5n-3)(%TFA)	2.1±1.96	1.96±0.95	0.146	NS
Na+(mmol/l)	136.6±3.99	137.1±2.83	0.260	NS
K+(mmol/l)	3.45±0.33	3.56±0.36	0.705	NS
Cl-(mmol/l)	116.9±0.89	116.8±0.64	0.257	NS
HCO3-(mmol/l)	20.4±7.19	22.5±5.68	0.623	NS
Urea(mg/dl)	18.5±7.96	16.1±7.34	0.656	NS

Table 13. Erythrocyte Membrane Fatty Acids, Plasma Electrolytes and Urea Concentrations in Male and Female Alzheimer's Disease Patients (Mean±SD)

Parameters	Male (n = 7)	Female (n = 8)	t – value	P
AA (20:4n-6)(%TFA)	14.3±2.63	13.7±1.09	0.533	NS
LA (18:2n-6)(%TFA)	9.04±1.71	10.4±2.28	1.034	NS
DHA (22:6n-3)(%TFA)	6.08±2.69	5.06±2.04	0.677	NS
EPA (20:5n-3)(%TFA)	4.18±1.01	2.5±1.20	0.239	NS
Na+(mmol/l)	136.2±4.26	135.4±3.08	0.314	NS
K+(mmol/l)	3.36±0.23	3.92±0.15	4.572	S*
Cl-(mmol/l)	116.7±0.20	117.2±0.29	2.875	S*
HCO3-(mmol/l)	16.9.4±9.50	21.9±10.71	0.768	NS
Urea(mg/dl)	18.9±3.35	20.6.1±2.16	0.311	NS

*significant @ $p < 0.05$

Table 14. Plasma Homocysteine, Folate, Vitamin E and Selenium concentrations in all subjects (Mean ± SD)

Parameters	Vascular dementia (N = 40)	Alzheimer's Disease (N = 15)	Control (N = 40)	F - value	p
Homocysteine (umol/L)	9.66±1.76	10.0±0.43	7.36±2.32	10.30	S*
Folate (nmol/L)	3.49±0.71	5.37±0.89	9.47±3.78	32.71	S*
Vitamin E (mg/dl)	1.63±0.74	1.73±0.86	1.78±0.68	0.760	NS
Selenium (umol/l)	1.39±0.72	1.48±0.57	2.06±0.53	3.017	S*

*significant @ $p<0.05$

Table 15. Statistical Comparisons of Plasma Homocysteine, Folate and Selenium Concentrations in Patients and Control (Mean ± SD)

Parameters	VD(N=40) vs AD	VD vs C(N=40)	AD(N=15) vs C
Homocysteine (umol/L)	VD:9.7±1.8 AD:10.0±0.4 t : 0.62 p : NS	VD:9.7±1.8 C : 7.4±2.3 t : 2.73 p : S*	AD:10.0±0.4 C : 7.36±2.3 t : 3.00 p : S*
Folate (ng/ml)	VD : 3.5±0.7 AD : 5.4±0.9 t : 5.81 p : S*	VD : 3.5±0.7 C : 9.5±3.8 t : 4.41 p : S*	AD : 5.4±0.9 C : 9.5±3.8 t : 4.22 p : S*
Selenium (umol/l)	VD : 1.4±0.7 AD : 1.5±0.6 t : 0.61 p : NS	VD : 1.4±0.7 C : 2.1±0.5 t : 2.87 p : S*	AD : 1.5±0.6 C : 2.1±0.5 t : 2.59 p : S*

*significant @ $p<0.05$

Table 16. Correlation Coefficients of Some Biochemical Parameters in VD Patients

Parameters	Homocysteine (umol/L)	Folate (nmol/L)	t PUFA (%TFA)	DHA (%TFA)	Se (umol/L)
Homocysteine	1	-0.67**	0.36*	-	-0.14**
Folate	-0.67**	1	-	0.49*	-
Total PUFA	0.36*	1	1	-	-
DHA	-	0.49*	-	1	-

*significant @ p = 0.001 ; **significant @ p = 0.000

PUFA – polyunsaturated fatty acid

DHA – docosahexanoic acid

4.5 TWO AND THREE – DIMENSIONAL GROUPINGS

The three – dimensional groupings of n-3 erythrocyte fatty acids (DHA and EPA), total cholesterol (TC) and homocysteine in each of the dementia subtypes and controls are represented in Figures 2 and 3. These groupings showed that the Alzheimer's disease (AD) and control (CTRL) groups had values that were displaced in space at the upper right hand corner (Figure 2) while the values for the vascular dementia (VD) group were distinctly separated from the control group, the few outliers notwithstanding (Figure 3). The two- and three – dimensional groupings of n-6 erythrocyte fatty acids (n6FA), triglycerides (TG) and homocysteine (HCY) in all subjects showed a sparse and scattered distribution in Figures 4, 5 and 6. However, from figure 7, the values for the vascular dementia group were separated in the lower limit while those for the control subjects were in the upper limit. The plots of two biochemical parameters in each instance in both VD and AD patients showed an interspersed distribution with no distinct variability in the two groups (Figures 8 – 10).

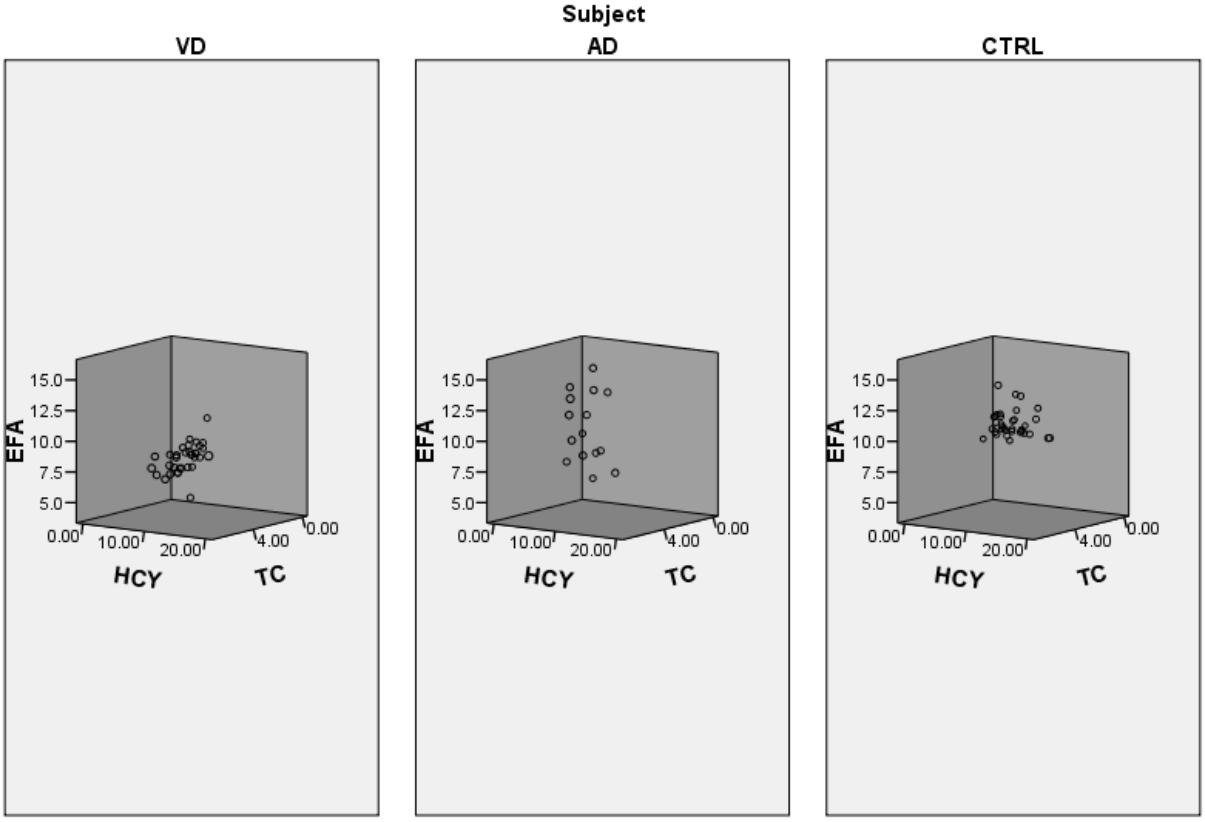

FIGURE 2 : Three dimensional grouping of n-3 erythrocyte fatty acids (EFA), plasma total cholesterol (TC) and homocysteine (HCY) in vascular dementia (VD), Alzheimer's disease (AD) patients and controls (CTRL)

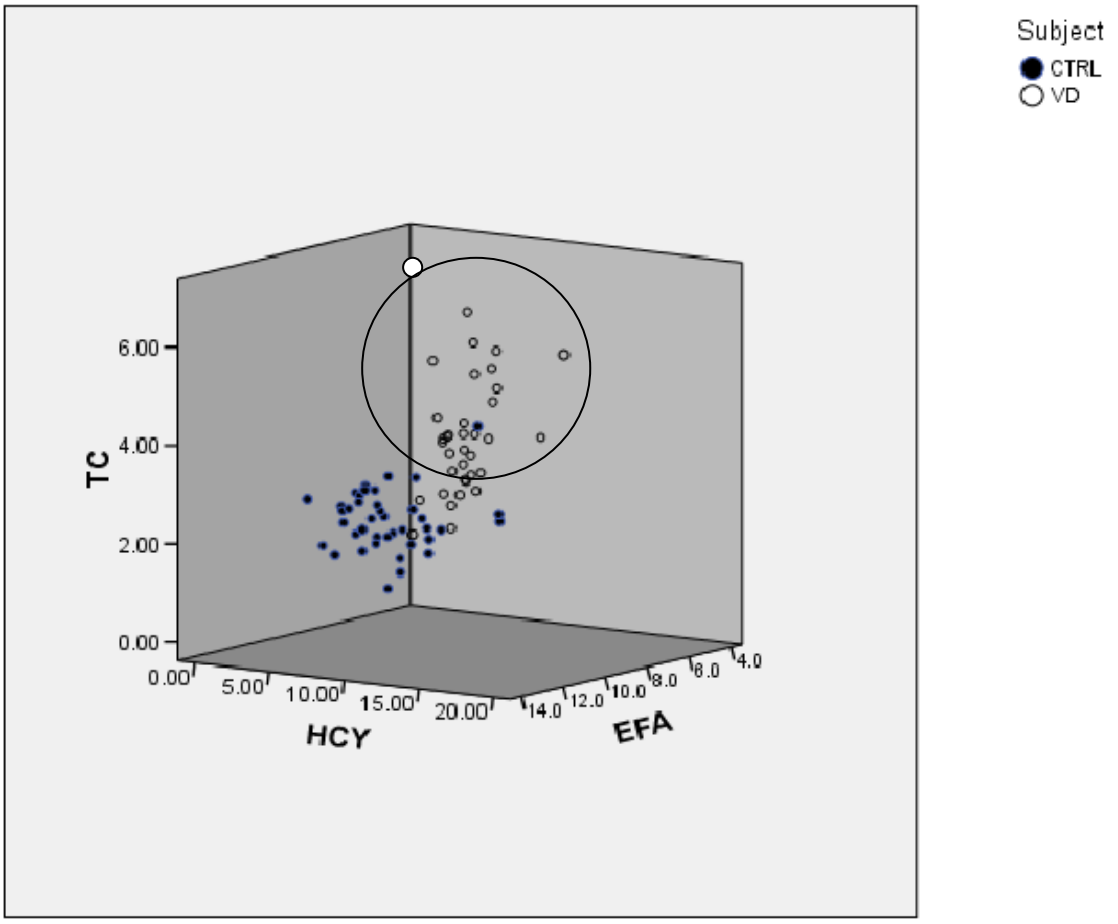

FIGURE 3: Three dimensional grouping of total cholesterol (TC), homocysteine (HCY) and n-3 erythrocyte fatty acids (EFA) in vascular dementia (VD) patients and controls (ctrl)

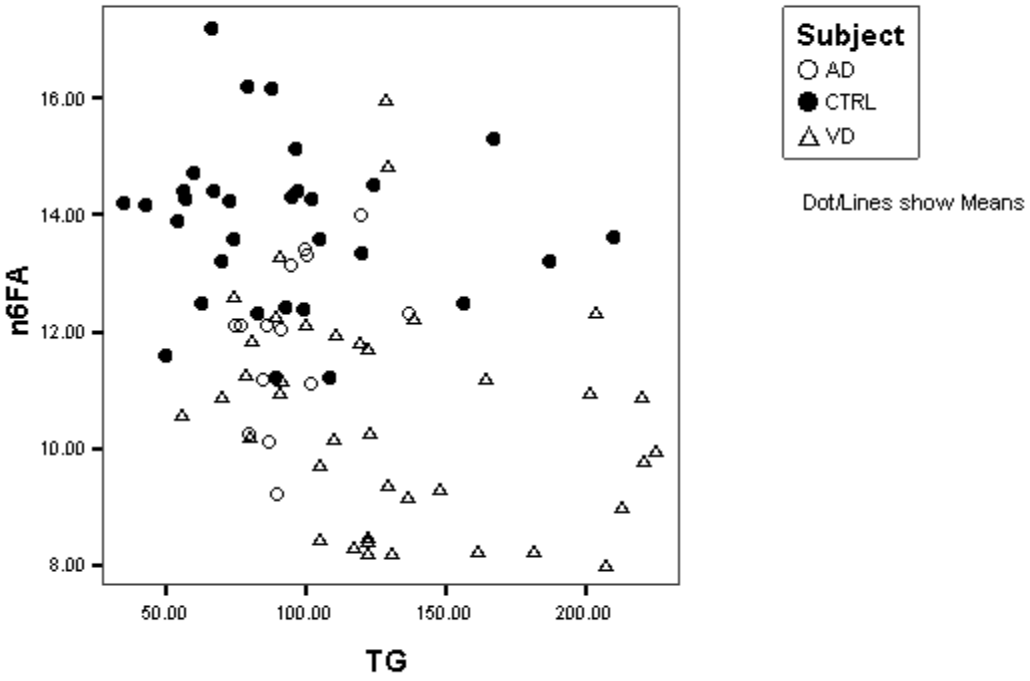

Figure 4 : Two dimensional plot of n-6 polyunsaturated fatty acids (n6FA) and triglyceride (TG) in all subjects

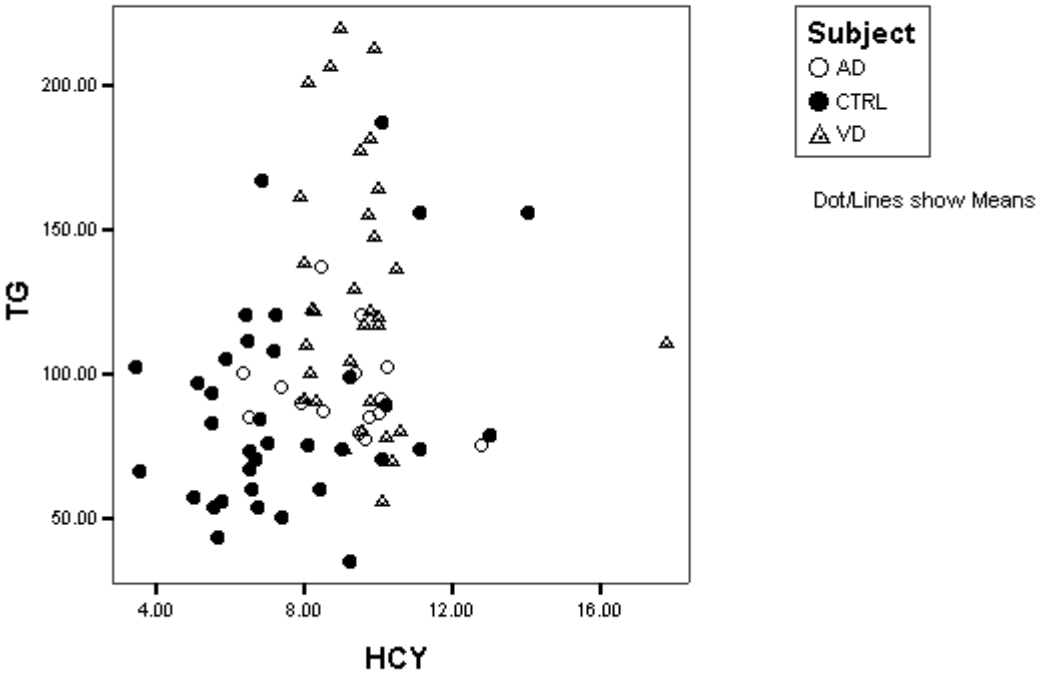

Figure 5 : Two dimensional plot of triglyceride (TG) and homocysteine (HCY) in all subjects

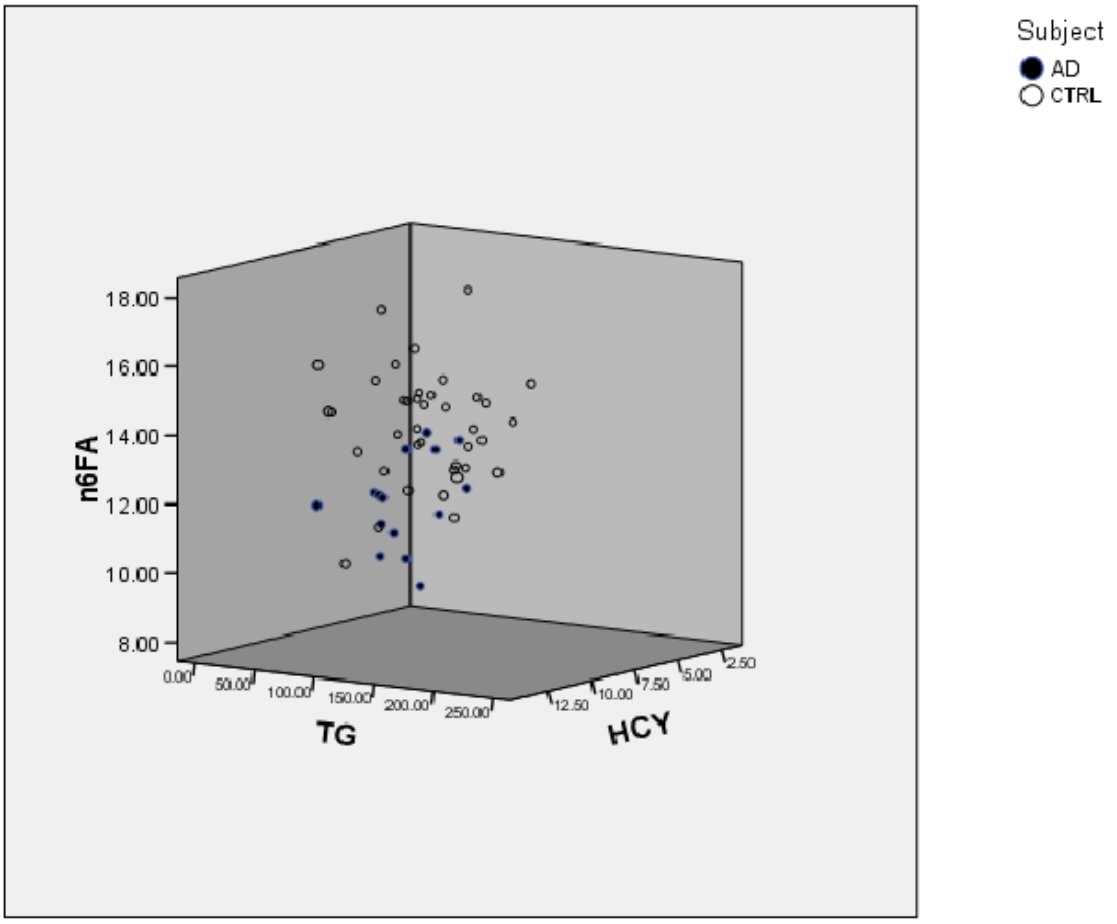

Figure 6 : Three dimensional grouping of n-6 erythrocyte fatty acids (n6FA), triglycerides(TG) and homocysteine (HCY) in Alzheimer's disease (AD) patients and controls (CTRL)

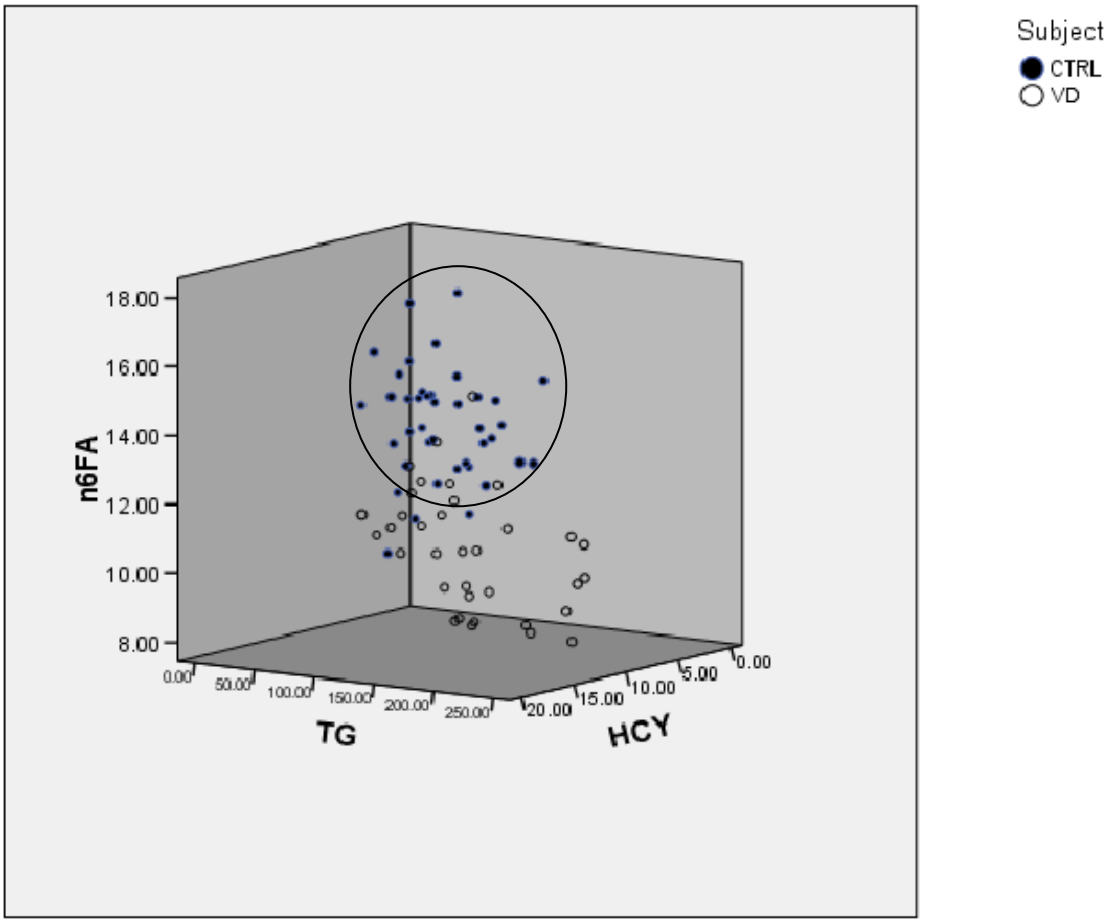

Figure 7 : Three dimensional grouping of erythrocyte n-6 fatty acids (n6FA), triglycerides (TG) and homocysteine (HCY) in vascular dementia (VD) patients and controls (CTRL)

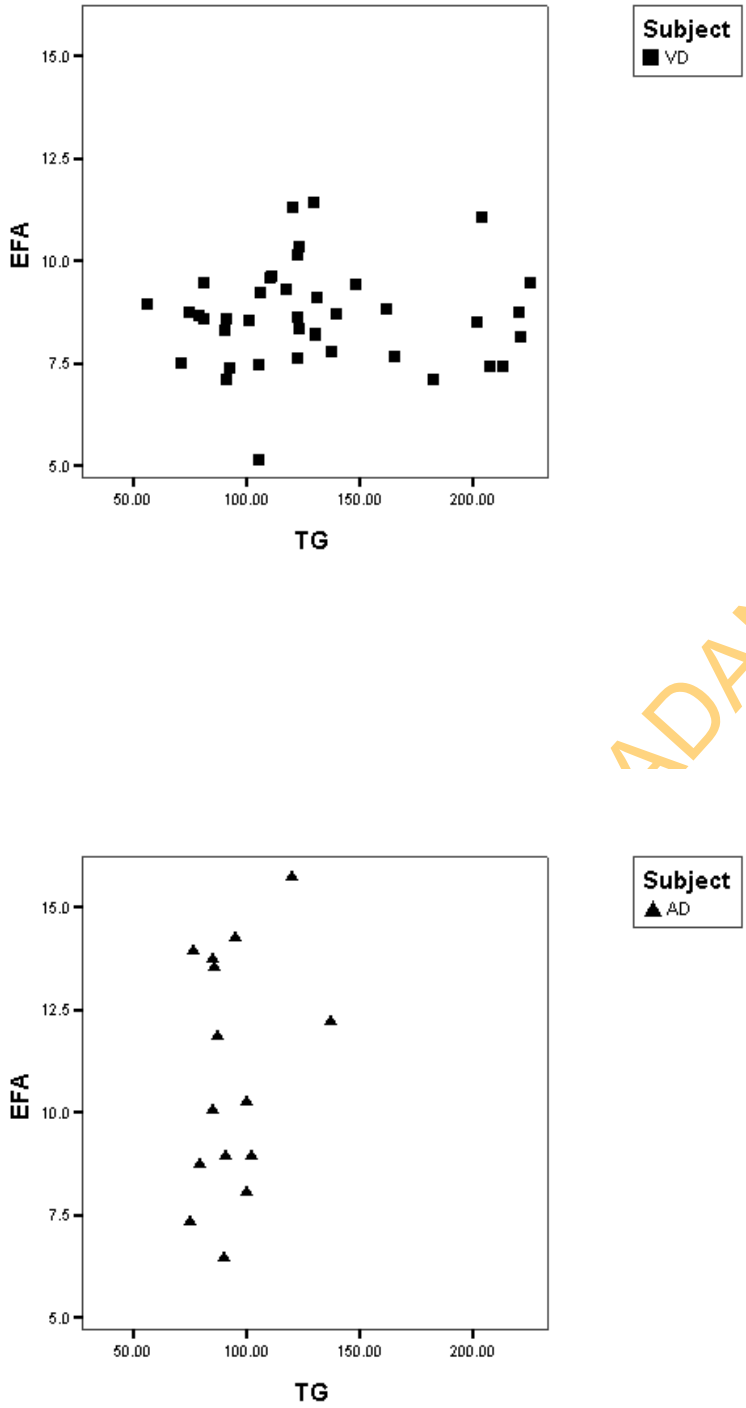

Figure 8 : Plot of n-3 erythrocyte fatty acids (EFA)(%TFA) and trigyceride (TG)(mg/dl) in VD and AD patients

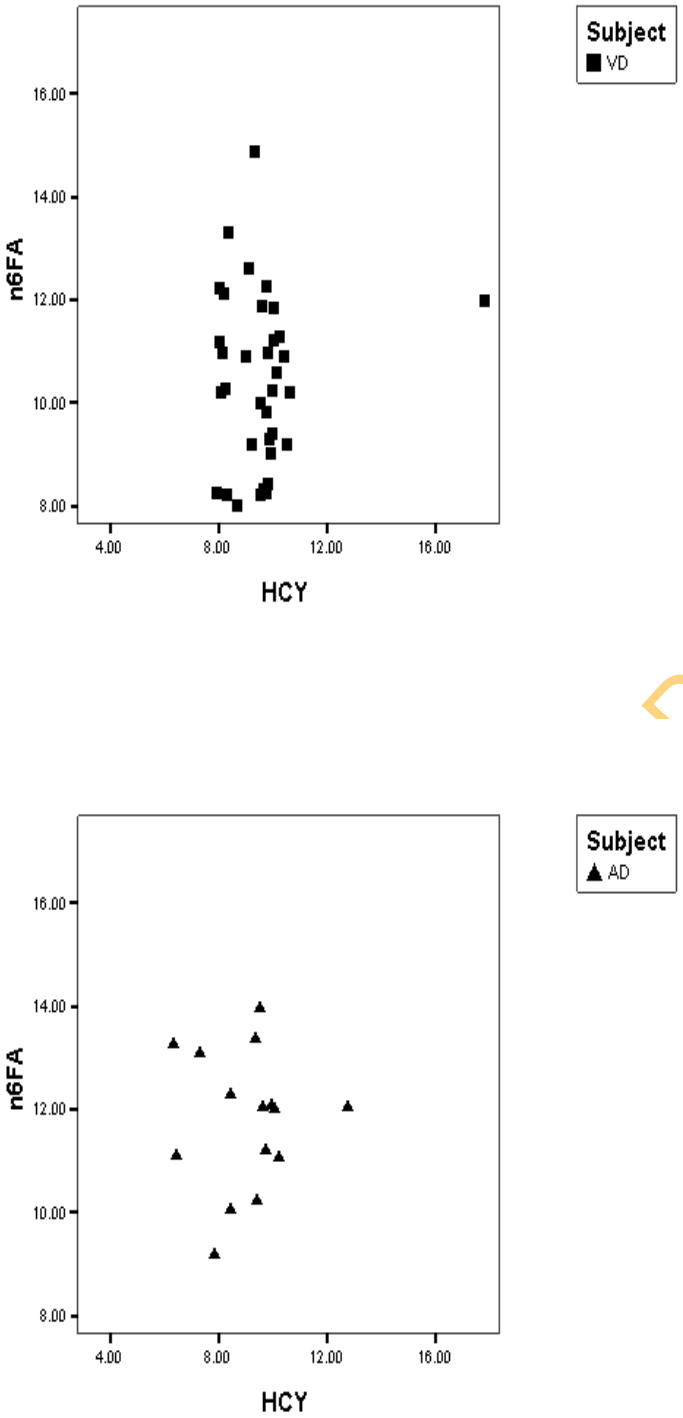

Figure 9 : Plot of n-6 erythrocyte fatty acids and homocysteine in VD and AD patients

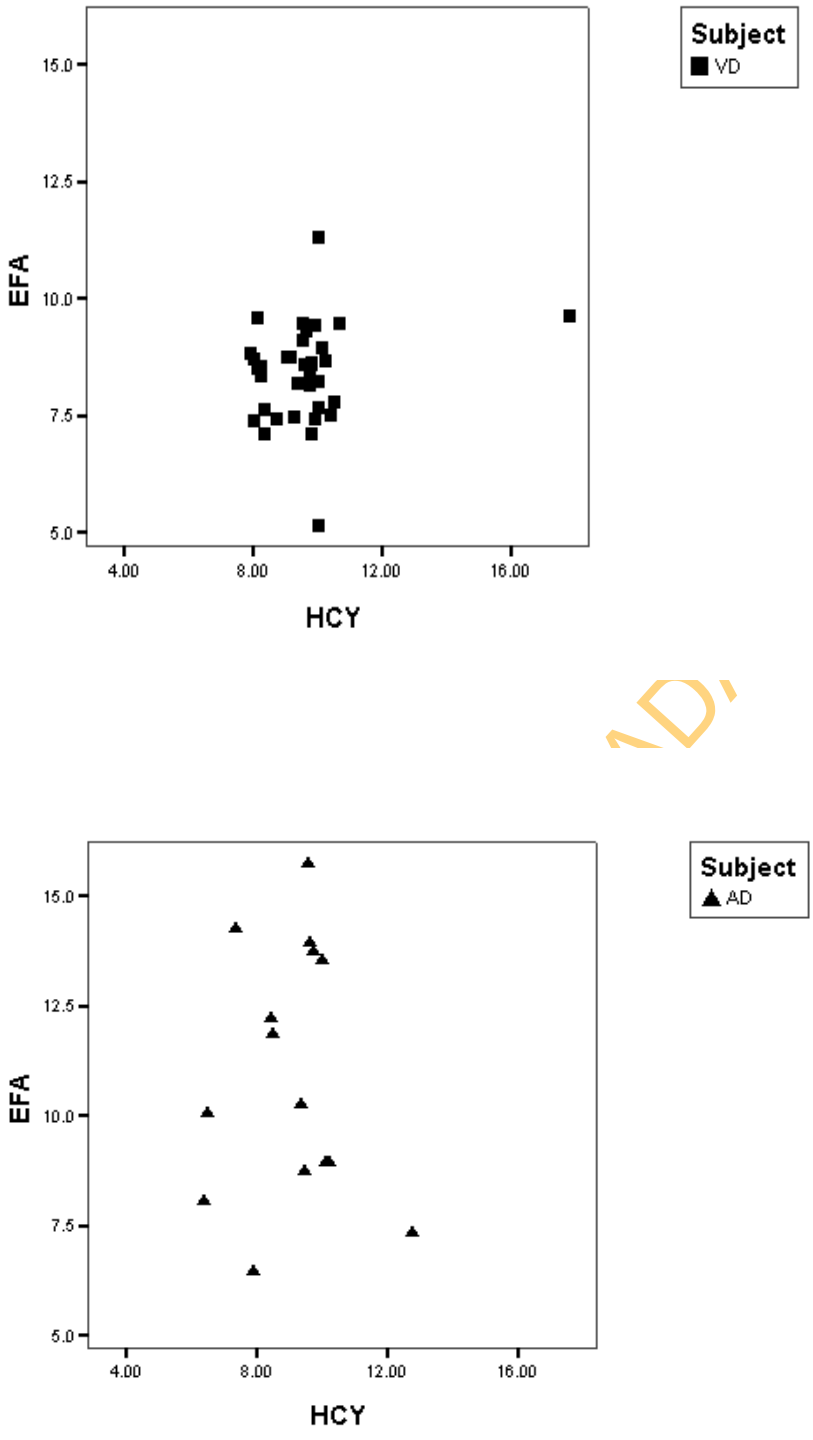

Figure 10 : Plot of n-3 erythrocyte fatty acids (EFA) and homocysteine (HCY) in VD and AD patients

CHAPTER FIVE

DISCUSSION

The primary purpose of the present study was to identify possible relations between the expression of specific putative atherogenic risk modulating factors and the incidence of dementia in this community. In the present study the patients were a mixed population of elderly male and female adults suffering from either vascular dementia (VD) or Alzheimer's disease (AD). Lifestyle factors including habitual diet, cigarette smoking and alcohol intake are believed to be major contributors to the development of VD and AD. The findings in this study showed that a larger percentage of VD patients (18.4%) were either current smokers or had smoked before when compared with the AD patients (3.1%). In a review of risk factors and post stroke dementia by Pasquier et al (1999), it was postulated that cigarette smoking was a common risk factor for both Alzheimer's and vascular disease.

Some of the key findings in the present study were that increased triglyceride, LDL-C, and decreased HDL-C concentrations in plasma were found in VD patients. In addition, total cholesterol concentration appeared to be elevated in VD and AD patients compared with control subjects. These findings also suggest that hypertriglyceridemia and elevated LDL-C may be among the intermediary mechanisms linking hyperlipidemia to VD. This observation is in agreement with some studies (Kuo et al, 1998; Moroney et al, 1999) and at variance with others of (Scacchi et al, 1998; Notkola et al, 1998). Hypertriglyceridemia, hypercholesterolemia, elevated LDL-C and decreased HDL-C were found in VD patients with BMI greater than 30kg/m^2. A previous study has reported that hyperlipidemia is associated with peripheral neuropathy and dementia especially in hyperlipidemic young subjects (Rogers et al, 1989). These workers suggested that the observation might be due to the adverse effects of hyperlipidaemia on neuronal membrane metabolism in man. In

addition, elevated serum lipids may enhance atherogenesis of the extracranial and intracranial arteries causing an increase in blood viscosity which in turn decreases cerebral perfusion and impair cognitive performance (Swank, 1956, Rogers et al, 1989).

Studies `reported an association between metabolic cardiovascular syndromes and dementia and it was suggested that possible neuroprotective therapy such as treatment against hypertension and other traditional vascular risk factors may help prevent vascular cognitive impairment in AD and VD (Kaiser, 2011; Kalmijn et al, 2000; Skoog,1994). Hyperlipidemia in particular has been identified as a risk factor for both VD and AD (Sparks, 1997;Jarvik et al, 1995, Notkola et al, 1998). This risk relationship is supported by animal models. For example, mice fed a high cholesterol diet were found to have a twofold increase in the β-amyloid protein and in the number and size of amyloid deposits (Refolo, 2000). Cross-national survey also showed that countries that have high dietary fat consumption tend to have a higher prevalence of AD (Kalmign et al, 1997). It is theoretically possible then that treatment of hyperlipidemia could lessen the risk of dementia and slow the progression of the disease. Infact, prior preliminary data have shown that individuals treated with (statins) have a lower prevalence of dementias of both vascular and Alzheimer's types (Refolo et al, 2000; Wolozin et al, 2000).

To operationalize the diagnosis of dementia for this study, a composite variable was developed in which a positive diagnosis was made when both a clinical diagnosis of dementia or one of its types were documented and the patient had a MMSE score less than 23. This cutoff has been shown to improve diagnostic accuracy (DSMMD, 1994). Covariates included age, gender, race, education, smoking, alcohol, blood pressure; medical diagnosis (stroke, hypertension, depression), family history of dementia or AD, cholesterol level, LDL, HDL and triglyceride. Both groups were similar in education, MMSE score, alcohol

consumption, tobacco use, stroke or depression. A limitation of this study could be the accuracy of the diagnosis of dementia. However, the high rate of concordance between clinical diagnosis and cognitive testing result suggest accuracy of dementia diagnosis.

There is increasing evidence to suggest an important role of cholesterol in the pathophysiology of dementia and AD (Jarvik et al, 1995; Notkola et al, 1998). For example, Jarvick et al (1994) found an increased risk of AD in patients with hypercholesterolemia. In the Rotterdam Study, hyperlipidemia was found to be associated with VD and AD (Hoffman et al, 1997). The role of statins in lipoprotein metabolism had been documented. These include inhibiting cholesterol synthesis, decreasing LDL circulation, modulating LDL receptor. Other effects include: that on nitric oxide, decreased endothelium and an anti-oxidant effect (Hess et al, 2000; de la Monte et al, 2000). Hajjar et al, (2002) also suggested that the use of statins was associated with a lower prevalence of VD and AD and that the use of statins could have a potential benefit on the progression of cognitive impairment in the elderly. However we did not assess the effect of lipid lowering drugs on cognitive function of the patients studied.

The systolic blood pressure was significantly higher in AD and VD patients than in controls while the change in diastolic blood pressure was not significant. It is noteworthy that the blood pressure values for AD and VD patients (156/89 and 148/85 respectively) were higher than the cut-off value of 140/85mmHg for diagnosis of hypertension (WHO/ISH, 1999). In their study on association of cognitive function with high blood pressure, hypertension and high pulse rate in persons aged 60 years and older, Obisesan et al (2008) reported that optimal blood pressure (120/80mmHg) was associated with best cognitive performance and that at age 70 years and older, high blood pressure, hypertension and uncontrolled blood pressure were independently associated with poorer cognitive function than normal blood

pressure. It is therefore suggested that optimal control of blood pressure may be useful in preventing neurocognitive loss in the aging population.

Increasing evidence points to an alteration in neurovascular function as key to the pathobiology of AD and cognitive impairment (Kaiser, 2011). In line with this, the causal role of vascular risk factors in different types of dementia has been linked to sclerosis of small cerebral arteries and arterioles which is considered to be responsible for diffuse periventricular white matter abnormalities, which play an important role in the development of VD (Reitz et al, 2004). Shadett et al (1994) also hypothesized that high concentrations of LDL-C and low levels of HDL-C are independent risk factors for coronary heart disease and carotid artery atherosclerosis, this in turn may lead to cognitive impairment through cerebral hypoperfusion or embolism. HDL-C particles have also been reported to play a role in the removal of excess cholesterol from the brain by interaction with ApoE and heparan sulfate proteoglycans in the subendothelial space of cerebral microvessels and this mechanism has been linked to low incidence of small-vessel disease (Zuliani et al, 2001).

The finding in this study shows a tendency of increased plasma cholesterol concentration been associated with the female gender suffering from VD and AD as well as increased LDL-C and triglyceride in the female AD group. The seemingly sex-linked difference was attributed to ApoE E4 genotype status that seems to have a greater deleterious effect on gross hippocampal pathology and memory performances in women compared with men (Azad et al, 2007). It is noteworthy that the triglyceride and LDL-C concentrations in male VD patients were also higher than in female patients. Some earlier studies showed different results for plasma lipids and the rate of incidence of VD for men and women (Reitz et al, 2004; Leys et al, 1998; Hendrie et al, 2001). These data showed that irrespective of sex, dyslipidemia is strongly associated with incidence of VD and AD.

There is increasing scientific interest in the hypothesis that very-long-chain n-3 PUFAs, as present in fish or fish oil and supplements rich in n-3 fatty acids are beneficial for the maintenance of cognitive performance in adults. This hypothesis is corroborated by the pattern of erythrocyte fatty acid changes in this study which shows that the percentage of total n-3 PUFAs, DHA and EPA, were significantly decreased in VD and AD patients compared to control subjects. Several observation studies, conducted among older adults reported that participants in those studies with high erythrocyte levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) - which are the most abundant n-3 PUFAs in erythrocyte - had a lower risk of experiencing cognitive decline (Heude et al, 2003; Conquer et al 2000). However others have reported no such associations (Engelhart et al, 2002; Laurin et al, 2003). Some of these studies also evaluated the association between fish consumption and cognitive performance and reported a lower incidence of AD (Morris et al, 2003) or a trend toward a lower risk of cognitive decline (Kalmijn et al, 1997) with increasing fish intake. In the present study the average percentage fish intake in patients with the dementia subtypes was slightly higher than the control subjects. Nevertheless there is the possibility that a more detailed cognitive assessment in a larger population may show significant associations in fish consumption and cognitive impairment in this community.

There were no significant differences in eythrocyte levels of DHA and EPA in vascular dementia as compared to those in Alzheimer's disease patients probably suggesting a possible relationship between n-3 PUFAs and low cognitive function irrespective of the subtypes of dementia. This is more likely as both VD and AD patients studied scored less than 24 points on the MMSE scale. Dietary n-3 PUFAs had been shown to improve brain functioning in animal studies and it was hypothesized that dietary intake of n-3 fatty acids and weekly consumption of fish may reduce the risk of incident Alzheimer's disease (Morris et al, 2003). Nevertheless, the findings in this present study are in line with earlier report by Heude et al

(2003) which showed that lower proportions of n-3 PUFAs in erythrocytes are associated with a higher risk of cognitive decline. Hence, there is the possibility that a more detailed cognitive assessment in a larger population may show significant associations in fish consumption and cognitive impairment in this community.

The erythrocyte concentration of linoleic acid was increased in VD and AD patients while the concentration of arachidonic acid tended to decrease in patients with vascular dementia when compared to the values in AD and control subjects. This is similar to the reports from studies where it was found that high dietary linoleic acid intake was associated with cognitive impairment and an increased risk of dementia (Kalmijn et al,2004; Heude et al, 2003). It was postulated that n-6 PUFAs including linoleic acid tend to "harden" brain cells and shifts the physiologic state to one that is more prothrombotic and proaggregatory (Heude et al, 2003; De Caterina et al, 2000).

A suggested mechanism for the cardio-protective effect of n-3 fatty acids focused on the influence of n-3 fatty acids on eicosanoid metabolism, inflammation, beta oxidation, endothelial dysfunction, cytokine growth factors, and gene expression of adhesion molecules (Das, 2000). Risk factors for cardiovascular and cerebrovascular diseases are similar. Cerebrovascular disease might play a role in vascular dementia (Zuliani et al, 2001; Reitz et al, 2004). N-3 fatty acids are also neuroprotective by suppressing the synthesis and release of interleukins and TNF-alpha (which is neurotoxic) and modulation of hypothalamic-pituitary-adrenal anti-inflammatory responses in the nervous system (Das, 2000).This shows a close association between the central nervous system and dietary n-3 fatty acids.

Interleukin-6 is detectable in early stage diffuse plaques formation in AD which is an acute-phase process crucial to pathogenesis of AD. Fish oil and gamma linolenic acid (GLA) have

also been known to suppress interleukin-1 production by stimulating monocytes and this phenomenon had been linked to low prevalence of AD in the elderly Japanese population (McCarty, 1999). High concentrations of DHA and EPA are associated with cardiovascular benefit. Trials showed reductions in cardiovascular events of 19-45% in subjects receiving n-3 fatty acid supplementation containing EPA and DHA. Patients with hypertriglyceridemia can also benefit from treatment with 3-4g daily intake of DHA and EPA (O'Keefe, 2008). It is recommended that more observational studies in this population within a normal range of erythrocyte n-3 PUFAs concentration could aid the understanding of the association between n-3 PUFAs with VD and AD. Randomized controlled trials with n-3 PUFA supplementation should help to clarify the importance of the observed associations.

The slight increase in plasma concentrations of Na^+ and K^+ as well as the decrease in Cl^-, HCO^-_3 and urea in VD and AD patients compared with the control group was not statistically significant. However there were significant decreases in plasma K^+ and Cl^- values in male AD than in female AD. These data is at variance with a study investigating the relationship between renal impairment and risk of dementia, Seliger et al (2004) reported that moderate renal insufficiency was associated with a 37% increase in the risk of dementia but not AD. While showing that ageing leads to a decline in renal function, a study by Cornelius (2004) further reported that muscle mass decreases with age making serum creatinine an unreliable measure of renal function in elderly people. The plasma electrolytes and urea concentrations measured in this study also provided a useful estimation of renal function.The relationship of renal dysfunction and dementia could be attributed to the association of renal impairment with an increased risk of carotid atherosclerosis and stroke, which are determinants of cognitive dysfunction and dementia (Seliger et al, 2004).

Previous literature from this community emphasized or studied the incidence and risk factors of VD and AD. With this study, the total plasma lipid profile, erythrocyte PUFAs , plasma homocysteine, folate, selenium and vitamin E were all determined in the two groups of neurodegenerative disorders. The statistically analyzed results from the present study showed significant associations between the occurrence of vascular dementia, Alzheimer's disease and hyperhomocysteinemia.This observation is similar to the findings of two case-control studies that reported significantly higher serum homocysteine levels in AD patients when compared with age-matched controls (McCaddon et al, 1998; Clark et al, 1998). An increased plasma homocysteine was equally reported as a strong independent risk factor for the development of dementia and AD in the Framingham cohort study (Seshadri et al, 2002). Dementia and hyperhomocysteinemia both seem to increase with increasing age, are common in the elderly and thus might occur coincidentally. Other recent studies however showed that plasma homocysteine levels were not associated with cognitive decline (Ravaglia et al,2000; Ariogul et al, 2005). The main confounding factors may be the nutritional habits as well as genetic differences of the population involved in different studies.

One of the main findings of the present study is that plasma folate deficiency is closely related to VD and AD. The mean plasma folate concentration was significantly lower in VD than in AD patients. Ramos et al (2005) also found an association of low folate status with impaired cognitive function and dementia in their Sacramento Area Latino Study on Aging. This observation is at variance with other studies that reported no significant relationship between folate and dementia (Crystal et al, 1994; Ariogul et al, 2005).

Several mechanisms have been proposed by which folate and homocysteine may affect brain function. Hyperhomocysteinemia is associated with carotid atherosclerosis and an increased

risk of stroke. Atherosclerosis and stroke increase the risk of clinical AD. Hyperhomocysteinemia has been related to cerebral microangiopathy, endothelial dysfunction, impaired nitric oxide activity, and increased oxidative stress – all factors associated with the aging of the brain. Hyperhomocysteinemia is also reported to be a risk factor for cerebrovascular disease and has been shown to predict incident dementia in the Framingham cohort study (Seshadri et al, 2002). More basic research has indicated that homocysteine induces excitotoxicity effects in brain through increased glutamate receptor activation. Folate deficiency may affect the brain by reducing synthesis of S-Adenosine Methionine (SAM) and thus inhibiting SAM-dependent methylation reactions. These include the synthesis and catabolism of many neurotransmitter, such as dopamine, norepinephrine, adrenaline and serotonin. Serotonin deficiency is associated with depression. Depression is a strong determinant of cognitive function in older adults. Therefore SAM, serotonin synthesis and depression may be related to low folate status and cognitive function (Ramos et al,2005).

Results from this study showed a negative association between homocysteine and folate and no association between homocysteine and n-3 PUFAs in dementia patients. This observation is in agreement with a recent study by Crowe et al (2008) that found that lowering plasma homocysteine concentrations by 4.4umol/l for two years with high intakes of folate, vitamin B12 and 6 did not alter the n-3 long- chain fatty acids (LCFAs) composition of plasma phosphatidylcholine. However, the result from the present study does not support the hypothesis that homocysteine affect the n-3 long chain PUFA composition of tissues (Durand et al, 1996) although a positive association between folate and DHA was recorded. This hypothesis (Durand et al, 1996) is based largely from evidence derived from experiments with animals and small observational studies in humans. It is noteworthy that the experimental animals were exposed to folate-deficient diets or extremely high concentrations

of homocysteine. The patients in the present study were however not exposed to such physiological conditions.

Proposed mechanisms through which folate and homocysteine may influence docosahexanoic acid include : (i) by reducing homocysteine concentrations, folate may reduce the generation of reactive oxygen species and thus spare docosahexanoic acid which is a major target for lipid peroxidation; (ii) a complex mechanism based on the involvement of folate in liver synthesis of phosphatidylcholine through its regulation of 1-carbon metabolism. Synthesis of phosphatidylcholine in the liver and secretion into lipoproteins is a major route for the appearance of docosahexanoic acid into the plasma (Durand et al, 1996).

Low level of plasma selenium was a constant feature in VD and AD patients but not in control subjects. In addition significant negative correlation was found between homocysteine and selenium. This observation is in agreement with the study by Gonzalez et al (2004) and in contrast with the result of the animal study by Uthus et al (2002) that found that selenium supplementation increased total homocysteine concentration in rats. The contrast in this study and theirs may be explained by the fact that selenium in foods may have a different effect in humans since selenite is the form used in the animal study, a form which does not occur in foods. Venn et al (2003) also observed that selenium did not influence plasma homocysteine. This is not comparable to the result from the present study because they performed a supplementation trial to study the effect of selenium intake, while this study focused on plasma selenium as an index of selenium status.

The effect of serum levels of folate on homocysteine has been established by several studies (Ford et al, 2002, Jacques et al, 2001) whereas the association of plasma selenium with homocysteine has not been studied in VD and AD patients. The results from this study may

be explained by homocysteine metabolism. There are 2 major metabolic pathways involving homocysteine: remethyation and transsulfuration. In remethylation, total homocysteine is converted to methionine by acquiring a methyl group from either N^5 –methyltetrahydrofolate or from betaine. This conversion is catalyzed by the enzymes methionine synthase (MS) and betaine homocysteine methyltransferase (BHMT). Although MS is a folate-dependent enzyme, BHMT activity is significantly decreased in selenium deficiency (Uthus et al, 2002), resulting in less homocysteine being remethylated to methionine. Both selenium and folate deficiency result in hypomethylation because these 2 nutrients influence 1-carbon metabolism (Davis & Uthus, 2003). However, the plasma selenium and folate levels were not correlated from this study.

The plasma levels of vitamin E was slightly reduced in the dementia subtypes in comparison with the control group in this study. Several studies found that low intake and reduced serum vitamin E was associated with higher risks of AD and vascular dementia (Grundman, 2000; Ryglewicz et al, 2002) and that Alzheimer' disease patients on vitamin E supplement survived longer than those not getting supplements (Lombard & Khalsa, 2009). There is good reason to believe that vitamin E can help prevent brain damage associated with VD and AD as a result of its protecting signal-sensitive neurons in the brain from free radical damage. Since vitamin E is fat-soluble, it has a free pass around the brain, interacts with cell membrane, traps free radicals and interrupts the rapid-fire chain reactions that produce more free radicals. Infact animal studies using vitamin E showed that it is an important antioxidant that reduces degeneration of cells in the hippocampus, the part of the brain hardest hit by AD (Lombard & Khalsa, 2009). Despite the lack of significant differences in plasma values of vitamin E observed in patients and controls in this study, low levels of this vitamin may still undoubtedly play an important role in the occurrence of these neurodegenerative disorders

but unlikely to be responsible for their severity and progression among Nigerian Africans when compared with the white populations of the developed countries.

It has been observed that information is scarce on the use of grouped data in the analysis of laboratory results of biochemical parameters often measured in patients suffering from Alzheimer's diease, vascular dementia and controls. It is worthy of note that until this time, there has been no useful diagnostic test to discriminate between both neurodegenerative disorders and control subjects, in addition, the clinical misdiagnosis of Alzheimer's disease has been reported to be approximatey 20 – 30% in the United States (Glenner & Wong, 1987). There exists a need for a definitive laboratory test which can be performed on individuals at risk and patients suspected of suffering from dementia and Alzheimer's disease to minimize if not eliminate misdiagnosis.

In this study, a technique that constructed from multidimensional data an optimal two - and three-dimensional projection plane showing the maximum class separation achievable was applied on results obtained from laboratory analysis of several biochemical parameters from VD, AD patients and control subjects according to the method of Boyd (1986). In line with this, a simple computer-based three-dimensional grouping of n-3 erythrocyte fatty acids, homocysteine and total cholesterol as well as n-6 erythrocyte fatty acids, triglycerides and homocysteine was applied to a set of data from vascular dementia, Alzheimer's disease patients and controls.Two distinct groupings were observed in patients suffering from vascular dementia and control subjects as depicted in Figures 3 and 7. Out of the forty vascular dementia patients studied, eleven had homocysteine values greater than 10umol/l, twenty were intermediate i.e had values between 8-9umol/l. Plasma cholesterol levels were elevated in twenty of the patients, (4.05-6.45mmol/l)(159.73-249.6mg/dl) very much above

the acceptable limit of normal when compared with the controls (1.90-3.18mmol/l)(73.53-123.07mg/dl). There were proportionate increases in erythrocyte n-3 fatty acids concentration in most VD patients when compared with the controls. Based on these results, it could be postulated that if a certain group of elderly individuals in a population were subjected to these three tests (n-3 fatty acids, total cholesterol and homocysteine), those that had values falling within the range of values obtained for VD patients could be suspected to be at risk for developing dementia. Those individuals with values within the lower limit of those obtained for control subjects may not be strictly at risk of developing vascular dementia although they may require further close monitoring. This representation suggests that grouped data could differentiate patients suffering from neurodegerative disorders from normal individuals in a small population of highly selected cases.The findings reported here could be explained based on the changes observed in the concentrations of erythrocyte polyunsaturated fatty acids, plasma total cholesterol, homocysteine and triglycerides in vascular dementia and Alzheimer's disease patients when compared with controls.

A two dimensional plot of erythrocyte n-6 fatty acids and triglyceride as well as triglyceride and homocysteine did not discriminate between VD and AD patients. It is possible that a distinct separation could be achieved in a larger population of subjects.The three – dimensional analysis achieved a better diagnostic separation of control subjects from vascular dementia patients. Attempts at plotting the total cholesterol, triglycerides and erythrocyte fatty acids with the rest of the biochemical parameters analysed did not achieve the desired seperations. Although the result obtained for Alzheimer's disease when compared to the control did not show any distinct variability, multiple variables have been demonstrated to yield more information when considered jointly than when analyzed separately. This is

demonstrated in some studies involving patients with hyperparathyroidism and hypothyroidism (Boyd, 1986; Dioka & Walsh, 1996).

Though the three-dimensional grouping of total cholesterol, triglycerides and erythrocyte fatty acids could help to discriminate vascular dementia from control subjects, this method is not perfect and is not available in many laboratories. It is therefore not intended to replace the conventional methods in use for the diagnosis of both neurodegenerative disorders.

CONCLUSIONS AND RECOMMENDATION

The data obtained from this case-control study showed no distinct variations in the values of the biochemical parameters studied between AD and VD patients. Hyperlipidemia, hyperhomocystenemia, deficient folate and selenium were strongly associated with the occurrences of VD and AD in elderly Nigerians. Low levels of erythrocyte DHA and EPA was also significantly related to cognitive impairment in the dementia subtypes compared with control subjects.A limitation of this study is the small sample size that may be responsible for the lack of statistical significance in some analyzed data. There is therefore the need for a detailed observational study in a larger population of the effect of omega-3-fatty acids and antioxidant supplementation on cognitive function that would aid a better understanding of the involvement of oxidative stress and lipid peroxidation in the occurrence of neurodegeneration in this community.

It is recommended that there should be increased awareness on the benefits of adequate consumption of fatty cold water fishes such as tuna, herring and mackerel as sources of omega-3 fatty acids that had been reported to alleviate the pathogenesis and progression of neurodegeneration and improve memory in the elderly. It is equally important that antioxidants nutrients supplementation complement increased intake of omega-3 fatty acids in order to prevent the deleterious effects of oxidative damage that may result from fatty acids peroxidation. Lowering lipid levels, weight reduction and regular exercise are important in maintaining good health in the general populace.

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QUESTIONAIRE

A 1. Name (initials only) : ……

2. Hospital number : ……

3. Age: ……..

4. Address: ……...

5. Blood Pressure: ……..

6. Weight (in kg): ……..

7. Height (in m): ……..

8. Sex: Female ☐ Male ☐

9. Marital Status: Married ☐ Single ☐ Divorced ☐

Widowed ☐ Others ☐

B. 10. **EDUCATIONAL STATUS**:

Illiterate ☐ Senior School Certificate ☐

Primary Six Certificate ☐ Graduate ☐

Junior School Certificate ☐ Others ☐

11. OCCUPATION: ☐

(a) Skilled (senior professionals, permanent secretaries, directors, top business executives, very senior military/police personnel etc)

(b) Intermediate (Graduates, Secretaries, Nurses, senior tutors, Technologist etc)

(c) Semi-skilled (junior clerks, mechanics, tailors, soldiers/policemen, apprentice etc)

(d) Unskilled (housewives, petty traders, subsistent farmers, messengers, housemaid etc)

(e) Unemployed

12. How often do you eat each of the following?

Key: a - Daily

b - At least once a week

c - At least once a month

d - Less than once a month

Starchy foods (rice, yams etc) ☐ Cooked vegetables ☐

Fresh vegetables ☐ Meat, fish, eggs ☐

Fresh fruits ☐ Bread ☐

13. Do you smoke? Yes ☐ No ☐

If yes, how many cigarettes do you smoke per day?

a) 1 b) 2 - 4 c) 5 - 7 d) >7

14. Have you smoked cigarette in the past one month? Yes ☐ No ☐

15a Do you drink alcohol? Yes ☐ No ☐

b. If yes, what type?

(i) Brandy (ii) Gin (Blended) (iii) Ogogoro (iv) Beer

(v) Others……..

c. What is your average consumption of alcohol (per bottle)?

(a) <3 (b) 3 – 6 (c) >7

(i) Daily ☐ (ii) weekly ☐ (iii) monthly ☐

16. Level of exercise:

(a) Regular (b) Occassional (c) Incidental

17. PAST MEDICAL HISTORY:

a) Dementia disorder

Date of first episode of illness…….

Were you hospitalized? Yes ☐ No ☐

b) Have you ever been diagnosed of having:

(i) Hypertension Yes No

(ii) Diabetes Mellitus Yes No

(iii) Cardiovascular disease Yes No

18 a. Date of diagnosis of dementia……

b. Type of dementia……

19 a. Date of sample collection……

b. Clinical findings:

(i) ……..

(ii)…….

(iii) …….

(iv) …….

c. Diagnosis ……..

d. Duration of the disease …….

20. Is anyone else in your family suffering from emotional problems / dementia?

Yes ☐ No ☐

a. Father b. Mother c. Brother d. Sister e. Cousin

f. Uncle g. Distant relative

21. Has any member of your family had:

a) Endocrine / metabolic disorders Yes or No

b) Immune disorders

c) Cardiovascular disorder

d) Renal disease

22 .Are you currently on any medication? Yes ☐ No ☐

If yes, state type:

Antipsychotic ……

Antidepressant ……

Oral contraceptive ……

Others ……

23. Other habits ……

24. Biochemical parameters:

a) Homocysteine

b) Folate

c) LDL – C

d) Triglyceride

e) HDL-C

f) Arachidonic acid

g) Eicosapentanoic acid

h) Docosahexanoic acid

i) Selenium

j) Vitamin E

k) Others……

The Mini-Mental State Examination (Folstein et al, 1975)

Patient_____Examiner_____Date_____

Maximum	Score	
		Orientation
5	( )	What is the (year)(season)(date)(day)(month)?
5	( )	Where are we (state)(country)(town)(hospital)(floor)?
		Registration
3	( )	Name 3 objects: 1 second to say each. Then ask the patient all

3 after you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. Count trials and record.

Trials _____

Attention and Calculation

5 ( ) Serial 7's. 1 point for each correct answer. Stop after 5 answers. Alternatively spell "world" backward.

Recall

3 ( ) Ask for the 3 objects repeated above. Give 1 point for each correct answer.

Language

2 ( ) Name a pencil and watch.

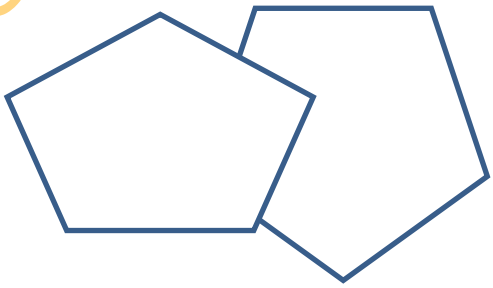
1 ( ) Repeat the following "No ifs, ands, or buts"

3 ( ) Follow a 3-stage command:
"Take a paper in your hand, fold it in half, and put it on the floor."

1 ( ) Read and obey the following: CLOSE YOUR EYES

1 ( ) Write a sentence

1 ( ) Copy the design shown.



_____ Total Score

ASSESS level of consciousness along a continuum _____

Alert Drowsy Stupor Coma

INFORMED CONSENT FORM

This study is investigating the associations of homocysteine, folate, lipoproteins, erythrocyte fatty acids and the incidence of dementia in this community. Your answers will be kept confidential. During the exercise, medical examination will be carried out on you to detect some signs of the disease. This will include taking blood from your veins. This will not cause you any injury. You have a right to withdraw from the study at any given time if you so wish.

CONSENT : Now that that the study has been well explained to me, I will be willing to take part in the study.

_____

Signature/ thumbprint of participant/date

_____

Signature of interviewer/ date