CHAPTER 4

RESULTS AND DISCUSSION

The results of the present study entitled, **“Vitamin B12 and Omega-3 fatty acid Interventions for Cognition in Elderly- a V.O.I.C.E. Trial”** are presented, discussed and deduced in this chapter. The results are presented into the three main phases according to the objectives of the study.

PHASE I: Baseline assessment of diet, nutrition and health status of cognitively impaired elderly with MCI.

PHASE II: Food product development using omega-3 fatty acid rich flaxseeds and sensory trials.

PHASE III: Intervention and impact evaluation of the MCI elderly group with omega-3 fatty acids and vitamin B12 supplementation.

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PHASE I: To assess the magnitude of cognitive and nutritional status of elderly population (aged 60 -85 years) with mild cognitive impairment in Vadodara city on dietary, cognitive and biochemical parameters

Mild cognitive impairment is a complex disorder which causes measurable cognitive decline with memory lapses and thinking disability. Seldom represented as an intermediate stage between the normal cognition levels and severe cognitive decline, MCI is the precursor portraying higher tendency of being advanced to Alzheimer's disease (AD) and other dementias. Indians are greatly susceptible to MCI owing to the multitude of contributory features genetic predisposition, nutritional inadequacies especially linked with Vitamin B12, Omega-3 fatty acids, anthropometry, blood pressure or cardiac–metabolic risk factors and diabetes. Higher economic out-of-pocket expenses are incurred by MCI associated morbidities, in turn raising the economic burden on health especially prevalent in low income developing countries like India, where health care resources and facilities are extremely scarce.

The early MCI prognosis targeting the elderly through neuropsychological battery can be efficient in sinking the disease progression. Recent studies have spotted towards this facet of prior detection of MCI for improvement of the cognitive and overall general health conditions of the geriatrics.

Hence, continuing with this as a backdrop, the present phase of the study commenced to identify the potent battery of existing screening tools and validate the current information. With this phase an attempt was made to perform the baseline examination and prevalence check of elderly at risk for MCI using a comprehensive neuropsychological test battery of cognitive tools, thereby assessing the socio-demographic, medical history, activity pattern, anthropometric measurements, bio-physical and dietary parameters succeeded by determination of association amidst these factors.

In favor of attaining the set objectives, the study sample of 402 subjects was enrolled from the Maharaja Sayajirao University Health Centre, Shri Vallabhacharyajee hospitals and private clinics of Baroda. The detailed methodology for eliciting the aforementioned parameters has been described in the Materials and Methods Chapter. The results of this phase are elucidated from the sections 4.1.1 to 4.1.11.

The results of this chapter have been segregated in the following sections:

4.1.1 Cognitive assessment of baseline based on neurological scoring

4.1.2 General characteristics of the baseline subjects

4.1.3 General characteristics of MCI patients

4.1.4 Medical history of the subjects

4.1.5 Activity pattern of the MCI patients

4.1.6 Anthropometric profile of the subjects

4.1. 7 Biophysical and Biochemical parameters of the subjects

4.1.8 Food habits and nutrient intake of the MCI patients

4.1.9 Frequency of consumption of vitamin B12 and omega-3 rich foods

4.1.10 Association between neurological test scores and other parameters in MCI patients

4.1.11 Relation between serum vitamin B12 levels and omega 3 intake with other parameters in MCI patients

4.1.1 Cognitive assessment of baseline elderly based on neurological scoring.

The neuropsychological test battery employed for detection of cognitive status of MCI patients (N=120) was analyzed for the best decisive cognitive test using the ROC curve analysis. As an illustration, the corresponding empirical ROC curve was drawn in Figure 4.1.1 by using SPSS software Area under the curve (AUC), C = 0.905. Since the AUC is more 0.9, therefore it is stated that ACE test is an excellent test to determine the neurological status of MCI patients.

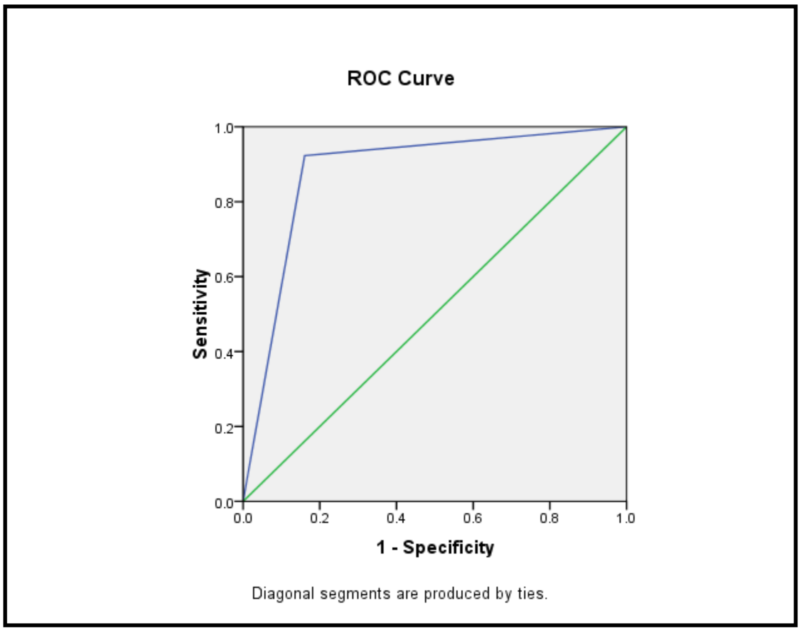


Figure 4.1.1: ROC curves of two diagnostic parameters (ACE test scores versus serum vitamin B12).

The ROC curve for figure by using SPSS software AUC is C = 0.819. As the AUC is more than 0.8 and less than 0.9 hence it could be verified that MMSE test is a good test to determine the neurological status of MCI patients.

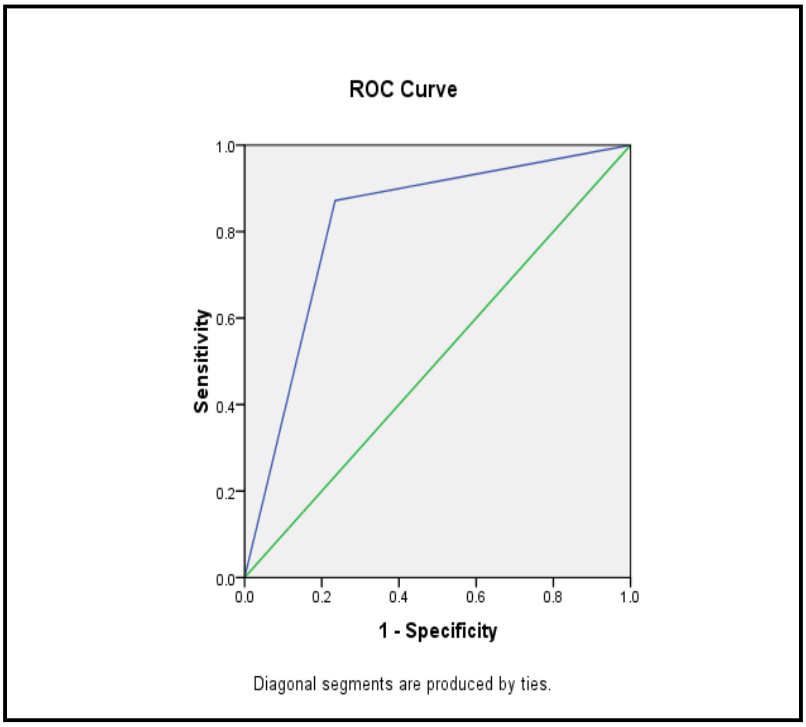


Figure 4.1.2: ROC curves of two diagnostic parameters (MMSE test scores versus serum vitamin B12).

Area under the curve (C) = 0.763. As the area under the curve is more than 0.7 and less than 0.8 therefore it is affirmed that YFPIT test is a fair test to determine the neurological status of MCI patients.

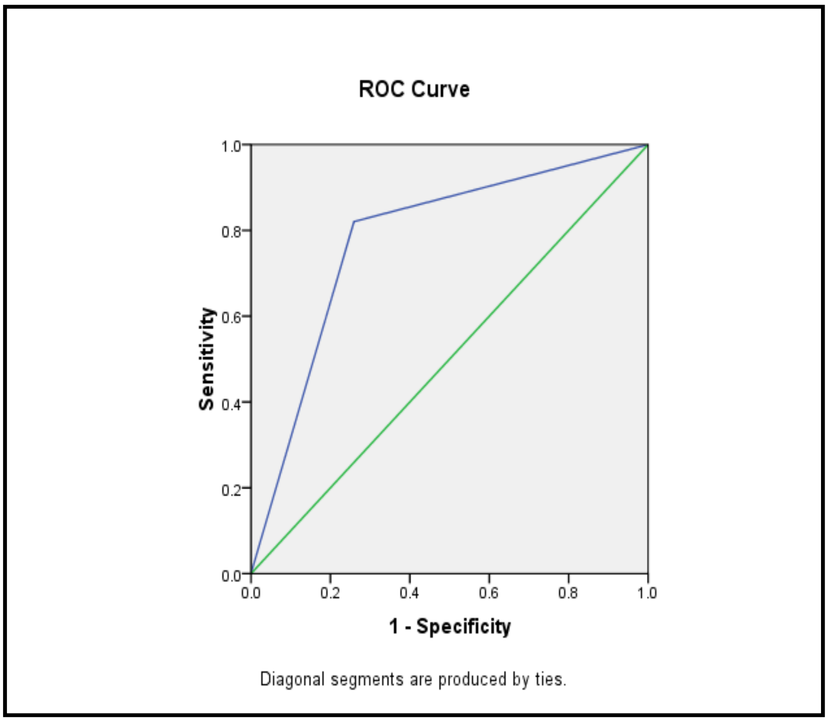


Figure 4.1.3: ROC curves of two diagnostic parameters (YFPIT test scores versus serum vitamin B12).

Succeeding this, the battery of neurological screening tests was conducted on baseline subjects (N=402). The results for ACE (Addenbrooke's Cognitive Examination) screening test on baseline sample suggested that 51% of subjects were in the normal range and 49% were cognitively impaired. Almost half of the males (46.6%) and females (50.9%) were cognitively impaired. In age group of 70-85 years (old –old) cognitive impairment was 61% compared to 43% in age group of 60-69 years (young-old) directing that incidences of dementia increase as the age progresses (Table 4.1.1.1). In the factual view that the ACE stood as the most precise in detection of MCI, it could be stated that 197 baseline patients were having MCI.

Table 4.1.1.1: Neurological status of baseline population detected by ACE screening test

Cognitive Status	Total (N= 402)	Males (n = 176)	Females (n = 226)	60-69 yrs (n=272)	70-85 yrs (n=130)
Normal	205 (51)	94 (53.4)	111 (49.1)	154 (56.6)	51 (39.2)
Cognitively impaired	197 (49)	82 (46.6)	115 (50.9)	118 (43.4)	79 (60.8)

Note: Numbers in parenthesis indicate percentage

With regard to screening the baseline subjects, MMSE (Mini-Mental State Examination) gave distinction of subjects based on the degree of cognitive impairment. MMSE test scores indicated that 58% of the subjects were cognitively impaired with 16% suffering from moderate cognitive impairment and 3% from severe cognitive impairment. In the two age groups, 14% more subjects in old-old elderly group were suffering from cognitive impairment than the other group. Also 27.7% had moderate cognitive impairment and 6.15% of subjects in 70-85 yrs group had severe cognitive impairment compared to 1.7% and 12.8% in 60-69yrs group (Table 4.1.1.2).

Table 4.1.1.2: Neurological status of baseline population detected by MMSE screening test

Cognitive Status	Total (N=402)	Males (n=176)	Females (n=226)	60-69 yrs (n=272)	70-85 yrs (n=130)
Normal	169 (42)	71 (40.3)	98 (43.4)	127 (46.7)	42 (32.30)
Mild Cognitive Impairment	156 (38.8)	68 (38.6)	88 (38.9)	112 (41.2)	44 (33.84)
Moderate Cognitive Impairment	65 (16.1)	32 (18.1)	33 (14.6)	29 (12.8)	36 (27.69)
Severe Cognitive Impairment	12 (2.9)	5 (2.8)	7 (3.1)	4 (1.7)	8 (6.15)

Note: Numbers in parenthesis indicate percentage

The Yamaguchi Fox-Pigeon Imitation Test (YFPIT) indicated that 44% of study population was cognitively impaired. In this test results explained that around 51% cognitive impairment was observed in males compared to 39% in females. Also cognitive impairment in the old-old subjects was 59% compared to 37% in the young-old age group (Table 4.1.1.3).

Table 4.1.1.3: Neurological status of baseline population detected by YFPIT screening test

Cognitive Status	Total (N= 402)	Males (n = 176)	Females (n = 226)	60-69 yrs (n=272)	70-85 yrs (n=130)
Normal	225 (55.9)	87 (49.4)	138 (61.1)	171 (62.9)	54 (41.5)
Cognitively impaired	177 (44.0)	89 (50.6)	88 (38.9)	101 (37.1)	76 (58.5)

Note: Numbers in parenthesis indicate percentage

Mini Nutritional Assessment (MNA) expressed that only 34% subjects had normal nutritional status. In the young-old age group 39% subjects had normal status however in the old-old group only 22% of subjects were lying in the normal category. Majority (57%) of the subjects with below normal nutritional status were in the category of at risk of malnutrition. In the old-old group it was 62% and 55% in young-olds. Around 9% of the subjects were malnourished. In the old-old elderly group percentage of subjects who were malnourished was thrice more than young-old elderly (Table 4.1.1.4).

Table 4.1.1.4: Neurological status of baseline population detected by MNA screening test

Nutritional Status	Total (N= 402)	Males (n = 176)	Females (n = 226)	60-69 yrs (n=272)	70-85 yrs (n=130)
Normal Nutritional status	135 (33.6)	61 (34.6)	74 (32.7)	107 (39.3)	28 (21.5)
At risk of malnutrition	230 (57.2)	98 (55.7)	132 (58.4)	149 (54.7)	81 (62.3)
Malnourished	37 (9.2)	17 (9.6)	20 (8.8)	16 (5.9)	21 (16.1)

Note: Numbers in parenthesis indicate percentage

Significant ($p < 0.01$) estimation was calculated between scores of old-old (70-85 yrs) and young-old (60-69 yrs) highlighting towards presence of the majority of subjects in the 'at risk of malnutrition' category. It represented that there was significant as well as negative impact on the nutritional status with the increasing age (Table 4.1.1.5).

Table 4.1.1.5: Impact of age on nutritional status

Age	Mean MNA Score
60-69 years	10.23 ± 1.76
70-85 years	9.51 ± 2.47
***z*-value**	**2.98****

**Significant at p<0.01

4.1.2 General characteristics of the baseline subjects (N=402).

Socio-demographic data of the baseline subjects revealed that majority of them were married (83%) and were Hindus (92%). Mean age of baseline subjects was approximately 68 years. Males accounted to be 41% and females to be 59%. The literacy rate among the subjects was high (96.3%). Almost half of the subjects were living in joint family (50%) and remaining (48%) were living in nuclear family along with their spouses. Majority (44%) of the population was leading a retired life and around 73% of the subjects had a family income more than 30,000 Rs. a month (Table 4.1.2.1).

Table 4.1.2.1: Socio-demographic characteristics of the baseline subjects

Parameters	Total baseline subjects (N=402)	Males (n=176)	Females (n= 226)
Age (Mean age – 67.6±5.99)			
60-69	272(67.6)	96(58.2)	176(74.2)
70-85	130(32.3)	69(41.8)	61(25.7)
Total	402(100)	165(41.0)	237(58.9)
Marital Status			
Unmarried	10(2.5)	3(1.7)	7(3.1)
Married	332(82.6)	154(87.5)	178 (78.8)
Widow/widower	57(14.2)	17(9.6)	40(17.7)
Separated	3(0.7)	2(1.1)	1(0.4)
Education level			
Post graduation	86(21.39)	52(29.54)	34(15.04)
Graduation	141(35.1)	65(36.9)	76(33.6)
High secondary school	32(7.9)	10(5.7)	22(9.7)
Secondary school	76(18.9)	24(13.6)	52(23.0)
Primary school	52(12.9)	13(7.4)	39(17.2)
Illiterate	15(3.7)	12(6.8)	3(1.3)
Occupation			
Service	37(9.2)	25(14.2)	12(5.3)
Self employed	39(9.7)	18(10.2)	21(9.3)
House wife	150(37.3)	0(0)	150(66.4)
Retired	176(43.8)	133(75.6)	43(19.0)
Religion			
Hindu	370(92.0)	157(95.1)	213(89.8)
Muslim	12(2.9)	7(4.2)	5(1.9)
Christian	4(0.9)	2(1.1)	2(0.9)
Jain	11(2.7)	3(1.7)	8(3.5)
Other	5(1.2)	2(1.1)	3(1.3)
Type of family			
Nuclear	193(48.0)	81(46.0)	112(49.5)
Joint	203(50.5)	92(52.3)	111(49.1)
Extended	1(0.2)	0(0)	1(0.4)
Single living	5(1.2)	3(1.8)	2(0.9)
Total monthly family income (Rs. / month)			
<10,000	28(4.4)	21(12.7)	7(3.1)
10001-30000	92(22.9)	30(18.2)	62(27.4)
30001-60000	134(33.3)	53(30.1)	81(35.8)
60001-100000	101(25.1)	49(27.8)	52(23.0)
>100000	47(11.7)	23(13.0)	24(5.9)
Care taker of the subject			
Family member	124(30.8)	52(29.5)	72(31.8)
Spouse	148(36.8)	34(19.3)	114(50.4)
Self	130(32.3)	90(51.1)	40(17.47)

Note: Numbers in parenthesis indicate percentage

4.1.3 Anthropometric and bio-physcial distribution of the baseline subjects (N=402)

The baseline subjects had the mean BMI of 24.7. Based on the Asia Pacific BMI cut-offs, a majority (42%) of the subjects fell in the normal BMI range.But, followed by second position of 41% subjects present in the pre-obese category. 11% subjects were in the Stage I of obesity (Figure 4.1.2.1). The subjects were then categorized for their bio-physical profile according to the JNC-8 classification. The results revealed that the maximum number (53%) of subjects were in the pre-hypertensive stage (136.28±13.79), 36% were in the Stage I of hypertension, 8% were in the normal category and 3% were in the Stage II of hypertension (Figure 4.12.2).

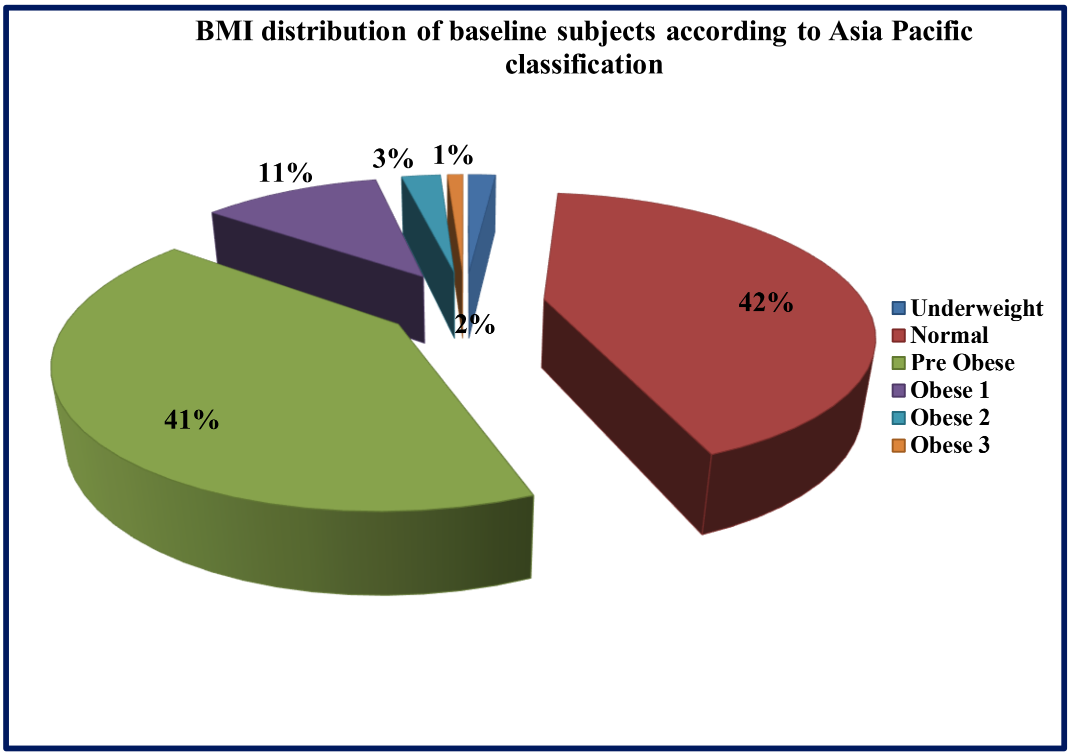


Figure 4.1.2.1: BMI distribution of baseline subjects (N=402)

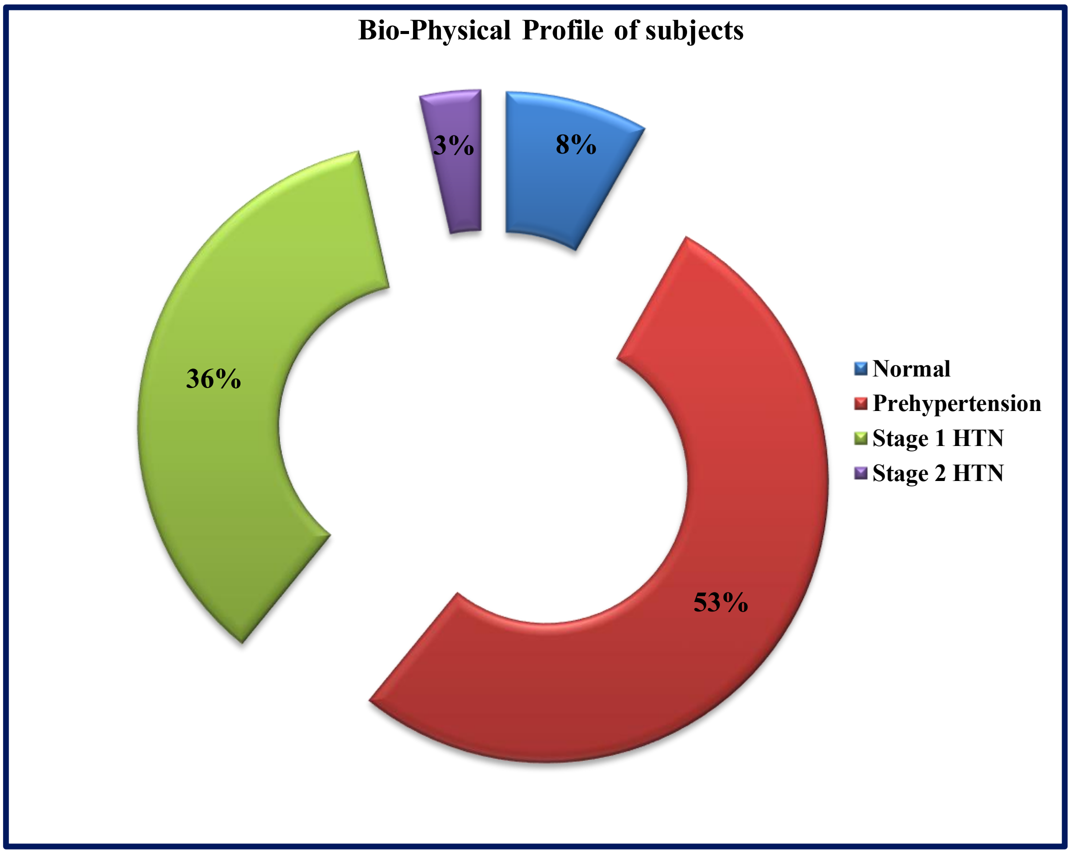


Figure 4.1.2.2: Blood pressure distribution of baseline subjects (N=402)

4.1.3 General characteristics of MCI patients (N=120)

Socio economic data of MCI subjects pointed that majority of them were married (88%) and were Hindus (94%). Mean age of baseline subjects was approximately 66 years. For the interventional part of the study, initially the patientsin the ae range of 76-85 were enrolled. However, deaths and other severe morbid conditions of patients restricted their entire inclusion. Percentage of males in the experiment sample was 44% and females amounted to be 56%. The literacy rate among the subjects was high (90%) and half of the population (50%) had studied higher than graduation. Almost half of the subjects were living in joint family (51%) and remaining (47%) were living in nuclear family. For 48% of the patients, spouse was the caretaker in the family. Majority (35%) of the patients had retired from their professions. Around 76% of the subjects had family income more than Rs 30,000 a month and 10% had income more than Rs 100,000 a month (Table 4.1.3.1).

Table 4.1.3.1: Socio economic characteristics of MCI patients

Parameters	Test subjects (N=120)	Males (n= 53)	Females (n= 67)
Age (Mean age – 66.1 ± 6.01)			
60-69 years	88 (73.3)	32 (60.4)	56 (83.6)
70-75 years	32 (26.7)	21 (39.6)	11 (16.4)
Total	120 (100)	53 (44.2)	67 (55.8)
Marital Status			
Unmarried	3 (2.5)	0 (0)	3 (4.5)
Married	105 (87.5)	50 (94.3)	55 (82.1)
Widow/widower	12 (10)	3 (5.7)	9 (13.4)
Education level			
Post graduation	22 (18.3)	13 (24.5)	9 (13.4)
Graduation	38 (31.7)	19 (35.8)	19 (28.3)
High secondary school	8 (6.7)	3 (5.7)	5 (7.4)
Secondary school	22 (18.3)	8 (15.1)	14 (20.9)
Primary school	17 (14.2)	3 (5.7)	14 (20.9)
Illiterate	13 (10.8)	7 (13.2)	6 (8.9)
Occupation			
Service	16 (13.3)	11 (20.7)	5 (7.46)
Self employed	15 (12.5)	5 (9.43)	10 (14.9)
House wife	47 (39.1)	0 (0)	47 (70.14%)
Retired	42 (35)	37 (69.8)	10 (14.9)
Religion			
Hindu	113 (94.2)	50 (94.3)	63 (94.0)
Muslim	0 (0)	0(0)	0 (0)
Christian	0 (0)	0(0)	0 (0)
Jain	6 (5)	2 (3.8)	4 (5.9)
Other	1 (0.8)	1 (1.9)	0 (0)
Type of family			
Nuclear	57 (47.5)	26 (49.0)	31 (46.3)
Joint	61 (50.8)	26 (49.0)	35 (52.2)
Extended	1 (0.8)	0 (0)	1 (1.5)
Single living	1 (0.8)	1 (1.8)	0 (0)
Total monthly family income (Rs./month)			
<10,000	5 (4.2)	2 (3.8)	3 (4.5)
10001-30000	35 (29.2)	12 (22.6)	23 (34.3)
30001-60000	39 (32.5)	16 (30.2)	23 (34.3)
60001-100000	29 (24.2)	15 (28.3)	14 (20.9)
>100000	12 (10)	8 (15.1)	4 (5.9)
Care taker of the subject			
Family member	24 (20)	9 (16.9)	15 (22.4)
Spouse	58 (48.3)	28 (52.8)	30 (44.8)
Self	38 (31.7)	16 (30.2)	22 (32.8)

Note: Numbers in parenthesis indicate percentage

4.1.4 Medical history of the MCI patients

Under the medical history, associated health problems prevalent in the MCI patients were studied. Majority (76%) of the MCI patients had anemia. More than half (59%) of the patients were obese and 58% hypertensive. Insomnia was also found to be another common trouble with 55% patients. One fourth (34%) patients were also suffering from balance problems. About 24% subjects complained of anorexia whereas 21% were dyslipidemic. Nearly 16% of the subjects had the prevalence of depression (Figure 4.1.4.1).

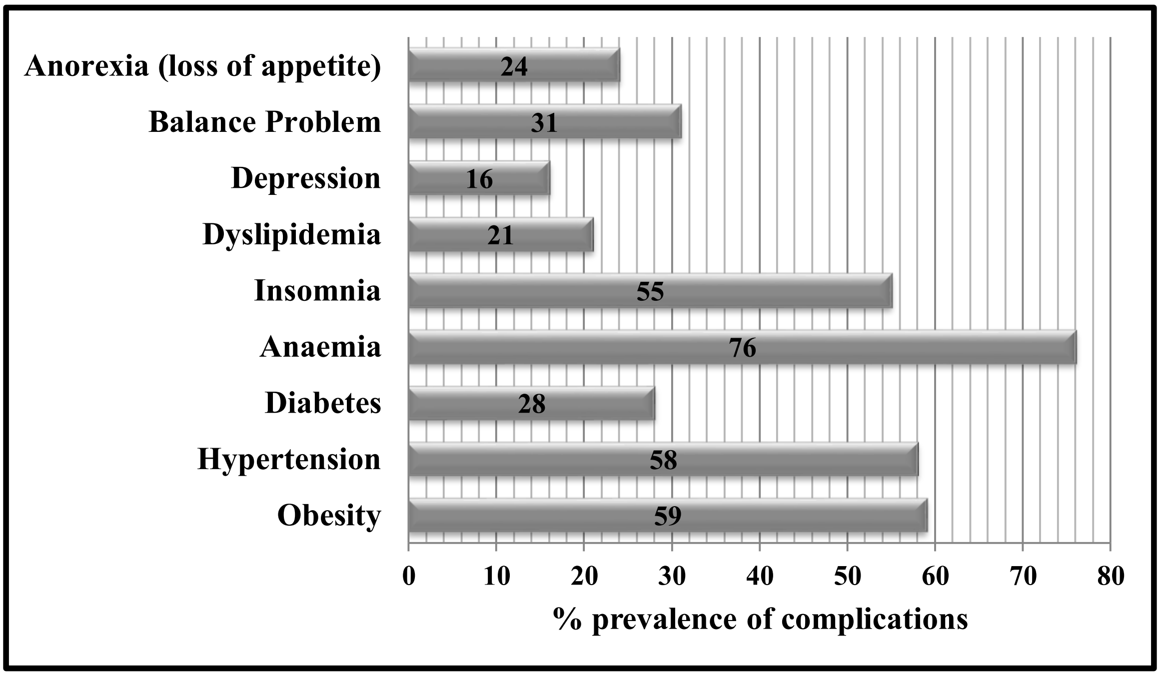


Figure 4.1.4.1: Associated health problems prevalent in MCI Subjects

4.1.5 Activity pattern of MCI patients

The subjects were divided into categories based on their physical activity pattern according to the WHO, Global Recommendations on Physical Activity on Health (2011). More than half (57%) of the subjects had low activity level pattern (minutes ≤200). Around 36% had moderate activity level whereas only 7% had high physical activity level. As compared to males (47%), more females were in the low activity level (65.6%). In addition to this, more number of old-old group were more sedentary than the young-olds. None of the patients in the old-old group stood in the high activity level range (Table 4.1.5.1, Figure 4.1.5.1).

Table 4.1.5.1: Distribution of MCI subjects according to their weekly physical activity pattern

Physical activity level (minutes per week)	Total (N=120)	Males (n = 53)	Females (n = 67)	60-69 yrs (n=88)	70-85 yrs (n=32)
Low (≤200)	69(57.5)	25(47.1)	44(65.67)	47(53.40)	22(68.7)
Moderate (200 - 500)	43(35.8)	22(41.5)	21(31.35)	33(37.5)	10(31.2)
High (≥ 500)	8(6.6)	6(11.32)	2(2.98)	8(12.5)	0(0)

Note: Numbers in parenthesis indicate percentage

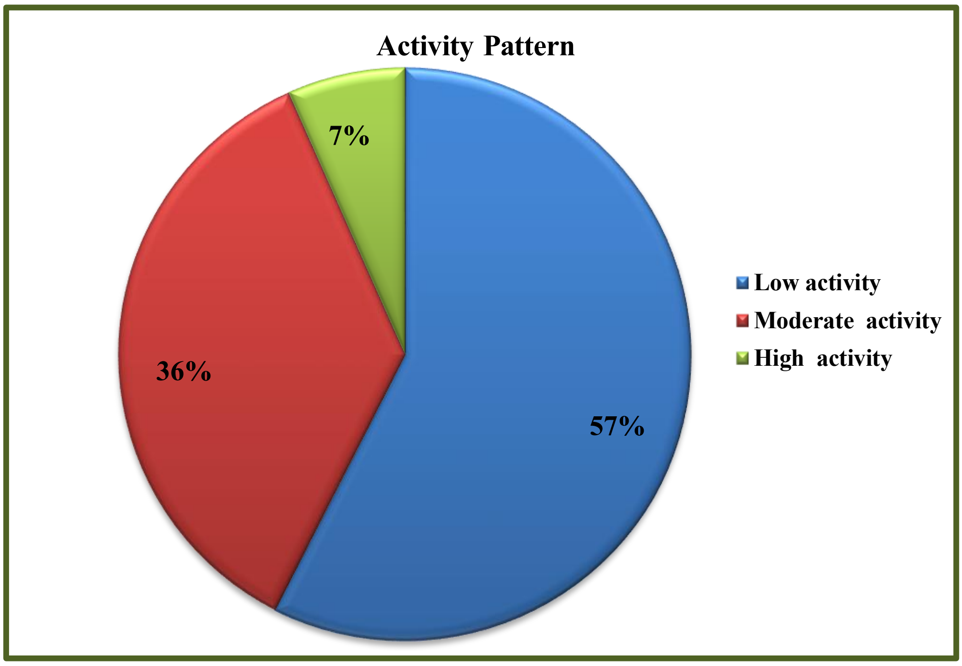


Figure 4.1.5.1: Physical activity pattern of MCI subjects

Daily activity pattern of MCI patients also specified that the old-old followed more sedentary lifestyle compared to young-old patients. Although old-old did include walking but spent lesser time in exercise and doing household chores. Active time period of young-olds was almost twice than that of old-old group (Table 4.1.5.2).

Table 4.1.5.2: Daily Activity Pattern of MCI Subjects (in minutes)

Age	Sleep (A)	Idle (B)	Occupational (C)	Household (D)	Walk (E)	Exercise (F)	Sedentary (A+B)	Active (C+D +E+F)
60-69 years	572	356	111	239	32	21	**907**	**403**
70-85 years	645	500	53	116	45	8	**1110**	**222**

4.1.6 Anthropometric profile of the subjects

The patients had the mean BMI of 26.20. BMI of females was significantly higher ($p<0.05$) than males. With regard to the waist circumference, there was an insignificant difference amongst both the genders. However, hip circumference was significantly higher ($p<0.001$) in females as compared to males and waist to hip ratio was significantly higher ($p<0.001$) in males rather than that of the females (Table 4.1.6.1).

The results obtained on comparing the BMI cut-offs for the grade-wise obesity between the males and females expounded that maximum (40%) of the subjects were under Obese I category with almost equal proportion in both genders, males (39.6%) and females (40.2%). Almost 22% of the total subjects were under normal BMI category as per the Asia Pacific BMI cut-offs (Table 4.1.6.2). Further, 19% of subjects were suffering from type II obesity, which was twice more prevalent in males (19.1%) than females (9.4%). Only 3% subjects were underweight (Figure 4.1.6.1).

Table 4.1.6.1: Mean values of anthropometric measurements of the MCI subjects

Variable	Total (N=120)	Males (n=53)	Females (n=67)	*t*- test value
Waist Circumference (cm)	93.42 ± 9.55	93.67 ± 9.02	93.23 ± 10.01	0.25^{NS}
Hip Circumference (cm)	99.4 ± 9.43	96.04 ± 6.80	102.06 ± 10.37	3.82***
Waist to Hip ratio	0.94 ± 0.07	0.98 ± 0.06	0.92 ± 0.06	4.93***
Body Mass Index (BMI)	26.20 ± 4.89	25.01 ± 4.25	27.15 ± 5.17	2.48*

*Significant at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, NS- Non- significant

Table 4.1.6.2: BMI Classification of MCI subjects according to Asia-Pacific classification

Category	BMI cut offs (Asia-Pacific)	Total (N=120)	Males (n=53)	Females (n=67)
Underweight	<18.5	4 (3.3)	2 (3.7)	2 (2.9)
Normal	18.5-22.9	26 (21.6)	14 (26.4)	12 (17.9)
Overweight	23-24.9	19 (15.8)	11 (20.7)	8 (11.9)
Obese I	25-29.9	48 (40)	21 (39.6)	27 (40.2)
Obese II	>30	23 (19.1)	5 (9.4)	18 (26.8)

Note: Numbers in parenthesis indicate percentage

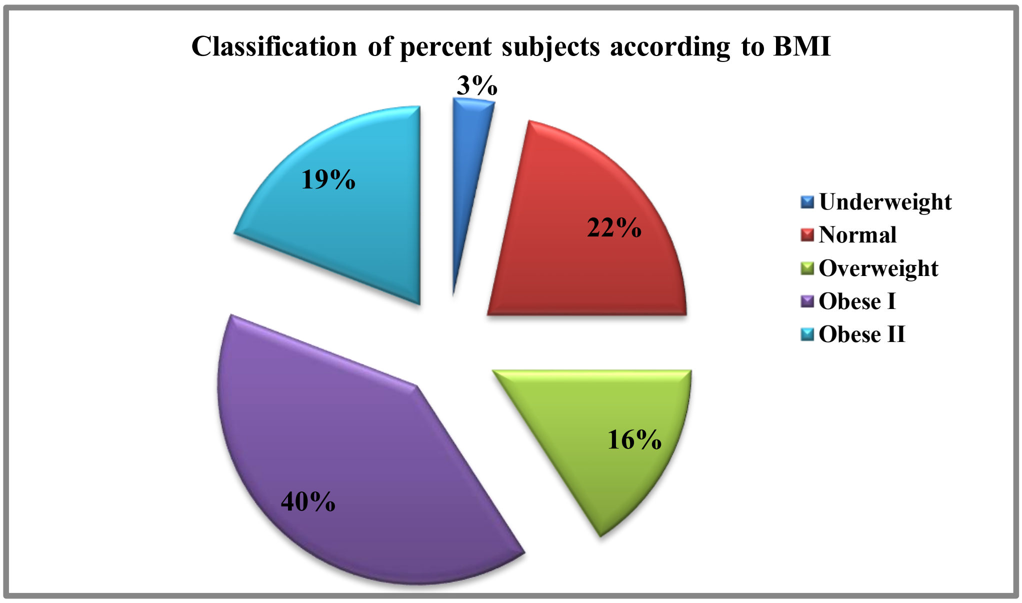


Figure: 4.1.6.1: Classification of MCI subjects according to BMI according to Asia Pacific Classification

4.1.7 Biophysical and Biochemical parameters of the patients

The mean systolic/diastolic blood pressure readings of the MCI patients were 138/86 mm Hg (Table 4.1.7.1). There was insignificant difference between males and females in blood pressure indicating hypertension was independent from one's gender. According to JNC VIII (2014) classification, 45% patients were stage I hypertensive, 37% subjects were pre-hypertensive, 13% were stage II hypertensive and 5 % were normal. (Table 4.1.7.2, Figure 4.1.7.1.a).

Table 4.1.7.1: Mean Values for Biophysical and Biochemical profile of MCI Subjects

Variables	Total (N=120)	Males (n=53)	Females (n=67)	*t*-test value
BP Systolic (mmHg)	138.66 ± 15.98	141.59 ± 15.90	136.32 ± 15.77	1.79^{NS}
BP Diastolic (mmHg)	85.61 ± 10.15	84.76 ±10.40	86.29 ± 9.96	0.81^{NS}
FBS (mg/dl)	98.08 ± 23.66	103.94 ± 30.99	93.44 ± 14.26	2.28*
HbA_{1c}	6.16 ± 1.38	6.36 ± 1.53	5.92 ± 1.21	1.69^{NS}
Hemoglobin (g/dl)	11.64 ± 1.68	12.39 ± 1.66	11.04 ± 1.44	4.68***
Serum Vitamin B12 (pg/ml)	185.76 ± 44.53	188.72 ± 45.91	183.42 ± 43.59	0.64^{NS}
MCV (fl.)	81.27 ± 12.87	81.32 ± 13.69	81.24 ± 12.28	0.03^{NS}
Total Cholesterol (mg/dl)	195.33 ± 33.49	189.15 ± 39.82	200.21 ± 26.81	1.73^{NS}
Triglycerides (mg/dl)	142.87 ± 60.96	138.32 ± 76.85	146.47 ± 44.90	0.68^{NS}
LDL (mg/dl)	120.44 ± 28.60	117.55 ± 34.38	122.72 ± 23.07	0.93^{NS}
VLDL (mg/dl)	29.57 ± 13.07	27.32 ± 14.43	33.35 ± 11.70	1.64^{NS}
HDL (mg/dl)	43.18 ± 8.48	41.36 ± 8.29	44.62 ± 8.40	2.12*
LDL/HDL ratio	2.89 ± 0.89	2.93 ± 1.00	2.85 ± 0.79	0.48^{NS}
Cholesterol/ HDL ratio	4.68 ± 1.20	7.73 ± 1.33	4.64 ± 1.09	0.38^{NS}

*Significant from the baseline value at p<0.05, ** at p<0.01, *** at p<0.001, NS- Non- significant

The mean fasting blood sugar of the patients was 98.08 mg/dl and the mean glycated hemoglobin was 6.1 (Table 4.1.7.1). The mean serum vitamin B12 levels were observed as 186 pg/ml. According to the criteria of American Diabetes Association (2007), 29% had a poor control of diabetes (Table 4.1.7.3, Figure 4.1.7.1.b). 82% had poor control of serum vitamin B12 (Figure 4.1.7.1.c).

The serum lipid outcome highlighted that the mean total cholesterol was 195 mg/dl (Table 4.1.7.1). 50% of the patients had normal cholesterol levels whereas 50% had borderline and poor levels (Table 4.1.7.3). About 62% of the patients had normal LDL levels. In the case of HDL majority (50%) had borderline values. According to the recommendations of the ADA (2016, 58% of the patients displayed good control on triglycerides (TG) with their mean TG values <150 mg/dl whereas only 8% patients had TG levels ≥ 200 mg/dl. The mean TC/HDL ratio of male patients was 7.7 and females was 4.64 showing an abnormal TC/HDL ratio i.e. >6.4 and >5.6 respectively in the males in comparison to the females (Table 4.1.7.1).

Table 4.1.7.2: Biophysical status of the MCI subjects

Categories	Total (N=120)	Males (n=53)	Females (n=67)
Normal (<120/80)	6 (5)	2 (3.8)	4 (5.9)
Pre-hypertension (120-139/80-89)	45 (37.5)	19 (35.8)	26 (38.8)
Stage I Hypertension (140-159/90-99)	54 (45)	14 (26.4)	30 (44.8)
Stage II Hypertension (≥160/100)	15 (12.5)	8 (15.1)	7 (10.4)

JNC VIII, 2014 classification
Numbers in parenthesis indicate percentage

Table 4.1.7.3: Glycemic and lipemic status of the MCI subjects categorized as

Parameters	Normal N (%)	Borderline N (%)	Poor N (%)
Fasting Blood Glucose N (%)	80-100 78 (65)	101-126 33 (27.5)	>126 9 (7.5)
H_bA_{1c} N (%)	≤5.4 44 (36.7)	5.4-6.4 41 (34.1)	≥6.5 35 (29.1)
Hemoglobin N (%)	≥12 49 (40.8)	10- 12 53 (44.2)	≤10 18 (15)
Serum Vitamin B12 N (%)	≥240 9 (7.5)	170-240 72 (60)	≤170 39 (32.5)
MCV N (%)	80-100 59 (49.2)	- -	≤80 or ≥100 61 (50.8)
Total Cholesterol N (%)	≤200 60 (50)	200-239 51 (42.5)	≥240 9 (7.5)
Triglycerides N (%)	≤150 70 (58.3)	150-199 40 (33.3)	≥200 10 (8.3)
LDL N (%)	≤130 74 (61.7)	130-159 37 (30.8)	≥160 9 (7.5)
VLDL N (%)	5-40 110 (91.7)	- -	>40 10 (8.3)
HDL N (%)	50-100 26 (21.7)	40-50 60 (50)	<40 34 (28.3)
LDL/HDL N (%)	≤2.5 49 (40.8)	- -	≥2.6 71 (59.2)
Cholesterol/HDL N (%)	≤4.2 54 (45)	- -	≥4.3 66 (55)

ADA, AHA 2009
Numbers in parenthesis indicate percentage

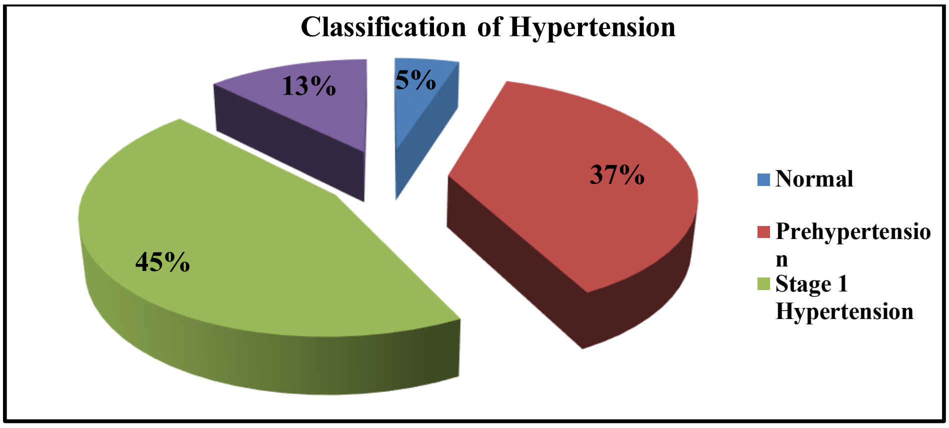


Figure 4.1.7.1.a: Percent distribution of patients with varying hypertensive levels

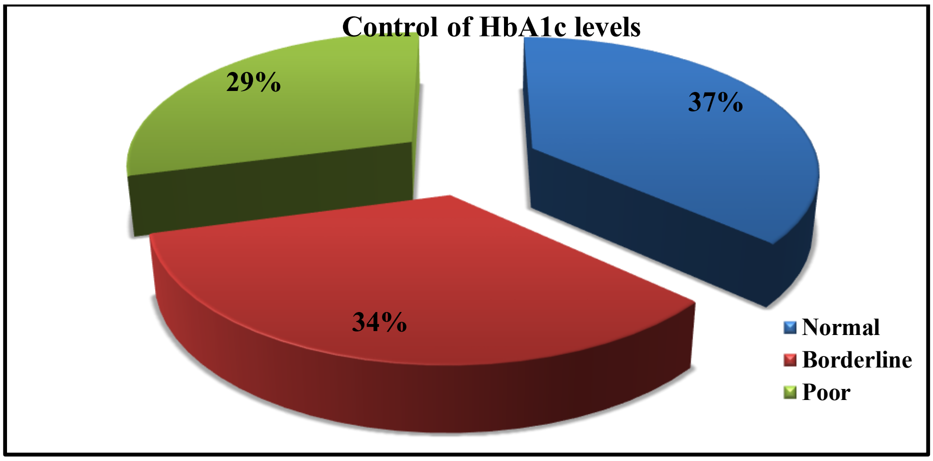


Figure 4.1.7.1.b: Percentage of patients with varying control of glycated hemoglobin

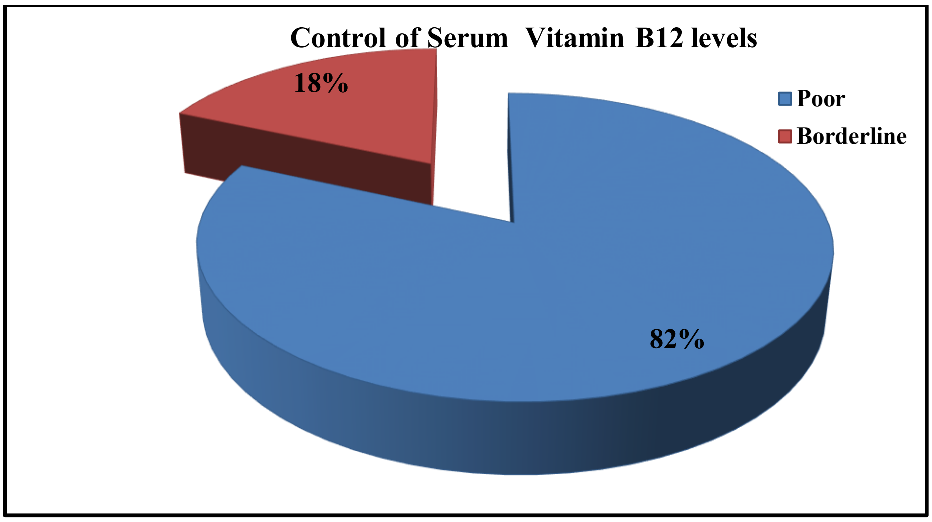


Figure 4.1.7.1.c: Percentage of MCI patients with varying control of serum vitamin B12 levels

Anthropometric, Biophysical and Biochemical profiles of the MCI subjects based on age groups

The patients were divided into two specific age groups; 60-69 years and 70-85 years to assess the differences in their age-wise anthropometric, biophysical and biochemical profiles. Results on sub-division revealed that in BMI there was significant difference ($p<0.001$) between the two age groups. Correspondingly, WHR, SBP and triglycerides also represented to be significantly different when observed between the age groups ($p<0.05$) in comparison to rest of the biophysical and biochemical factors which illustrated an insignificant differences (Table 4.1.7.4).

Table 4.1.7.4: Anthropometric, Biophysical and Biochemical profiles of the MCI subjects based on age groups

Variables	60-69 years (n= 88)	70-85 years (n=32)	*t*-test
BMI (kg/m^2)	27.03 ± 4.86	23.92 ± 4.23	8.98***
WHR	0.93 ± 0.069	0.98 ± 0.07	1.87*
FBS (mg/dl)	97.96 ± 25.17	98.41 ± 19.27	0.89^{NS}
Hemoglobin	11.61 ±1.52	11.72 ± 2.07	0.56^{NS}
Serum Vitamin B12 (pg/ml)	784.73 ± 356.77	781.56 ± 296.49	0.77^{NS}
SBP (mmHg)	135.95 ± 16.94	146.34 ± 18.98	1.56*
DBP (mmHg)	86.75 ± 10.31	82.39 ± 9.04	0.98^{NS}
HbA_{1C}	6.08 ± 1.37	6.21 ± 1.40	0.79^{NS}
Total Cholesterol (mg/dl)	194.32 ± 31.07	198.09 ± 38.87	0.95^{NS}
Triglycerides (mg/dl)	144.55 ± 62.49	138.28 ± 57.98	2.1*
LDL (mg/dl)	119.79 ± 26.47	122.24 ± 34.40	1.1^{NS}
HDL (mg/dl)	42.95 ± 8.91	43.83 ± 7.23	0.65^{NS}
TC/HDL	4.69 ±1.09	4.67 ± 1.48	1.2^{NS}

*Significant from the baseline value at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, NS- Non- significant

Prior initiation of the supplementation phase, a sub-set of MCI patients were stratified for profile-matching based on the glycated hemoglobin (HbA1c) status so as to ascertain any abnormal trend in the diabetic versus the non-diabetic MCI patients The mean FBS values showed a significant difference between the diabetic and non-diabetic MCI patients being representative of poor status of fasting blood sugar in case of diabetics. Also, the significant difference was found in the Cholesterol/HDL ratio between both the groups depicting a high TC/HDL ration in the diabetic MCI patients.An insignificance was evaluated for the remaining variables (Table 4.1.7.5)**.**

Table 4.1.5.: Mean values for biophysical and biochemical profile of MCI subjects based on their glycated hemoglobin status

Variables	HbA1c<8 (n=86)	HbA1c≥8 (n=34)	*t*-test value
BP Systolic (mmHg)	138.59 ± 17.04	146.43 ± 17.99	0.87^{NS}
BP Diastolic (mmHg)	85.56 ±10.59	85.95 ± 10.33	0.02^{NS}
FBS (mg/dl)	91.08 ± 10.56	146.72 ± 33.53	4.98***
Hemoglobin (g/dl)	11.56 ± 1.64	12.18 ± 1.83	1.65^{NS}
Serum Vitamin B12 (pg/ml)	185.42 ± 43.61	187.15 ± 38.54	0.55^{NS}
Total Cholesterol (mg/dl)	194.57 ± 31.49	200.32 ± 45.11	0.98^{NS}
Triglycerides (mg/dl)	143.62 ± 66.81	136.62 ± 48.98	0.87^{NS}
LDL (mg/dl)	120.68 ± 26.43	118.82 ± 41.61	0.35^{NS}
VLDL (mg/dl)	29.75 ± 11.74	33.18 ± 9.98	1.43^{NS}
HDL (mg/dl)	43.58 ± 8.24	40.56 ± 9.52	1.25^{NS}
LDL/HDL ratio	2.85 ± 0.49	3.07 ± 1.27	1.1^{NS}
Cholesterol/ HDL ratio	4.6 ± 1.05	5.22 ± 1.84	2.01*
ACE Score	69.74 ± 7.04	67.85 ± 7.72	0.95^{NS}
MMSE Score	24.81 ± 2.73	24.18 ± 3.29	0.09^{NS}
MNA Score	10.01 ± 1.77	9.81 ± 1.75	0.26^{NS}

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from value at p<0.001, NS- Non- significant

A similar profile-matching assessment of the total cholesterol status was performed before initiating the supplementation phase in order to determine any abnormal trend in the cholesterolemic versus the non- cholesterolemic of the segregated MCI patients ((Table 4.1.7.6)**.**

Table 4.1.7.6: **Mean values for biophysical and biochemical profile of MCI subjects based on their total cholesterol levels**

Variables	TC <200 (n=60)	TC >200 (n=60)	*t*-test value
BMI	26.18 ± 5.39	26.14 ± 4.39	0.21^{NS}
BP Systolic (mmHg)	139.65 ± 17.42	138.82 ± 32.11	0.56^{NS}
BP Diastolic (mmHg)	85.94 ± 11.01	83.67 ± 9.27	1.76^{NS}
FBS (mg/dl)	97.60 ± 23.27	94.01 ± 24.87	2.1*
Hemoglobin (g/dl)	11.62 ± 1.67	11.54 ± 1.66	0.7^{NS}
Serum Vitamin B12 (pg/ml)	189.67 ± 39.53	180.98 ± 48.06	2.25*
HbA_{1c}	6.01 ± 0.77	5.83 ± 1.37	1.1^{NS}
Triglycerides (mg/dl)	128.87 ± 67.07	153.73 ± 50.37	2.87**
LDL (mg/dl)	101.98 ± 19.74	140.65 ± 23.28	3.99***
VLDL (mg/dl)	27.98 ± 16.05	29.91 ± 9.06	1.35^{NS}
HDL (mg/dl)	42.48 ± 8.19	45.95 ± 8.77	1.64^{NS}
LDL/HDL ratio	2.48 ± 0.70	3.18 ± 0.87	4.46***
ACE Score	68.32 ± 7.99	68.57 ± 9.23	.019^{NS}
MMSE Score	24.45 ± 3.09	24.31 ± 3.23	0.13^{NS}
MNA Score	10.03 ± 1.79	10.78 ± 1.75	0.07^{NS}

*Significant from the baseline value at p<0.05, ** at p<0.01, *** at p<0.001, NS- Non- significant

4.1.8 Food habits and nutrient intake of the MCI patients

Maximum (76%) patients were vegetarians followed by ovo-lacto vegetarians (14%) and non vegetarians (10%). Almost 63% of the patients consumed 2-3 cup of beverages daily and 15% of the patients were in habit of taking more than 3 cups. 54% patients reported to be having 3 meals a day (breakfast, lunch and dinner) whereas 26% were eating only twice a day. Majority (90%) patients were teetotallers and only 3.33% reported to be consuming alcohol and 6.66% said they used to smoke occasionally (Table 4.1.8.1).

Table 4.1.8.1: Food habits of the MCI Subjects

Category	Total subjects (N=120)
Type of Diet	
Vegetarian	91 (75.8)
Non Vegetarian	12 (10)
Ovo-lacto vegetarian	17 (14.2)
Number of Tea/Coffee day	
1 cup	26 (21.7)
2-3 cups	76 (63.3)
> 3 cups	18 (15)
Meals per day	
≤ Two times	31 (25.8)
Three times	65 (54.2)
≥ Four Times	24 (12)
Addiction	
Alcohol	4 (3.3)
Tobacco	2 (1.7)
Smoking	8 (6.7)

Numbers in parenthesis indicate percentage

4.1.8.2 Nutrient intake of the MCI subjects

Table 4.1.8.2 is representative of the macro-nutrients and micro- nutrients. The mean energy intake was 2110 calories/day. Nutrient intake of protein was slightly more (14%-18%) and fat consumption was almost twice than the RDA in both genders. Vitamin C consumption was also double than RDA. ALA consumption was around 85% less than the RDA levels

Table 4.1.8.2: Mean intake of nutrients of MCI subjects as per 24 hour dietary recall

Nutrients	Range	Total (N=120)	RDA for Females	Females (n=67)	% RDA	RDA for Males	Males (n=57)	% RDA	*t*-test
Energy (Kcal)	1381-2894	2110 ± 321	1875^	1950 ± 264	4 ↑	2425 π	2296 ± 291	5.3 ↓	3.53***
Protein (gm)	40-91	63.8 ± 10.5	50	58.8 ± 8.8	17.6 ↑	60	68.9 ± 9.6	14.8 ↑	4.26***
Fat (gm)	37-106	70.6 ± 14.7	20	66.0 ± 14.7	230 ↑	20	75.4 ± 13.3	277 ↑	2.27*
Iron (mg)	10- 42	20.57 ± 5.89	30	18.9 ± 5.29	37 ↓	28	22.2 ± 6.0	20.7 ↓	2.12*
Calcium (mg)	306 -1052	681.3 ± 160.6	400	646 ± 169	61.5 ↑	400	717 ± 144	79.2 ↑	1.40 NS
Vitamin A (mg)	151- 1813	434.8 ± 316.3	600	430 ± 306	28.2 ↓	600	439 ± 330	26.8 ↓	0.80 NS
Vitamin B12 (mg)	0 – 1.4	0.30 ± 0.08	1	0.28 ± 0.08	72 ↓	600	0.32 ± 0.08	68 ↓	1.30 NS
Vitamin C (mg)	24 - 394	126.28 ± 77.3	40	121.2 ± 83.6	202 ↑	40	131.7 ± 70.6	227 ↑	2.65*
ALA (-3) g	0 – 1.2	0.17 ± 0.03	1.1	0.15 ± 0	86.3 ↓	1.6	0.2 ± 0.04	87.5 ↓	1.00 NS
Zinc (mg)	3- 11	6.72 ± 1.60	NA	6.0 ± 1.43	NA	NA	7.44 ± 1.45	NA	4.80***
Phosphorus (mg)	895 -2210	1452 ± 282	NA	1330 ± 249	NA	NA	1578 ± 261	NA	3.70***
Thiamine (mg)	0.93 – 0.67	1.58 ±0.33	1.2^	1.42 ± 0.27	1.4 ↑	0.9 π	1.75 ± 0.30	94 ↑	2.90**
Riboflavin (mg)	0.39 - 1.36	0.86 ± 0.18	1.4^	0.79 ± 0.17	43.5 ↓	1.1 π	0.94 ± 0.17	14.5 ↓	3.50***
Niacin (mg)	7.3 – 23.4	13.73 ± 2.9	14	12.33 ± 2.34	11.9 ↓	16	15.18 ± 2.86	5.12 ↓	3.01***
Folic Acid (mg)	130 - 430	271.6 ±63.6	100	255.1 ± 58.9	155 ↑	100	288.7 ± 64.8	188 ↑	1.20 NS

*Significant from the baseline value at p<0.05, ** at p<0.01, *** at p<0.001, NS- Non- significant, NA- Not applicable , NIN RDA for Indians 2009

Vitamin B12 rich non vegetarian food consumption was very negligible and only half of the subjects (53%) were frequently taking milk to fulfill their vitamin B12 needs to some levels. For the geriatric population, ideally short and frequent meals are recommended in which uniform distribution of energy at the proper timing is very essential. Behavior less than ideal was observed in the subjects with most of the patients having a few infrequent and large meals to fulfill their energy requirements (Figure 4.1.8.1).

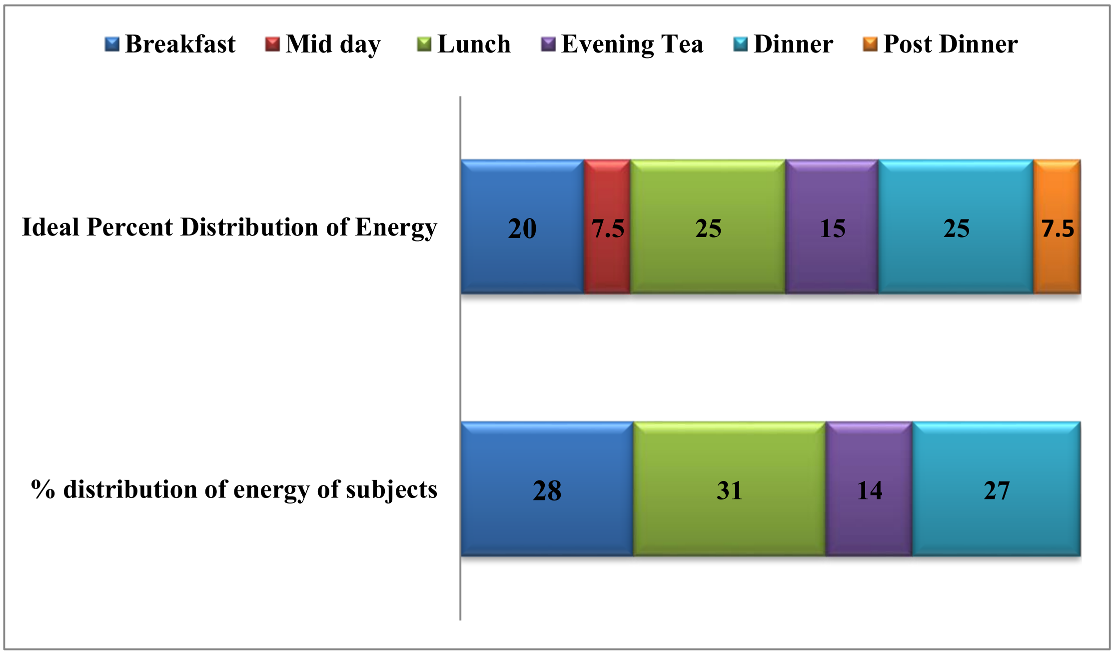


Figure 4.1.8.1: Ideal % distribution of energy vs % distribution of energy by the subjects

4.1.9 Food Frequency Questionnaire for food consumption of MCI patients

Table 4.1.9.1 reveals the frequency of various food groups as per the food frequency method. The results showed that more than 90% subjects were frequently consuming cereals, pulses, roots and tubers. Whole wheat flour was the most commonly consumed food item followed by rice. 91% of the subjects were consuming pulses on the daily basis. Consumption of green leafy vegetables was on lower level of the scale with majority (79%) of the subjects eating them less frequently. 94% of the patients gave higher importance to other vegetables like brinjal, cauliflower etc. in their palate having them on frequent basis in comparison to the green leafy vegetables. Almost half of the people (49%) were consuming fruits infrequently. Milk products were also very popular in the patients with 88% frequently consuming them especially milk in the tea form.

Table 4.1.9.1: Frequency of consumption of food groups by the subjects as per food frequency method

Food Groups	Frequent N (%)	Less Frequent N (%)
Cereals (whole wheat, rice, rice flakes, bajra, semolina)	116 (96.7)	4 (3.3)
Pulses (red gram dal, green gram dal, bengal gram, lentil)	109 (90.8)	11 (9.2)
Green Leafy Vegetables (cabbage, spinach, fenugreek leaves, colocasia leaves)	25 (20.8)	95 (79.2)
Roots and Tubers (potato,onion, carrot, beet root)	116 (96.7)	4 (3.3)
Other Vegetables (brinjal, cauliflower, ash gourd, ladies finger)	112 (93.3)	8 (6.7)
Fruits (banana, apple, orange, papaya, tomato)	62 (51.7)	58 (48.3)
Nuts and oil seeds (groundnut, sesame seeds, walnut, almond, coconut)	17 (14.2)	103 (85.8)
Fats (groundnut oil, ghee, butter, cottonseed oil)	70 (58.3)	50 (41.6)
Milk products (milk, buttermilk, curd, paneer, shreekhand)	105 (87.5)	15 (12.5)
Snacks (khakra, bhajiya, papad, dabeli, samosa)	69 (57.5)	51 (42.5)
Sweets (kheer, basundi, peda, icecream, gulabjamun)	14 (11.7)	106 (88.3)

Numbers in parenthesis indicate percentage

The chief food sources of Vitamin B12 included in our food frequency questionnaire were milk, curd, butter, cheese, and non-vegetarian foods as eggs, shrimp, liver, mutton. Omega-3 rich sources included were fenugreek, walnut, fish and soybeans. Milk was the most popular source of Vitamin B12 among the patients, 58% of the patients were consuming it frequently, followed by 14% to be consuming curd. The non- vegetarian foods like liver, mutton etc. were not consumed in our almost vegetarian patient sample (Table 4.1.9.2).

Table 4.1.9.2 illustrates that fenugreek seeds were consumed very frequently and were the only source of Omega-3 in the diet of the 70% patients. Rest common sources of Omega-3 like walnut, fish, soybeans etc. were not being taken on a frequent basis. Thus, daily requirement of omega-3 was insufficient in almost all the patients.

Table 4.1.9.2: Frequency of consumption of Vitamin B12 and Omega 3 rich food sources by the patients as per food frequency questionnaire

Food Groups		Frequent N (%)	Less Frequent N (%)	Rarely N (%)
Vitamin B12	Milk	69 (57.5)	26 (21.7)	25 (20.8)
	Curd	17 (14.2)	51 (42.5)	52 (43.3)
	Eggs	1 (0.8)	7 (5.8)	102 (85)
	Shrimp	0 (0)	1 (0.8)	119 (99.2)
	Liver	0 (0)	0 (0)	120 (100)
	Mutton	0 (0)	3 (2.5)	117 (97.5)
Omega -3	Butter	4 (3.3)	22 (18.3)	94 (78.3)
	Cheese	2 (1.7)	5 (4.1)	113 (94.2)
	Soybean	5 (4.16)	4 (3.33)	111 (92.5)
	Walnut	0 (0)	5 (4.1)	115 (95.8)
	Fish	1(0.8)	5 (4.1)	114 (95)
	Fenugreek seed	84 (70)	13 (10.8)	23 (19.2)

Numbers in parenthesis indicate percentage

4.1.10 Association between neurological test scores and other parameters in MCI patients

As observed from the Table 4.1.10.1, a negative correlation was found between age and neurological score which indicated that as the age of the patients progressed, their cognitive abilities reduced. ACE as well as MNA scores had significant ($p<0.01$) negative correlation and MMSE too, had a significant ($p<0.05$) negative correlation with the age. In the present analysis, it was found that MNA ($p<0.001$) and ACE ($p<0.05$) scores also had a significant positive correlation with BMI. In addition, MNA significantly (p<0.001) correlated with the waist circumference (WC) and the hip circumference (HC). MMSE (p<0.05) and MNA (p<0.01) had positive relation with hemoglobin at significant levels. ACE (p<0.01), MMSE (p<0.05) and MNA (p<0.05) scores were significantly correlated with serum Vitamin B12 levels. RBC count was significantly positively correlated with MNA scores (p<0.01). MCV also depicted positive correlation with ACE and MMSE scores (p<0.05). MCH revealed to be positively correlated with ACE score (p<0.05). Furthermore, the total serum cholesterol values significantly positively correlated with MNA scores (p<0.05). When the association between HDL levels and ACE scores was examined, it suggested towards a significant positive correlation with ACE scores (p<0.05).

Table 4.1.10.1: Correlation between neurological test scores and other parameters in MCI patients

Parameters	ACE	MMSE	MNA
Age	-0.215**	-0.165*	-0.216**
Physical Activity	0.118	0.125	0.079
BMI	0.176*	0.116	0.488***
WC	0.065	0.022	0.409***
HC	0.11	0.13	0.35***
WHR	- 0.058	- 0.149	0.105
DBP	0.042	0.024	0.021
SBP	- 0.078	- 0.077	- 0.079
HbA_{1c}	0.133	0.109	- 0.001
FBS	0.047	0.094	- 0.028
Hemoglobin	0.054	0.174*	0.232**
Serum vitamin B12	0.232**	0.191*	0.156*
RBC Count	0.025	0.064	0.225**
WBC Count	0.007	0.024	0.059
MCV	0.175*	0.152*	- 0.043
MCH	0.157*	0.131	- 0.029
Total Cholesterol	- 0.050	-0.062	-0.152*
HDL	0.182*	0.100	0.119
TG	-0.127	-0.073	-0.061
LDL	-0.019	-0.047	-0.110
VLDL	-0.118	-0.113	-0.140

*Correlation values are significant at p<0.05, *** correlation values are significant at p<0.001

4.1.11 Relation between omega 3 intake and serum vitamin B12 levels with other parameters in MCI patients

As described in the Table 4.1.11.1, the results indicated that BMI had a significant inverse correlation with serum vitamin B12 levels ($p<0.001$). Negative correlation was also observed between diastolic blood pressure (DBP) with both omega-3 intake and serum vitamin B12 levels ($p<0.05$) respectively. Results also directed towards the significant positive association ($p<0.05$) between omega 3 intake and hemoglobin. Serum vitamin B12 levels and RBC count were also found to be significantly ($p<0.05$) positively correlated. Similarly, HDL showed to have significant positive correlation ($p<0.001$) with serum vitamin B12 levels. Triglycerides were negatively correlated with serum vitamin B12 levels ($p<0.05$) (Table 4.1.11.1).

Table 4.1.11.1: Correlation between omega 3 intake and B12 serum levels with other parameters in MCI patients

Parameters	Serum Vitamin B12	Omega-3 intake
Age	-0.054	-0.032
BMI	-0.370***	-0.057
WHR	-0.143	-0.098
DBP	-0.231*	-0.156*
SBP	-0.101	-0.141
HbA_{1c}	0.0290	0.0421
FBS	0.0176	-0.0215
Hemoglobin	0.087	0.151*
RBC Count	0.157*	0.989
WBC Count	0.012	0.178
Total Cholesterol	0.043	-0.198*
HDL	0.260***	0.134
TG	-0.167*	-0.065
LDL	0.056	-0.129
VLDL	-0.198*	-0.095

*Correlation values are significant at $p<0.05$, *** correlation values are significant at $p<0.001$

RESULT HIGHLIGHTS

- ***Almost half (49%) of baseline subjects were diagnosed with MCI. Maximum (89.17%) MCI subjects were literates followed Hinduism (94.16%) and practiced vegetarian dietary (75.83%) pattern.***

- ***69% of MCI subjects were obese, 15.8% overweight and 57% patients had a sedentary lifestyle.***

- ***Majority (58%) were hypertensive, 37% pre-hypertensive, 82% had poor serum B12 level and 20% at borderline.***

- ***Significant differences found for FBS ($p<0.05$), hemoglobin ($p< 0.001$) and HDL ($p<0.05$) values***

- ***More than 90% frequently consumed cereals, pulses, roots and tubers. Protein intake was slightly more (14%-18%) and fat consumption was almost twice than the RDA in both genders. Consumption of iron was 20% less and prime reason behind 76% of anemic patients.***

- ***ALA consumption was around 85% less than RDA levels. Vitamin B12 rich food consumption was also very negligible and only half (53%) of patients frequently took milk to fulfil their Vitamin B12 requirement to some extent.***

DISCUSSION:

For undertaking the present study, the sample of 402 elderly subjects was enrolled from the Maharaja Sayajirao University Health Centre and Vallabhacharyajee hospital, Baroda to obtain their baseline profiles and establish the associations amongst the various factors involved. Baseline information was elicited from the subjects employing the neuropsychological test battery of cognitive tools and thereafter socio-demographic, medical history, activity pattern, anthropometric measurements, bio-physical and dietary parameters. Findings of the study for neuropsychological screening tests signified that cognitive impairment in the elderly was 49% by ACE, 58% by MMSE, 44% by YFPIT and 66% subjects had an abnormal nutritional status.

The function of neuropsychological test battery for prognosis of Alzheimer's disease (AD) in elderly has been long studied. In the Italian Multicentre Study on Dementia, a neuropsychological test battery was developed for screening, staging and monitoring cognitive impairment in AD patients and for delineating their pattern of cognitive decline in the mild, moderate, severe and very severe mental impairment stages. The sub-tests of the battery were significantly accurate enough to screen early demented from non-demented elderly measuring higher cortical functions primarily involved as short- and long-term memory, orientation, language, and praxis (Bracco et al 1990). Another study indicated that a diagnostic instrument enabling general practitioners for distinguishing the early dementia, particularly Alzheimer's disease, from normal aging contributes to the distinction between normal aging and early dementia (Jonker et al 1990).

Similarly, the cohort study reported that the semantic verbal fluency and delayed recall subtest scores of the brief neuropsychological battery best discriminated dementia and MCI groups from the healthy control group (Serna et al 2015). Evidences have established the dire need for early clinical diagnosis of MCI using domain-wise detailed neuropsychological test battery posing highly sensitive in identification of elderly cognitive impairment through significant correlation with radiological scores and suggested that early therapeutic interventions are highly rewarding and carry very good long term prognosis for cognitive morbidity (Jura 1992; Issac et al 2016).

The male and female patients in the present study were largely obese (69%). In the year 2014, the prevalence of obesity (BMI ≥30 kg/m^2) in India was 4.9% (WHO, 2014). India was home to over a million (4.1 million) population with dementia in 2015. The mean attributable cost was estimated to be 1764 dollars for dementia and 764 dollars for diabetes, both of which remained statistically significant ($p<0.05$). Prevalence of obesity is causally associated with dementia and corresponding upward changes are expected to be observed in dementia incidence. The epidemic of obesity resulted in upward trends in the incidence and prevalence of dementia in many low and middle income countries (ADI, 2015). This postulates obesity as a strong link, especially with regard to cognitive decline. Evidence from numerous studies confirmed that obesity is associated with an increased incidence of MCI and progression to dementia (Tanamas et al 2016; Lara et al 2016; Yao et al 2016).

Majority patients had central obesity representative of higher risk of NCDs. According to the ICMR-INDIAB Study [ICMR- NDIAB-3], the prevalence of abdominal obesity was high in India with 153 million individuals (Pradeepa et al 2015). Central adiposity is considered an equal if not more important independent risk factor of cardio-metabolic diseases (Sniderman et al 2007). The greater abdominal adiposity prevalent in Indians is referred to as the "Asian Indian Phenotype" (Mohan et al 2007). Several studies have described of the association of central obesity with an increased risk of cognitive disorders such as dementia independent of diabetes and cardiovascular co-morbidities (Whitmer et al 2008; Gustafson 2008).

The undertaken study also pointed that majority of the patients were leading a sedentary lifestyle. Physical inactivity was independently associated with MMSE-based MCI in elderly (Lee et al 2016; Chen et al 2016; Yao et al 2016). Similarly, various other studies have also reported that involvement in physical activities by the older groups seems to reduce the risk of MCI (Wang et al 2015; Schlosser Covell et al 2015; Zhao et al 2015; Grande et al 2014).

The present study also found that majority of the MCI patients were hypertensive. Researches carried out have explicitly directed towards the inter-linkage of hypertension with MCI (Li et al 2013; Zou et al 2014; Goldstein et al 2013).In a research study, the discriminant function analysis showed that only age and hypertension are potential factors which may have an influence on progression to dementia in the MCI group within one year of prospective observation (Siuda et al 2009.Hypertension (OR=2.075; 95% CI: 1.170-3.678) showed a significant association with the risk of MCI (Yao et al 2016). Similarly, observations have depicted higher cognitive scores in hypertensive elderly to be associated with decrease in systolic blood pressure (Lacruz et al 2016).

Even though, a multitude of factors govern MCI but vitamin B12 may affect its progression and complications. The patients in the study were largely vitamin B12 deficient. Study results have suggested that vitamin B12 intake is associated with cognitive function in cognitively impaired AD and MCI elderly, and the association is stronger in AD patients (Kim et al 2014; Silva et al 2013). de Jager et al (2012) also in their small intervention trial established that vitamin B12 appeared to slow cognitive and clinical decline in people with MCI, in particular in those with elevated homocysteine. Moreover, a prospective study has confirmed that vascular risk factors were found significantly associated with cognitive impairment more in the MCI group (p =0.041), including vitamin B12 deficiency (p =0.012) and folate deficiency (p =0.023) with a likelihood of progressing to dementia in an year (Siuda et al 2009). Further, mechanistic studies, epidemiologic analyses, and randomized controlled intervention trials provide insight to the positive effects of micronutrients such as the vitamin B12 in helping neurons to cope with aging and for individuals at risk in particular, is a viable alternative approach to delaying brain aging and for protecting against the onset of AD pathology (Mohajeri et al 2014).

There is ample scarcity of studies conducted in relation to vitamin B12 supplementation and MCI. However, a three –year longitudinal study in India by Dhikav et al (2014) declared that vitamin B12 deficiency is common in MCI and dementia and is observed in a significantly more number of subjects with dementia as compared to MCI.

In our study, significant differences were found for FBS (p<0.05), hemoglobin (p< 0.001) and HDL (p<0.05) values amongst the male and female MCI patients. This is parallel to the study by Parnowski and Kaluza (2013) wherein correlations were found between HDL cholesterol (p = 0.036, OR = 1.061, <0.99; 1.13>), hyperglycaemia (p = 0.008, OR = 0.97, <0.95; 0.99>), metabolic syndrome (p = 0.03; OR = 0.34; <0.12; 0.91>) with age (p = 0.001; OR = 0.9; <0.84; 0.95>) and dementia. The correlations of glucose levels with age (r = -0.47, p = 0.000001), fasting glucose (r =-0.33, p = 0.0024), HDL levels (r = 0.32; p = 0.05) too were determined confirming that the metabolic-cognitive syndrome seemed to be a part of pathogenesis of dementia. In another cross-sectional study, Mortimer et al (2010) suggested that a high normal level of fasting blood glucose (FBG) may be a risk factor for dementia. The higher FBG was associated with dementia (vs. amnestic MCI) independent of vascular risk factors and MRI indicators of vascular disease, and remained a significant risk factor when analyses were restricted to subjects with normal FBG.

In this study, the MCI patients were consuming a diet which was slightly more (14%-18%) in the protein intake and fat consumption was almost twice than the RDA in both the genders. The subjects with a lower intake of mono-unsaturated fatty acids (MUFA), saturated fatty acids (SFA) and cholesterol and higher intakes of total calories, fresh fruit, carbohydrate, thiamine, foliate, vitamin C, and minerals (iron and zinc), had the best performance in cognitive tests (MMSE score >28 points), with a statistical significance after adjustment for age and sex (Ortega et al 1997). Higher SFA intake was associated with an increased risk of impairment in memory function, psychomotor speed, and cognitive flexibility by 15–19%, although not significantly (Solfrizzi et al 2009). Findings from the Chicago Health and Aging Project (CHAP), on 2,560 persons aged 65 years and older, showed that in a large population-based sample, a high intake of saturated and trans-unsaturated fat was associated with a greater cognitive decline over a 6-year follow-up. Intake of MUFA was inversely associated with cognitive change among persons with good cognitive function at baseline and among those with stable long-term consumption of margarine, a major food source. Slower decline in cognitive function was associated with higher intake of

PUFA, but the association appeared to be due largely to its high content of vitamin E and which is inversely related to cognitive decline (Morris et al 2004).

Present study elicited that the consumption of iron was 20% less and prime reason behind 76% of anemic patients. There is increasing evidence regarding the association of anemia with cognitive impairment. A study reported that out of 4,157 participants (50% men, 50-80 years) of the second examination (t1) of a cohort study over last five years (baseline (t0) 2000-2003), 4,033 participants were included for availing hemoglobin information and complete cognitive assessment. Amongst them the anemia prevalence was defined as hemoglobin <13 g/dl in men (n=84) and <12 g/dl in women (n=79). Group comparisons were used to compare the cognitive subtests. 579 participants were included with MCI and 1,438 cognitively normal participants out of the total cohort. Anemic participants showed lower performances in verbal memory and executive functions. The fully adjusted odds ratios (OR) for MCI, aMCI, and naMCI in anemic versus non-anemic participants were 1.92 (95% -CI, 1.09-3.39), 1.96 (1.00-3.87), and 1.88 (0.91-3.87). Anemia at both times points showed a non-significant association with naMCI (OR 3.74, 0.94-14.81, fully adjusted). These results implied that anemia is associated with an increased risk of MCI independent of traditional cardiovascular risk factors. The association of anemia and MCI has important clinical relevance, because many causes of anemia can be treated effectively (Dlugaj et al 2015). In the Australian Imaging Biomarker and Lifestyle (AIBL) study (a community-based, cross-sectional cohort comprising 768 healthy controls (HC), 133 participants with mild cognitive impairment (MCI) and 211 participants with AD) it was revealed that individuals with AD had significantly lower hemoglobin, mean cell hemoglobin concentrations, packed cell volume and higher erythrocyte sedimentation rates (adjusted for age, gender, APOE-ε4 and site). In AD, plasma iron, transferrin, transferrin saturation and red cell folate levels exhibited a significant distortion of their customary relationship to hemoglobin levels. There was a strong association between anemia and AD (adjusted odds ratio (OR) =2.43, confidence interval (CI) (1.31, 4.54)). Moreover, AD emerged as a strong risk factor for anemia on step-down regression, even when controlling for all other available explanations for anemia (adjusted OR=3.41, 95% CI (1.68, 6.92)). These data indicated that AD is complicated by anemia, which may itself contribute to cognitive decline (Faux et al 2014).

The present study results drew that the dietary ALA consumption of the MCI patients was around 85% less than RDA levels. O'Callaghan et al (2014) in their RCT study on thirty-three adults aged > 65 y with MCI receiving a supplement rich in the long-chain ω-3 PUFAs for 6 months demonstrated that the telomeric shortening may be attenuated by ω-3 PUFA supplementation. The excessive shortening of the telomeric ends of chromosomes is a marker of accelerated aging. Oxidative stress and nutritional deficiency may influence this process. Numerous studies have directed that accelerated cognitive decline and MCI correlate with lowered tissue levels of DHA/EPA and maintained towards the phenomenal function of omega-3 fatty acids in MCI which can effectively counteract these processes, e.g., by promoting membrane formation and synaptogenesis, enhancing memory/behavior, improving endothelial function, and cerebrovascular health (Engelborghs et al 2014;; Rondanelli et al 2012; Kamphuis and Scheltens 2010; Panza et al 2009; Kidd 2007).In a Southern Italian elderly population from the Italian Longitudinal Study on Aging (ILSA), a clear reduction of risk of age-related cognitive decline (ARCD) has been established with elevated intake of PUFA and MUFA. Furthermore, in the ILSA, high PUFA intake appeared to have borderline non-significant trend for a protective effect against the development of MCI. These epidemiological findings on pre-dementia syndromes, i.e. MCI or ARCD, advocated for a possible role of fatty acids intake in maintaining adequate cognitive functioning and possibly in preventing or delaying the onset of dementia (Solfrizzi et al 2008).

Results from our study indicate that the vitamin B12 rich food consumption was also very negligible and only half (53%) of MCI patients frequently took milk to fulfill their Vitamin B12 requirement to a miniscule extent. It has been very well documented in large body of data that low vitamin B12 concentrations within the normal range are associated with poorer memory performance, which is an effect that is partially mediated by the reduced microstructural integrity of the hippocampus. Vitamin B12 act as cofactor to enhance the supply of precursors required to make neuronal membranes and synapses (Kobe et al 2016; Silva et al 2013; Kim et al 2013;

Kim et al 2014). In the Framingham study, participants with plasma vitamin B12 concentrations of 257 pmol/L or less had a significantly faster cognitive decline than individuals with higher concentrations, and the cognitive disadvantages linked to low vitamin B12 were increased at high folate levels (Rubenstein et al 2001). In a similar fashion, a UK study identified that B-vitamin supplementation was associated with a decline of cerebral atrophy in the grey matter regions of the brain that are vulnerable to Alzheimer's disease (Henry 2005).

One of the few India-based studies report that folic acid and vitamin B12 intervention in people with elevated hcy levels in India could prove to be effective in lowering hcy levels and help maintain or improve cognitive function (Agrawal et.al 2015). However, a multi-target approach using combinations of (micro) nutrients might have beneficial effects on cognitive function in neurodegenerative brain disorders like AD leading to synaptic degeneration (Engelborghs et al 2014). Additionally, some of the prospective and case–control studies have shown that low dietary intake of B12 vitamin was associated with cognitive decline or an increased risk of AD (Tucker et al 2005; Mizrahi et al 2003; Corrada et al 2005).

CONCLUDING REMARKS

Almost half (49%) of baseline subjects were diagnosed with MCI. Maximum (89.17%) MCI subjects were literates, followed Hinduism (94.16%) and practiced vegetarian dietary (75.83%) pattern. 69% of MCI subjects were obese, 15.8% overweight, 58% hypertensive, 37% pre-hypertensive, 82% had poor serum B12 level and 20% at borderline. Significant differences found for FBS ($p<0.05$), hemoglobin ($p< 0.001$) and HDL ($p<0.05$) values. More than 90% frequently consumed cereals, pulses, roots and tubers. Protein intake was slightly more (14%-18%) and fat consumption was almost twice than the RDA in both genders. Consumption of iron was 20% less and prime reason behind 76% of anaemic patients. ALA consumption was around 85% less than RDA levels. Vitamin B12 rich food consumption was also very negligible and only half (53%) of patients frequently took milk to fullfill their Vitamin B12 requirement to some extent.

PHASE II: To develop food product from omega-3 fatty acid source (flaxseeds) and conduct acceptability trials by sensory function tests

With the rapid expansion of the functional food industry, the unconventional role of flaxseeds being a rich dietary source of α-linolenic acid (ALA), lignans, fibre and with a number of positive health benefits (Khalesi et al 2015) is reigning its status being to the fore even for the neurological disorders (Simopoulos 2000; Gogus and Smith 2010). In addition to these features, flaxseeds incorporation usually yields higher taste and aroma acceptance scores (Aliani et al 2012). Hence, this phase of the research study was framed to conduct acceptability trials of food products developed using flaxseeds *viz.* ***Khichdi, Porridge, Globs*** *(laddoo)* and ***Mukhwaas*** at various substitution levels.

The results of this phase are divided into the sub-categories as following:

Section 4.2.1: Proximate analysis and fatty acid profile estimation of raw versus roasted flaxseeds

Section 4.2.2: Effect of percentage substitution of flaxseeds in food products *viz. khichdi, porridge, globs* and *mukhwaas* at varying levels

4.2.1: Proximate analysis and fatty acid profile estimation of raw versus roasted flaxseeds

The fatty acid profile was estimated through gas chromatography technique to assess that which of the two flaxseed forms, raw or roasted, has higher percentage of alpha-linolenic acid (ALA) and in what amounts. For achieving this purpose, the hundred grams amount of both the samples were taken and thereafter they were subjected to the proximate analysis to determine their nutrient content namely for energy, protein, fat, carbohydrate, crude fiber, moisture and ash. The intercomparative values of both the raw and roasted flaxseeds based on the results of the proximate analysis have been represented below as (Table 4.2.1.1):

Table 4.2.1.1: Proximate analysis of raw versus roasted flaxseeds

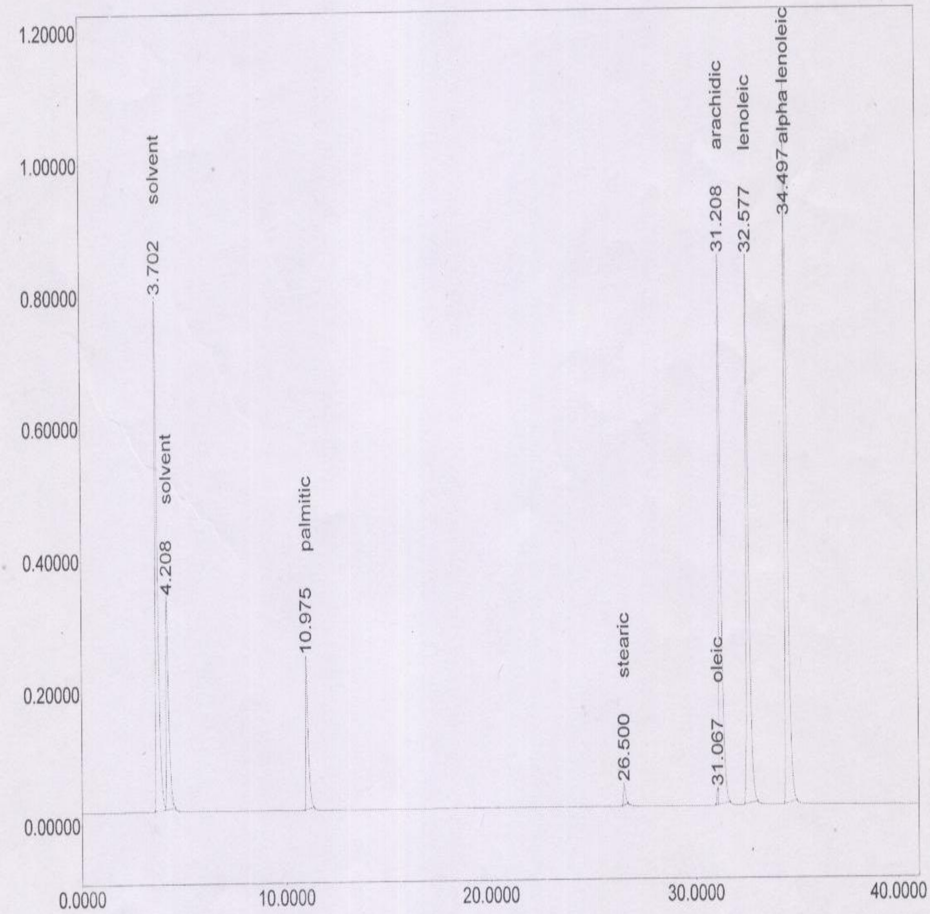
Nutrients Analysed (per 100g)	Raw Flaxseeds	Roasted Flaxseeds
Energy (Kcal)	539.6	546.4
Protein (g)	25.7	25.9
Fat (g)	33.2	33.2
Carbohydrate (g)	34.5	36
Crude Fiber (g)	4.15	4.28
Moisture (g)	3.9	2.1
Ash (g)	2.7	2.8

The proximate analysis results indicated that flaxseeds in both the forms in hundred grams of sample have same amount of fat i.e. 33.2 grams. This fat was extracted in oil form from the flaxseed for the purpose of loading it into the gas chromatogram (GC). The small oil samples of both the forms, roasted and raw were separately injected in the GC. As the liquid sample passed through GC tube its vaporisation into gas took place and subsequently came across with inert gas, which acted as a carrier. Many constituents of the sample gas travelled through the long coiled tube at different rates and reached the detector at the different points of time. The detector was able to take all the signals and showed them on the chromatograph depicting the amount in which a particular constituent was received and the times taken by each to finally reach there.

The chromatograph of both the flaxseeds samples are given below.

sample name :- flex seed oil
oven temp :-140 to 260 prog.
inj temp :- 260
det temp :- 260

< Chromatogram >

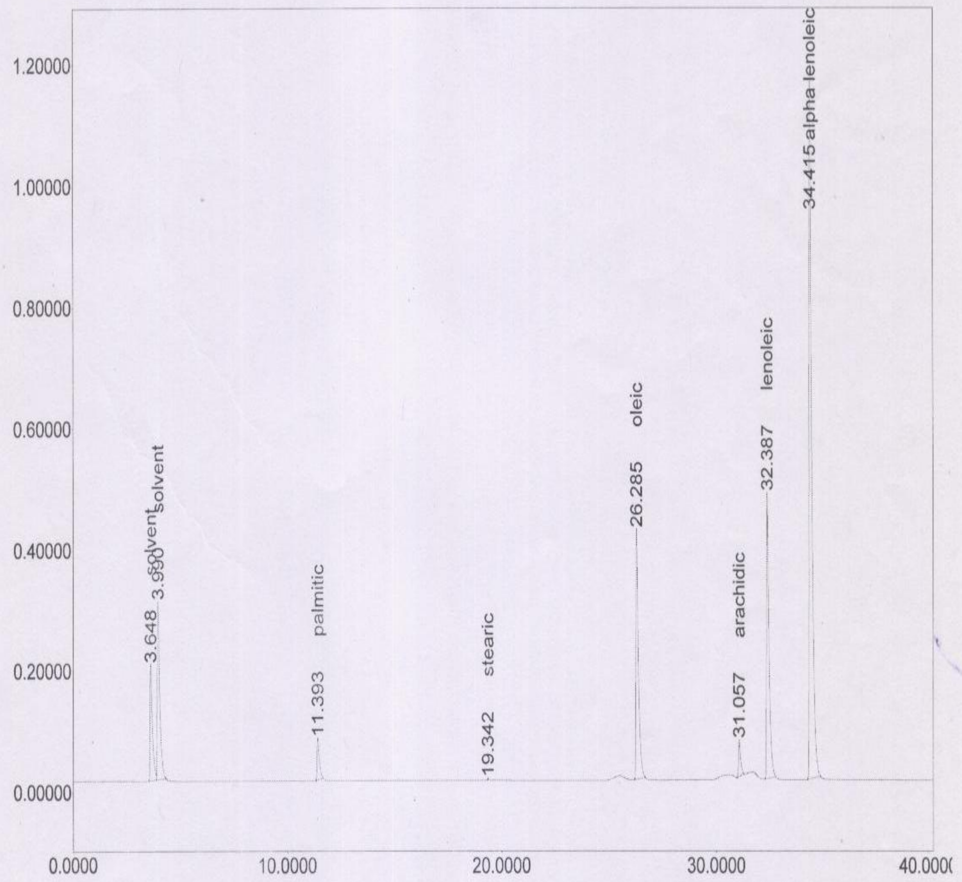


#	RT(min)	Peak name	Area(mV*sec)	Height(mV)	Area%
1	3.702	solvent	6383.301	777.465	17.537
2	4.208	solvent	2838.478	320.619	7.798
3	10.975	palmitic	1864.181	229.645	5.122
4	26.500	stearic	222.915	32.060	0.612
5	31.067	oleic	110.547	24.059	0.304
6	31.208	arachidic	7295.005	829.965	20.042
7	32.577	lenoleic	7664.691	827.691	21.058
8	34.497	alpha lenoleic	10019.255	1092.209	27.527

Figure 4.2.1.1: Chromatograph depicting fatty acid profile of raw flaxseeds

sample name :- flex seed oil roasted
oven temp :-140 to 260 prog.
inj temp :- 260
det temp :- 260

< Chromatogram >



#	RT(min)	Peak name	Area(mV*sec)	Height(mV)	Area%
1	3.648	solvent	1450.606	189.429	6.709
2	3.990	solvent	2337.738	291.594	10.811
3	11.393	palmitic	500.779	66.943	2.316
4	19.342	stearic	0.571	0.546	0.003
5	26.285	oleic .	3228.282	412.308	14.930
6	31.057	arachidic	392.217	59.035	1.814
7	32.387	lenoleic	3729.475	470.954	17.248
8	34.415	alpha lenoleic	9983.328	1154.227	46.170

Figure 4.2.1.2: Chromatograph depicting fatty acid profile of roasted flaxseeds

Each peak represents a component present in the sample. Retention time is time interval between sample injection and the maximum of the peak. It is characteristic of the identity of the component under the operating conditions. Identity of the component can be confirmed by making injections of reference material under the same operational conditions. The matching of retention time of reference material and the component peak confirms the identity of the unknown sample component.

The gas chromatogram results indicates that flaxseed oil consists of following components, arranged in order of increasing retention time: palmitic, stearic, oleic, arachidic, lenoleic, alpha-linolenic. Among these components alpha-linolenic acid (ALA) is the main source of omega 3 nutrient and most relevant in the study. ALA also has maximum retention time of approx 34.5 minutes and observed last on the detector.

From the area measurement the concentration of ALA as a percentage of total is calculated. The gas chromatograph results though show area % separately but reported value of chromatogram also factor in value of solvent in the mixture. As solvents are not part of flaxseed oil therefore their values need to be factored out while calculating concentration of ALA in flaxseed oil.

ALA Area % in Raw Flaxseed = $\dfrac{\text{Area of Peak (alpha-linolenic acid) x 100}}{\text{Area of Peak (alpha-inolenic+linoleic+arachidonic+oleic+stearic+palmitic)}}$

$$= \frac{10019 \times 100}{10019 + 7664 + 7295 + 111 + 223 + 1865}$$

$$= 36.86\%$$

ALA Area % in Roasted Flaxseed = $\dfrac{\text{Area of Peak (alpha-linolenic acid) x 100}}{\text{Area of Peak (alpha-inolenic+linoleic+arachidonic+oleic+stearic+palmitic)}}$

$$= \frac{9983 \times 100}{9983 + 3729 + 392 + 3228 + 0.5 + 501}$$

$$= 55.97\ \%$$

As both the forms had same 33.2 grams of oil in 100 grams of flaxseeds thus the amount of ALA in 100 grams of flaxseeds were

ALA in raw flaxseeds/ 100 g = 33.2 x (36.86/100) = 12.23 g.

ALA in roasted flaxseeds/ 100 g = 33.2 x (55.96 /100) = 18.57 g.

The observed value of ALA in roasted form was 34.11% higher than the one in raw form therefore in the interest of MCI patients roasted form of flaxseed were preferred over raw form because of higher percentage of ALA within these flaxseeds.

4.2.2: Effect of percentage substitution of flaxseeds in food products *viz. khichdi, porridge, laddoo and mukhwaas* at varying levels

Under this section of the second phase, four products selected for flaxseed substitution were *khichdi*, *porridge*, *laddoo* and *mukhwaas* and thereafter subjected to organoleptic assessment using the 9-point hedonic scale and the composite scoring test. These food items are consumed on regular basis in the Gujarat region and so were deemed as potentially vital for flaxseeds substitution. The base ingredients for *khichdi*, *porridge* and *laddoo* (rice, green gram, wheat gruel, sugar, milk and jaggery) were incorporated with flaxseeds at the three (10 gm, 15gm and 20 gm) varying levels respectively. Each of the flaxseed amounts indicate cooked roasted weighed portion being added to the base.

4.2.2.1: Effect of percentage substitution of flaxseeds in *khichdi* at varying levels

The results under this sub-section of the second phase involved for percentage substitution of flaxseeds at the varying levels are presented in the Table 4.2.2.1 to 4.2.2.2 and presented graphically in Figure 4.2.2.1-4.2.2.2.

a) Organoleptic evaluation of the khichdi

The organoleptic scoring of *khichdi* prepared by substitution of flaxseeds at varying levels is mentioned in the Table 4.2.2.1. The mean hedonic scores of the semi-trained as well as the untrained group of panelists revealed significant ($p<0.05$) reduction of 10.24% and 9.55% respectively indicating a decrease in the scores with the increase of flaxseeds substitutions in prepared *khichdi*. The variant K1 emerged as a top scorer in terms of likeability and variant K3 fared the least as adjudged by both the panelists (Figure 4.2.2.1).

Table 4.2.2.1: Effect of substitution of roasted flaxseeds at various levels with base material in *khichdi* using mean hedonic scores of panelists

Variations	Varied flaxseeds amount in base	Hedonic score (semi-trained)	Hedonic score (untrained)
Variant I (K1)	10	6.64 ± 1.09	6.70 ± 0.90
Variant II (K2)	15	6.33 ± 0.99	6.15 ± 1.20
Variant III (K3)	20	5.96 ± 0.85	6.06 ± 0.82
Percent Increase/ Decrease		10.24 ↓	9.55 ↓
ANOVA		**3.43***	**3.52***

*Significant at p<0.05

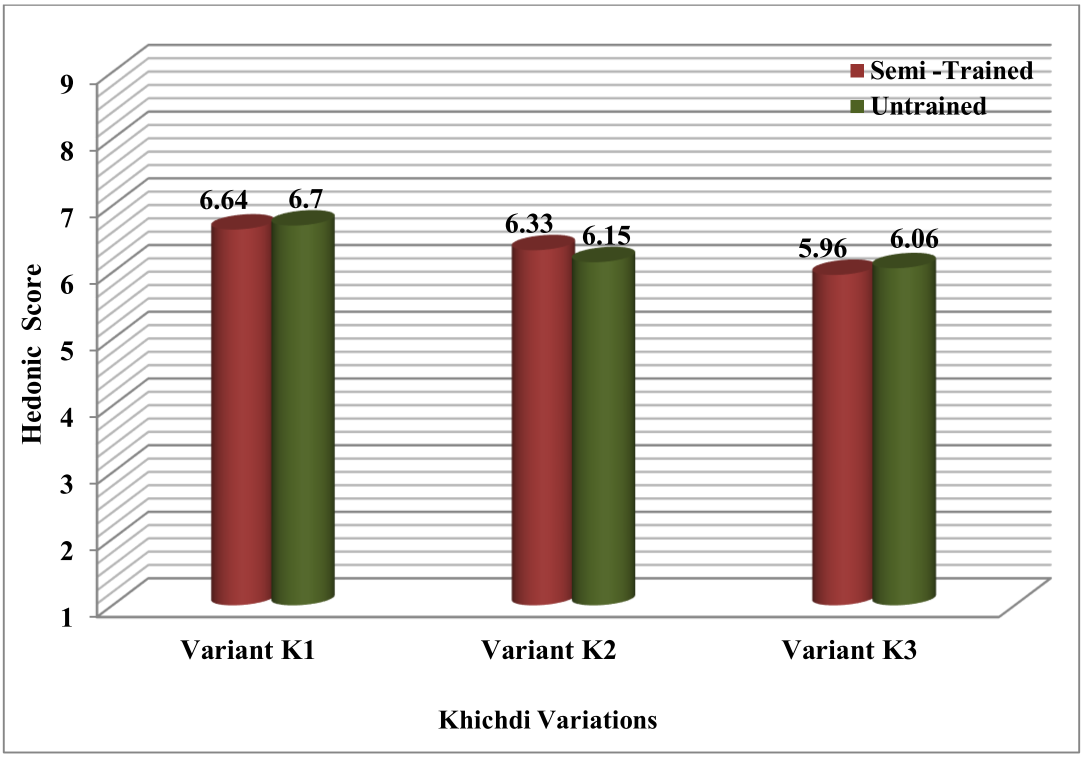


Figure 4.2.2.1: Mean hedonic scores of flaxseed variations in *khichdi* by semi-trained and untrained panelists

The composite scoring by semi-trained panelists yielded the following results which are depicted in the Table 4.2.2.2 and also in the Figure 4.2.2.2.

i) **Taste:** The taste attribute of *khichdi* significantly ($p<0.001$) reduced by 23.6% upon substitution of flaxseeds up to 20 gms. Variant K1 scored highest score of 12.7 for taste amongst all of the variants.

ii) **Appearance:** Appearance scores increased insignificantly by 4.51% as the levels of flaxseeds increased. When there was an increase in the levels of flaxseed substitution, all of the samples exhibited a pleasant dark brown tinge. The highest score for appearance was of the Variant K2 with 7.6.

iii) **Odour:** There was presence of undesirable odour with the increase of flaxseed levels at an insignificant 8.5%. Variant K1 stood as top grosser for odour with a score of 8.5.

iv) **Texture:** For the texture scores, an insignificant 10.7% was observed with increasing levels of flaxseeds in the khichdi samples. Variant K2 had the maximum score of 9.6 for this attribute.

v) **Absence of defects:** Absence of defects was another vital attribute to be considered for studying the organoleptic attributes of *khichdi*. The significant ($p<0.01$) decrease of 15.9% was found with increasing the flaxseeds substitution levels. Variant K1 fared highly with a 7.7 score.

vi) **Suitability of serving:** Suitability of serving is the ease with which the given amount of flaxseeds can be entirely consumed at a particular amount of time. A very slight insignificant reduction of 2.67% was found with increasing levels of flaxseeds in terms of the serving suitability. Variant K1 scored highly with 12.2.

vii) **Overall acceptability:** On increasing the levels of flaxseeds, overall acceptability scores of *khichdi* were largely affected with significant ($p<0.001$) reduction to 24.38% due to the significant decreasing of the organoleptic scores namely taste ($p<0.001$) and absence of defects ($p<0.01$). Variant K1 secured the highest 12.9 score for this attribute.

viii) **Total Score:** Based on the overall scores, the total scores were significantly ($p<0.05$) obtained in the highest favor for the Variant 1 and least favored acceptability scores for the Variant 3 of khichdi. Variant 1 emerged to be foremost with the total score of 70.6.

Table 4.2.2.2: Effect of variations of roasted flaxseeds on organoleptic qualities of *khichdi* using Composite scores

Variations with substitution levels	Organoleptic Attributes							
	Taste (20)	Appearance (10)	Odor (10)	Texture (15)	Absence of defects (10)	Suitability of serving (15)	Overall acceptability (20)	Total Score (100)
Variant I (K1) 10g	12.7^{a} ± 2.3	7.2^{NS} ± 1.5	8.5^{NS} ± 1.8	9.2^{NS} ± 2.1	7.7^{a} ± 1.3	12.2^{NS} ± 1.7	12.9^{a} ± 2.5	70.6^{a} ± 5.8
Variant II (K2) 15g	11.4^{a} ± 2.0	7.6^{NS} ± 1.5	8.4^{NS} ± 2.0	9.6^{NS} ± 2.6	7.0^{b} ± 1.4	11.9^{NS} ± 2.7	11.2^{b} ± 2.8	67.2^{a}± 6.7
Variant III (K3) 20g	9.7^{b} ± 1.7	7.5^{NS} ± 1.5	7.8^{NS} ± 1.7	8.3^{NS} ± 1.6	6.5^{b} ± 1.3	11.9^{NS} ± 2.3	9.8^{c} ± 1.9	61.3^{b} ± 6.1
Percent Increase/ Decrease	23.6 ↓	4.1 ↑	8.5 ↓	10.7 ↓	15.9 ↓	2.6 ↓	24.3 ↓	13.4 ↓
ANOVA	**19.56*** **	**0.54^{NS}**	**1.15^{NS}**	**2.69^{NS}**	**7.05** **	**0.17^{NS}**	**12.38*** **	**5.1***

Note – values in parenthesis indicate maximum score under each attribute, *Significant at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, NS- Non- significant,

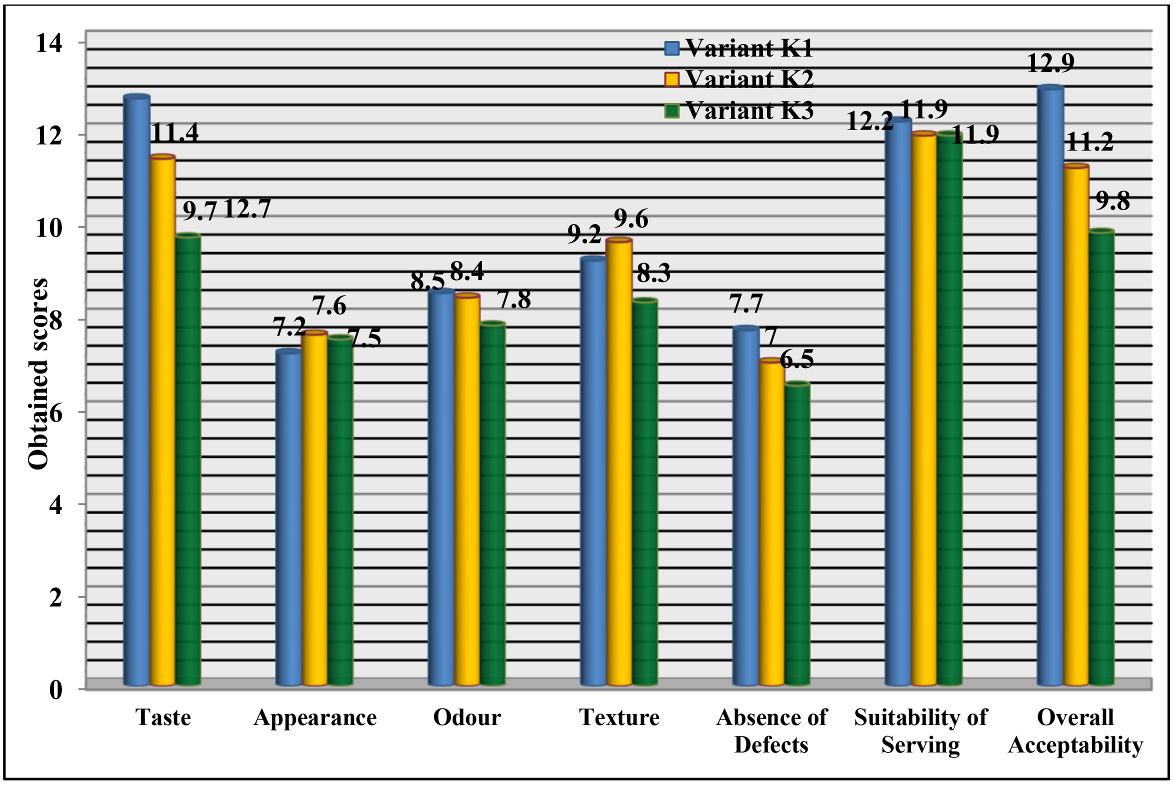


Figure 4.2.2.2: Mean composite scores of flaxseed variations in *khichdi* by semi-trained panelists

Khichdi **was well accepted with the 10 g levels of flaxseeds substitution by 66% of semi-trained panelists as adjudged by the Composite scoring test. There was a significant gradual decrease in the organoleptic attribute scores viz. taste, absence of defects and overall acceptability with the increase in the substitution levels of the flaxseeds. Moreover, Hedonic scores of 60% semi-trained and 53% untrained panelists favored towards variant with 10g of flaxseeds.**

4.2.2.2: Effect of percentage substitution of flaxseeds in *porridge* at varying levels

The results under this sub-section are presented in the Table 4.2.2.2.1 to 4.2.2.2.2 and presented graphically in Figure 4.2.2.2.1- 4.2.2.2.2.

a) Organoleptic evaluation of the *porridge*

The organoleptic scoring of *porridge* prepared by substitution of flaxseeds at varying levels is described in the Table 4.2.2.2.1. The mean hedonic scores of the semi-trained panelists depicted significant ($p<0.001$) reduction of 1.51% and insignificant 5.29% for the untrained panelists directing an increase in the scores with the increase of flaxseeds substitutions in porridge. The variant P3 resulted being superior most in terms of acceptability and variant P1 scored the least as affirmed by both the panelists (Figure 4.2.2.2.1).

Table 4.2.2.2.1: Effect of substitution of roasted flaxseeds at various levels with base material in *porridge* using mean hedonic scores of panelists

Variations	Varied flaxseeds amount in base	Hedonic score (semi trained)	Hedonic score (untrained)
Variant I (P1)	10	5.96 ± 0.85	6.23 ± 0.52
Variant II (P2)	15	6.56 ± 0.67	6.7 ± 0.87
Variant III (P3)	20	6.86 ± 1.00	6.56 ± 0.77
Percent Increase/ Decrease		15.1 ↑	5.29 ↑
ANOVA		**8.59*** **	**2.74^{NS}**

*** Significant at $p<0.001$, NS- Non- significant

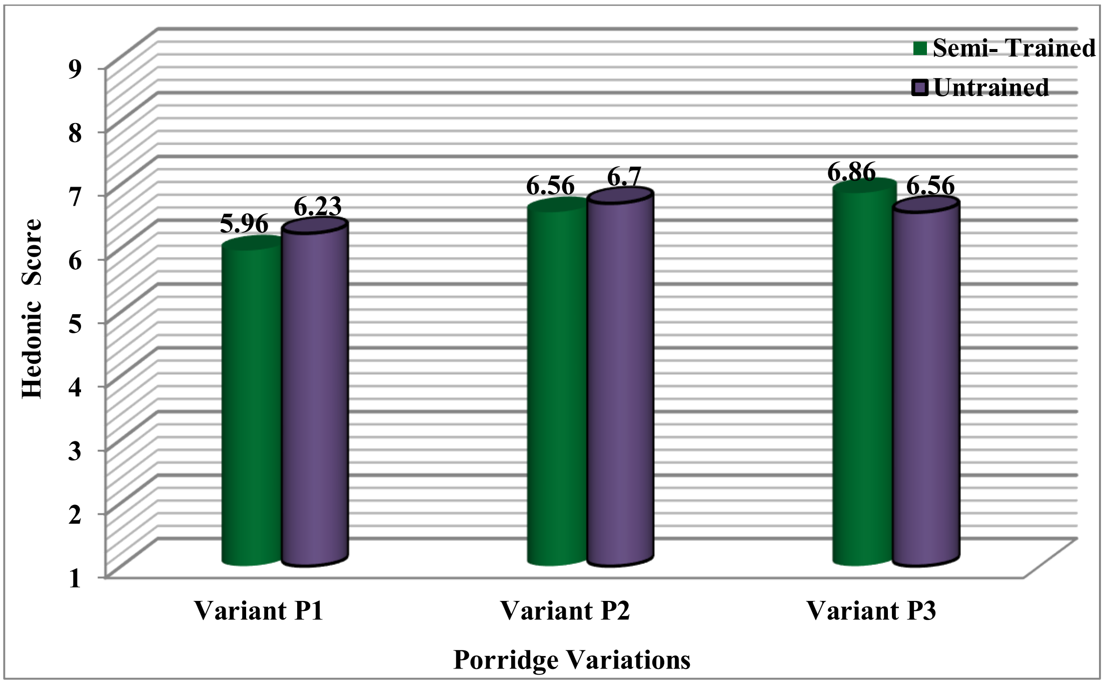


Figure 4.2.2.2.1: Mean hedonic scores of flaxseed variations in *porridge* by semi- trained and untrained panelists

The composite scoring performed by semi-trained panelists brought the following results which are presented in the Table 4.2.2.2.2 and also in the Figure 4.2.2.2.2

i) **Taste:** Significant (p<0.001) improvement by 10.56% was observed for the taste attribute of *porridge* upon substitution of flaxseeds up to 20 gms. Variant P3 scored highest with a score of 14.0 for taste amongst the entire variants (Figure 4.2.2.2.2).

ii) **Appearance:** Appearance scores decreased insignificantly by 2.9% except for the variant P2. On increasing the substitution of flaxseeds, the samples were accepted for having a pleasant dark brown tinge up to the variant P2 and receded for the variant P3. The highest score for appearance was of the variant P2 with 7.9.

iii) **Odour:** Presence of desirable odour was detected with the increase of flaxseed levels with an insignificant 3.2%. Variant P3 secured the top position for odour with a score of 8.0 (Figure 4.2.2.2.2).

iv) **Texture:** For the texture scores, an insignificant increase of 7.02% was observed with increasing levels of flaxseeds in the *porridge* samples. Variant P2 had the maximum score of 9.9 for this attribute.

v) **Absence of defects:** Absence of defects attribute of *porridge* displayed a significant (p<0.001) increase of 16.1% with increasing the flaxseeds substitution levels. Variant P3 achieved a high score of 8.0 (Figure 4.2.2.2.2).

vi) **Suitability of serving:** Suitability of serving paved for a significant (p<0.01) increase of 12.5% with increasing the substitution levels of flaxseeds. Variant P3 scored highly with 13.4 (Figure 4.2.2.2.2).

vii) **Overall acceptability:** In terms of overall acceptability, the scores of *porridge* were largely affected with significant (p<0.001) increase to 16.1% due to the significant increasing of the organoleptic scores namely taste (p<0.001), absence of defects (p<0.001) and suitability of serving (p<0.05). Variant P3 secured the highest 13.4 score for this attribute (Figure 4.2.2.2.2).

viii) **Total Score:** As far as the total score is concerned, significant (p<0.001) increase by 10.6% were obtained in the highest favor for the Variant 3 and least favored acceptability scores for the Variant 1 of *porridge*. Variant 3 emerged to be foremost with the total score of 74.2 (Figure 4.2.2.2.2).

Table 4.2.2.2.2: Effect of variations of roasted flaxseeds on organoleptic qualities of *porridge* using Composite scores

Variations with substitution levels	Organoleptic attributes							
	Taste (20)	Appearance (10)	Odor (10)	Texture (15)	Absence of defects (10)	Suitability of serving (15)	Overall Accept-ability (20)	Total Score (100)
Variant I (P1) 10g	12.1^{a} ± 1.5	7.7^{NS} ± 1.3	7.8^{NS} ± 1.2	9.0^{a} ± 1.4	6.7^{a} ± 1.4	11.7^{a} ± 1.8	11.2^{a} ± 2.6	66.4^{a} ± 3.6
Variant II (P2) 15g	13.2^{a} ± 1.6	7.9^{NS} ± 1.3	7.9^{NS} ± 1.3	9.9^{b} ± 2.4	7.3^{b} ± 1.0	12.8^{b} ± 1.9	12.4^{b} ± 2.9	72.1^{b} ± 5.0
Variant III (P3) 20g	14.0^{c} ± 2.2	7.5^{NS} ± 1.4	8.0^{NS} ± 1.2	9.7^{b} ± 2.0	8.0^{c} ± 0.9	13.4^{b} ±1.7	13.4^{b} ± 3.9	74.2^{b} ± 4.0
Percent Increase/ Decrease	13.5 ↑	2.9 ↓	3.2 ↑	7.02 ↑	16.12 ↑	12.46 ↑	16.15 ↑	10.56 ↑
ANOVA	**7.96*** **	**0.62^{NS}**	**0.32^{NS}**	**1.72^{NS}**	**9.81*** **	**7.06** **	**9.13*** **	**7.7*** **

*Significant from the baseline value at p<0.05, ** at p<0.01, *** at p<0.001, NS- Non- significant

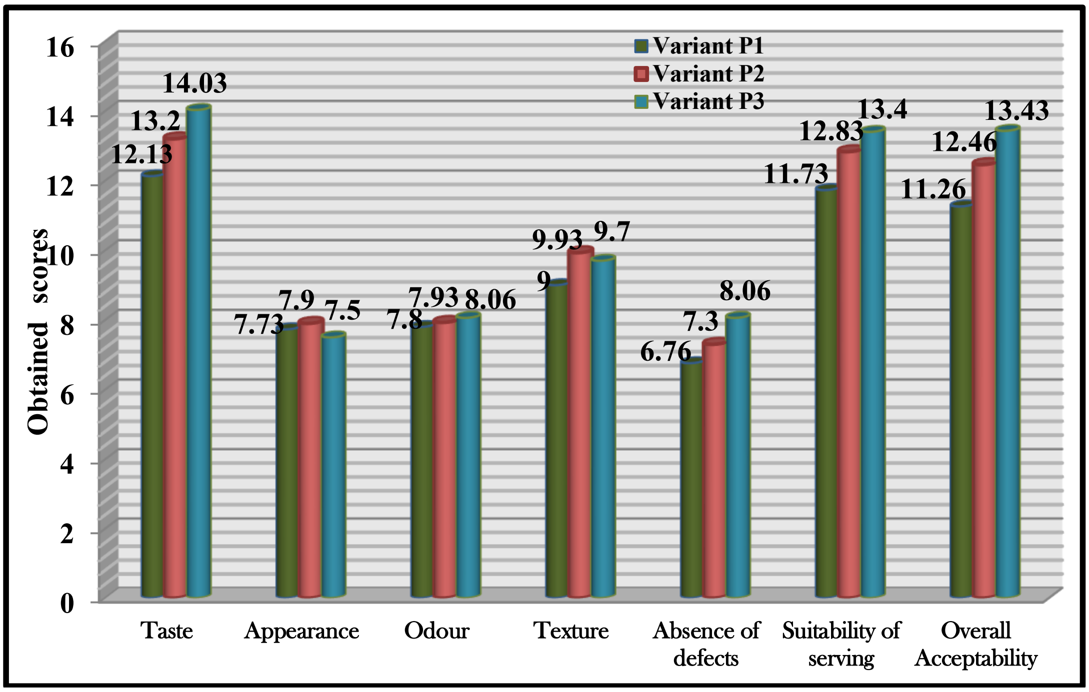


Figure 4.2.2.2.2: Mean composite scores of flaxseed variations in *porridge* by semi-trained panelists

***Porridge* was well accepted with the 20 g levels of flaxseeds substitution. With the increase in the substitution levels of the flaxseeds, there was a significant increase confirmed from the collective attributes of the Composite scoring test. A majority (57%) of semi trained panelists preferred variant III with 20% of flaxseeds over other variants. As per Hedonic scores, maximum number (66.6%) of semi trained panelists gave variant P3 as their first preference. However for 46.6% untrained panelists variant P2 was the first choice.**

4.2.2.3: Effect of percentage substitution of flaxseeds in *globs* (*laddoo)* at varying levels

The results under this sub-section are presented in the Table 4.2.2.3.1 to 4.2.2.3.2 and presented graphically in Figure 4.2.2.3.1 - 4.2.2.3.2.

a) Organoleptic evaluation of the *globs*

The organoleptic scoring of *globs* prepared by substitution of flaxseeds at varying levels is described in the Table 4.2.2.3.1. The mean hedonic scores of the semi-trained and untrained panelists remained significantly ($p<0.01$) indicative of the rise by 18.2% and 13.5% respectively as the flaxseeds substitution levels in the *globs* soared. The variant G3 resulted being superior most in terms of acceptability and variant G1 scored the least as affirmed by both the panelists (Figure 4.2.2.3.1).

Table 4.2.2.3.1: Effect of substitution of roasted flaxseeds at various levels with base material in *globs (laddoo)* using mean hedonic scores of panelists

Variations	Varied flaxseeds amount in base	Jaggery	Hedonic score (semi trained)	Hedonic score (untrained)
Variant I (G1)	10	15	6.2 ± 0.92	6.87 ± 0.63
Variant II (G2)	15	15	6.93 ± 0.98	7.67 ± 0.96
Variant III (G3)	20	15	7.33 ± 0.92	7.8 ± 0.89
Percent Increase/ Decrease			18.22 ↑	13.53 ↑
ANOVA			**11.15****	**10.91****

** F value significant at $p < 0.01$ level

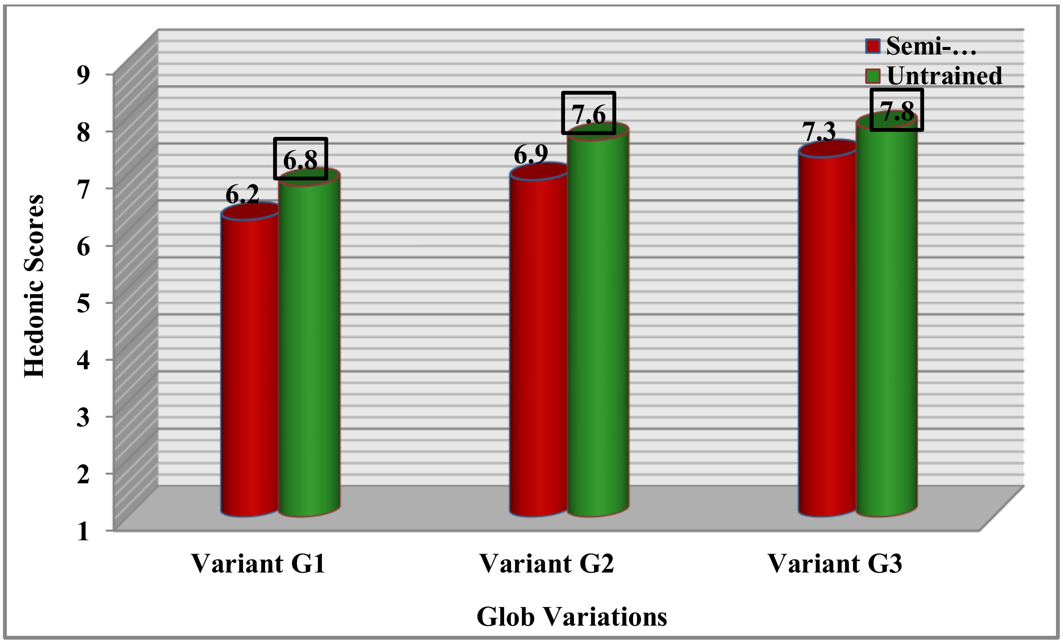


Figure 4.2.2.3.1: Mean hedonic scores of flaxseed variations in *globs* by semi-trained and untrained panelists

The composite scoring performed by semi-trained panelists expounded the following results which are presented in the Table 4.2.2.3.2 and also in the Figure 4.2.2.3.2.

i) **Taste:** The taste attribute of *globs* showed insignificant improvement by 7.4% upon increasing the substitution levels of flaxseeds. Variant G3 scored highest with a score of 15.5 for taste amongst the entire variants (Figure 4.2.2.3.2).

ii) **Appearance:** Appearance scores increased insignificantly by 5.1% for all of the variants. On increasing the substitution of flaxseeds, the samples were accepted for having a pleasant dark brown tinge up to the variant G3. The highest score for appearance was of the variant G3 with 8.8.

iii) **Odour:** Presence of desirable odour was detected with the increase of flaxseed levels with an insignificant 2.7%. Variant G3 secured the top position for odour with a score of 8.8 (Figure 4.2.2.3.2).

iv) **Texture:** For the texture scores, an insignificant increase of 2.9% was observed with increasing levels of flaxseeds in the *globs* samples. Variant G3 had the maximum score of 12.1 for this attribute.

v) **Absence of defects:** Absence of defects attribute of *globs* displayed an insignificant increase of 12.6% with increasing the flaxseeds substitution levels. Variant G2 and G3 achieved a high score of 8.2 each (Figure 4.2.2.3.2).

vi) **Suitability of serving:** Suitability of serving resulted for an insignificant increase of 3.5% with increasing the substitution levels of flaxseeds. Variant G2 scored very slightly high with 11.8 in comparison to G3 (Figure 4.2.2.3.2).

vii) **Overall acceptability:** In terms of overall acceptability, the scores of *globs* were largely affected with insignificant increase to 11.2% due to increase in all of the organoleptic scores. Variant G3 secured the highest 16.1 score for this attribute (Figure 4.2.2.3.2).

viii) **Total Score:** As far as the total score is concerned, an insignificant increase by 6.9% too was obtained in the highest favour for the Variant 3 and least favoured acceptability scores for the Variant 1 of *globs*. Variant 3 emerged to be foremost in acceptability with the total score of 81.1 (Figure 4.2.2.3.2).

Table 4.2.2.3.2: Effect of variations of roasted flaxseeds on organoleptic qualities of *globs* using mean composite scores

Variations with substitution levels	Organoleptic attributes							
	Taste (20)	Appearance (10)	Odor (10)	Texture (15)	AD (10)	SS (15)	OA (20)	Total Score (100)
Variant I (G1) 10g	14.4 NS ± 3.3	8.3 NS ± 1.3	8.6 NS ± 1.4	11.7 NS ± 1.9	7.2 NS ± 2.0	11.0 NS ± 2.5	14.4 NS ± 3.1	75.8 NS ± 8.8
Variant II (G2) 15g	15.4 NS ± 2.9	8.2 NS ± 1.4	8.3 NS ± 1.5	11.8 NS ± 2.2	8.2 NS ± 1.6	11.8 NS ± 2.1	15.9 NS ± 2.8	79.7 NS ± 9.8
Variant III (G3) 20g	15.5 NS ± 3.3	8.8 NS ± 1.6	8.8 NS ± 1.8	12.1 NS ± 2.1	8.2 NS ± 2.2	11.4 NS ± 2.7	16.1 NS ± 2.9	81.1 NS ± 8.2
Percent Increase/ Decrease	7.4 ↑	5.1 ↑	2.7 ↑	2.9 ↑	12.6 ↑	3.5 ↑	11.2 ↑	6.9 ↑
ANOVA	**1.02** NS	**1.34** NS	**0.82** NS	**0.25** NS	**2.65** NS	**0.77** NS	**2.99** NS	**0.96** NS

AD- Absence of defects, SS- Suitability of serving, OA- Overalll acceptability
*Significant from the baseline value at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, NS- Non- significant

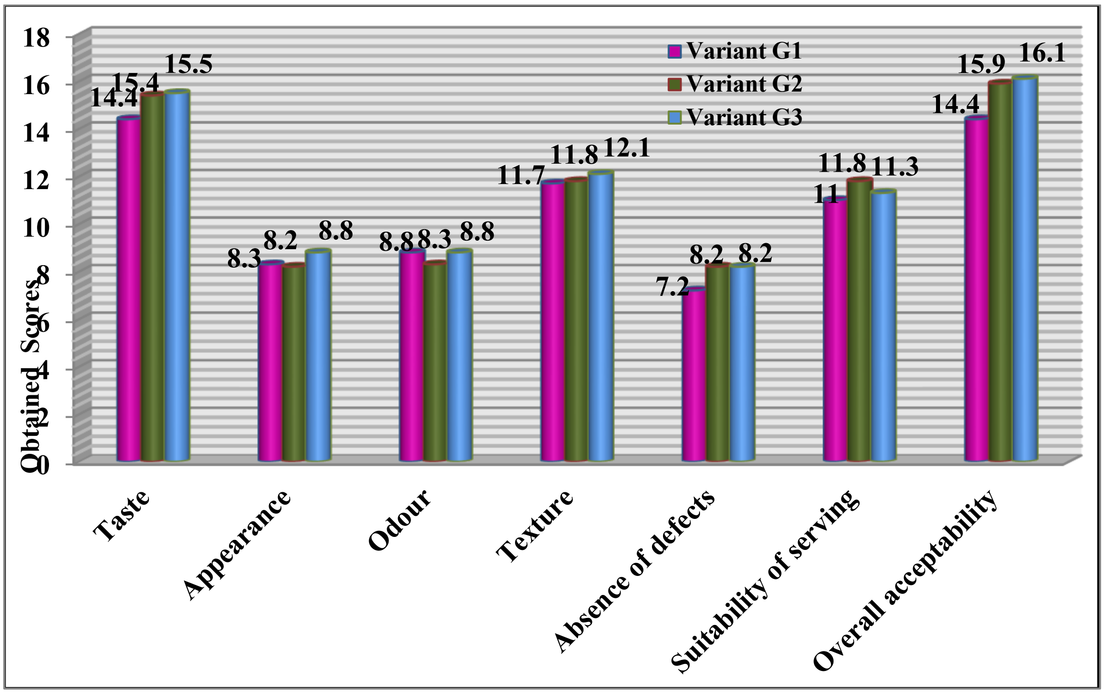


Figure 4.2.2.3.2: Mean composite scores of flaxseed variations in *globs* by semi-trained panelists

***Globs* were well accepted with the 20 g levels of flaxseeds substitution. An insignificant increase was observed from the Composite scoring test results on increasing the substitution levels of the flaxseeds. 50% of semi trained panelists preferred variant III over other variants on consideration of all the attributes simultaneously. As per Hedonic scores, maximum number (53%) of semi trained panelists preferred variant G3 over others and 47% of untrained panelists liked variant G3 the most.**

Section 4.2.2.4: Effect of 20g substitution levels of flaxseeds in *mukhwaas*

The results under this sub-section are presented in the Table 4.2.2.4.1 to 4.2.2.4.2 and presented graphically in Figure 4.2.2.4.1.

a) Organoleptic evaluation of the *mukhwaas*

The organoleptic scoring of mukhwaas substitution of flaxseeds at 20 g levels is described in the Table 4.2.2.4.1. Flaxseeds mukhwaas (20 g) depicted significance ($p<0.05$) in terms of the mean hedonic scores of semi-trained and untrained panelists.

Table 4.2.2.4.1 composite scoring of variant M1 resulted being significantly superior in terms of texture ($p<0.001$), absence of defects ($p<0.05$), overall acceptability ($p<0.05$) and total score ($p<0.05$) as confirmed by both the panelists. The rise in acceptability scores of the 20 g flaxseeds substitution levels is indicative (Figure 4.2.2.4.1).

The composite scoring performed by semi-trained panelists brought about the following results which are presented in the Table 4.2.2.4.2 and also in the Figure 4.2.2.4.1.

i) **Taste:** The taste attribute of *globs* showed insignificant improvement by 7.4% upon increasing the substitution levels of flaxseeds. Variant G3 scored highest with a score of 15.5 for taste amongst the entire variants (Figure 4.2.2.4.1).

ii) **Appearance:** Appearance scores increased insignificantly by 5.1% for all of the variants. On increasing the substitution of flaxseeds, the samples were accepted for having a pleasant dark brown tinge up to the variant G3. The highest score for appearance was of the variant G3 with 8.8.

iii) **Odour:** Presence of desirable odour was detected with the increase of flaxseed levels with an insignificant 2.7%. Variant G3 secured the top position for odour with a score of 8.8 (Figure 4.2.2.4.1).

iv) **Texture:** For the texture scores, an insignificant increase of 2.9% was observed with increasing levels of flaxseeds in the *globs* samples. Variant G3 had the maximum score of 12.1 for this attribute.

v) **Absence of defects:** Absence of defects attribute of *globs* displayed an insignificant increase of 12.6% with increasing the flaxseeds substitution levels. Variant G2 and G3 achieved a high score of 8.2 each (Figure 4.2.2.4.1).

vi) **Suitability of serving:** Suitability of serving resulted for an insignificant increase of 3.5% with increasing the substitution levels of flaxseeds. Variant G2 scored very slightly high with 11.8 in comparison to G3 (4.2.2.4.1).

vii) **Overall acceptability:** In terms of overall acceptability, the scores of *globs* were largely affected with insignificant increase to 11.2% due to increase in all of the organoleptic scores. Variant G3 secured the highest 16.1 score for this attribute (Figure 4.2.2.4.1).

viii) **Total Score:** As far as the total score is concerned, an insignificant increase by 6.9% too was obtained in the highest favour for the Variant 3 and least favoured acceptability scores for the Variant 1 of *globs*. Variant 3 emerged to be foremost in acceptability with the total score of 81.1 (Figure 4.2.2.4.1).

Table 4.2.2.4.1: Effect of 20g roasted flaxseeds mukhwaas using mean hedonic scores of panelists

Assessors	Flaxseed (gms)	Hedonic score
Semi Trained panelists	20	7.4 ± 0.85
Untrained panelists	20	8.3 ± 0.88
***t*-test**		**2.42***

*Significant at $p<0.05$

Table 4.2.2.4.2: Effect on organoleptic qualities of 20g roasted flaxseeds mukhwaas using mean Composite scores

Variations	Taste (20)	Appearance (10)	Odor (10)	Texture (15)	AD (10)	SS (15)	OA (20)	TS (100)
Semi-trained Variant M1	15.63 ± 4.21	8.23± 1.28	8.6 ± 1.6	13.3± .24	8.17± .78	12.93± 2.04	17.13± 2.95	84.16 ± 12.35
Untrained Variant M2	16.46 ± 3.47	7.76± 1.98	7.96±2 .16	11± 2.02	9.23± 1.5	12.6± 2.5	16.53± 2.87	81.56 ± 10.68
***t* test**	**0.92**^{NS}	**0.69**^{NS}	**0.78**^{NS}	**4.12*****	**1.69***	**0.89**^{NS}	**1.17***	**1.4***

AD- Absence of defects, SS- Suitability of serving, OA- Overalll acceptability, TS-Total Score
*Significant from the baseline value at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, NS- Non- significant

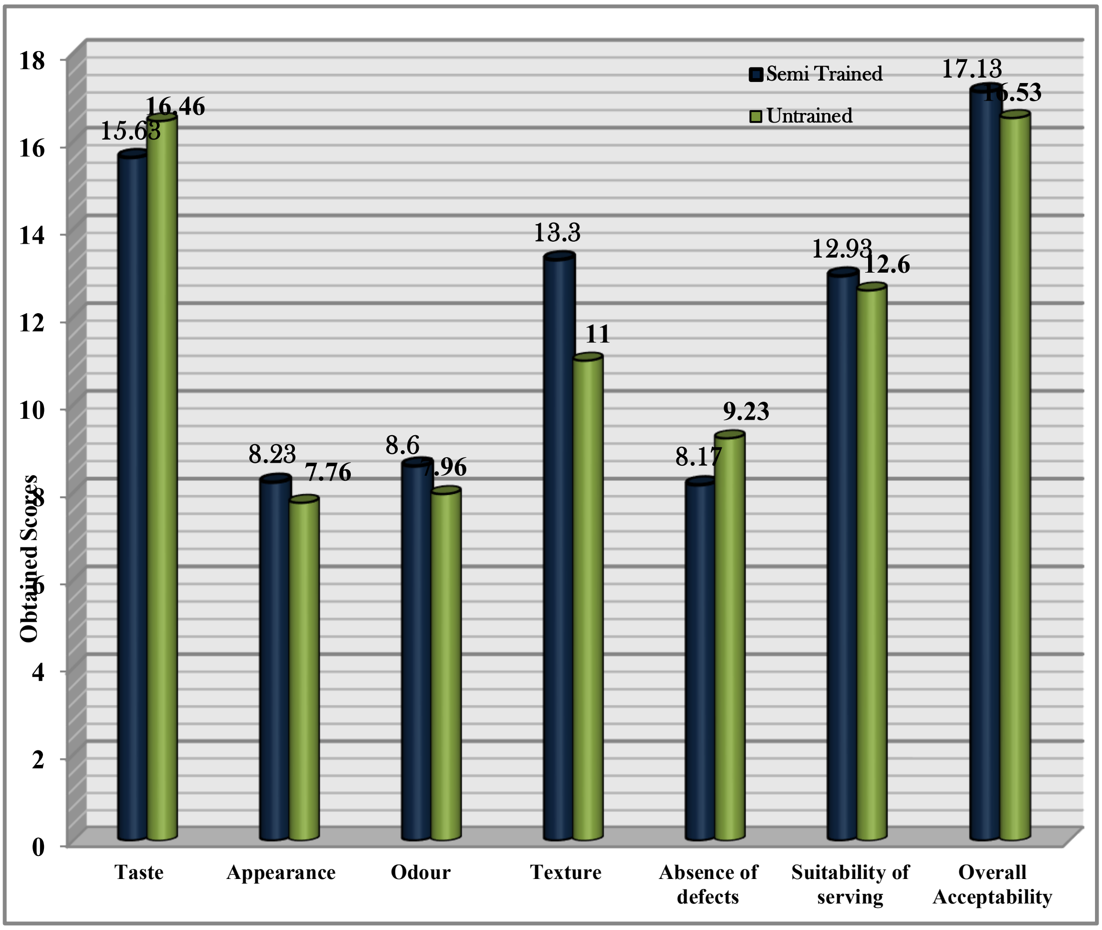


Figure 4.2.2.4.1: Mean composite scores of 20g flaxseed *mukhwaas* (M1) by semi-trained and untrained panelists

Variant M1 of *Mukhwaas* significantly scored in the well accepted limits having the 20 g levels of flaxseeds substitution. A significant difference in the attributes viz. texture, absence of defects, overall acceptability and total score was perceived from the Composite scoring test results of 20 g flaxseeds mukhwaas indicative of its suitability as a preferred food product by the acceptability scores of semi-trained and untrained panelists in comparison to rest of the food formulations. Hence, confirming the 20 g flaxseeds *mukhwaas* (M1) to be the end determination point for intervention.

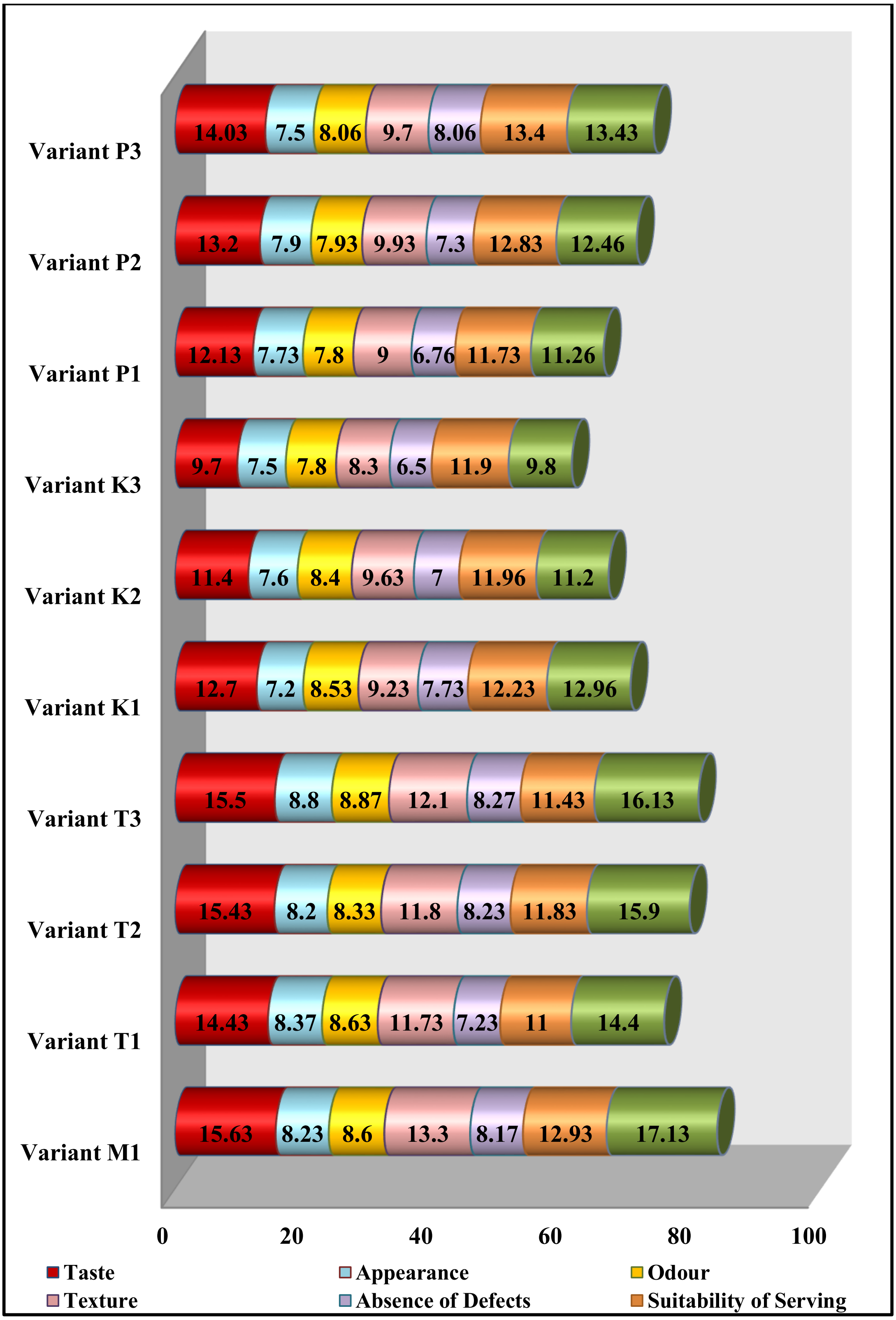


Figure 4.2.2.5: Score card of mean composite scores of overall formulated flaxseed variations assessed by semi-trained panelists

RESULT HIGHLIGHTS

- ***Mukhwaas significantly scored in the well accepted limits having the 20 g levels of flaxseeds substitution.***
- ***A significant difference in the attributes viz. texture, absence of defects, overall acceptability and total score was perceived from the Composite scoring test results of 20 g flaxseeds mukhwaas indicative of its suitability as a preferred food product by the acceptability scores of semi-trained and untrained panelists in comparison to rest of the food formulations.***
- ***Globs were well accepted with the 20 g levels of flaxseeds substitution. 50% of semi trained panelists preferred variant III over other variants on consideration of all the attributes simultaneously. As per Hedonic scores, maximum number (53%) of semi trained panelists preferred variant G3 over others and 47% of untrained panelists liked variant G3 the most.***
- ***Porridge was well accepted with the 20 g levels of flaxseeds substitution. A majority (57%) of semi trained panelists preferred variant III with 20% of flaxseeds over other variants. As per Hedonic scores, maximum number (66.6%) of semi trained panelists gave variant P3 as their first preference. However for 46.6% untrained panelists variant P2 was the first choice.***
- ***Khichdi was well accepted with the 10 g levels of flaxseeds substitution by 66% of semi-trained panelists as adjudged by the Composite scoring test. There was a significant gradual decrease in the organoleptic attribute scores viz. taste, absence of defects and overall acceptability with the increase in the substitution levels of the flaxseeds. Moreover, Hedonic scores of 60% semi-trained and 53% untrained panelists favoured towards variant with 10g of flaxseeds.***

DISCUSSION

The present phase was conducted with the prime objective to analyse the proximate values of raw versus roasted flaxseeds and examine the effect of varying substitution levels of roasted flaxseeds pertaining to the organoleptic characteristics of various food products viz. *Khichdi*, *Porridge, Laddoo* and *Mukhwaas.*In our study, the roasted form of flaxseeds were estimated to be having 33.2 g per 100g of fat and 18.6 g of ALA per 100 g.There is a severe shortage of studies reporting about ALA content in the Indian flaxseeds variety. Meanwhile, few parallel studies in relation to our findings reveal the beneficial traits of roasting of flaxseeds and thereby reducing the threat of cyanogenic compounds. According to Morris (2007), flaxseeds are a renewable and rich source of ALA. Using flax in food preparation, whether at home or in commercial settings increases the omega-3 fat content of popular foods. Further it was stated that one tablespoon (8 g) of milled flax contains 1.8 g of ALA. In an experimental study, the raw flaxseed meal due to significant amount of anti-nutritional compounds, lipid and α-linolenic acid content was tested using the extrusion processing at high barrel exit temperature (140°C).Significant reduction was found in the the cyanogenic compounds (84 %), tannin (73%) and mucilage (27%) in the flaxseed meal (Imran et al 2015).

The roasted flaxseeds incorporated *khichdi* showed significant gradual decrease in the specific organoleptic attribute scores namely taste, absence of defects and overall acceptability with the increase in the substitution levels of the flaxseeds. Similar study depicted that a declining trend was examined in cookies having up to 15% flaxseed flour incorporation. The thickness and diameter of cookies increased as flaxseed flour was increased which restricted the spread of cookies. In another study, the hardness and fracturability of experimental cookies increased with this amount (Ganorkar and Jain 2014). An experimental study using roasted and ground flaxseeds showed marginal decrease in the cookie baking test but substitution beyond 15% flaxseeds had an adverse effect on the texture and flavour of the cookies (Rajiv et al 2012). Furthermore, the findings similar to that stated in our study could be found from the numerous experimental studies assessing the sensory quality of the bread enriched with flaxseed demonstrating a bitter and reduced sweetness and a lower overall flavor score (Alpaslan and Hayta 2006; Aliani et al 2012; Bartkiene et al 2012). There is uncertainty regarding the level of flaxseed enrichment in bread products at which changes in the sensory properties become significant. Differences in the methodology of the sensory analysis could contribute to this heterogeneity. Methodologies of sensory evaluation differ in terms of the number, training, age, origin, and recruitment procedure of the panel, all of which can affect the results of the analysis. Sensory evaluations can also be affected by the type, freshness, and processing history of the bread and the flaxseed ingredient (Mercier et al 2014). For descriptive sensory evaluations, Hussain et al (2011) evaluated 9 quality parameters of pan bread enriched with 0%, 4%, 8%, 12%, 16%, and 20% of full-fat or partially defatted ground flaxseed by a trained panel of 6 judges. The detrimental effect of flaxseed for most quality attributes was significant at or above 4% enrichment level. In contrast, Conforti and Davis (2006) reported no significant effect for the aroma, aftertaste, and softness of white bread with 15% flaxseed flour enrichment, while Koca and Anil (2007) reported no significant effect for the flavor, texture, and color of white bread with 20% flaxseed enrichment. Other studies have provided mixed results. Alpaslan and Hayta (2006) evaluated the consumer acceptance of bagels enriched with 0%, 5%, 10%, and 15% ground flaxseed with a panel of 8 trained judges. At 5% flaxseed enrichment, the scores for enriched breads were similar to the control bread, but at 10% enrichment, a negative effect for the flavor, mouthfeel, and crumb appearance was reported. Studies on the consumer perception of functional foods indicated that the likelihood of consumption is inversely related to the presence of off-flavor and that consumers are generally unwilling to compromise on the taste of the foods for health benefits (Tuorila and Cardello 2002; Verbeke 2006). These results elaborate that flaxseed-enriched products need to be developed with similar sensory attributes and consumer acceptance than products without flaxseeds to ensure their commercial success (Mercier et al 2014).

In the present study, with the increase in the substitution levels of the flaxseeds in *porridge*, there was a significant increase confirmed from the collective organoleptic attributes. In the study conducted by Roozegar et al (2015) coated and uncoated ground flaxseed were added to wheat flour in 5, 15 and 25 % levels in order to produce fortified Taftoon bread. Rheological, physical and organoleptic tests showed that with increasing coated and uncoated ground flaxseed percentages, a decrease in water absorption and an increase in stability, dough development and relaxation time of dough occurred. The highest dough development and dough stability time were observed by adding 25 % coated ground flaxseed with Arabic gum. Results indicated that coated and uncoated ground flaxseed had a good effect on decreasing the staling rate compared to the control bread. Results of organoleptic test showed that bread with 5 and 15 % coated and uncoated ground flaxseeds had better acceptability.

Similar observations were noted in the study where the scores assigned to all the sensory parameters of breads affected significantly with the 10 and 15% replacements of both flaxseed flours on variation in levels of flaxseed fortified in wheat flours resulting in an acceptable product (Mervat et al 2005). In other instance, the acceptance of flaxseed as a dietary functional food ingredient in cakes assessed at structured nine point hedonic scale revealed consumer acceptance up to 30% supplementation level (Moraes et al 2010). Meanwhile, the organoleptically acceptable cookies were prepared by supplementing 20% flax in foods as an ingredient (Hussain et al 2006).

In the undertaken study the globs were well accepted with the 20 g levels of flaxseeds substitution. The overall scores in terms of taste, appearance, texture, odour, texture, absence of defects and overall acceptability were slightly affected increase on increasing the substitution levels of the flaxseeds. The findings from a research based study demonstrated that flaxseeds at 30–50% substitution in flour greatly enhanced the nutritional qualities of some nutrients without affecting the overall acceptability of bakery products. The sensory scores of breads containing 30% and muffins containing 50% flaxseed flour were acceptable. The linolenic acid, fiber and folate contents were improved by 28 g, 16 g and 387 mg dietary folate equivalents (DFE), respectively in 30% flaxseed bread compared to control. In 50% flaxseed muffins compared to control, α-linolenic acid, fiber and folate contents were improved by 21 g, 17 g and 341-mg DFE, respectively (Elina and Vijay 2009). Susheelamma (1989) studied the polysaccharide preparations from raw and roasted flaxseed in the preparation of idly type steamed puddings and found that the porous texture of the pudding was stabilized satisfactorily by the polysaccharides, which can satisfactorily replace the black gram polysaccharide. Oomah and Mazza (1993) too concluded that flaxseed can be incorporated in a wide range of potential products.

At the same time the available literature reported on the development and standardization of several food adjuncts namely traditional chutneys instant chutneys and chutney powders based on the various raw materials available during different seasons (Balaswamy et al. 2005; Satyanarayana et al. 2001; Balaswamy et al. 2004; Prabhakara Rao et al. 2005; Jyothirmayi et al. 2006; Narsing Rao et al. 2008). Interestingly, there was no literature available on utilization of flaxseed in such food adjuncts. Therefore, it was a prerogative in the present study to focus on roasted flaxseeds incorporation in the food products and then evaluate them on the basis of the organoleptic characteristics.

Variant M1 of *Mukhwaas* significantly scored in the highly acceptable limits having the 20 g levels of flaxseeds substitution. A significant difference altogether in the attributes viz. texture, absence of defects, overall acceptability and total score was perceived from the sensory function tests Composite scoring test results indicate the suitability of 20 g flaxseeds mukhwaas as a preferred food product by the acceptability scores of semi-trained and untrained panelists in comparison to rest of the food formulations. Hence, confirming the 20 g flaxseeds *mukhwaas* (M1) to be the end determination point for intervention. In line with our findings few studies depicted that addition of flaxseed flour at the level of 15% was good and at higher levels up to 25% it was found satisfactory in bread (Mente 2008). An optimum number of the sensory evaluation studies have determined the overall organoleptic quality of roasted flaxseeds. The 20 g flaxseed ladoo were accepted at the higher levels with a total scoring of 85 on the hedonic scale by the semi-trained and untrained panellists (Chauhan and Kansara 2012). Prabhakara Rao et al (2013) evaluated the overall organoleptic quality of flaxseed chutney powder having the standardized recipe consisting of roasted and ground flaxseed powder and other ingredients in the ratio of 50: 50 which yielded a highly acceptable product. In addition to this, the sensory quality on storage as analysed from the hedonic scores on 0 day was 8.4 (excellent), and after 6 months the score was 7.4 (good).

CONCLUDING REMARKS

Roasting profoundly enhanced the ALA content to 56% as compared to 37% of the raw form. Roasted flaxseeds can be effectively incorporated in the four food formulations undertaken in this study. Noteworthy feature was the well acceptability of porridge, globs and mukhwaas at the higher (20g) substitution levels in these formulations. Owing to the rich ALA content in flaxseeds emerging as a beneficial key for preventing neurological disorders , it may thus be recommended for consumption on regular basis in any of the developed forms for the geriatrics.

PHASE III: Intervention and impact evaluation of the MCI elderly group with Vitamin B12 and Omega -3 fatty acids supplementation.

Mild Cognitive Impairment is a really silent and complex neurological disorder that presents an array of biochemical and dietary abnormalities, a heterogeneous clinical picture often associated with a genetic linkage. The prolonged impaired state going undetected generates multiple pathological conditions subsequently resulting in macro-vascular aberrations. Thus, it can be stated that improvement of the cognition levels eventually retards the associated complications. In the current scenario, the nutraceutical as well as therapeutic doses can work wonders for enhancing cognitive health.

The methylcobalamin injectable doses are the only efficient means for enhancing the vitamin B12 levels in the geriatrics as it's readily absorbed with no losses in relation to their inconsistent dietary counterparts. Flaxseeds are functional foods of prime concern being the rich source of alpha-linolenic acid (ALA), possessing anti-cholesterolemic and anti-glycemic properties. With this as the background, the present study was devised to observe the effects of vitamin B12 and flaxseeds supplementation on the improvement of cognition, vitamin B12, and control of lipemic as well as glycemic control in the MCI patients.

The results falling under this phase are represented under the following sub-sections:

- 4.3.1 Anthropometric and Biophysical profile of the patients before and after the supplementation trial.
- 4.3.2 Nutrient intake of the patients
- 4.3.3 Glycemic response of the mild cognitively impaired subjects before and after the vitamin B12 and flaxseed supplementation.
- 4.3.4 Lipemic response of the patients
- 4.3.5 Atherogenic indices of the patients before and after both the supplementations.
- 4.3.6 Serum vitamin B12 and Cell Blood Counts (CBC) before and after the vitamin B12 and flaxseed supplementation.

4.3.7 Neuropsychological test battery of the MCI patients conducted pre and post supplementation.

4.3.8 Relationship between the neuropsychological test battery, serum Vitamin B12, glycemic response and serum lipid parameters of the MCI patients

4.3.9 Associations between serum vitamin B12, cell blood counts and lipemic responses with the anthropometric, biophysical, biochemical and dietary parameters.

4.3.1 Anthropometric and Biophysical profile of the patients before and after the supplementation trial.

Insignificant odds in the number of hypertensive patients were observed between the groups of baseline subjects with MCI patients (Table 4.3.1.1).

Table 4.3.1.1: Odds of hypertension between baseline and MCI subjects

Status	Baseline subjects (N= 402)	MCI patients (N= 120)	Odds ratio	p-value
Normal Blood Pressure	26	6	**1.92**	**0.152**^{NS}
Hypertension	226	114		

NS- Non- significant

Results indicated that the baseline subjects had 3.71 times more chances to have normal BMI as compared to MCI patients. Significance in number of abnormal BMI patients was observed between the groups of baseline subjects with MCI patients (Table 4.3.1.2).

Table 4.3.1.2: Odds of abnormal BMI values between baseline and MCI subjects

Status	Baseline subjects (N= 402)	MCI patients (N= 120)	Odds ratio	*p*-value
Normal BMI	143	26	**3.71**	**0.00067*****
Abnormal BMI	139	94		

*** Significant from the baseline value at p<0.001

As depicted in the Table 4.3.1.3 an insignificant difference was found between both the experimental Group 1 and Group 2 for the anthropometric and biophysical measurements. On the other hand, a significant reduction ($p<0.001$) in the BMI, waist circumference, hip circumference, systolic and diastolic blood pressure except WHR remaining insignificantly different was observed within both the Group 1 and Group 2 MCI patients.

Table 4.3.1.3: Anthropometric and Biophysical profile of MCI subjects before and after supplementation

Parameters		Group1(B12) (n=60)	Group2 (Flaxseeds+B12) (n=60)	*t*-test
BMI (kg/m²)	**Pre**	25.98 ± 5.33	26.42 ± 4.52	**0.03**^{NS}
	Post	25.08 ± 4.77	25.52 ± 4.41	
	% difference	3.46 ↓	3.78 ↓	
	Paired *t* test	**6.40*** **	**4.85*** **	
WC (cm)	**Pre**	92.50 ± 10.09	94.34 ± 8.95	**0.78**^{NS}
	Post	90.24 ± 9.00	91.41 ± 9.94	
	% difference	2.44 ↓	3.10 ↓	
	Paired *t* test	**4.40*** **	**4.23*** **	
HC (cm)	**Pre**	99.63 ± 9.87	99.17 ± 9.03	**1.15**^{NS}
	Post	96.75 ± 9.19	96.93 ± 8.82	
	% difference	2.89 ↓	2.25 ↓	
	Paired *t* test	**7.12*** **	**5.98*** **	
WHR	**Pre**	0.93 ± 0.075	0.95 ± 0.068	**1.77**^{NS}
	Post	0.93 ± 0.073	0.94 ± 0.069	
	% difference	0	1.05 ↓	
	Paired *t* test	**0.97**^{NS}	**1.49**^{NS}	
Systolic BP (mmHg)	**Pre**	138.96 ± 17.2	137.93 ± 15.0	**0.38**^{NS}
	Post	130.54 ± 14.4	129.31 ± 12.6	
	% difference	6.05 ↓	6.24 ↓	
	Paired *t* test	**6.12*** **	**5.48*** **	
Diastolic BP (mmHg)	**Pre**	85.78 ± 10.90	84.70 ± 9.46	**0.47**^{NS}
	Post	78.8 ± 8.71	79.37 ± 9.52	
	% difference	8.13 ↓	6.29 ↓	
	Paired *t* test	**6.17*** **	**6.26*** **	

*Significant from the baseline value at $p<0.05$, ** Significant from the baseline value at $p<0.01$,
*** Significant from the baseline value at $p<0.001$, NS- Non- significant

Further, when gender and age-wise comparisons were made, the BMI reduction of Group 1 patients came to be 3.42% in males, 3.57% females, 4.16% young-old and

1.14% old-old categories respectively. Waist circumference reduced by 2.86% in males, 2.21% in females, 2.30% in young-old and 2.80% in old-old. The positive significant shift of the supplementation was encountered from the improved systolic blood pressure being reduced by 8.71% in males (p<0.001), 7.62% in females (p<0.001), 8.28% for young-old (p<0.001) and 7.75% for old-old (p<0.05) (Table 4.3.1.4).

Table 4.3.1.4: Anthropometric and Biophysical profile of MCI subjects before and after supplementation in Group1 (B12)

Parameter		Group 1 Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
BMI (kg/m^2)	**Pre**	25.9±5.3	25.1±5.2	26.9±5.5	27.2±5.0	22.8±4.7
	Post	25.1±4.8	24.2±4.9	25.2±4.7	26.0±4.6	22.5±4.1
	% difference	3.46 ↓	3.42 ↓	3.57 ↓	4.16 ↓	1.14 ↓
	Paired *t* test	**6.40*** **	**4.72*** **	**4.39*** **	**7.44*** **	**0.96^{NS}**
WC (cm)	**Pre**	92.5±10.1	93.6±10.1	91.6±10.3	93.1±10.1	90.8±10.1
	Post	90.2±9.0	90.9±9.6	89.5±8.6	90.9±8.5	88.2±10.2
	% difference	2.44 ↓	2.86 ↓	2.21 ↓	2.30 ↓	2.80 ↓
	Paired *t* test	**4.40*** **	**4.22*** **	**2.53***	**3.61*** **	**2.45***
HC (cm)	**Pre**	99.6±9.9	96.6±9.7	102.5±10.9	101.6±9.6	94.1±8.6
	Post	96.8±9.2	94.5±8.1	98.9±9.8	98.5±9.1	91.9±7.7
	% difference	2.89 ↓	2.18 ↓	3.50 ↓	3.07 ↓	2.35 ↓
	Paired *t* test	**7.12*** **	**4.79*** **	**5.46*** **	**6.35*** **	**3.22** **
WHR	**Pre**	0.93±0.08	0.96±0.06	0.89±0.07	0.91±0.07	0.96±0.07
	Post	0.93±0.07	0.96±0.06	0.90±0.08	0.92±0.07	0.96±0.08
	% difference	0	0	1.11 ↑	1.09 ↑	0
	Paired *t* test	**0.97^{NS}**	**0.83^{NS}**	**2.03^{NS}**	**1.42^{NS}**	**0.52^{NS}**
Systolic BP (mmHg)	**Pre**	138.9±17.2	144.2±17.1	134.0±16.1	135.9±16.0	147.4±18.0
	Post	130.5±14.4	134.9±15.8	126.4±11.7	129.1±14.8	134.6±12.7
	% difference	6.05 ↓	6.39 ↓	5.72 ↓	5.00 ↓	8.72 ↓
	Paired *t* test	**6.12*** **	**3.76*** **	**5.54*** **	**4.36*** **	**4.90*** **
Diastolic BP (mmHg)	**Pre**	85.8±10.9	84.9±11.2	86.6±10.8	86.7±10.98	83.2±10.6
	Post	78.8±8.7	77.5±7.5	80.0±9.7	79.5±8.7	76.8±8.8
	% difference	8.13 ↓	8.71 ↓	7.62 ↓	8.28 ↓	7.75 ↓
	Paired *t* test	**6.17*** **	**3.81*** **	**5.24*** **	**5.58*** **	**2.67***

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

Table 4.3.1.5 is indicative of the gender and age-wise comparisons with the BMI reduction of Group 2 patients to be 3.88% in males, 3.06% females, 3.08% young-old and 4.18% old-old categories respectively. Waist circumference reduced by 3.04% in males, 3.14% in females, 2.60% in young-old and 4.53% in old-old. Moreover, a significant positive modification of the supplementation was examined from the improved systolic blood pressure being reduced by 6.01% in males (p<0.001), 6.40% in females (p<0.001), 5.52% for young-old (p<0.001) and 8.15% for old-old (p<0.01).

Table 4.3.1.5: Anthropometric and Biophysical profile of MCI subjects before and after supplementation in Group2 (Flaxseeds plus B12)

Parameters		Group 2 Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
BMI (kg/m^2)	**Pre**	26.4±4.5	24.9 ± 3.0	27.4±4.9	26.9±4.7	25.1±3.2
	Post	25.5±4.4	23.9 ± 2.6	26.5±5.1	26.1±4.8	24.0±2.8
	% difference	3.78 ↓	3.88 ↓	3.06 ↓	3.08 ↓	4.18 ↓
	Paired *t* test	**4.85*****	**5.31*****	**2.98****	**3.67*****	**3.48****
WC (cm)	**Pre**	94.3±8.9	93.9±7.9	94.7±9.7	94.9±9.4	92.6±7.7
	Post	91.4±9.9	91.0±7.8	91.7±11.2	92.5±10.4	88.4±8.2
	% difference	3.10 ↓	3.04 ↓	3.14 ↓	2.60 ↓	4.53 ↓
	Paired *t* test	**4.23*****	**3.13****	**3.00****	**4.36*****	**2.00^{NS}**
HC (cm)	**Pre**	99.2±9.0	95.4±5.8	101.7±9.9	101.3±9.1	93.2± 5.6
	Post	96.9±8.8	93.6±6.9	99.2±9.3	99.2±8.5	90.8± 6.7
	% difference	2.25 ↓	1.91 ↓	2.47 ↓	2.15 ↓	2.59 ↓
	Paired *t* test	**5.98*****	**2.85****	**5.51*****	**5.65*****	**2.55***
WHR	**Pre**	0.95±0.07	0.98±0.07	0.93±0.06	0.94±0.06	0.99±0.06
	Post	0.94±0.07	0.97±0.06	0.92±0.06	0.93±0.07	0.97±0.07
	% difference	1.05 ↓	1.02 ↓	1.07 ↓	1.06 ↓	2.02 ↓
	Paired *t* test	**1.49^{NS}**	**1.18^{NS}**	**0.97^{NS}**	**0.96^{NS}**	**1.12^{NS}**
Systolic BP (mmHg)	**Pre**	137.9±15.0	138.4±14.3	137.6±15.8	136.7±14.8	141.2±15.4
	Post	129.3±12.6	130.1±12.5	128.8±12.8	129.1±13.8	129.7±9.0
	% difference	6.24 ↓	6.01 ↓	6.40 ↓	5.52 ↓	8.15 ↓
	Paired *t* test	**5.48*****	**4.15*****	**3.87*****	**4.27*****	**3.48****
Diastolic BP (mmHg)	**Pre**	84.7±9.4	83.6±9.6	85.8±9.2	86.8±9.7	81.3±7.0
	Post	79.4±9.5	79.3±9.8	79.4±9.5	80.50±9.9	76.2±7.5
	% difference	6.29 ↓	5.14 ↓	7.47 ↓	7.24 ↓	6.21 ↓
	Paired *t* test	**6.26*****	**3.70****	**5.04*****	**5.88*****	**2.46***

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

Table 4.3.1.6 showcases a composite picture of the biophysical profile of the patients. The results display that the patients in both supplementation groups increased to the normal levels of blood pressure by 15% and 18.33% respectively. Similarly, a positive trend was noticeable with the reduction of patients from the Stage1 and Stage 2 in both the groups thereafter leading to an increase in the pre-hypertensive category. (Figure 4.3.1.1, Figure 4.3.1.2).

Table 4.3.1.6: Biophysical profile according to stages of hypertension in Group 1 (B12) and Group 2 (Flaxseeds plus B12) MCI patients

Stages of Hypertension	Group 1 Pre	Group 1 Post	Group 2 Pre	Group 2 Post
Normal	2 (3.3)	9 (15.0)	4 (6.7)	11 (18.3)
Pre-hypertension	27 (45.0)	36 (60.0)	18 (30.0)	33 (55.0)
Stage 1	22 (36.7)	13 (21.7)	32 (53.3)	13 (21.7)
Stage 2	9 (15.0)	2 (3.3)	6 (10.0)	3 (5.0)

Numbers in parenthesis indicate percentage

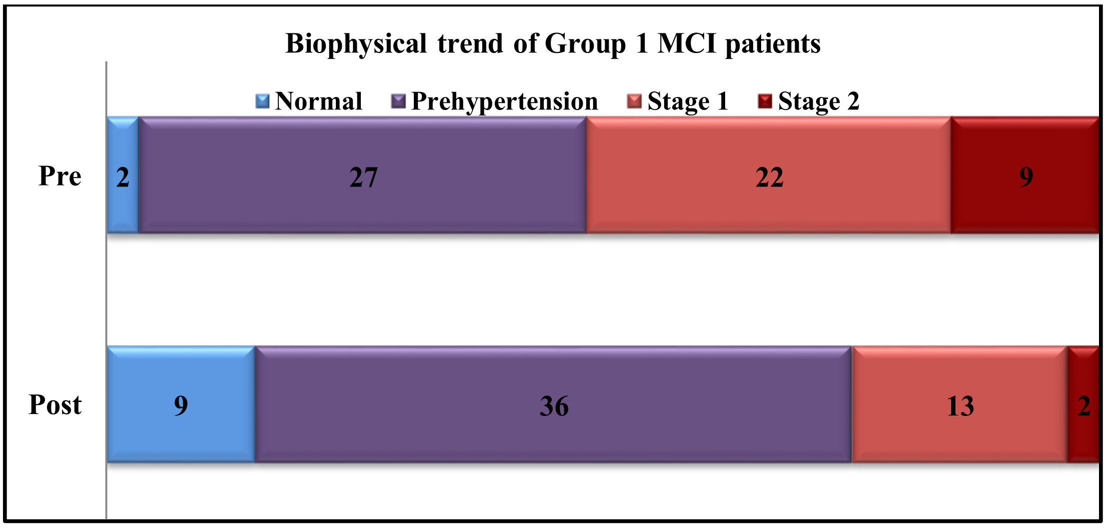


Figure 4.3.1.1: Biophysical trend in Group 1 (B12) MCI patients before and after supplementation

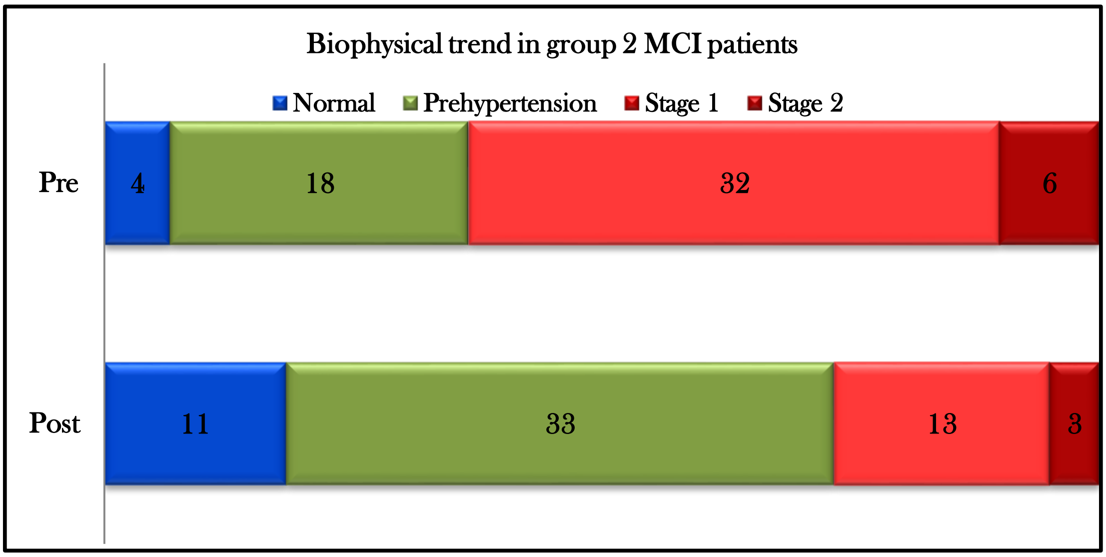


Figure 4.3.1.2: Biophysical trend in Group 2 (Flaxseeds plus B12) MCI patients before and after supplementation

4.3.2 Nutrient intake of the patients

In the Table 4.3.2.1, the dietary analysis of the subjects is depicted. Results are representative that energy, protein, iron, vitamin B12, zinc, phosphorus and folic acid increased in both the groups. A significant increase ($p<0.001$) in the fat and omega- 3 fatty acid from the baseline values was observed only for the Group 2. An insignificant increase was found in the omega -3 for Group 1 after the supplementation.

Table 4.3.2.1: Nutrient intake of MCI patients before and after supplementation

Parameters		Group 1 (B12)	Group 2 (Flaxseeds + B12)	*t*-test
Energy (Kcal)	**Pre**	2116 ± 308	2104 ± 341	**1.7^{NS}**
	Post	2282 ± 340	2184 ± 390	
	Paired *t* test	**2.43***	**0.98^{NS}**	
	% difference	7.8 ↑	3.80 ↑	
Protein (g)	**Pre**	63.3 ± 10.5	64.3 ± 10.6	**2.5***
	Post	76.2 ± 14.1	72.7 ± 13.9	
	Paired *t* test	**5.08*****	**3.08****	
	% difference	20.3 ↑	13.06 ↑	
Fat (g)	**Pre**	70.7 ± 15.1	70.6 ± 14.1	**4.23****
	Post	68.5 ± 15.4	74.16 ± 15.9	
	Paired *t* test	**0.62^{NS}**	**1.1^{NS}**	
	% difference	3.11 ↓	5.04 ↑	
Iron (mg)	**Pre**	19.5 ± 4.5	21.8 ± 7.0	**3.27****
	Post	23.1 ± 8.2	22.5 ± 6.7	
	Paired *t* test	**2.73****	**0.4^{NS}**	
	% difference	18.46 ↑	3.21 ↑	
Vitamin B12 (µg)	**Pre**	0.27 ± 0.06	0.32 ± 0.08	**1.85^{NS}**
	Post	0.31 ± 0.08	0.34 ± 0.07	
	Paired *t* test	**2.29***	**1.6^{NS}**	
	% difference	14.8 ↑	6.2 ↑	
Vitamin C (mg)	**Pre**	115 ± 61	139 ± 92	**3.12****
	Post	105 ±53	104 ± 72	
	Paired *t* test	**0.8^{NS}**	**1.8^{NS}**	
	% difference	8.69 ↓	25.17 ↓	
Zinc (mg)	**Pre**	6.8 ± 1.6	6.61 ± 1.61	**3.5*****
	Post	7.2 ± 1.8	6.64 ± 1.44	
	Paired *t* test	**1.15^{NS}**	**0.8^{NS}**	
	% difference	5.88 ↑	0.45↑	
Phosphorus (mg)	**Pre**	1443 ± 279	1464 ± 291	**2.38***
	Post	1762 ± 328	1670 + 278	
	Paired *t* test	**5.02*****	**3.09****	
	% difference	22.10 ↑	14.07 ↑	
Folic Acid (mg)	**Pre**	264 ± 59	281 ± 68	**3.9*****
	Post	313 ± 98	293 ± 98	
	Paired *t* test	**3.00****	**0.67^{NS}**	
	% difference	18.56 ↑	4.27 ↑	
ALA (Omega 3) (g)	**Pre**	0.29 ± 0.07	0.24 ± 0.05	**892*****
	Post	0.32 ± 0.06	2.85 ± 0.14	
	Paired *t* test	**1.6^{NS}**	**791*****	
	% difference	10.34 ↑	1087.50 ↑	

*Significant from the baseline value at $p<0.05$, ** Significant from the baseline value at $p<0.01$,
*** Significant from the baseline value at $p<0.001$, NS- Non- significant

4.3.3 Glycemic response of mild cognitively impaired subjects before and after the vitamin B12 and flaxseed supplementation.

As shown in the Table 4.3.3.1, the glycemic response of the patients in both Group 1 and Group 2 significantly reduced by 2.65% and 13.93% for the FBS ($p<0.001$) and HbA_{1c} of group 2 by 20.32% ($p<0.001$) and a slight increase of HbA_{1c} by 3.68% in case of Group 1. The reduction in the mean FBS for both the groups and mean HbA_{1c} values of Group 2 can be observed (Figure 4.3.3.1 [a], [b]).

Table 4.3.3.1: Glycemic response of MCI subjects before and after supplementation

Parameters		Group 1 (B12)	Group 2 (Flaxseeds + B12)	*t*-test
FBS (mg/dl)	**Pre**	95.79 ± 18.4	100.37 ± 27.8	**5.23*** **
	Post	93.25 ± 14.8	86.38 ± 15.7	
	% difference	2.65 ↓	13.93 ↓	
	Paired *t* test	**2.99***	**7.17*** **	
HbA_{1c}	**Pre**	5.97 ± 1.64	6.25 ± 1.46	**8.04*** **
	Post	6.19 ± 0.71	4.98 ± 0.69	
	% difference	3.68 ↑	20.32 ↓	
	Paired *t* test	**1.44**^{NS}	**8.22*** **	

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001, NS- Non- significant

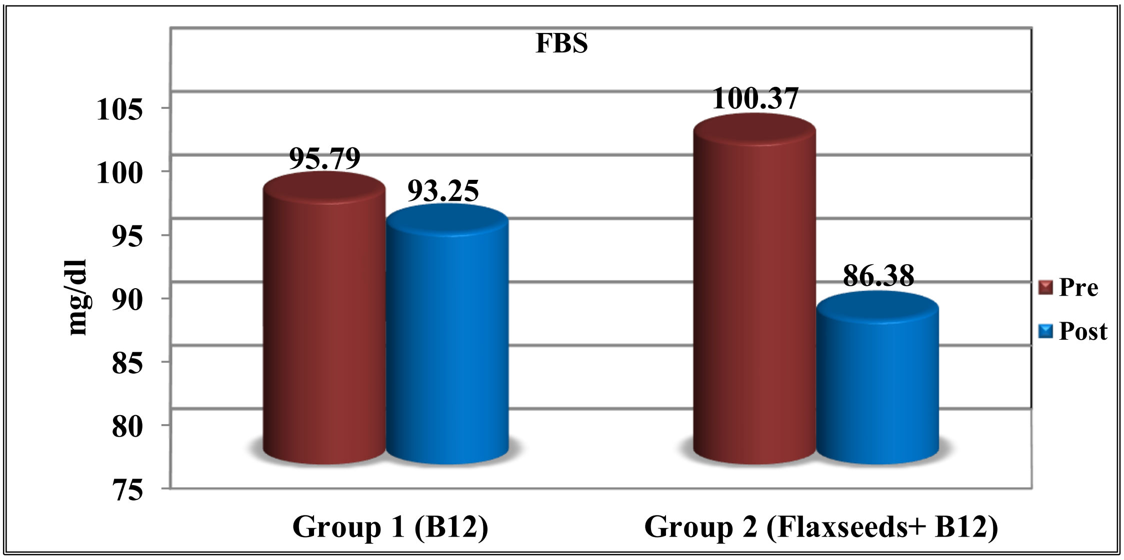


Figure 4.3.3.1(a): Glycemic response of MCI patients before and after supplementation

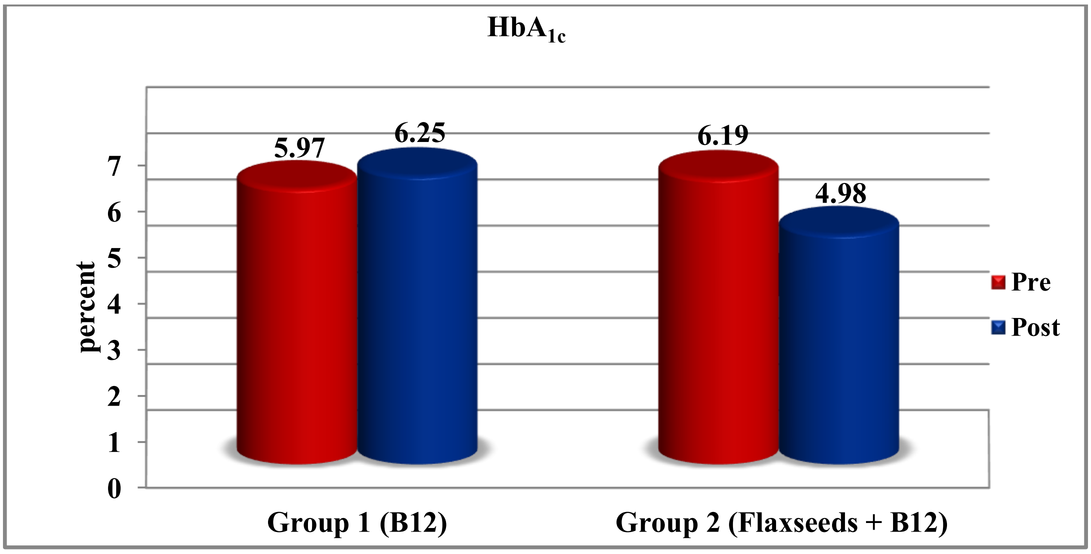


Figure 4.3.3.1(b): Glycemic response of MCI patients before and after supplementation

Table 4.3.3.2 shares a glimpse of significant reduction in FBS values of the total patients ($p<0.05$) in females ($p<0.01$), young –old ($p<0.05$) and HbA_{1c} to be slightly significantly increased ($p<0.05$) in females belonging to the Group 1.

Table 4.3.3.2: Glycemic profile of MCI subjects before and after supplementation in Group 1 (B12)

Parameters		Group 1 Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n =44)	70-85 yrs (n=16)
FBS (mg/dl)	**Pre**	95.8±18.4	99.4±22.4	92.4±13.2	96.1±18.8	94.8±18.1
	Post	93.2±14.8	96.7±17.6	90.0±10.9	94.0±15.5	91± 12.8
	% difference	2.65 ↓	2.77 ↓	2.53 ↓	2.17 ↓	4.01 ↓
	Paired *t* test	**2.99***	**1.74**NS	**2.83****	**2.22***	**2.01**NS
HbA_{1c}	**Pre**	5.9±1.6	6.1±1.4	5.9±1.1	5.9±1.3	5.9±1.3
	Post	6.2±0.7	6.2±0.6	6.2±0.7	6.1±0.6	6.3±0.9
	% difference	3.68 ↑	0.98 ↑	5.97 ↑	2.50 ↑	6.39 ↑
	Paired *t* test	**1.44**NS	**0.22**NS	**2.53***	**0.28**NS	**2.11**NS

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

As mentioned, the Table 4.3.3.3 presents a significant reduction in both the FBS ($p<0.001$) and HbA1c ($p<0.001$) values with respect to each of the males, females, young-old and old-old patients in the Group 2 of the supplementation.

Table 4.3.3.3: Glycemic profile of MCI subjects before and after supplementation in Group 2 (flaxseeds plus B12)

Parameters		Group 2 Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-75 yrs (n=16)
FBS (mg/dl)	Pre	100.4±27.8	109.4±38.7	94.2±15.2	101.1±33.6	102.0±20.2
	Post	86.4±15.7	90.8±20.7	83.4±10.6	85.3±16.9	89.9±16.6
	% difference	13.93 ↓	16.98 ↓	11.38 ↓	15.64 ↓	13.43 ↓
	Paired *t* test	**7.17*** **	**4.23*** **	**8.70*** **	**4.99*** **	**8.87*** **
HbA_{1c}	Pre	6.2±1.5	6.7±1.6	5.9±1.3	6.2±1.5	6.5±1.5
	Post	4.9±0.7	5.1±0.7	4.9±0.6	4.9±0.7	4.9±0.6
	% difference	20.32 ↓	23.72 ↓	17.75 ↓	19.44 ↓	22.87 ↓
	Paired *t* test	**8.22*** **	**5.81*** **	**5.98*** **	**6.79*** **	**4.57*** **

*** Significant from the baseline value at p<0.001

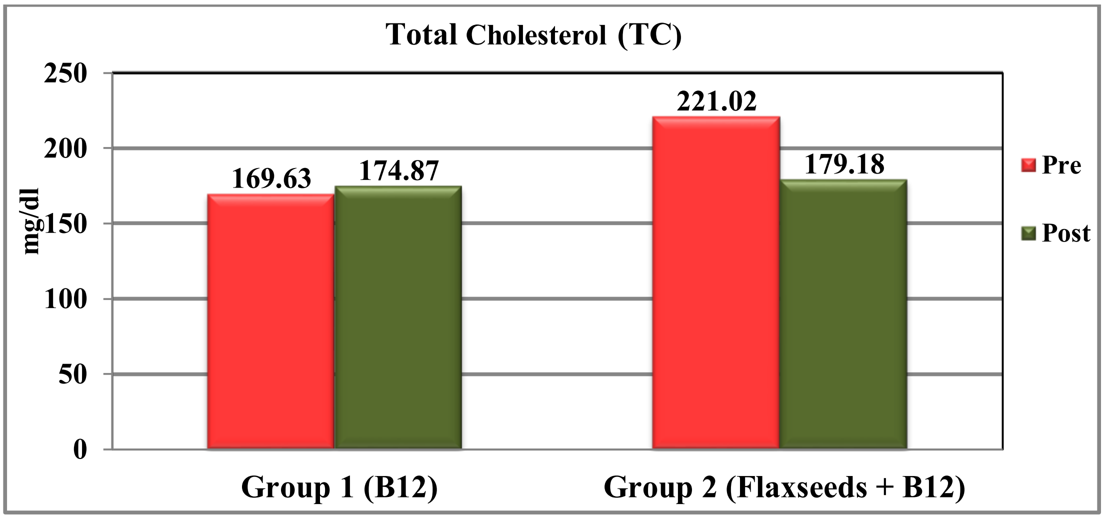
4.3.4 Lipemic response of the patients

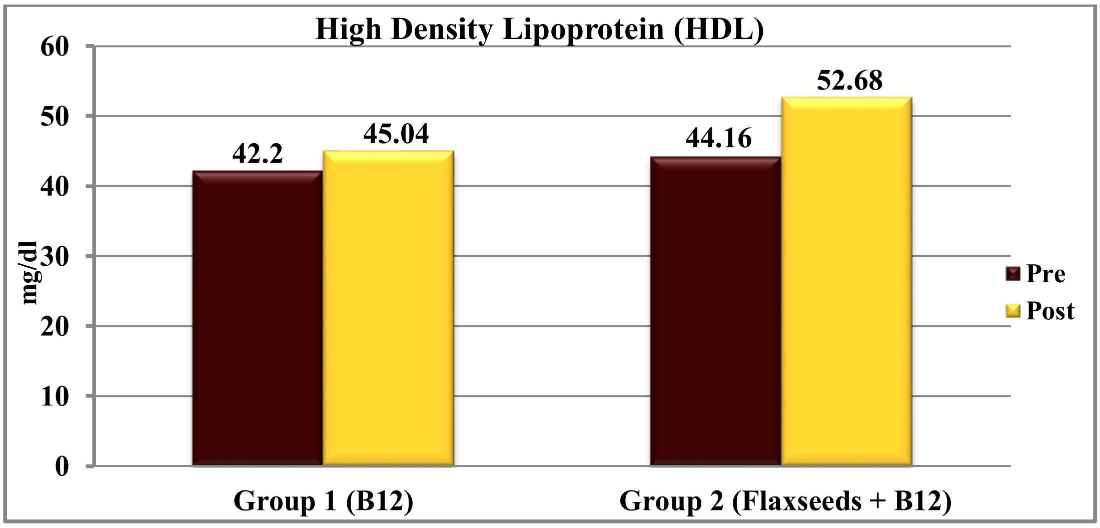
A significant reduction (p< 0.001) in the TC, TG, LDL and VLDL values by 18.93%, 23.44%, 20.18% and 33.94% was examined as resultant of the flaxseeds plus B12 supplementation in Group 2 respectively. An increasing trend was significantly noticeable in the TC (p<0.05), LDL (p<0.001) and VLDL (p<0.05) values by 2.99%, 7.40% and 6.31% for the Group 1. Furthermore, a significant increase (p< 0.001) in the HDL values by 19.29% and 6.73% for both the Group 2 and 1 was detected (Table 4.3.4.1).

Table 4.3.4.1: Lipemic response of MCI subjects before and after supplementation

Parameters		Group 1 (B12)	Group 2 (Flaxseeds+B12)	*t*-test
TC (mg/dl)	Pre	169.63 ± 21.23	221.02 ± 21.66	**11.35*** **
	Post	174.87 ± 14.46	179.18 ± 17.94	
	% difference	2.99 ↑	18.93 ↓	
	Paired *t* test	**2.34***	**11.63*** **	
HDL (mg/dl)	Pre	42.20 ± 8.03	44.16 ± 8.85	**7.56*** **
	Post	45.04 ± 8.50	52.68 ± 7.92	
	% difference	6.73 ↑	19.29 ↑	
	Paired *t* test	**5.60*** **	**13.02*** **	
TG (mg/dl)	Pre	128.40 ± 66.92	157.34 ± 50.90	**4.67*** **
	Post	124.38 ± 57.46	120.45 ± 34.21	
	% difference	3.23 ↓	23.44 ↓	
	Paired *t* test	**1.31**^{NS}	**5.77*** **	
LDL (mg/dl)	Pre	102.64 ± 19.31	138.24 ± 25.16	**10.91*** **
	Post	110.24 ± 16.24	110.33 ± 22.10	
	% difference	7.40 ↑	20.18 ↓	
	Paired *t* test	**4.17*** **	**9.26 *** **	
VLDL (mg/dl)	Pre	28.04 ±15.99	31.11 ± 9.17	**5.24*** **
	Post	29.93 ± 14.35	20.55 ± 7.33	
	% difference	6.31 ↑	33.94 ↓	
	Paired *t* test	**2.11***	**5.88*** **	

*Significant at p<0.05, *** Significant at p<0.001, NS- Non- significant





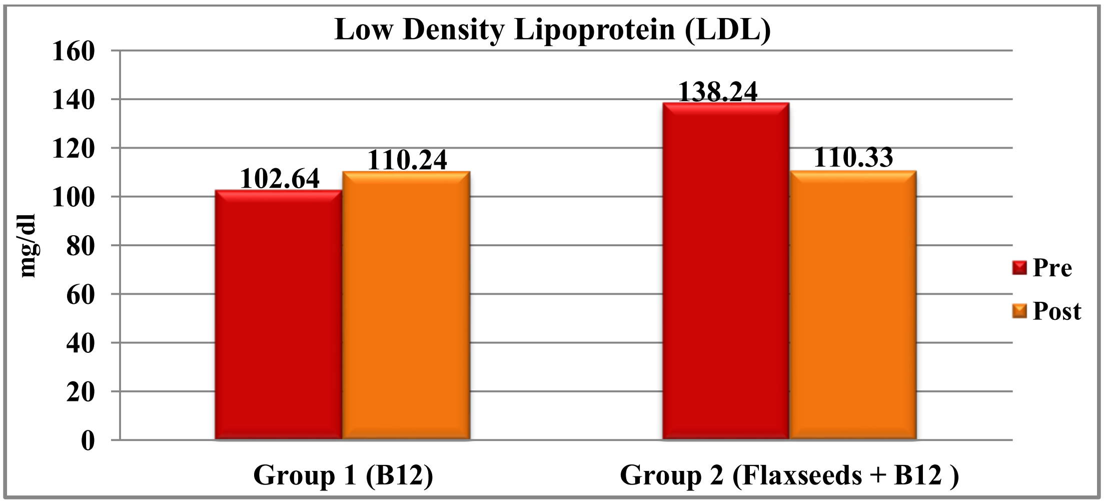


Figure 4.3.4.1: Lipemic response of MCI subjects before and after supplementation

As evidenced from the Table 4.3.4.2, a significant increase ($p < 0.001$) in HDL by 8.02% in females, 5.77% in males, 7.91% in young-old and 3.66% in old-old groups was confirmed in the Group 1. Further, significant decrease ($p<0.05$) in TG by 6.86% in males and 4.97 % for young –old could be noted. Meanwhile, the TC values showed a significant increase ($p<0.05$) with 5.14% in males and 6.93% in old-old. Significant increase in the LDL by 9.88% in the males ($p < 0.01$), 5.35% in females ($p<0.05$), 6.53% in the young-old ($p < 0.01$) and 9.72% old-old ($p<0.05$) along with significant increase ($p < 0.05$) in VLDL to 15.13% in males and 20.72% in old- old groups was focused.

Table 4.3.4.2: Lipemic response of MCI subjects before and after supplementation in Group 1 (B12)

Parameters		Group 1 Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
TC (mg/dl)	**Pre**	169.6±21.2	162.2±24.6	176.8±15.2	169.8±20.6	169.2±23.6
	Post	174.9±14.5	170.5±15.9	178.3±12.1	172.7±14.8	180.9±11.8
	% difference	2.99 ↑	5.14 ↑	0.88 ↑	1.69 ↑	6.93 ↑
	Paired *t* test	**2.34***	**2.22***	**0.63**^{NS}	**1.17**^{NS}	**2.44***
HDL (mg/dl)	**Pre**	42.2±8.0	39.4± 8.4	44.5±7.1	41.6±8.5	43.9±6.3
	Post	45.0±8.5	41.7±8.5	48.1±7.6	44.8±9.2	45.6± 6.4
	% difference	6.73 ↑	5.77 ↑	8.02 ↑	7.91 ↑	3.66 ↑
	Paired *t* test	**5.60*****	**5.58*****	**4.08*****	**4.98*****	**3.34****
TG (mg/dl)	**Pre**	128.4±66.9	117.3±43.9	128.1±37.9	135.4±73.6	109.1±39.3
	Post	124.4±57.5	116.5±33.9	119.3±29.5	128.7±64.6	112.6±28.4
	% difference	3.23 ↓	0.72 ↓	6.86 ↓	4.97 ↓	3.16 ↑
	Paired *t* test	**1.31**^{NS}	**0.17**^{NS}	**2.55***	**2.04***	**0.49**^{NS}
LDL (mg/dl)	**Pre**	102.6±19.3	98.5±22.2	106.1±16.1	101.8±18.4	104.8±22.1
	Post	110.2±16.2	108.2±17.5	111.8±15.3	108.5±15.6	115.0±17.6
	% difference	7.40 ↑	9.88 ↑	5.35 ↑	6.53 ↑	9.72 ↑
	Paired *t* test	**4.17*****	**3.51****	**2.30***	**3.24****	**2.62***
VLDL (mg/dl)	**Pre**	28.0±15.9	23.1±8.8	29.9±14.3	29.8±17.8	23.2±7.9
	Post	29.9±14.3	26.6±7.2	30.2±11.7	30.6±16.3	28.0±6.6
	% difference	6.31 ↑	15.13 ↑	0.68 ↑	2.77 ↑	20.72 ↑
	Paired *t* test	**2.11***	**2.38***	**0.18**^{NS}	**0.83**^{NS}	**2.64***

*Significant from the baseline value at $p<0.05$, ** Significant from the baseline value at $p<0.01$,
*** Significant from the baseline value at $p<0.001$, NS- Non- significant

The flaxseeds plus B12 supplementation caused the HDL to be significantly increased (p< 0.001) by 21.49% in males, 17.86% in females, 18.65% in young-old and 21.07% in old-old. Meanwhile, significant lowering (p<0.001) in the values by 19.62% 18.46%, 18.62% and 19.58% of TC , 23.97% (p< 0.01), 23.11%, 21.20% and 29.10% (p<0.01) for TG , 21.22% , 19.47%, 20.59% and 19.04 (p<0.01) for LDL, 33.04%, 34.48%, 33.51% and 35.23% for VLDL in males, females, young-old and old-old respectively (Table 4.3.4.3).

Table 4.3.4.3: Lipemic response of MCI subjects before and after supplementation in Group 2 (Flaxseeds plus B12)

Parameters		Group 2 Total(n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
TC (mg/dl)	**Pre**	221.0±21.7	221.9±28.6	220.4±15.9	218.8±17.3	227.0±30.6
	Post	179.2±17.9	178.4±20.7	179.7±16.1	177.9±15.0	182.6±24.7
	% difference	18.93 ↓	19.62 ↓	18.46 ↓	18.62 ↓	19.58 ↓
	Paired *t* test	**11.63*** **	**6.42*** **	**10.14*** **	**12.09*** **	**4.44 *** **
HDL (mg/dl)	**Pre**	44.2±8.8	43.3±7.9	44.7±9.5	44.3±9.2	43.7± 8.0
	Post	52.7±7.9	52.6±7.3	52.7± 8.4	52.6±8.2	52.9±7.3
	% difference	19.29 ↑	21.49 ↑	17.86 ↑	18.65 ↑	21.07 ↑
	Paired *t* test	**13.02*** **	**11.43*** **	**8.46*** **	**9.90*** **	**10.43*** **
TG (mg/dl)	**Pre**	157.3±50.9	149.9±59.1	162.3±44.8	153.7±48.1	167.4±58.4
	Post	120.4±34.2	113.9± 35.3	124.8±33.2	121.1±35.2	118.7±32.4
	% difference	23.44 ↓	23.97 ↓	23.11 ↓	21.20 ↓	29.10 ↓
	Paired *t* test	**5.77*** **	**3.76** **	**6.52*** **	**6.54*** **	**3.67** **
LDL (mg/dl)	**Pre**	138.2±25.2	140.0±33.5	137.0±18.0	137.7±20.5	139.6± 35.8
	Post	110.3±22.1	110.3±24.1	110.3±21.0	109.4±19.7	113.0± 28.2
	% difference	20.18 ↓	21.22 ↓	19.47 ↓	20.59 ↓	19.04 ↓
	Paired *t* test	**9.26 *** **	**4.78*** **	**9.14*** **	**10.42*** **	**3.06** **
VLDL m(g/dl)	**Pre**	31.1±9.2	28.9±9.3	32.6±8.9	31.5±9.3	30.1±9.1
	Post	20.5±7.3	19.4±7.5	21.3±7.2	20.9±7.6	19.5±6.7
	% difference	33.94 ↓	33.04 ↓	34.48 ↓	33.51 ↓	35.23 ↓
	Paired *t* test	**5.88*** **	**5.82*** **	**8.84*** **	**8.77*** **	**5.81*** **

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

4.3.5 Atherogenic indices of the patients before and after both the supplementations.

Table 4.3.5.1 represents a significant reduction (p<0.001) in the TC/HDL, LDL/HDL, TG/HDL and Atherogenic Index of the Group 2 by 33.27%, 33.88%, 37.27% and 41.20% respectively. In Group 1 patients too, a significant lowering (p<0.01) of

TG/HDL by 10.40% succeeded by insignificant decline of the TC/HDL by 3.44%, Atherogenic Index by 4.54% and very minute increase of 0.35% in LDL/HDL was detected. In addition to these changes, a sharp drop off to 2.48 for the Atherogenic Coefficient value of Group 2 patients was noticeable in comparison to value 3 of the Group 1 (Figure 4.3.5.1).

Table 4.3.5.1: Atherogenic indices of MCI patients before and after supplementation.

Parameters		**Group 1 (B12)**	**Group 2 (Flaxseeds + B12)**	***t*-test**
TC/HDL	**Pre**	4.1±0.86	5.2±1.25	**14.44*** **
	Post	4.0±0.72	3.5±0.65	
	% difference	3.44 ↓	33.27 ↓	
	Paired *t* test	**1.57**^{NS}	**12.36*** **	
LDL/HDL	**Pre**	2.5±0.69	3.3±0.91	**11.79*** **
	Post	2.5±0.58	2.3±0.59	
	% difference	0.35 ↑	33.88↓	
	Paired t test	**0.14**^{NS}	**12.31*** **	
TG/HDL	**Pre**	3.3±2.79	3.8±1.63	**3.56*** **
	Post	2.9±2.36	2.4±0.87	
	% difference	10.40 ↓	37.27↓	
	Paired t test	**3.13** **	**8.47*** **	
Atherogenic Index	**Pre**	3.1± 0.86	4.2±1.25	**11.84*** **
= (TC-HDL)/HDL	**Post**	3.0± 0.72	2.5±0.65	
	% difference	4.54 ↓	41.20 ↓	
	Paired t test	**1.58**^{NS}	**12.36*** **	

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

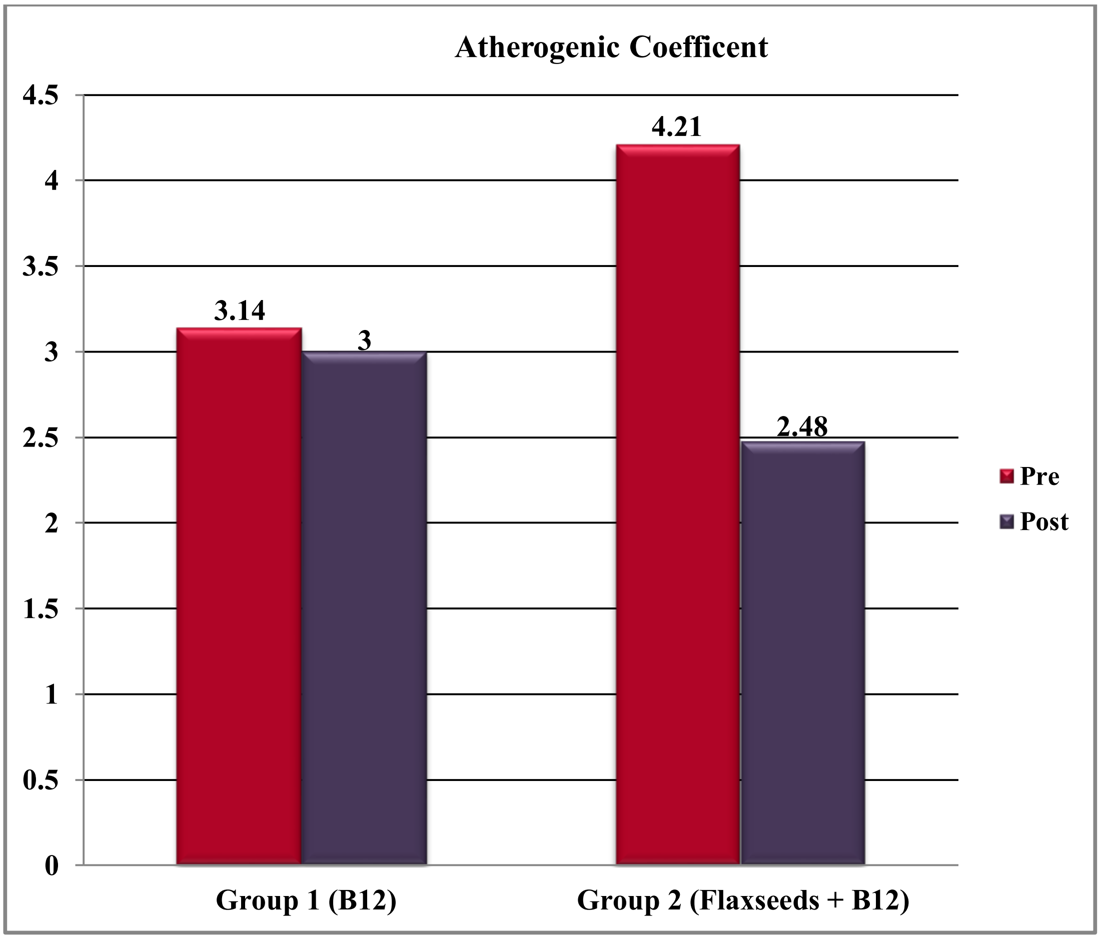


Figure 4.3.5.1: Atherogenic Coefficient of the of MCI subjects before and after supplementation

The Group 1 values in females and young-old were significantly lessened in TC/HDL by 7.08% and 5.85% ($p<0.05$), TG/HDL by 15.91% and 12.83% ($p<0.01$) and Atherogenic Index by 9.40% and 7.65% ($p<0.05$). Moreover, LDL/HDL values for these insignificantly went down by 3.22% and 1.64%, (Table 4.3.5.2).

Table 4.3.5.3 points towards the Group 2 values in case of males, females, young-old and old-old to be significantly lessened ($p<0.001$) in TC/HDL by 35.34%, 31.83%, 32.46% and 35.37% , LDL/HDL by 36.07%, 32.34%, 33.73% and 34.28% ,TG/HDL by 39.26%, 36%, 34.99% and 42.98%, and atherogenic index by 43.52%, 39.56%, 40.32% and 43.42% respectively.

Table 4.3.5.2: Atherogenic indices of MCI subjects before and after supplementation in Group 1 (B12)

Parameters		Group 1 Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
TC/HDL	**Pre**	4.1±0.86	4.2±0.92	4.1±0.81	4.2±0.90	3.89±0.68
	Post	4.0±0.72	4.2±0.76	3.8±0.63	3.9±0.76	4.04±0.62
	% difference	3.44 ↓	0.21 ↑	7.08 ↓	5.85 ↓	3.78 ↑
	Paired t test	**1.57**^{NS}	**0.10**^{NS}	**2.34***	**2.27***	**1.04**^{NS}
LDL/HDL	**Pre**	2.5±0.69	2.6±0.72	2.5±0.66	2.6±0.72	2.4±0.62
	Post	2.5±0.58	2.7±0.59	2.4±0.54	2.5±0.60	2.6±0.53
	% difference	0.35 ↑	4.01 ↑	3.22 ↓	1.64 ↓	6.13 ↑
	Paired t test	**0.14**^{NS}	**1.15**^{NS}	**0.94**^{NS}	**0.55**^{NS}	**1.51**^{NS}
TG/HDL	**Pre**	3.3±2.79	3.6±3.81	3.0±1.28	3.6±3.17	2.5±1.00
	Post	2.9±2.36	3.4±3.28	2.5±0.73	3.1±2.72	2.5±0.68
	% difference	10.40 ↓	5.55 ↓	15.91 ↓	12.83 ↓	0.89 ↓
	Paired t test	**3.13****	**1.17**^{NS}	**3.41****	**3.45****	**0.13**^{NS}
Atherogenic Index = (TC-HDL)/HDL	**Pre**	3.1± 0.86	3.2±0.91	3.1±0.81	3.2±0.90	2. 9±0.69
	Post	3.0± 0.72	3.2±0.76	2.8±0.63	2.9±0.76	3.0±0.63
	% difference	4.54 ↓	0.43 ↑	9.40 ↓	7.65 ↓	4.99 ↑
	Paired t test	**1.58**^{NS}	**0.10**^{NS}	**2.35***	**2.27***	**1.02**^{NS}

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, NS- Non- significant

Table 4.3.5.3: Atherogenic indices of MCI subjects before and after supplementation in Group 2 (Flaxseeds + B12)

Parameters		Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
TC/HDL	**Pre**	5.2±1.25	5.4±1.50	5.1±1.07	5.1±1.08	5.5±1.66
	Post	3.4±0.65	3.5±0.67	3.5±0.65	3.5±0.63	3.5± 0.73
	% difference	33.27 ↓	35.34 ↓	31.83 ↓	32.46 ↓	35.37 ↓
	Paired *t* test	**12.36*****	**7.21*****	**10.44*****	**12.07*****	**5.20*****
LDL/HDL	**Pre**	3.2±0.91	3.4±1.13	3.2±0.75	3.2±0.79	3.3±1.22
	Post	2.2±0.59	2.1±0.61	2.1±0.59	2.1±0.57	2.2±0.68
	% difference	33.88↓	36.07 ↓	32.34 ↓	33.73 ↓	34.28 ↓
	Paired *t* test	**12.31*****	**6.42*****	**12.77*****	**14.25*****	**4.25*****
TG/HDL	**Pre**	3.8±1.63	3.7±1.93	3.8±1.43	3.8±1.53	4.0±1.92
	Post	2.4±0.87	2.2±0.87	2.5±0.86	2.4±0.88	2.3±0.84
	% difference	37.27↓	39.26 ↓	36.00 ↓	34.99 ↓	42.98 ↓
	Paired *t* test	**8.47*****	**4.54*****	**7.59*****	**7.70*****	**4.13*****
Atherogenic Index = (TC-HDL)/HDL	**Pre**	4.2±1.25	4.4±1.50	4.1±1.07	4.1±1.08	4.5±1.66
	Post	2.5±0.65	2.5±0.67	2.5±0.65	2.5±0.63	2.5±0.72
	% difference	41.20 ↓	43.52 ↓	39.56 ↓	40.32 ↓	43.42 ↓
	Paired *t* test	**12.36*****	**7.23*****	**10.40*****	**12.05*****	**5.22*****

*** Significant from the baseline value at p<0.001, NS- Non- significant

4.3.6 Serum vitamin B12 and Cell Blood Counts (CBC) before and after the vitamin B12 and flaxseed supplementation.

As suggestive from the Table 4.3.6.1, the significantly remarkable rise ($p<0.001$) in Serum vitamin B12 was apparent to be 370% and 276% in Group 2 and Group 1 patients respectively. Significantly, the CBC profile of the MCI patients in Group 2 and 1 improved for hemoglobin by 12.23% and 10.88% ($p<0.001$), RBC by 22.33% and 19.75% ($p<0.001$), WBC by 10.68% and 10.98% ($p<0.001$), PCV by 14.36% and 13.95% ($p<0.001$), MCV by 4.22% and 3.13% ($p<0.05$), MCH by 6.05% and 5.44% ($p<0.001$) respectively. The noteworthy features were significant increases ($p<0.001$) in values of Serum Vitamin B12 of Group 1 males, females, young-old and old-old sub-groups by 308%, 249%, 255% and 339%, hemoglobin by 9.81%, 12.14%, 9.58% and 14.61%, PCV by 10.63%, 17.15%, 13.83% and 14.27% while MCV although being insignificant reached upwards by 2.27%, 3.17%, 3.60% ($p<0.05$) and 1.90% respectively (Table 4.3.6.2).

Meanwhile, Table 4.3.6.3 heralds significant increases ($p<0.001$) of Serum Vitamin B12 in Group 2 males, females, young-old and old-old sub-groups by 390%, 345%, 397% and 310% ,hemoglobin by 10.60%, 13.30%, 11.88% and 13.00%, PCV by 13.03%, 15.34%, 14.54% and 13.95% respectively while MCV except in case of females to be significantly increasing to 5.15% ($p<0.05$) insignificantly remained in improved condition for other subgroups.

Table 4.3.6.1: Serum vitamin B12 and CBC profile of MCI subjects before and after supplementation

Parameters		Group 1 (B12)	Group 2 (Flaxseeds+B12)	*t*-test
Serum B12 (pg/ml)	**Pre**	190.5±40.34	181.0± 48.21	**2.58***
	Post	715.4±243.48	852.4± 406.27	
	% difference	276 ↑	370 ↑	
	Paired *t* test	**16.33*** **	**12.41*** **	
		CBC Profile		
Hemoglobin (G/UL)	**Pre**	11.7± 1.69	11.6± 1.68	**0.61**^{NS}
	Post	12.9± 0.99	13.0± 0.87	
	% difference	10.88 ↑	12.23 ↑	
	Paired *t* test	**8.07*** **	**9.22*** **	
PCV (%)	**Pre**	37.5± 4.53	37.4±4.19	**0.23**^{NS}
	Post	42.8± 2.70	42.8±7.54	
	% difference	13.95 ↑	14.36 ↑	
	Paired *t* test	**12.51*** **	**12.48*** **	
MCV (fl)	**Pre**	81.91±12.77	80.63±3.04	**0.39**^{NS}
	Post	84.48±5.72	84.04±3.66	
	% difference	3.13 ↑	4.22 ↑	
	Paired *t* test	**2.24***	**2.16***	
RBC Count (mil/Ul)	**Pre**	4.2±0.55	4.1±0.86	**0.83**^{NS}
	Post	4.9±0.53	5.0±0.54	
	% difference	19.75 ↑	22.33 ↑	
	Paired *t* test	**11.42*** **	**9.23*** **	
WBC Count (k/Ul)	**Pre**	6.3±1.63	6.3±1.61	**0.16**^{NS}
	Post	6.9±1.38	6.9±1.30	
	% difference	10.98 ↑	10.68 ↑	
	Paired *t* test	**7.57*** **	**6.42*** **	
MCH (pg)	**Pre**	27.5±3.97	27.2±3.84	**0.25**^{NS}
	Post	29.0±3.43	28.9±1.24	
	% difference	5.44 ↑	6.05 ↑	
	Paired *t* test	**4.08*** **	**3.86*** **	

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

Table 4.3.6.2: Serum Vitamin B12 and CBC profile of MCI subjects before and after supplementation in Group 1 (B12)

Parameters		Group 1 Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
Serum B12 (pg/ml)	**Pre**	190.5±40.3	184.7±39.9	195.9±40.5	194.4±37.8	179.7±46.1
	Post	715.3±243.4	751.1±320.4	681.8±135.5	689.2±175.3	787.2±370
	% difference	276 ↑	308 ↑	249 ↑	255 ↑	339 ↑
	Paired *t* test	**16.33*** **	**9.43*** **	**18.6*** **	**18.19*** **	**6.48*** **
Hemoglobin (g/Ul)	**Pre**	11.7±1.69	12.4±1.62	10.9±1.44	11.7±1.51	11.6±2.16
	Post	12.9±0.99	13.6±0.87	12.3±0.52	12.8±0.97	13.3±0.96
	% difference	10.88 ↑	9.81 ↑	12.14 ↑	9.58 ↑	14.61 ↑
	Paired *t* test	**8.07*** **	**5.79*** **	**5.59*** **	**7.39*** **	**4.10*** **
PCV (%)	**Pre**	37.5±4.53	39.0±4.01	36.2±4.59	37.6±3.91	37.4±6.00
	Post	42.8±2.70	43.2±3.11	42.4±2.24	42.8±2.47	42.7±3.34
	% difference	13.95 ↑	10.63 ↑	17.15 ↑	13.83 ↑	14.27 ↑
	Paired *t* test	**12.51*** **	**8.96*** **	**9.79*** **	**12.10*** **	**5.02*** **
MCV (fl)	**Pre**	81.9±12.77	81.8±12.71	82.6±13.00	81.1±12.23	84.1±14.33
	Post	84.5±5.72	83.7±5.71	85.2±5.72	84.0±5.90	85.7±5.17
	% difference	3.13 ↑	2.27 ↑	3.17↑	3.60 ↑	1.90 ↑
	Paired *t* test	**2.24***	**1.51**^{NS}	**1.63**^{NS}	**2.47***	**0.55**^{NS}
RBC Count (mil/Ul)	**Pre**	4.2±0.55	4.4±0.60	3.9±0.44	4.1±0.57	4.2±0.50
	Post	4.9±0.53	5.0±0.57	4.9±0.51	4.9±0.51	5.1± 0.62
	% difference	19.75 ↑	15.17 ↑	24.18 ↑	19.41 ↑	20.28 ↑
	Paired *t* test	**11.42*** **	**6.52*** **	**10.11*** **	**9.41*** **	**6.38*** **
WBC Count (k/Ul)	**Pre**	6.3±1.63	6.5±1.77	6.1±1.49	6.1±1.42	6.7±2.07
	Post	6.9±1.38	7.0±1.41	6.9±1.37	6.9±1.31	7.1±1.61
	% difference	10.98 ↑	9.13 ↑	13.11 ↑	13.23 ↑	5.50 ↑
	Paired *t* test	**7.57*** **	**3.58*** **	**8.83*** **	**10.58*** **	**1.43**^{NS}
MCH (pg)	**Pre**	27.5±3.97	27.6±3.39	27.5±4.49	27.5±3.43	27.4±5.30
	Post	29.0±3.43	28.8±1.18	29.3±1.56	29.1±1.93	29.0±1.68
	% difference	5.44 ↑	4.19 ↑	6.65 ↑	5.48 ↑	5.40 ↑
	Paired *t* test	**4.08*** **	**2.96** **	**2.98** **	**4.54*** **	**1.40**^{NS}

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

Table 4.3.6.3: Serum Vitamin B12 and CBC profile of MCI subjects before and after supplementation in Group 2 (Flaxseeds plus B12)

Parameters		Group 2 Total(n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
Serum B12 (pg/ml)	**Pre**	181.0±48.2	193.5±52.6	172.6±43.7	177.7±47.5	189.9±50.4
	Post	852.4±406.2	946.4±372.5	789.6±420.6	880.2±456.2	775.8±209.0
	% difference	370 ↑	390 ↑	345 ↑	397 ↑	310 ↑
	Paired *t* test	**12.41*** **	**9.36*** **	**8.58*** **	**9.95*** **	**10.40*** **
Hemoglobin (g/Ul)	**Pre**	11.6±1.68	12.3±1.74	11.1±1.46	11.5±1.55	11.8±2.03
	Post	13.0±0.87	13.7±0.72	12.6±0.68	12.9±1.78	13.4±1.00
	% difference	12.23 ↑	10.60 ↑	13.30 ↑	11.88 ↑	13.00 ↑
	Paired *t* test	**9.22*** **	**5.01*** **	**7.84*** **	**7.81*** **	**4.78*** **
PCV (%)	**Pre**	37.4±4.19	38.9±4.46	36.4±3.72	37.4±3.62	37.6±5.60
	Post	42.8±7.54	44.0±2.78	42.0±2.43	42.8±2.52	42.8±3.38
	% difference	14.36 ↑	13.03 ↑	15.34 ↑	14.54 ↑	13.95 ↑
	Paired *t* test	**12.48*** **	**7.77*** **	**9.68*** **	**11.24*** **	**5.52*** **
MCV (fl)	**Pre**	80.6±13.04	81.5±15.08	80.0±11.67	80.1±12.76	82.1±14.1
	Post	84.0±3.66	83.8±3.18	84.2±3.99	83.6±3.67	85.3±3.42
	% difference	4.22 ↑	2.85 ↑	5.15 ↑	4.25 ↑	3.90 ↑
	Paired *t* test	**2.16***	**0.83**NS	**2.20***	**1.88**NS	**1.01**NS
RBC Count (mil/Ul)	**Pre**	4.1±0.86	4.4±0.76	3.9±0.88	4.0±0.84	4.4±0.91
	Post	5.0±0.54	5.2±0.64	4.9±0.45	4.9±0.43	5.3±0.74
	% difference	22.33 ↑	18.18 ↑	25.38 ↑	22.52 ↑	21.83 ↑
	Paired *t* test	**9.23*** **	**5.43*** **	**7.46*** **	**8.24*** **	**4.23*** **
WBC Count (k/Ul)	**Pre**	6.3±1.61	6.5±1.60	6.1±1.63	5.9±1.42	7.1±1.88
	Post	6.9±1.30	7.2±1.32	6.8±1.27	6.8±1.24	7.4±1.41
	% difference	10.68 ↑	11.76 ↑	10.09 ↑	13.73 ↑	4.24 ↑
	Paired *t* test	**6.42*** **	**5.27*** **	**4.19*** **	**7.46*** **	**1.25**NS
MCH (pg)	**Pre**	27.3±3.84	28.6±4.11	26.4±3.43	26.9±3.55	27.9±4.6
	Post	28.9±1.24	29.3±1.07	28.6±1.28	28.8±1.33	29.0±0.96
	% difference	6.05 ↑	2.55 ↑	8.57 ↑	6.92 ↑	3.75 ↑
	Paired *t* test	**3.86*** **	**0.89**NS	**5.16*** **	**4.52*** **	**0.91**NS

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01,
*** Significant from the baseline value at p<0.001, NS- Non- significant

4.3.7 Neuropsychological test battery of the MCI patients conducted pre and post supplementation.

The vitamin B12 supplementation brought forth a significant (p<0.001) improvement in the MMSE scores of the Group 1 patients with a rise of 9.63% in the total patients. The gender-wise division also highlighted a significant increase (p<0.001) in the scores by 6.79% and 12.46% in males and females whereas 10.20% and 8.24% for young-old (60-69 yrs) and old-old (70-85 yrs) categories respectively (Table 4.3.7.1).

Table 4.3.7.1: Pre and post MMSE score of MCI patients in supplementation Group 1 (vitamin B12)

MMSE Score in Group1(B12)	Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
Pre	24.5±3.28	25.3±2.79	23.7±3.55	24.3±3.29	25.0±3.20
Post	26.9±3.10	27.0±2.52	26.7±3.59	26.8±3.26	27.0±2.63
Percentage ↑ / ↓	9.63 ↑	6.79 ↑	12.46 ↑	10.20 ↑	8.24 ↑
Paired *t*-test	**10.26*** **	**6.82*** **	**8.51*** **	**8.33*** **	**7.34*** **

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001, NS- Non- significant

As indicated in the Table 4.3.7.2, the ACE scores too significantly (p<0.001) escalated to 12.73% in Group 1 patients. Cross comparative analysis also depicted a significant increase (p<0.001) in the scores by 10.57% and 14.89% in males and females whereas 14.39% and 8.25% for young-old (60-69 yrs) and old-old (70-85 yrs) categories respectively.

Table 4.3.7.2: Pre and post ACE score of MCI patients in the supplementation Group 1

ACE Score in Group1(B12)	Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
Pre	68.4±9.58	70.5±7.59	66.5±10.90	68.2±9.72	68.9± 9.17
Post	77.1±7.95	77.9±7.59	76.3±8.33	78.0±7.16	74.6± 9.33
Percentage ↑ / ↓	12.73 ↑	10.57 ↑	14.89 ↑	14.39 ↑	8.25 ↑
Paired *t*-test	**8.73*** **	**7.12*** **	**5.97*** **	**7.68*** **	**5.69*** **

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001, NS- Non- significant

Table 4.3.7.3 illustrates a significant (p<0.001) percent change in the MNA score of Group 1 patients. A 7.19% increase was significantly (p<0.001) observed in total patients with 5.44% (p<0.01) and 8.76% (p<0.001) for males and females respectively.

Significant increase of 6.42% (p<0.001) and 9.34% (p<0.01) was noted in the case of young-old and old-old groups.

Table 4.3.7.3: Pre and post MNA score of MCI patients in Group 1

MNA Score in Group1(B12)	Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
Pre	10.0±1.78	10.10 ± 1.65	9.93 ± 1.93	10.27 ± 1.53	9.31 ± 1.29
Post	10.7±1.42	10.65 ± 1.37	10.80 ± 1.49	10.93 ± 1.24	10.18 ± 1.70
Percentage ↑ / ↓	7.19 ↑	5.44 ↑	8.76 ↑	6.42 ↑	9.34 ↑
Paired *t*-test	**5.24*** **	**3.26** **	**4.12*** **	**3.90*** **	**3.95** **

** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001

As depicted in the Table 4.3.7.4, an overall significant ($p<0.001$) change of 17.06% in the MMSE score of MCI patients belonging to the experimental Group 2 with flaxseeds plus vitamin B12 was observed with a positive shift towards normal cognition ability after having received this twofold supplementation. When a gender-wise comparison was drawn, it depicted that the MMSE scores took an overall significant ($p<0.001$) net raise of 14.44 % for males and 18.84 % for females. Similarly, the age-wise categorization showed that the young –old patients had a 15.81% increased MMSE score whereas old-old demonstrated a 20.82% increase.

Table 4.3.7.4: Pre and post MMSE score of MCI patients in supplementation Group 2 (flaxseeds plus Vitamin B12)

MMSE Score in Group2 (flaxseeds + B12)	Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85yrs (n=16)
Pre	24.0±3.18	24.5±2.57	23.7±3.53	24.5±2.88	22.8±3.72
Post	28.1±1.78	28.0±1.75	28.2±1.83	28.3±1.68	27.6±1.99
Percentage ↑ / ↓	17.06 ↑	14.44 ↑	18.84 ↑	15.81 ↑	20.82 ↑
Paired *t*-test	**12.52*** **	**7.79*** **	**9.98*** **	**11.05*** **	**6.24*** **

*** Significant from the baseline value at p<0.001

The Table 4.3.7.5 reveals that the experimental Group 2 MCI patients had a significant (p<0.001) improvement of 20.10% in the total ACE score after flaxseeds plus vitamin B12 supplementation.

The trend from these gender and age stratifications was depictive of a significant (p<0.001) positive shift towards healthy cognition levels resultant from the Group 2 supplementation programme. Moreover, the ACE scores rose to 20.29 % for males and 19.97 % increase for females. Correspondingly, the patients in the age range of 60-69 yrs had a 17.63 % increased ACE score while for 70-85 yrs it was an increase of 27.46%.

Table 4.3.7.5: Pre and post ACE score of MCI patients in the supplementation Group 2

ACE Score in Group2 (flaxseed+B12)	Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
Pre	67.9±9.19	67.8±8.27	67.9±9.86	69.2±8.40	64.2±10.47
Post	81.5±6.81	81.5±7.08	81.5±6.73	81.4±6.34	81.8±8.19
Percentage ↑ / ↓	20.10 ↑	20.29 ↑	19.97 ↑	17.63 ↑	27.46 ↑
Paired *t*-test	**12.55*** **	**8.23*** **	**9.36*** **	**11.60*** **	**6.52*** **

*** Significant from the baseline value at p<0.001

As revealed from the Table 4.3.7.6, the MNA scores too brought about a significant (p<0.001) change in the intervened subjects. The overall 12.36 % significant rise in the scores of total subjects with 12.30 % in males and 11.58 % in females was noticed. The 11.82 % and 14.34 % increase was observed for the both age groups.

Table 4.3.7.6: Pre and post MNA score of MCI patients in supplementation Group 2

MNA Score in Group2 (flaxseeds + B12)	Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
Pre	9.9±1.76	9.8±1.92	10.1±1.66	10.0±1.73	9.6±1.85
Post	11.1±1.06	11.0±1.19	11.3±0.97	11.3±1.01	11.0±1.21
Percentage ↑ / ↓	12.36 ↑	12.30 ↑	11.58 ↑	11.82 ↑	14.34 ↑
Paired *t*-test	**7.46*** **	**4.38*** **	**5.99*** **	**6.03*** **	**4.37*** **

*** Significant from the baseline value at $p<0.001$

Table 4.3.7.7 signifies the improvement of total patients rising to normal cognition by 65% total patients in Group 2 using MMSE. Further, 67% of the males, 64% in females, 66% young-old and 62% old-old shifted to normality. 42% of total patients in Group 1 improved to normal side. Similarly, 35% males, 48% females, 32% young-old and 69% old-old progressed to normal cognitive status. The MMSE scores of patients represented that the number of 44 in Group 2 and 27 from Group 1 progressed towards the normal category from the MCI state (Figure 4.3.7.1).

Table 4.3.7.7: Improvement in MCI patients to normal cognition levels aided through MMSE detection

Number of MCI subjects detected by MMSE tool			Males (n= 53)	Females (n= 67)	60-69 yrs (n= 88)	70-85 yrs (n= 32)
Group 1 (B12) (n=60)	**Pre**	58 (96.7)	28 (96.5)	30 (96.8)	42 (95.5)	16 (100.0)
	Post	33 (55.0)	18 (62.0)	15 (48.4)	28 (63.6)	5 (31.2)
	MCI Shifted to Normal	25 (41.7)	10 (34.5)	15 (48.38)	14 (31.8)	11 (68.75)
Group 2 (Flaxseeds + B12) (n=60)	**Pre**	55 (91.7)	21 (87.5)	34 (94.4)	39 (88.6)	16 (100.0)
	Post	16 (26.7)	5 (20.8)	11 (30.5)	10 (22.7)	6 (37.5)
	MCI Shifted to Normal	39 (65.0)	16 (66.7)	23 (63.9)	29 (65.9)	10 (62.5)

Numbers in parenthesis indicate percentage

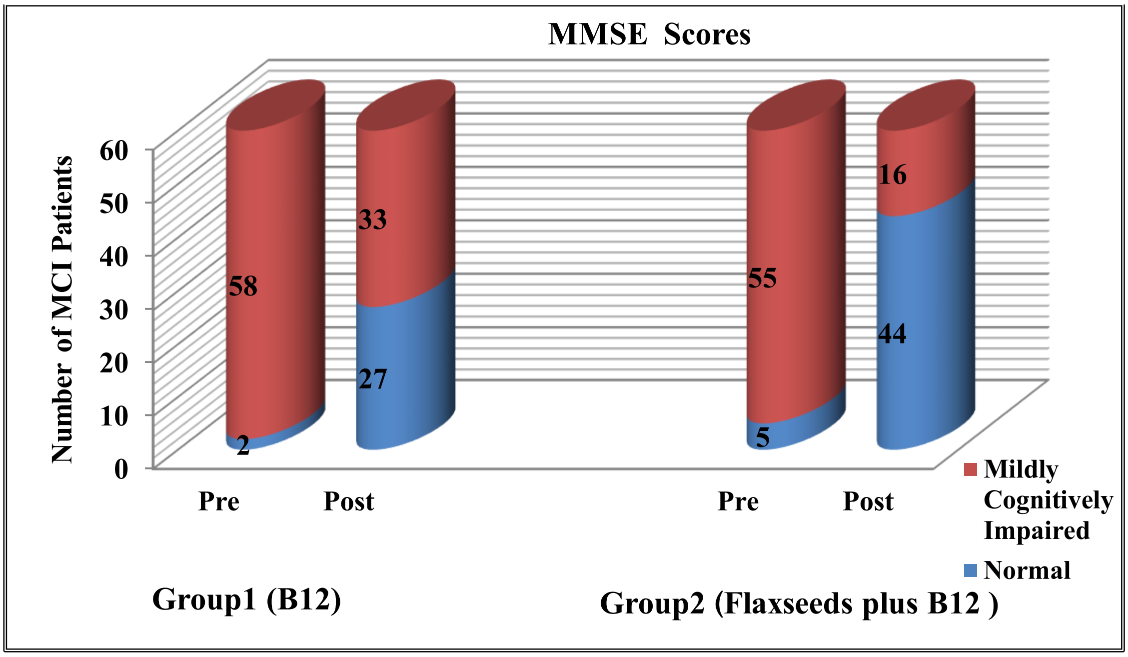


Figure 4.3.7.1: MMSE Scores of MCI patients before and after in both supplementation groups

The improvement of MCI patients raised to normal cognition levels by 45% in Group 2 with males being 50%, females 42%, young-old 45% and old-old to 44% using the ACE. Likewise, the number changed to 23% in Group 1 where 28% males, 19% females, 22% young–old and 25% in old-old reversed to normal condition (Table 4.3.7.8). As symbolized from the ACE scores, Group 2 had 27 MCI patients reverted to normal condition and 14 patients in the case of the Group 1(Figure 4.3.7.2).

Table 4.3.7.8: Improvement in MCI patients to normal cognition levels aided through ACE detection

Number of MCI subjects detected by ACE tool			Males (n= 53)	Females (n= 67)	60-69 yrs (n= 88)	70-85 yrs (n= 32)
Group 1 (B12) (n=60)	Pre	60 (100.0)	29 (100.0)	31 (100.0)	44 (100.0)	16 (100.0)
	Post	46 (76.7)	21 (72.4)	25 (80.6)	34 (77.3)	12 (75.0)
	MCI Shifted to Normal	14 (23.3)	8 (27.6)	6 (19.3)	10 (22.7)	4 (25.0)
Group 2 (Flaxseed + B12) (n=60)	Pre	60 (100.0)	24 (100.0)	36 (100.0)	44 (100.0)	16 (100.0)
	Post	33 (55.0)	12 (50.0)	21 (58.3)	24 (54.5)	9 (56.3)
	MCI Shifted to Normal	27 (45.0)	12 (50.0)	15 (41.7)	20 (45.6)	7 (43.7)

Numbers in parenthesis indicate percentage

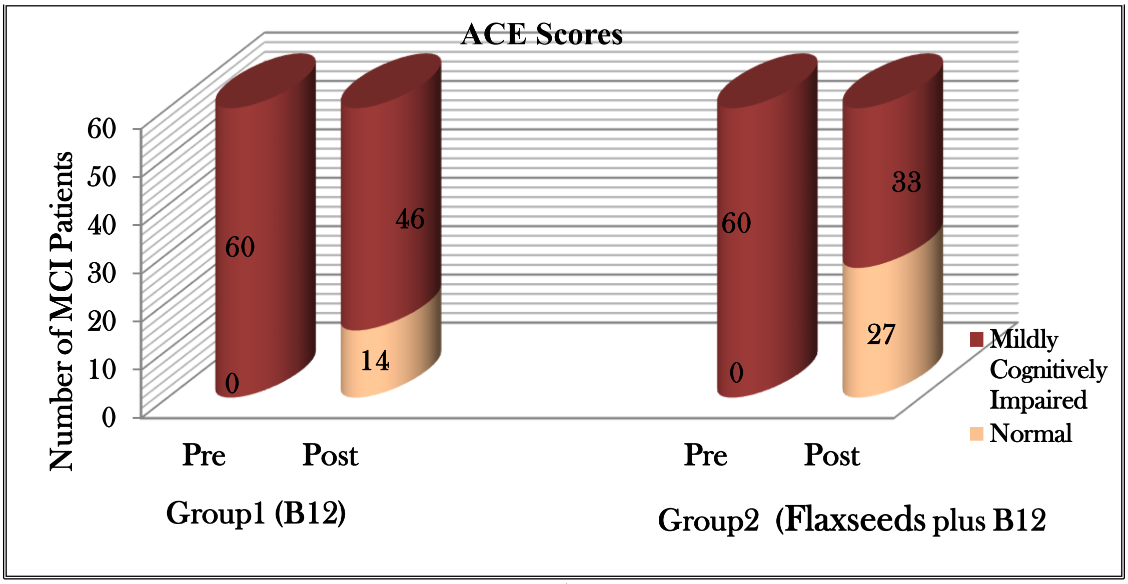


Figure 4.3.7.2: ACE Scores of MCI patients before and after in both supplementation groups

Table 4.3.7.9 characterizes the normal condition of MCI patients increasing to 60% in Group 2 with males being 58%, females 61%, young-old 64% and old-old to 50% using the YFPIT. In the same manner, 38% normal increase in Group 1 along with 35% males, 42% females, 41% young–old and 31% old-old moved to normal status. A number of 39 patients in Group 2 and 28 patients in Group 1 progressed to normal condition according to YGPIT (Figure 4.3.7.3).

Table 4.3.7.9: Improvement in MCI patients to normal cognition levels aided through YFPIT detection

Number of MCI subjects detected by YFPIT tool			Males (n= 53)	Females (n= 67)	60-69 yrs (n= 88)	70-85 yrs (n= 32)
Group 1 (B12) (n=60)	**Pre**	55 (91.7)	28 (96.5)	27 (87.1)	42 (95.5)	13 (81.3)
	Post	32 (53.3)	18 (62.0)	14 (45.2)	24 (54.5)	8 (50.0)
	MCI Shifted to Normal	23 (38.3)	10 (34.5)	13 (41.9)	18 (40.9)	5 (31.2)
Group 2 (Flaxseeds + B12) (n=60)	**Pre**	57 (95.0)	22 (91.7)	35 (97.2)	43 (97.7)	14 (87.5)
	Post	21 (35.0)	8 (33.3)	13 (36.1)	15 (34.1)	6 (37.5)
	MCI Shifted to Normal	36 (60.0)	14 (58.3)	22 (61.1)	28 (63.6)	8 (50.0)

Numbers in parenthesis indicate percentage

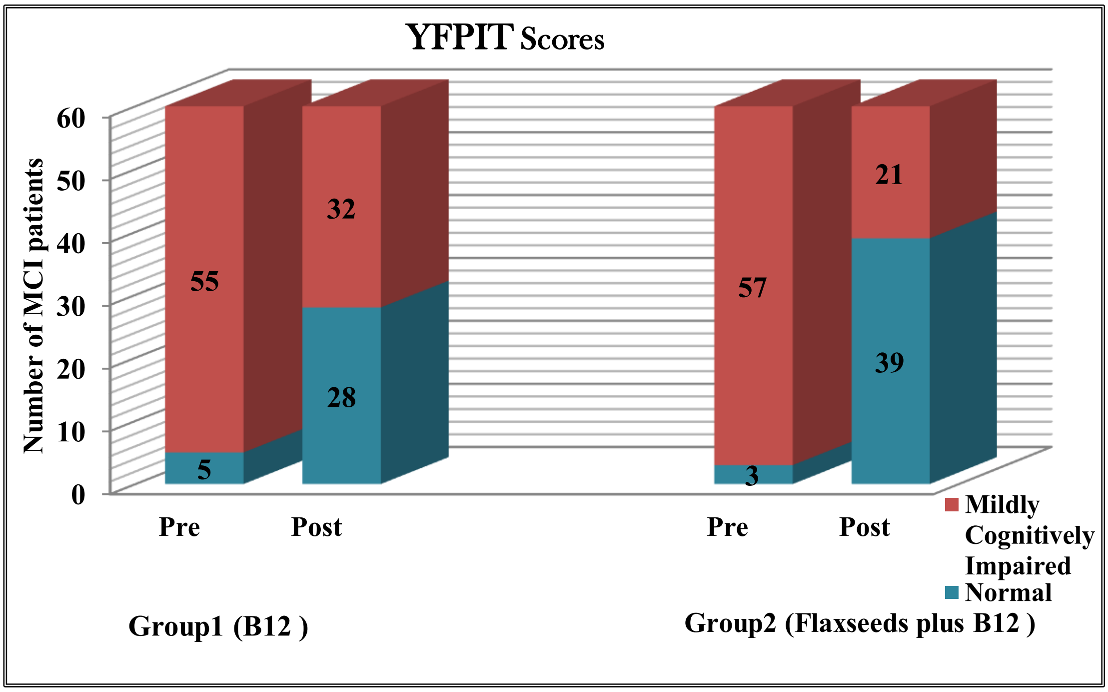


Figure 4.3.7.3: YFPIT Scores of MCI patients before and after in both the supplementation groups

As apparent from the Table 4.3.7.10, an increase of 45% was observed in the normal nutritional status of Group 1 patients with a reduction in at risk of malnutrition and malnourished categories amounting to 51.66% and 3.33% using the MNA scores. Further 11 males, 16 females, 21 young–old and 6 old-old regained towards normal status (Figure 4.3.7.4).

Table 4.3.7.10: Improvement in Group 1 MCI patients to normal nutrition levels aided through MNA detection

MNA Status	Group 1 (B12)	Total (n=60)	Males (n=29)	Females (n=31)	60-69 yrs (n=44)	70-85 yrs (n=16)
Normal Nutritional Status	Pre	19 (31.7)	9 (31.0)	10 (32.2)	15 (34.1)	4 (25.0)
	Post	27 (45.0)	11 (37.9)	16 (51.6)	21 (47.7)	6 (37.5)
At risk of Malnutrition	Pre	37 (61.7)	18 (62.1)	19 (61.3)	27 (61.4)	10 (62.5)
	Post	31 (51.7)	17 (58.6)	14 (45.2)	22 (50.0)	9 (56.3)
Malnourished	Pre	4 (6.7)	2 (6.9)	2 (6.5)	1 (2.3)	3 (18.7)
	Post	2 (3.33)	1 (3.4)	1 (3.2)	1 (23.3)	1 (6.2)

Numbers in parenthesis indicate percentage

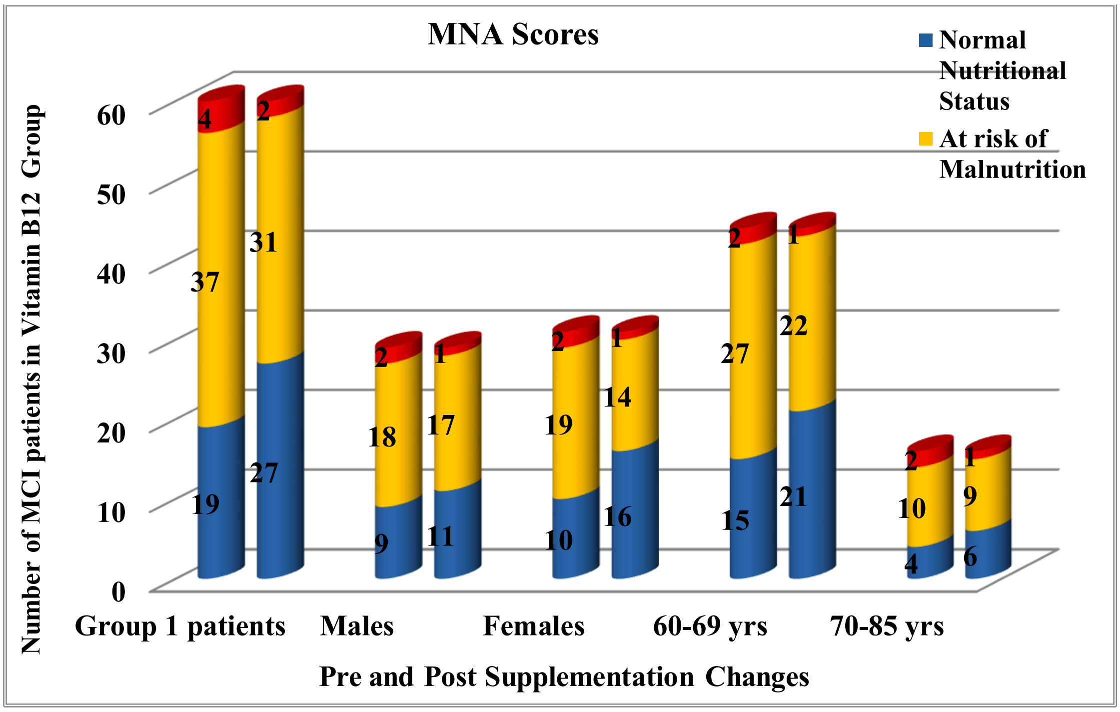


Figure 4.3.7.4: MNA Scores of MCI patients before and after in the Group 1 of supplementation

Table 4.3.7.11 is suggestive of an increase of 53.33% brought in the normal nutritional status of Group 2 patients alongside a reduction in at risk of malnutrition and malnourished categories to be 46.66% and 0% using the MNA scores. Moreover, 11 males, 21 females, 25 young–old and 7 old-old patients recovered back the normal status (Figure 4.3.7.5).

Table 4.3.7.11: Improvement in Group 2 MCI patients to normal nutrition levels aided through MNA detection

MNA Status	Group 2 (Flaxseeds + B12)	Total (n=60)	Males (n=24)	Females (n=36)	60-69 yrs (n=44)	70-85 yrs (n=16)
Normal Nutritional Status	Pre	18 (30.0)	8 (33.3)	10 (27.8)	14 (31.8)	4 (25.0)
	Post	32 (53.3)	11 (45.8)	21 (58.3)	25 (56.8)	7 (43.7)
At risk of Malnutrition	Pre	37 (61.7)	13 (54.2)	24 (66.7)	27 (61.4)	10 (62.5)
	Post	28 (46.7)	13 (54.2)	15 (41.7)	19 (43.2)	9 (56.3)
Malnourished	Pre	5 (8.3)	3 (12.5)	2 (5.6)	3 (6.8)	2 (12.5)
	Post	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Numbers in parenthesis indicate percentage

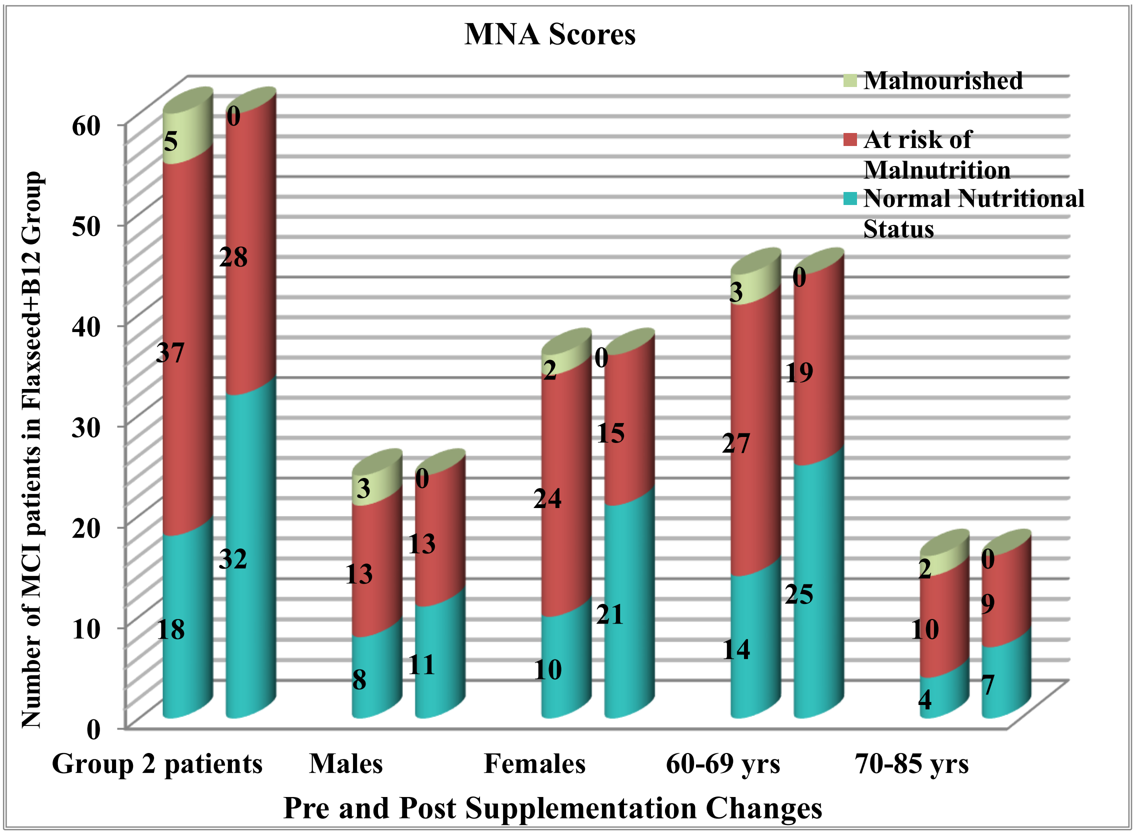


Figure 4.3.7.5: MNA Scores of MCI patients before and after in the Group 2 of supplementation

4.3.8 Relationship between the neuropsychological test battery, serum Vitamin B12, glycemic response and serum lipid parameters of the MCI patients

Table 4.3.8.1 recapitulates the association between the change in glycemic factors with change in serum vitamin B12 and dietary Omega-3 levels. Serum vitamin B12 was significantly ($p < 0.05$) and inversely associated with FBS whereas change in serum B12 with HbA_{1c}, dietary omega-3 levels with the changes of FBS and HBA_{1c} proved to be inversely associated with insignificance.

Table 4.3.8.1: Correlation amongst change in glycemic factors with change in serum vitamin B12 and dietary Omega-3 levels

Parameters	Change in Serum Vitamin B12	Change in Omega 3 intake
Change in FBS	- 0.147*	- 0.077 ^{NS}
Change in HbA_{1c}	- 0.046 ^{NS}	- 0.010 ^{NS}

*Significant from the baseline value at p<0.05, NS- Non- significant

4.3.9 Associations between serum vitamin B12, cell blood counts and lipemic responses with the anthropometric, biophysical, biochemical and dietary parameters

As described in the Table 4.3.9.1, the change in serum vitamin B12 demonstrated a significant ($p<0.05$) positive correlation with the hip circumference (HC) change. Dietary Omega-3 was significantly ($p<0.05$) positively correlated to the change in the waist circumference (WC). However, serum vitamin B12 insignificantly positively correlated to BMI as well as SBP and insignificantly negatively correlated with WC, WHR and DBP. Meanwhile, the Omega-3 too showed an insignificant positive correlation for BMI, HC, WHR, DBP and insignificant negative correlation with SBP.

Table 4.3.9.1: Correlation amongst change in anthropometric and biophysical factors with change in serum Vitamin B12 and Omega-3 levels

Parameters undergone change	Change in serum B12	Change in omega-3 intake
BMI	0.039 ^{NS}	0.005 ^{NS}
WC	- 0.014 ^{NS}	0.184*
HC	0.166*	0.116 ^{NS}
WHR	- 0.122 ^{NS}	0.124 ^{NS}
SBP	0.010 ^{NS}	- 0.020 ^{NS}
DBP	-0.037 ^{NS}	0.071 ^{NS}

*Significant from the baseline value at $p<0.05$, NS- Non- significant

An association was determined between the ACE and MMSE neurological scores, nutritional assessment, serum vitamin B12 and Omega-3 levels as evident from the Table 4.3.9.2. The Omega-3 levels significantly ($p<0.05$) positively correlated to the ACE and MMSE neurological scores and insignificantly positively correlated to the MNA score. On the other hand, serum vitamin B12 established to be insignificantly positively correlated to all of the ACE, MMSE and MNA scores.

Table 4.3.9.2: Correlation amongst change in neurological and nutritional assessment scores with change in B12 and omega-3 intake levels

Parameters for change	Change in Serum vitamin B12	Change in omega-3 intake
ACE Score	0.112 ^{NS}	0.161*
MMSE Score	0.132 ^{NS}	0.174*
MNA Score	0.040 ^{NS}	0.121 ^{NS}

*Significant from the baseline value at $p<0.05$, NS- Non- significant

RESULT HIGHLIGHTS

- ***Anthropometric parameters revealed that there was a significant reduction in the BMI by 3.4% and 3.8% in Group 1 and 2 post the B12 and B12 plus flaxseed supplementation for a period of six months respectively.***
- ***Biophysical analysis showed significant decrease in systolic blood pressure readings of Group 1 and 2 by 6.0% and 6.2%.***
- ***The dietary protein intake of the patients significantly increased with slight significant increase of energy for Group 1 whereas significantly higher intakes were depicted than the baseline with 14.8% for vitamin B12 in Group 1 and 1087.5% for omega-3 in Group 2.***
- ***Glycemic response significantly diminished to 2.6% and 14% for FBS and HbA_{1c} values in Group 1 and further amounted to 20.3% for HbA_{1c} in Group 2 with flaxseeds plus B12 supplementation.***
- ***Amongst the lipid parameters, significant drop in TC (19%), TG (23.4%), LDL (20.2%) and VLDL (34%) was observed for Group 2 whereas the HDL levels significantly escalated in Group 2 (19.3%) and Group 1(6.8%).***
- ***A significant decline was detected for TC/HDL, TG/HDL and Atherogenic Index by 33.3%, 37.3%, 41.2% in the MCI patients of Group 2 respectively. Moreover, a significant fall in LDL/HDL by 34% was also ascertained in Group 2 patients.***
- ***Serum vitamin B12 directed towards a significant increase by 370% and 276% in case of Group 2 and 1 patients. The CBC profile also showed a significant increase in terms of hemoglobin by 12.2%, PCV by 14.4% and MCV by 4.2% in Group 2 and in Group 1 by 10.9%, 13.9% and 3.1%.***
- ***Improvement shift to normal cognition levels by ACE score depicted an increase of 45% normals in Group 2 and likewise 23% in Group1. MMSE scores adjudged 65% to be cognitively healthy in Group 2 and 41% in Group1. The MNA scores determined that 53% subjects in Group 2 regained normal nutritional status and 45% in Group 1.***

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DISCUSSION

In the present research trial of 20 g roasted flaxseeds plus vitamin B12 supplementation on MCI patients for a period of six months significant improvements in dietary ALA (omega-3 fatty acid) and serum vitamin B12 were brought forth. In continuation with these findings, the elevated omega-3 levels caused significantly increased percentage of patients for normality in ACE as well as MMSE scores and noteworthy significant improvements were found by marked reduction in the glycemic and lipemic responses.

Post supplementation, the dietary omega-3 of Group 2 patients increased by 1087% along with serum vitamin B12 shooting up by 370%.

Comprehensive reviews focusing on the similar lines signified that flaxseeds are one of the richest plant sources of the ω-3 fatty acid i.e. α-linolenic acid (ALA) rich source of ω-3 fatty acid: α-linolenic acid (ALA) (Gebauer et al. 2006; Tonon et al. 2011. α-linolenic acid is the major fatty acid which ranged from 39.00 to 60.42 % among all of the lipids present in flaxseed oil followed by oleic, linoleic, palmitic and stearic acids (Guimaraes et al 2013;Pellizzon et al 2007).

Evidence based trials indicate that flaxseeds abundantly possess relatively high concentrations of a-Linolenic Acid (18:3 n-3) accruing about 58.7 (g fatty acid/100-g oil) in comparison to the other fatty acid compositions of seed oils fruits, herbs and seeds. The Iranian linseed oil (*Linum usitatissimum*) when studied for its fatty acid content, total lipid, refractive index, peroxide, iodine, acid and saponification values, provided a significant difference in the average amount of peroxide value only on the first 7 days of storage, and its increase (8.30%) conformed to the international standard.(Gunstone et al 1994, Hassan-Zadeh et al 2008).

In a recent mouse model study the ALA supplementation in diet was better than intravenous treatment in improving motor coordination. Both types of ALA supplementation i.e by dietary supplementation or injections improved spatial learning and memory after stroke. This cognitive improvement correlated with higher survival of hippocampal neurons. These results supported of clinical investigations establishing therapeutic plans using ALA supplementation (Bourourou et al 2016).

In addition to this, another recent study has shown omega-3 PUFA as ALA to be effective in treating and preventing various diseases. ALA may act as therapeutic agents as well and their significant role against inflammatory diseases, such as cardiovascular and neurodegenerative diseases has been described. ALA among the omega-3 PUFA are a significant modifiers, which can influence brain elasticity and thus, effect on central nervous system functioning. Therefore, appropriate dietary management appears to be a non-invasive and effective approach to counteract neurological and cognitive disorders (Wysoczański et al 2016).

Several other animal studies conducted in the analogous position established that dietary sources rich in a-linolenic acid such as linseed (flaxseed) encouragingly increased the EPA, DPA, DHA, and linolenic acid contents to 100%, 29%, 35%, and 55%, respectively (Cunnane et al. 1990; Cherian and Sim 1995; Romans et al. 1995).

Further, the results using the animal models have in the past indicated that the growing pigs fed on 5% flaxseeds feed for 8 weeks had significantly increased amounts of desaturation-elongation products of alpha-linolenic acid in liver, heart, kidney and brain than pigs fed the same diet containing no flax. The 18:3n-3 (ALA) in flaxseeds attributed to be both absorbed and readily available for being metabolized to longer chain products (Cunnane et al 1990). Ahn et al. (1996) found that dietary α-linolenic acid (LNA) significantly ($p < 0.05$) led to the increased proportion of n−3 fatty acids and these increases mainly consisted of C18:3n3 (ALA), C20:5n3 (EPA) and/or C22:6n3 (DHA).

Concurrently, support has been garnered for the efficacy of flaxseeds in neurocognitve disorders. Pan et al (2015) have forwaded towards this direction portrarying that the nutraceutical alpha-linolenic acid (LIN) is an essential omega-3 polyunsaturated fatty acid that has a wide safety margin. In their animal model, the administration of three intravenous injections of alpha-linolenic acid over a 7 day period significantly improved motor performance on the rotarod, enhanced memory retention, exerted an anti-depressant-like activity and increased animal survival. This dosing schedule significantly reduced soman-induced neuronal degeneration in four major vulnerable brain regions up to 21 days. Taken together, alpha-linolenic acid reduces the profound behavioral deficits induced by soman possibly by decreasing neuronal cell death, and increases animal survival.

A total of 181 consecutive, demented (DSM-III or DSM-III-R criteria and score below 24 on the Mini-Mental State Examination [MMSE]) outpatients (mean age 77.5 years) were prospectively evaluated and had their vitamin B12 level measured. The frequency of vitamin B12 deficiency (less than 200 pg/mliter) was 25% (46 patients). Treatment outcome was obtained in 19 patients (19 of 46). Despite cobalamin replacement, 16 of 19 patients persisted in showing progressive decline during follow-up visits (3 to 24 months). The non-response to vitamin B12 replacement in most cases seems to reflect the presence of associated irreversible dementia or a follow-up of shorter duration in a few patients. All of the patients who showed some improvement (MMSE returned to normal values) had mild dementia with a history of less than 2 years. Thus, screening for B12 deficiency should be considered in patients with recent onset of mild mental status changes (Cunha et al 1995).

Furthermore, randomised clinical trials (RCTs) have demonstrated that vitamin B12 (cobalamin) treatment was effective and also better tolerated in elderly patients aged above 60 years when treated for serum vitamin B12 deficiency (Bolaman et al 2003). Vitamin B12 intervention in people with elevated hcy levels in India could prove to be effective in lowering hcy levels and help maintain or improve cognitive function (Agrawal et al 2015). Another study has validated that long-term supplementation of daily oral 400 μg folic acid plus 100 μg vitamin B12 promoted improvement in cognitive functioning after 24 months particularly in the cases of immediate and delayed memory performance (Walker et al 2012).

In an overview, Bourre (2006) has evaluated that vitamin B12 delays the onset of signs of dementia (and blood abnormalities), provided it is administered in a precise clinical timing window, before the onset of the first symptoms. Supplementation with cobalamin improves cerebral and cognitive functions in the elderly; it frequently improves the functioning of factors related to the frontal lobe, as well as the language function of those with cognitive disorders.

Results of this V.O.I.C.E. trial showed that marked improvement in the MCI patients was evident with more than half (53%) in Group 2 and 43% of Group 1 elderly to have been placed in the normal cognition levels as determined from the MNA sores According to the Alzheimer's Disease International (ADI, 2014) report "nutritional screening aims to identify those who are malnourished or 'at risk' of malnutrition, and composite screening tools have been developed, e.g. the widely used six item mini nutritional assessment (MNA-SF)".

In the undertaken trial, the anthropometric parameters revealed that there was a significant reduction in the BMI by 3.4% and 3.8% in Group 1 and Group 2 post the B12 and B12 plus flaxseed supplementation for a period of six months respectively. Also, there was significant decrease with both groups ($p<0.001$) and non-significant difference between the groups. It was safely assumed that flaxseed supplementation had little to contribute in decrease in BMI and maximum of this reduction could be attributed to B12 supplementation. The follow-up BMI decrease in both the groups, suggested that nutritional supplementation has a beneficial effect in improving the overall nutritional status of the patient. In an independent study, a DNA methylation variant was associated with BMI changes through interactions with total or supplemental vitamin B2, vitamin B12, and folate. (Huang et al 2015). Also many studies including one conducted by Denise Evans in 2013 suggested that nutritional supplementation helps bring BMI at more desirable levels.

Biophysical analysis showed significant decrease in systolic blood pressure readings of Group 1 and 2 by 6.0% and 6.2%. Again no significant difference was found between the groups suggesting more contribution of B12 supplementation in bringing down the blood pressure. According to the American Heart Association, plasma homocysteine has association with the high blood pressure in large community studies. In evidence taken from the Framingham Heart Study and published in 2003, researchers found that those individuals with hypertension had elevated homocysteine levels. Homocysteine levels could be brought under control by ample supplementation on Vitamin B12. An experimental study conducted on rats by Franca and Vianna in 2010 confirmed that B12 vitamin therapy was effective for the control of systolic blood pressure and oxidative stress. Hence, it could be thought as one of the alternative therapies to prevent the occurrence of stroke.

Glycemic response significantly diminished to 2.6% and 14% for FBS and HbA_{1c} values in Group 1 and further amounted to 20.3% for HbA_{1c} in Group 2 with flaxseeds plus B12 supplementation. The possible role of vitamin B12 in glycaemic control was confirmed by another study conducted for four weeks by Narayanrao (2015) on type 2 diabetic patients, where they found significant fall in HbA_{1c} levels from 21.5 ± 2.6 to 15.4 ± 6.4 (p=0.04) after B12 supplementation.

In Group 2 flaxseeds further helped in the cause of controlling glycemic factors. Flaxseed intake decreased glucose and insulin and improved insulin sensitivity. Flaxseed contains alpha-linolenic acid, an important plant-based omega-3 fatty acid. Hutchins and her colleagues (2013) in a 12-week long study observed a group of 25 overweight men and postmenopausal women with pre-diabetes subjects who consumed 0, 13, or 26 grams of ground flaxseed daily. They monitored glucose, insulin, homeostatic model assessment (HOMA-IR), and normalized percent of alpha-linolenic fatty acid (ALA) and ultimately observed that 13 gm/day of flaxseed significantly decreased insulin resistance.

In Group 1 no significant difference was found between pre-intervention and post-interventional parameters. However, in Group 2 significant drop in TC (19%), TG (23.4%), LDL (20.2%) and VLDL (34%) was observed whereas the HDL levels significantly escalated in Group 2 (19.3%). Giving additional flaxseed supplementation could be attributed for this improvement in lipemic factors. Similar results have been found on a study conducted on rats (Khalesi et al 2011) where for 30 days rats were fed with flaxseed rich diet and it was concluded that consumption of flaxseed may significantly reduce total cholesterol and increase high density lipoprotein cholesterol in blood. Another study (Lucas et al 2002) concluded that flaxseed supplementation lowered ($p<0.05$) both serum total cholesterol and non-high-density lipoprotein cholesterol by 6%.

V.O.I.C.E. trial had resulted into serum vitamin B12 directed towards a significant increase by 370% and 276% in case of Group 2 and Group 1 patients. CBC profile also showed a significant increase in terms of hemoglobin by 12.2%, PCV by 14.4% and MCV by 4.2% in Group 2 and in Group 1 by 10.9%, 13.9% and 3.1%. Vitamin B-12 contributes to hemoglobin synthesis by activating succinyl CoA, a chemical required to make heme. Succinyl CoA serves as a precursor for heme, and it undergoes several chemical modifications to eventually form an active hemoglobin protein. Vitamin B12 deficiency can cause macrocytic anemia. This type of anemia causes the production of fewer but larger red blood cells, decreasing the amount of hemoglobin in the blood. Low vitamin B12 levels lead to lower hemoglobin and those with lower hemoglobin levels are at higher risk of Alzheimer's disease as suggested by a current study (Min et al 2016) which further specified that just by increasing vitamin B12 serum levels mortality rate of AD can be reduced to some extent. Also in another study conducted by Allain in 1997 on a sample size of 233 patients on rural and urban population of Zimbabweans it was discovered that vitamin B12 was significantly lower in those who were anaemic (290 vs 413 pg/ml, $p<0.05$).

Present V.O.I.C.E. trial revealed that there was weak but positive correlation between omega-3 intake and neurological scores. It could be implied here, that an increase in the consumption of omega-3 fatty acid rich foods could contribute in the prevention of dementia and lowering down the negative impact of aging on the central nervous system. Similar results were introduced by Dangour (2009) in the inhabitants of Latin American countries and China possessing better cognitive functions in old age on consuming more of the omega-3 rich diets inclusive of fish and meat. In a meta-analysis study by Lin et al (2013) demonstrated that the concentration of omega-3 PUFA (EPA, DHA) significantly reduced in older people with dementia compared to those without the disease. In a similar instance, the Chicago Health and Aging Project (CHAP) validated that individuals having the higher intake of fish and diets rich in omega-3 had lower rate of incident dementia. Concludingly, a recent study has fostered that ALA supplementation by modification of the daily diet prevented mortality and cerebral damage in a rodent model of ischemic stroke. The pleiotropic ability of ALA triggered responses which resulted in neuronal protection, stimulation of neuroplasticity, and brain artery vasodilation. Overall, this proposed a promising therapeutic opportunity by integrating a nutritional-based approach focusing on enriching the daily diet in ALA to prevent the devastating damage (Blondeau 2016).

Results also indicated an inverse relation of serum vitamin B12 levels of the MCI patients with respect to their fasting blood sugar levels. A similar study conducted to find the evaluation of serum vitamin B12 levels in type 1 diabetic patients (Koshy et al 2012) initiated that there was a noteworthy presence of low serum B12 levels in 45.12 % of type 1 diabetic patients.

CONCLUDING REMARKS

In the Group 2, serum vitamin B12 increased (276%), HDL (19.3%) and dietary ALA (1087.50%) too escalated whereas BMI (3.8%), SBP (6.2%), HbA_{1c} (20.3%), TC (19%), TG (23.4%), LDL (20.2%), VLDL (34%), TC/HDL (33.3%), TG/HDL (37.3%), LDL/HDL (34%) and Atherogenic Index, (41.2%) diminished. ACE depicted 45% increase, MMSE of 65% and 53% regained normal nutritional status from MNA and ALA (flaxseeds) significantly positively correlated to WC, ACE, and MMSE scores.

When observed in the case of Group 2, serum vitamin B12 (370%), HDL escalated (6.8%). BMI (3.4%), SBP (6.0%), FBS (2.6%) and HbA_{1c} (14%) reduced. 23% MCI patients by ACE, 41% from MMSE and 45% by MNA were in the normal category. Serum vitamin B12 significantly and inversely associated with FBS whereas significantly positively with HC.