Chapter 4

Results and Discussion

The results of the research plan exhibited in the preceding chapter are portrayed and discussed in this section under the following heads and subheads as per the objectives of the study:

PHASE I

Nutritional status of pre and post-menopausal women (30-60y) of urban Vadodara

- Life style, health care and dietary behaviours of pre and post-menopausal women
- Physiological and metabolic aberrations in pre and post-menopausal women
- Effect of age and obesity on the association of menopause with metabolic profile
- Causal factors of Vitamin B12 and Folic acid deficiency and their relationship with risk factors of non-communicable diseases in women
- Association of inflammation with NCD risk factors in women
- Association of insulin resistance with NCD risk factors in non-diabetic women

PHASE II

Identification of flaxseed variety for supplementation and estimation of its nutritive profile

PHASE III

Metabolic and inflammatory response to supplementation of whole roasted flaxseeds in pre-menopausal overweight/obese women

- Screening and baseline characteristics of the women
- Randomised control trial to study the impact of whole roasted flaxseeds on lipid profile, insulin resistance and inflammation in pre-menopausal overweight/obese women.

PHASE I

NUTRITIONAL STATUS OF PRE AND POST-MENOPAUSAL WOMEN (30-60Y) OF URBAN VADODARA

Menopause can bring about various physiological, endocrinological, metabolic and psychological changes. Nutrition and lifestyle related habits of the women can affect the extent and severity of these changes or can be altered vice versa. Since the last few decades as India has been facing nutrition transition, a revolutionary change in the lifestyle habits have been observed affecting women who still remain the most neglected member of the family. The recent trends indicating a steep rise in the prevalence of non-communicable diseases (NCDs) in women are disturbing and need to be focussed. As NCDs are chronic in nature and develop due to faulty lifestyle and dietary habits over a period of time, there is a need to explore the role of menopause and other possible risks for development of NCDs in women.

Therefore the first phase of the research was dedicated to study the nutritional status and major risk factors for NCDs in pre and post-menopausal women. A screening followed by 2*2 factorial design was used for selection of the women for the study. Non-invasive risk analysis was performed on 131 women who gave consent followed by biochemical estimation on 90 women.

The results of this phase have been divided into six sections. First section deals with the non-invasive risk analysis focussing on the lifestyle, health and diet related practices of the women. Second section comprises biophysical and biochemical profile of pre and post-menopausal women including anthropometric indices, blood pressure, lipid profile, glycemic profile, thyroid profile, nutritional anemia, liver function test and kidney function test. Third section explores the relationship of menopause and metabolic aberrations irrespective of age and obesity. The causal factors of vitamin B12 deficiency and folate deficiency and their association with NCD's risk have been discussed in the fourth section. Fifth and sixth section deals with prevalence of inflammation and insulin resistance among women and their associated risk factors for NCDs respectively.

4.1. LIFE STYLE, HEALTH CARE AND DIETARY BEHAVIOURS IN PRE AND POST-MENOPAUSAL WOMEN

4.1.1 Characteristics of screened women

A total of 408 women were screened from the five zones of urban Vadodara. The basic characteristics of the women are presented in Table 4.1. The age distribution showed almost equal number of women between the three age group categories. Around 32.4% of the women were between 30-40y, 32.4% between 41-50y and 35.3% between 51-60y of age. The information on menopause revealed that about 46% of the women were pre-menopausal, 7.8% were going through peri-menopausal phase, 36% were in post-menopausal condition and 10% had undergone hysteractomy. No case of pregnancy was reported by the women. Out of total 408 women screened 4.7% were underweight, 15.9% had normal weight. The prevalence of overweight and obesity was 17.4% and 62% respectively. This is indicative of the presence of dual burden of malnutrition in the women. However the scenario of overweight/obesity seemed to be far worse than under-nutrition.

4.1.2 Background information of the women for non-invasive risk analysis

A pre-tested semi-structured questionnaire was used to assess the socio economic status of the women which included information regarding religion, education, marital status, occupation and income. Data on religion (Table 4.2) reflected that all of the women were Hindus. Majority of the women had education ranging from secondary to graduation. The percentage of higher education in women was higher in pre-menopausal women (50.8%) than that in post-menopausal ones (35.4%). Most of the women (92.4%) were married with a higher per cent of widows in the post-menopausal group. Pre-menopausal women were involved more in occupational activities like unskilled labour (4.6% v/s 1.5%), service (16.9% v/s 3%) and business (6.2% v/s 4.5%) than pre-menopausal women. However majority of the women in both the categories were housewives (81.7%). More than half of the women had nuclear families (62.6%), followed by joint families (26.7%) and extended nuclear families (10.7%). About 62% of the women had per capita income >Rs.5000. Post-

TABLE 4.1: BASIC CHARACTERISTICS OF THE SCREENED WOMEN (n=408); (n,%)

Parameters	n (%)
Age Distribution	
30-40y	132 (32.4)
41-50y	132 (32.4)
51-60y	144 (35.2)
Menopausal Status	
Pre Menopause	187 (45.8)
Peri Menopause	32 (7.8)
Post Menopause	148 (36.3)
Hysteractomy	41 (10.1)
Pregnancy	0 (0)
Prevalence of Obesity	
Underweight	19 (4.7)
Normal	65 (15.9)
Overweight	71 (17.4)
Obesity	253 (62)

Values in parenthesis indicate percentages

menopausal women had significantly higher proportion ($p<0.01$) of subjects with high per capita income. The decade wise categorization of age (Table 4.3) depicted more or less similar representation from all three decades i.e. 30-40y (32.2%), 41-50y (28.9%) and 50-60y (38.9%). The mean age of the women was 46.4±9.97 years. When compared between the two groups, post-menopausal women had significantly higher mean age ($p<0.001$) than pre-menopausal ones for obvious reasons (Figure 1).

4.1.3. Health profile of the women:

a) Obstetric History:

Mean age of menarche and pregnancy in women was 14.44±1.8y and 22.9±3.1y respectively (Table 4.4). The mean age of menopause was 45.45±4.8y. Obstetric history of the women showed that around 45% had more than 2 pregnancies in their life and only 16.8% had more than 2 children. The trend indicated high prevalence of abortions and was supported by information collected on abortions (40.5%) in the women. About 6.1% of the women reported still birth or death after birth of child. Merely 6% pre-menopausal women had >2 children in comparison to 27.3% in post-menopausal women, showing strong preference of having just 1 or 2 children in younger population ($p<0.001$).

During menopausal transition various menopausal symptoms are faced by women which can affect their health, nutritional status and quality of life. As reported in Figure 4.2, 21.1% of the post-menopausal women experienced vasomotor symptoms like hot flushes and night sweats and 28.8% experienced somatic symptoms like headache, tingling of muscle, joint pain, lack of energy with varying intensity from mild to severe. Psychological symptoms were the most common symptoms (33.3%) experienced by women. However, a low incidence of urogenital symptoms (3%) was seen in the women.

TABLE 4.2: BACKGROUND INFORMATION OF THE WOMEN (n, %)

Background Information	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	χ^2 Value
Religion				
Hindu	131 (100)	65 (100)	66 (100)	--
Education				
Illiterate	1 (0.8)	0 (0)	1 (1.5)	--
Primary	9 (6.9)	2 (3.1)	7 (10.6)	
SSC	32 (24.4)	12 (18.5)	20 (30.3)	
HSC	32 (24.4)	17 (26.2)	15 (22.7)	
Graduate	44 (33.6)	25 (38.5)	19 (28.8)	
Postgraduate	12 (9.2)	8 (12.3)	4 (6.1)	
PhD	1 (0.8)	0 (0)	1 (1.5)	
Marital Status				
Unmarried	1 (0.8)	0 (0)	1 (1.5)	--
Married	121 (92.4)	64 (98.5)	57 (86.4)	
Widow	9 (6.9)	1 (1.5)	8 (12.1)	
Occupation				
Unskilled Labour	4 (3.1)	3 (4.6)	1 (1.5)	8.95
Housewife	107 (81.7)	47 (72.3)	60 (90.9)	
Service	13 (9.9)	11 (16.9)	2 (3)	
Business	7 (5.3)	4 (6.2)	3 (4.5)	
Type of Family				
Nuclear	82 (62.6)	38 (58.5)	44 (66.7)	1.17
Joint	35 (26.7)	20 (30.8)	15 (22.7)	
Extended	14 (10.7)	7 (10.6)	7 (10.8)	
Per Capita Income (n=120)				
>Rs.5000	75 (62.5)	30 (50)	45 (75)	8.0**
<Rs.5000	45 (37.5)	30 (50)	15 (25)	

Values in parenthesis indicate percentages

Two tailed significance **p<0.01

TABLE 4.3: AGE-WISE DISTRIBUTION OF THE WOMEN (n,%; Mean±SD)

Age (Y)	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)
30-40	29 (32.2)	29 (64.4)	0 (0)
41-50	26 (28.9)	15 (33.3)	11 (24.4)
51-60	35 (38.9)	1 (2.2)	34 (75.6)
Mean Age (Mean±SD)	46.4±9.97	38.22±6.1	54.45±5.4

Values in parenthesis indicate percentages

TABLE 4.4: REPRODUCTIVE INFORMATION OF THE WOMEN (Mean±SD; n,%)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	Students' 't' Value
	Mean±SD	**Mean±SD**	**Mean±SD**	
Age of Menarche (y)	14.44±1.8	14.37±1.82	14.5±1.76	0.42
Age of First Pregnancy (y)	22.91±3.1	23.08±3.18	22.75±2.98	0.61
Age of Menopause (y)	--	--	45.45±4.8	--
	n (%)	**n (%)**	**n (%)**	**χ^2 Value**
>2 Pregnancies	59 (45.04)	25 (38.5)	34 (51.5)	2.24
>2 Children	22 (16.8)	4 (6.2)	18 (27.3)	10.37***
Abortions (Yes)	53 (40.5)	27 (41.5)	26 (39.4)	0.06
Still Birth or Death After Birth	9 (6.1)	5 (7.7)	4 (4.5)	0.13

Values in parenthesis indicate percentages

Two tailed significance***p<0.001

FIGURE 4.1: COMPARISON OF MEAN VALUES OF AGE BETWEEN PRE AND POST-MENOPAUSAL WOMEN

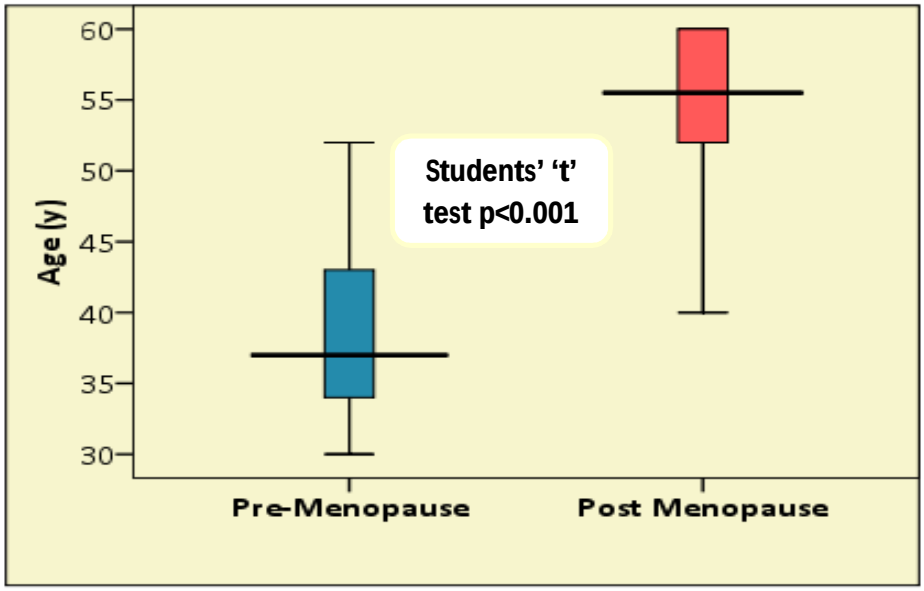
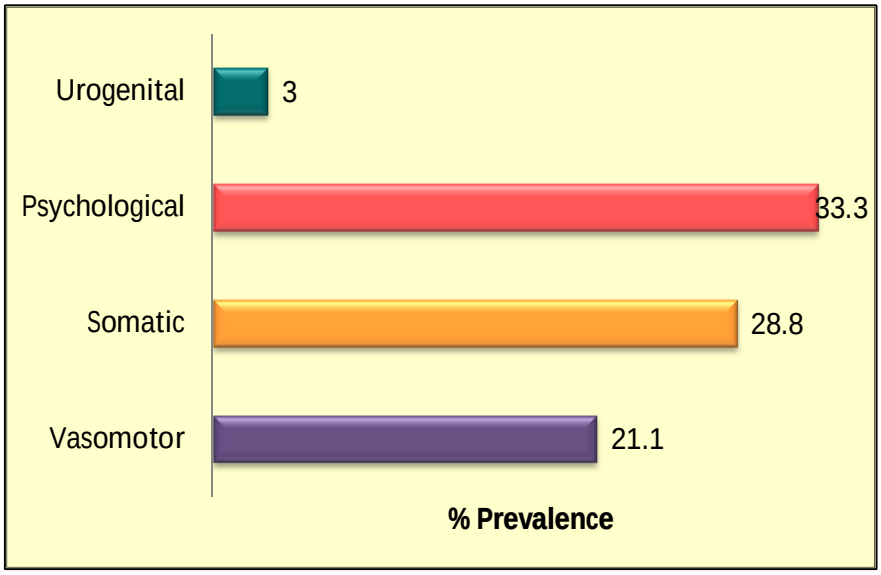


FIGURE 4.2: MENOPAUSAL SYMPTOMS EXPERIENCED BY POST-MENOPAUSAL WOMEN (%)



b) General gastrointestinal ailments and nutritional deficiency symptoms:

Table 4.5 illustrates the prevalence of gastrointestinal ailments in the women. The data revealed that around 21% of the women suffered from acidity, 10% from gastritis and 3% from constipation. General nutritional deficiency symptoms showed highest prevalence for fatigue (35%). Cramps/muscle weakness was found in 26% of the women. About 16% of the women were suffering from frequent headaches, 4.6% from loss of appetite, 8.4% from breathlessness and 9.9% from numbness in feet. A significantly higher number of the pre-menopausal women were suffering from frequent headaches ($p<0.05$) however the complaint of having cramps/muscle weakness (<0.01) was significantly higher in post-menopausal women. Around 8.4% of the women reported sudden weight gain and 3% observed sudden weight loss of more than 5 kg at any point of time in their life. These results showed that the weight gain in these women was a gradual process and it could be controlled through lifestyle modifications.

4.1.4. Life style habits and healthcare practices of the women:

The life style habits of the women, depicted in Table 4.6, revealed that none of the women consumed alcohol and only <1% had habit of tobacco chewing. Physical activity pattern of the women showed that around 70% of the women were moderately physically active and only 6% were having low physical activity level. The high prevalence of moderate or vigorous physical activity in the women was in contrast with risks assessed through anthropometric indices and one of the reasons for the same, might be, over reporting by the women. No significant difference was seen in the physical activity levels of pre and post-menopausal women. Nearly 78% of the women had normal sleep pattern. Disturbed sleep was more prevalent in post-menopausal women ($p<0.05$). Average sleeping and sitting hour in the women were 7.22±1.12 and 4.90±1.87 respectively, with a significantly high mean sitting hours in post-menopausal women ($p<0.05$) than pre-menopausal ones.

TABLE 4.5: GENERAL GASTROINTESTINAL AILMENTS AND NUTRITIONAL DEFICIENCY SYMPTOMS REPORTED BY THE WOMEN (n, %)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	χ^2 Value
Gastritis	13 (9.9)	8 (12.3)	5 (7.6)	0.82
Acidity	27 (20.6)	13 (20)	14 (21.2)	0.3
Constipation	4 (3.1)	2 (3.1)	2 (3)	0.0
Fatigue	46 (35.1)	25 (38.5)	21 (31.8)	0.63
Headache	21 (16)	15 (23.1)	6 (9.1)	4.76*
Loss of Appetite	6 (4.6)	4 (6.1)	2 (3.1)	0.67
Breathlessness	11 (8.4)	4 (6.1)	7 (10.8)	0.94
Paleness	3 (2.3)	0 (0)	3 (4.5)	--
Cramps/ Muscle Weakness	34 (26)	10 (15.4)	24 (36.4)	7.9**
Numbness in Feet	13 (9.9)	9 (13.8)	4 (6.1)	2.22
Sudden Weight Gain	11 (8.4)	4 (6.2)	7 (10.6)	0.84
Sudden Weight Loss	4 (3.1)	2 (3.1)	2 (3.0)	0.0002

Values in parenthesis indicate percentages
Two tailed significance*p<0.05, **p<0.01

TABLE 4.6: LIFE STYLE HABITS REPORTED BY THE WOMEN (n,%; Mean±SD)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	χ^2 Value
Alcohol Intake	0 (0)	0 (0)	0 (0)	--
Tobacco Consumption	1 (0.8)	0 (0)	1 (1.5)	--
Low Physical Activity	8 (6.1)	2 (3.1)	6 (9.1)	2.05
Disturbed Sleeping Pattern	29 (22.1)	9 (13.8)	20 (30.3)	5.11*
Average Sleep <7 Hours	31 (23.7)	10 (15.4)	21 (31.8)	4.9*
	Mean±SD	**Mean±SD**	**Mean±SD**	**Students' 't' Value**
Average Sleep (Hours)	7.22±1.12	7.4±0.98	7.04±1.2	1.9
Sitting (Hours)	4.90±1.87	4.5±1.8	5.3±1.9	2.5*

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

With regard to the health care practices, around 20% of the women reported regular health check-up with a significantly higher proportion ($p<0.05$) of post-menopausal women going for regular health check-up (Table 4.7). The women were asked regarding awareness of self-breast examination and the results showed that about

32% of the women were aware of the practice and out of these women 90% practiced it with varying frequency from twice a month to once in 6 months. Very few women had undergone PAP smear (6.9%) or mammography (8.4%). Of these, majority belonged to post-menopausal group. None of the post-menopausal women had ever received hormonal replacement therapy (HRT). About 61% of the women used Reverse Osmosis or any other kind of water purifier at home. Nearly one fourth of the women used nutritional supplements and the proportion of women consuming supplements was significantly higher ($p<0.05$) in post-menopausal women than pre-menopausal ones.

4.1.5. Dietary practices of the women:

The diet pattern of the women (Table 4.8) showed that most of the women were lacto-vegetarian (81.7%) with significantly higher number ($p<0.05$) of non-vegetarians and ovo-lactarians in the pre-menopausal women. Majority (90.8%) of the women consumed full fat milk of different varieties. Cotton seed oil was the first choice for cooking for almost 50% of the women followed by groundnut oil (15.3%) and only 17.6% used combination of oil for preparing food (Figure 4.3). As illustrated in Table 4.8, around 15% of the women reused the oil for deep frying. The mean consumption of oil, sugar, salt was 45.2±18.6 g, 32.5±17.9 g and 9.8±4.5 g respectively showing high consumption in women. The intake of all three items was significantly higher in households of post-menopausal women as compared to pre-menopausal women (Figure 4.4 (a) (b) (c)).

TABLE 4.7: HEALTH CARE PRACTICES REPORTED BY THE WOMEN (n, %)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	χ^2 Value
Health Check-up				
No Regular Health Check-up	105 (80.2)	57 (87.7)	48 (72.7)	4.6*
Frequency of check-up >6 months	13 (50)	4 (50)	9 (50)	0.0
Breast Cancer Preventive Practices				
No Awareness Regarding Breast Examination	89 (67.9)	41 (63.1)	48 (72.7)	1.4
No Breast Examination Practice in Aware Women	4 (9.5)	4 (16.7)	0 (0)	--
Frequency of Practice of Breast Examination >1 Month	25 (59.6)	12 (50)	13 (72.2)	0.03
No PAP Smear Done	122 (93.1)	64 (98.5)	58 (87.9)	5.7*
No Mammography Done	120 (91.6)	62 (95.4)	58 (87.9)	2.4
No Hormonal Replacement Therapy (For Post-menopausal Women)	--	--	66 (100)	--
Use of Water Purifiers				
Reverse Osmosis (RO) Technique	45 (34.4)	18 (27.7)	27 (40.9)	3.41
Other Water Purifiers	35 (26.7)	17 (26.2)	18 (27.3)	
Use of Nutritional Supplements	32 (24.4)	10 (15.4)	22 (33.3)	5.71*

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

TABLE 4.8: DIETARY PRACTICES OF THE WOMEN (n,%; Mean±SD)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	χ^2 Value
Ovo-lactarian or Non-vegetarian	24 (8.4)	17 (26.2)	7(10.3)	5.23*
Cow, Buffalo or Full Fat Packed Milk	119 (90.8)	57 (87.7)	62 (93.9)	1.5
Use of Single Oil Throughout Year	108 (82.4)	54 (83.1)	54 (81.8)	0.04
Re-use of Oil for Deep Frying	20 (15.3)	10 (15.4)	10 (15.15)	0.001
Per Capita Consumption of Oil >30g/day at Household Level	105 (80.1)	50 (76.9)	55 (83.3)	0.84
Per Capita Consumption of Salt >5g/day at Household Level	107 (81.7)	51 (78.5)	56 (84.8)	0.89
Per Capita Consumption of Sugar >30g/day at Household Level	65 (49.6)	28 (43.1)	37 (56.1)	2.2
	Mean±SD	**Mean±SD**	**Mean±SD**	**Students' 't' Value**
Per Capita Consumption of Oil/day (g) at Household Level	45.2±18.6	41.8±15.7	48.7±20.6	2.16*
Per Capita Consumption of Salt/day (g) at Household Level	9.8±4.5	8.7±3.9	10.8±4.9	2.7**
Per Capita Consumption of Sugar/day (g) at Household Level	32.5±17.9	28.8±13.5	36.2±20.9	2.4*

Values in parenthesis indicate percentages
Two tailed significance*p<0.05, **p<0.01

FIGURE 4.3: PER CENT FREQUENCY DISTRIBUTION OF VARIOUS OILS USED BY THE WOMEN (%)

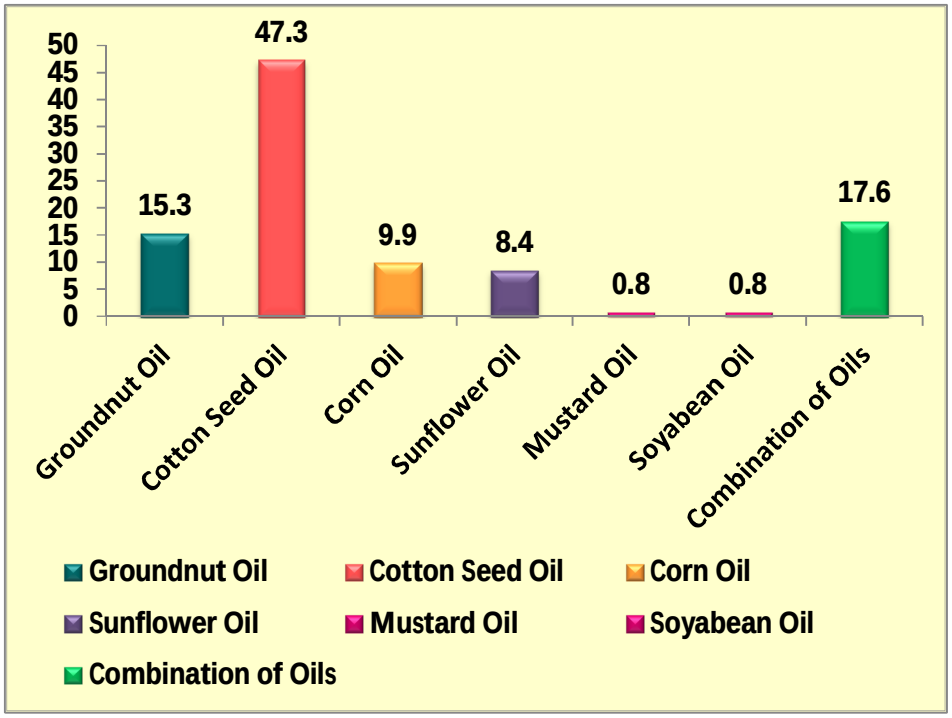
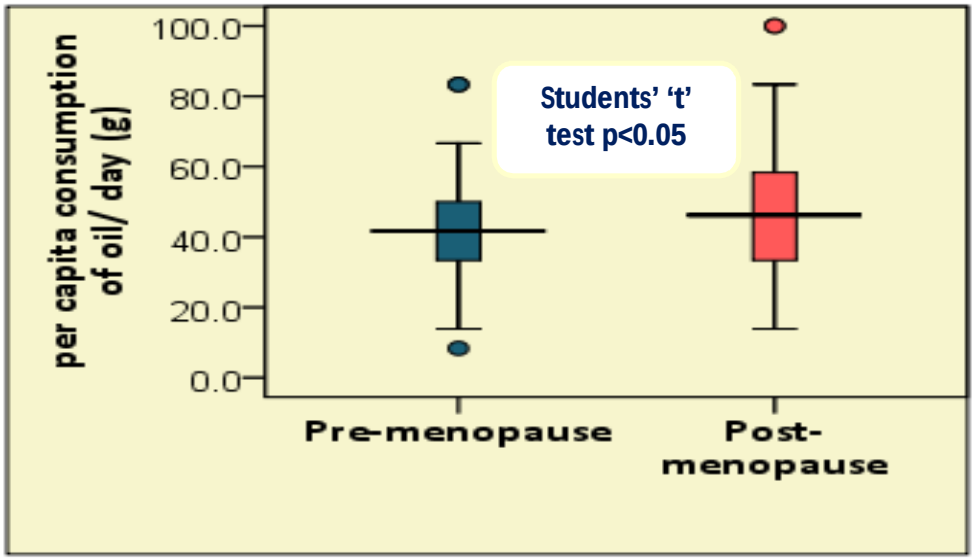
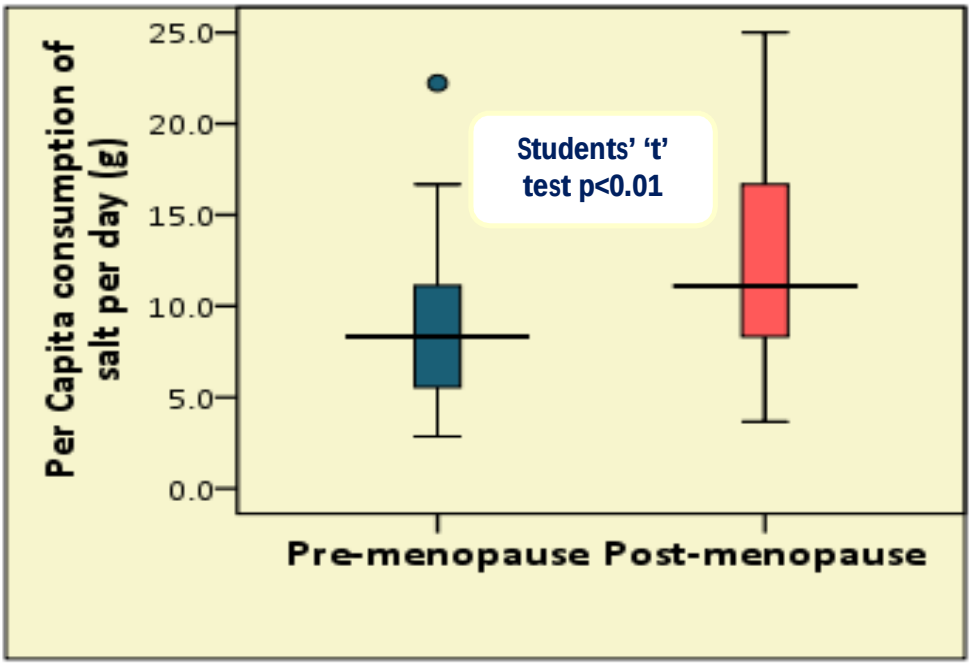


FIGURE 4.4: COMPARISON OF MEAN PER CAPITA CONSUMPTION OF OIL, SALT AND SUGAR IN GRAMS BETWEEN PRE AND POST-MENOPAUSAL WOMEN HOUSEHOLD

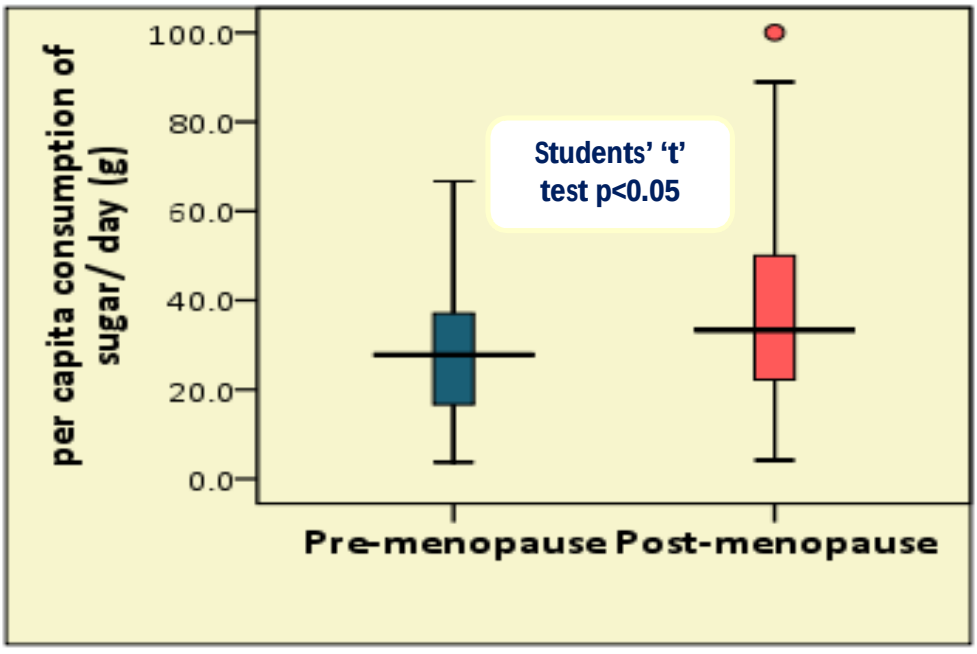
(a)



(b)


Per Capita consumption of salt per day (g)
25.0
20.0
15.0
10.0
5.0
0.0
Students' 't' test p<0.01
Pre-menopause
Post-menopause

(c)


per capita consumption of sugar/ day (g)
100.0
80.0
60.0
40.0
20.0
0.0
Students' 't' test p<0.05
Pre-menopause
Post-menopause

a) Average daily nutrient intake of the women:

The information on nutrient intake was collected by 24 hour dietary recall method. The mean value for each nutrient and its corresponding % RDA was calculated as per ICMR 2009 guidelines. The Table 4.9 depicts the average daily nutrient intake of various macro and micro nutrients in the women. The data showed that the average daily energy intake of the women was 1504±415 Kcal, which was below the RDA (79%) for a sedentary adult woman. The mean protein intake (42.8g) was also below the recommended daily intake (77.8%). However visible fat intake was much higher than the RDA (178%). The women were adequately consuming calcium, thiamine and Vitamin C. However daily intake of various other key nutrients like iron (54%), retinol (25.1%), vitamin B12 (35%), β carotene (44%), pyridoxine (5.5%) and zinc (42%) was half or below half of the daily requirement of RDA. Total vitamin A intake was calculated using conversion factor of 1:12 for retinol and β carotene (Food and Nutrition Board, IOM, 2001). Average intake of vitamin A in the subjects was found to by half of the RDA (54.4%). Fatty acid profile of the diet of the women was calculated from only those food items for which standard values of 100g are provided by National Institute of Nutrition. It showed that the daily diet of the women had higher ratio of polyunsaturated fatty acid, contributed mostly by n-6 fatty acid. The dietary fiber intake (49.3%) of the women was found to be low when compared with the recommended intake by WHO (2003).

When the contribution of each macronutrient towards the total energy intake was calculated (Figure 4.5) it revealed that CHO contributed around 55.4% followed by fats (34%) and protein (11.6%). Therefore, it is evident that although the total energy intake of the women was below RDA, the per cent contribution of fats was higher than the recommendation and the fatty acid profile of daily fat intake fat was also not favourable.

A comparison of daily nutrient intake between pre and post-menopausal women was performed, details of which are illustrated in Table 4.10. No significant difference in the micro and macronutrient intake between two groups was observed.

TABLE 4.9: AVERAGE DAILY NUTRIENT INTAKE OF THE WOMEN (Mean±SD)

Nutrients	Daily Intake (Mean±SD) n=131	Daily RDA	%RDA
Energy (Kcal)	1504±415	1900	79.2
Protein (g)	42.8±12.9	55	77.8
Total Fat (g)	56±22	--	--
Visible Fat (g)	35.6±14.8	20	178
Calcium (mg)	678.9±493.8	600	113
Iron (mg)	11.99±5.5	21	54.1
Retinol (µg)	150.7±116.2	600	25.12
β Carotene (µg)	2110±3752 (Range 0-18200)	4800	43.96
Vitamin A (Retinol + β Carotene) (µg)	326.5	600	54.4
Thiamine (mg)	1.04±0.32	1.0	104
Riboflavin (mg)	0.8±0.4	1.1	72.7
Niacin Equivalent (mg)	8.1±2.7	12	67.5
Pyridoxine (mg)	0.11±0.12	2	5.5
Ascorbic Acid (mg)	94.5±97.03	40	236.2
Dietary Folate (µg)	167.5±82.6	200	83.8
Vitamin B12 (µg)	0.35±0.3	1	35
Magnesium (mg)	276.6±95	310	89.2
Zinc (mg)	4.25±1.38	10	42.5
Total Dietary Fiber (g)	14.8±6.9	30^	49.3
Insoluble Dietary Fiber (g)	11.1±5.4	--	--
Soluble Dietary Fiber (g)	3.6±1.7	--	--
Carbohydrates (g)	201.7±52.3	--	--

^WHO, 2003

FIGURE 4.5: PER CENT DISTRIBUTION OF CALORIES FROM MACRONUTRIENTS

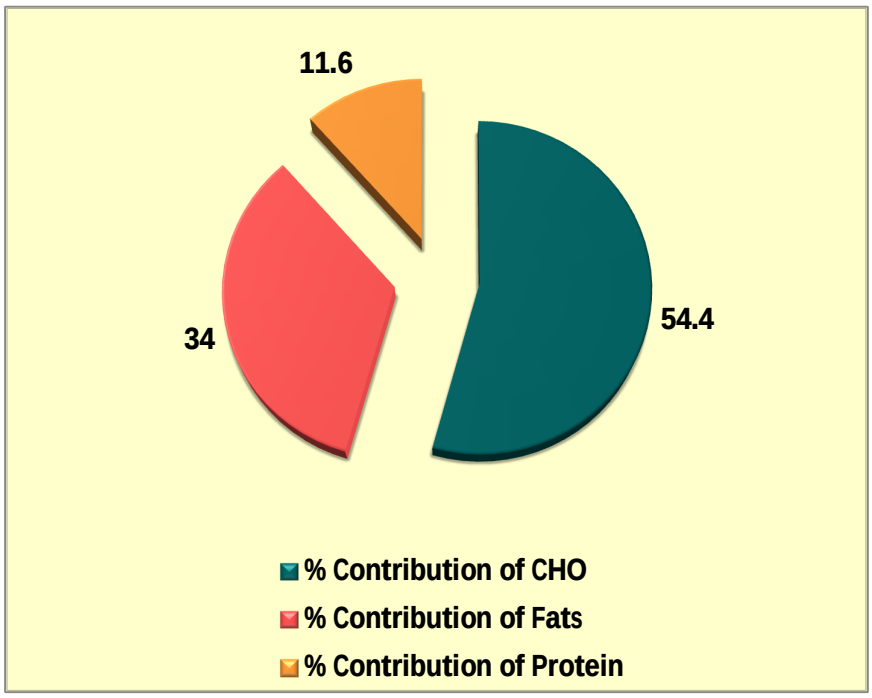


TABLE 4.10: MEAN VALUES OF VARIOUS NUTRIENTS IN PRE AND POST-MENOPAUSAL WOMEN (MEAN±SD)

Nutrients	Pre Menopause (n=65)	Post Menopause (n=66)	Students' 't' Value
Energy (Kcal)	1503±433	1507±401	0.5
Protein (g)	42.4±12.1	43.2±13.7	0.4
Total Fat (g)	54±22	57±21	0.7
Visible Fat (g)	34.8±14	36.4±15.7	0.61
Calcium (mg)	680.9±546.7	676.8±439.7	0.5
Iron (mg)	12.8±6.5	11.2±4.3	1.6
Retinol (µg)	151.8±128.3	149.6±103.8	0.11
β Carotene (µg)	2226.3±3917.4	1995.1±3607.3	0.35
Thiamine (mg)	1.05±0.27	1.04±0.36	0.1
Riboflavin (mg)	0.82±0.3	0.79±0.4	0.4
Niacin Equivalent (mg)	8.2±2.5	8.0±±2.9	0.56
Pyridoxine (mg)	0.10±0.12	0.11±0.11	0.6
Ascorbic Acid (mg)	87.8±54.3	101.2±97.5	0.98
Dietary Folate (µg)	166.1±78.7	168.8±86.8	0.18
Vitamin B12 (µg)	0.36±0.33	0.34±±0.26	0.26
Magnesium (mg)	283.4±83.2	270±105.6	0.7
Zinc (mg)	4.3±1.3	4.2±1.5	0.2
Total Dietary Fiber (g)	13.9±5.97	15.7±7.6	1.6
Insoluble Dietary Fiber (g)	10.4±4.5	11.8±6.0	1.5
Soluble Dietary Fiber (g)	3.5±1.6	3.8±1.8	1.1
Carbohydrate (g)	204.4±52.3	198.9±52.5	0.60

b) Frequency of consumption of trans-fat rich food items:

A semi-quantitative questionnaire was used to assess the frequency of consumption of trans-fat rich food items and their amount consumed per serving. As depicted in the Table 4.11 biscuits (55.6%) were found to be consumed most frequently in the women followed by sev/namkeen (42.7%) and khari/nankhatai (16%). Cake/pastry (97.7%), fried sweets (9.8%), popcorn (90%), puff (96.2%), french fries (98.5%), burger/vadapav (93%) and pizza (99%) were least frequently consumed trans-fat rich food items. Women consumed average 3 pieces of biscuits, 2 pieces of khari/nankhatai and about 62g of sev/namkeen in one serving. Similar trends were noted when the data was segregated based on the menopausal status of the women (Table 4.12 and 4.13). Table 4.14 depicts the comparison between pre and post-menopausal women for lifestyle variables studied.

TABLE 4.11: FREQUENCY OF CONSUMPTION OF TRANS FAT RICH FOOD ITEMS (n=131)

Food Item	High Frequency n (%)	Moderate Frequency n (%)	Low Frequency n (%)	Average Amount (pc.) Consumed per Serving (Mean±SD)
Samosa	3 (2.3)	22 (16.8)	106 (80.9)	1.3±0.6
Pakoda/ Bhajiya	1 (0.8)	47 (35.9)	83 (63.4)	6.1±2.3
Biscuits	75 (55.6)	19 (14.5)	37 (28.2)	3±1.3
Cake/ Pastry	1 (0.8)	2 (1.5)	128 (97.7)	1.1±0.3
Milk Based Sweets	4 (3.1)	13 (9.9)	114 (87.02)	1.4±0.7
Fried Sweets	2 (1.5)	2 (1.5)	127 (97.95)	1.4±0.7
Chips	10 (7.6)	34 (25.95)	87 (66.4)	69.9±41.6g
Sev/ Namkeen	56 (42.7)	26 (19.9)	49 (37.4)	61.6±46.1g
Popcorn	3 (2.3)	10 (7.6)	118 (90.1)	97.8±71.6g
Puff	0 (0)	5 (3.8)	126 (96.2)	1.04±0.2
Maggie	4 (3.1)	21 (16.03)	106 (80.9)	69.3±34.1g
Khari/ Nankhatai	21 (16.03)	16 (12.2)	94 (71.8)	2.1±0.9
French Fries	0 (0)	2 (1.5)	129 (98.5)	11±5.5
Burger/ Vada Pav	1 (0.8)	8 (6.1)	122 (93.1)	1.3±0.6
Pizza	0 (0)	1 (0.8)	130 (99.2)	1.1±0.6

Values in parenthesis indicate percentages

TABLE 4.12: FREQUENCY OF CONSUMPTION OF TRANS FAT RICH FOOD ITEMS IN PRE- MENOPAUSAL WOMEN (n=65)

Food Item	High Frequency n (%)	Moderate Frequency n (%)	Low Frequency n (%)	Average Amount Consumed per Serving (Mean±SD)
Samosa	3 (4.6)	13 (20)	49 (75.4)	1.38±0.5
Pakoda/ Bhajiya	1 (1.5)	22 (33.9)	42 (64.6)	6.23±2.4
Biscuits	34 (52.3)	10 (15.4)	21 (32.3)	2.9±1.2
Cake/ Pastry	1 (1.5)	2 (3.1)	62 (95.4)	1.1±0.3
Milk Based Sweets	1 (1.5)	9 (13.9)	55 (84.6)	1.3±0.6
Fried Sweets	0 (0)	1 (1.5)	64 (98.5)	1.3±0.6
Chips	5 (7.7)	17 (26.2)	43 (66.2)	74.9±42.7
Sev/ Namkeen	26 (40)	16 (24.6)	23 (35.4)	72.8±53.9
Popcorn	1 (1.5)	6 (9.2)	58 (89.2)	109.1±79.9
Puff	0 (0)	4 (6.2)	61 (93.9)	1±0
Maggie	4 (6.2)	15 (23.1)	46 (70.8)	71.5±36.7
Khari/ Nankhatai	8 (12.3)	5 (7.7)	52 (80)	2.3±1.1
French Fries	0 (0)	1 (1.5)	64 (98.5)	12.5±5
Burger/ Vada Pav	1 (1)	1 (1.5)	63 (96.9)	1.3±0.6
Pizza	0 (0)	1 (1.5)	64 (98.5)	1.2±0.8

Values in parenthesis indicate percentages

TABLE 4.13: FREQUENCY OF CONSUMPTION OF TRANS FAT RICH FOOD ITEMS IN POST-MENOPAUSAL WOMEN (n=66)

Food Item	High Frequency n (%)	Moderate Frequency n (%)	Low Frequency n (%)	Average Amount Consumed per Serving (Mean±SD)
Samosa	0 (0)	9 (13.6)	57 (86.4)	1.27±0.6
Pakoda/ Bhajiya	0 (0)	25 (37.9)	41 (62.1)	6±2.3
Biscuits	41 (62.1)	9 (13.6)	16 (24.2)	3.04±1.4
Cake/ Pastry	0 (0)	0 (0)	66 (100)	0.9±0.2
Milk Based Sweets	3 (4.6)	4 (6.1)	59 (89.4)	1.5±0.8
Fried Sweets	2 (3.03)	1 (1.5)	63 (95.5)	1.5±0.7
Chips	5 (7.6)	17 (25.8)	44 (66.7)	65.7±40.7
Sev/ Namkeen	30 (45.5)	10 (15.1)	26 (39.4)	50±32.8
Popcorn	2 (3.03)	4 (6.1)	60 (90.9)	73.8±45.02
Puff	0 (0)	1 (1.5)	65 (98.5)	1.1±0.3
Maggie	0 (0)	6 (9.1)	60 (90.9)	60±19.2
Khari/ Nankhatai	13 (19.7)	11 (16.7)	42 (63.6)	2±0.8
French Fries	0 (0)	1 (1.5)	65 (98.5)	9±6.6
Burger/ Vada Pav	0 (0)	7 (10.6)	59 (89.4)	1.3±0.6
Pizza	0 (0)	0 (0)	66 (100)	1±0.2

Values in parenthesis indicate percentages

Table 4.14: HIGHLIGHTS SHOWING DIFFERENCES BETWEEN LIFE STYLE, BEHAVIOURAL AND DIETARY PRACTICES IN PRE AND POST-MENOPAUSAL WOMEN

Variables	High prevalence in Pre-menopausal women	High prevalence Post-menopausal women
More than two children		***
Headaches	*	
Cramps/ Muscle weakness		*
Disturbed sleeping pattern		*
Average sleep <7 hours		*
Mean Sitting Hours		*
No regular health check-up	*	
No PEP smear done	*	
Use of Supplements		*
Ovo Lactarian/ Non-vegetarian	*	
Per Capita consumption of oil/day (g) at household level		*
Per Capita consumption of salt/day (g) at household level		*
Per Capita consumption of sugar/day (g) at household level		*

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

Discussion

According to Third Consensus meeting of Indian Menopause Society (2008) the estimated menopausal population will be around 103 million in India by 2026 (Unni, 2008). Menopause is the most neglected phase of a women's life and issues related to menopause have been ignored over a period of time. The hormonal changes during menopause can lead to various menopausal symptoms as well as can bring about various health related changes like effect on cardiovascular health, deteriorated body composition, mental health, risk of cancer, bone health etc. Poor lifestyle and dietary practices can aggravate the risk of adverse health conditions.

The average age of menopause varies by geographical regions and ethnicity. A review done by Palacios et al (2010) revealed that the median age of menopause in Europe ranges from 50.1 to 52.8 years, in North America from 50.5 to 51.4 years, in Latin America from 43.8 to 53 years, and in Asia from 42.1 to 49.5 years. In the present study the mean age of onset of menopause was found to be 45.45±4.8 y. Various studies from different parts of northern India have shown the range of 44.5-47.5y for the onset of menopause (Mahajan et al, 2012; Kapur et al, 2009; Sharma et al, 2007; Kriplani and Banerjee, 2005). In Asia, women of poorer socioeconomic status are said to be having significantly earlier onset of menopause (Palacios et al, 2010). An Indian study performed in Uttrakhand (Kapur et al, 2009) also supported the findings stating that socioeconomic status and lifestyles significantly affect the age at the onset of menopause. However a North Indian study performed at All India Institute of Medical Sciences (Kriplani and Banerjee, 2005) contradicted the fact showing no association of onset of menopause with socio demographic and reproductive factors.

The prevalence of menopausal symptoms was around 21% for vasomotor symptoms, 29% for somatic symptoms, 33% for psychological symptoms and 3% for urogenital symptoms in the free living population of the study. The results were comparable with those reported by Elayath and Iyer (2013) in the population of Vadodara for somatic (21.8%) and vasomotor (28%) symptoms; however a substantial difference for the urogenital symptoms (16.4%) and psychological symptoms (19.3%) was

observed. According to Kakkar et al (2007), age, level of education and working/non-working status may contribute to significant variations in menopausal symptoms. The fact can partly explain the difference between the two studies undertaken within the same population as the subjects had differences in mean age of menopause and education level. The women attending the health check-up facility experienced higher prevalence of menopausal symptoms than the free-living population (Elayath and Iyer, 2013).

Menopause is known to affect the sleeping pattern of the women. According to Eichling and Sahni (2005) estrogen have an antidepressant as well as direct sleep effect and the presence of vasomotor symptoms during menopause can lead to disturbed sleep. Present study indicated that post-menopausal women had a significantly higher prevalence of disturbed sleep pattern ($p<0.05$) with average sleep <7hours ($p<0.05$). A community based study performed by Young et al (2003) also reported post-menopausal being less satisfied with their sleep quality than the pre-menopausal ones.

The prevalence of breast and lung cancer is highest of all the cancers in women and around half of the cancer cases occurring in women are from developing countries (NCD Alliance, 2011). The awareness regarding detection of breast cancer was found very low in the women of present study. A Delhi based study on 400 women (15 years and above) also showed inadequate awareness regarding breast examination in the women with only 11% being aware of breast self-examination. Only two of them had ever done breast self-examination (Somdatta and Baridalyne, 2008). In a large cross sectional study which included 1796 women in the age group 30-59 years of rural India, the knowledge of self-breast examination was only 16.4% with none of the women practicing it. The main source of knowledge was some training provided by NGOs, television and print media (Rao et al, 2005). However the knowledge and behaviours of American Asian Indian women regarding breast cancer and adherence rate of self-breast examination (40.7%) was quite high in a study performed by Sadler et al (2001). The annual mammography screening rate was also high in the American Asian Indian women (61- 70%) in different age groups (Sadler et al, 2001).

In the present study this rate was merely 9.4% showing very low rate of mammography in comparison to migrated women.

Tobacco (smoking and chewing) and alcohol intake have been two major modifiable lifestyle risk factors identified by WHO. According to WHO's report on women's NCD health (2011) the proportion of female smokers is estimated to rise from 12% in 2010 to 20% by 2025. Passive smoking is one of the major concern in countries where women are not empowered enough to demand for smoke free space in public as well as at home. Alcohol consumption in developing countries is also on a rise. Presence of these risk factors in the studied population (both pre and post-menopausal women) was almost nil, though passive smoking was not assessed. NFHS-3 reported the prevalence of smoking and alcohol as 11% and 2% respectively in Indian women which is quite high than the present study. Low socio economic status and education have found to be two major causal factors for tobacco and alcohol intake (NFHS-3) in Indian women which describes the disparities between the results.

Another major lifestyle risk factor for NCDs and metabolic aberrations related to them is physical inactivity. The prevalence of physical activity in women has been ranged from 48% to 83.2% (Singh et al, 2015; Anjana et al, 2014; Elayath and Iyer, 2013) in various studies. The results from ICMR-INDIAB study conducted in four regions of India (Tamilnadu, Maharashtra, Jharkhand and Chandigarh) using WHO-GPAQ, reported physical inactivity prevalence lowest in Jharkhand and highest in Chandigarh in urban women (n=4208) depicting regional variations. The variation in physical activity levels have also been noted due to use of different methods, questionnaires and sample selection in different studies. In the present study prevalence of physical inactivity was very low for both pre (3.1%) and post-menopausal women (9.1%). Mean sitting hours were significantly higher ($P<0.05$) in post-menopausal women. The major reason behind low prevalence of physical inactivity may be over reporting of the subjects. IPAQ-S was used for assessment of physical activity in women in the present study. Various studies have demonstrated that use of IPAQ can lead to over reporting of physical activity when compared to the direct methods of assessment (Warner et al, 2012; Prince et al, 2008; Rzewnicki

et al, 2003). When compared IPAQ with GPAQ in a review of population-based prevalence studies of physical activity, median MET-minutes varied from 1461 using IPAQ to 356 median MET-minutes using GPAQ (Macniven el al, 2012).

Regular screening through health check-ups can help in prevention and development of complications of various non communicable diseases. Government of India established national program for prevention and control of cancer, diabetes, cardiovascular diseases and stroke (NPCDCS) during 2010-11 (Srivastava and Bachani, 2011). For implementation of such programs Reddy (2003) stressed to focus on both 'opportunistic' and 'targeted' screening. Under NPCDCS persons above 30 years are intended to undergo opportunistic screening during initial contact at any health care facility in 12th five year plan 2013-17. The program is currently running only in 100 districts and it is a long way ahead to implement it in the entire country (NRHM). Therefore awareness in population regarding regular health check-up is important. In the present study Post-menopausal women had significantly higher ratio of going for health check-up though the overall prevalence of regular health check-up was very low. In our country where maternal health and check-ups are focused so much, strategies should be made to encourage women to go for at least biannually health check-ups.

Intervention through hormonal replacement therapy (HRT) has been studied during last two decades to prevent post-menopausal adverse health conditions like osteoporosis, CVD's, cognition etc. (Panay et al, 2013). However the effect of HRT has been controversial and it is thought to exert more harm than benefits according to women's health initiative trial (Rossouw et al, 2002). The British Menopause Society has recommended women to have fully informed choice weighting the pros and cons of HRT (Panay et al, 2013). In the present study no post-menopausal women reported having undergone hormonal replacement therapy.

Use of nutritional supplements in the women population has rarely been studied. According to a study performed in US on different ethnic groups regarding use of supplements, it was concluded that the vulnerable populations that might benefit most from use of supplements generally use them the least. The reason behind it lies

in various cultural beliefs, attitudes and sociodemographic determinants (Jasti et al, 2003). The prevalence of nutritional supplement's used by women in present study was very low.

Poor dietary practices like high intake of salt, sugar and fat are well established as dietary risk factors for developing obesity, hypertension, diabetes and cardio-vascular diseases (Brown et al, 2009; Johnson et al, 2009; Reddy and Katan, 2004). The study depicted high levels of per capita consumption of oil, sugar and salt at household level. Mean salt intake (9.8 g) observed in the present study was higher than reported (8.5g) by Radhika et al (2007) and recommended salt intake levels (<5g). There is dearth of data on average consumption of these dietary components at national level.

The mean energy intake of the subjects in present study was below the RDA (1504±415) however fat intake was high contributing 35% of total energy. Visible fat consumption was quite high than RDA (178%) and intake of major fat antioxidant B-carotene was not even half of the recommendations. Results were comparable with departmental study performed by Elayath and Iyer (2013) which reported average energy consumption of women around 1562kcal, 32.8% of total energy coming from fats and β-carotene consumption meeting only 30.8% of RDA. According to NSS (2007) report per capita consumption of energy and total fats in urban Gujarat was 1991Kcal and 63.7gm respectively.

Along with the total fat consumption, type of fat consumed also plays a detrimental role in safeguarding cardiac health of a person. In the present study almost half of the subject regularly used Cotton seed oil. Cotton seed oil is rich in omega-6 fatty acid (55%) and SFA (27%) especially omega-6 fatty acid (Agarwal et al, 2003). Imbalanced ratio of n-3 and n-6 may have potentially adverse metabolic and glycemic consequences in population (Simopoulos, 2008). Trans fat has been found to be most unsafe and WHO has recommended to limit trans-fat intake <1% of total energy (WHO, 2003). Trans fat was consumed by the subjects most frequently in the form of biscuits, sev/namkeen and khari/nankhatai in the current study.

SUMMARY

- Equal representation of women from three decades of age range (30-60y) was noticed.
- Dual burden of malnutrition was observed in the screened subjects.
- Psychological symptoms were the most experienced in post-menopausal women followed by somatic, vasomotor and urogenital symptoms.
- Most of the women reported having moderate physical activity as derived by using International Physical Activity Questionnaire.
- Only 20% of the women underwent regular health check-up with a significant higher proportion of post-menopausal women going for health check-ups.
- The awareness regarding self-breast examination was low in the women.
- None of the post-menopausal women had undergone HRT.
- About 82% of the women were lacto-vegetarians.
- Cotton seed oil was the most preferred choice of oil in the women.
- Mean consumption of oil, sugar and salt was higher than the recommendations with post-menopausal women consuming significantly higher amounts than the pre-menopausal ones.
- Mean energy and protein intake was below the recommended intake by ICMR. Total fat contributed to about 34% of the total energy intake which is higher than recommendation.
- No significant difference between nutrient intake of pre and post-menopausal women was observed.
- Traditional food like biscuits, sev/namkeen and khari/nankhatai were the major source of trans fat in pre and post-menopausal women.

Conclusions

The subjects in the present study followed a fairly healthy lifestyle as indicated by low prevalence of physical inactivity, tobacco or alcohol intake. Both pre and post-menopausal women were not much attentive towards their health, though good health care practices were more pronounced in post-menopausal women probably due to escalated health issues with increase in age. Dietary practices were poor in both pre and post-menopausal women. Thus, there is a need to create awareness regarding improving health practices and dietary behaviours in post-menopausal as well as premenopausal women.

4.2. PHYSIOLOGICAL AND METABOLIC ABERRATIONS IN PRE AND POST-MENOPAUSAL WOMEN

4.2.1 Family history and self-reported disease profile:

The information on family history (Table 4.15) of various non-communicable diseases (n=131) showed that the highest prevalence of family history was for hypertension (52.5%) followed by diabetes (39.7%) and CHD (27.5%). Lower prevalence of family history of cancer (16.1%), asthma (14.4%), hyperlipidemia (11.4%), and hyper/hypo-thyroidism (10%) was there.

Similar trends were seen in the self-reported disease profile (Table 4.15) with highest prevalence for hypertension (21.4%) followed by diabetes (10.7%) in the women. The prevalence of self-reported CHD (1.5%), hyperlipidemia (3.8%) and hypo/hyperthyroidism (6.1%) was found to be on the lower side. None of the women reported having cancer or asthma. On comparing between pre and post-menopause, it was seen that post-menopausal women had significantly higher odds of developing diabetes (OR 7.0; 95%CI 1.4-66.3) and hypertension (OR 4.9; 95%CI 1.8-13.1) as compared to pre-menopausal women. Prevalence of other chronic diseases like CVD, hyperlipidemia and hypo/hyperthyroidism was also higher in post-menopausal women though it was not statistically significant.

4.2.2. Prevalence of overweight/obesity

Table 4.16 illustrates the mean values of various anthropometric indices of the women. The mean weight and height of the women were 60.02±10.7 kg and 152.5±5.99 cm respectively. All the anthropometric indices i.e. mean WC, WHR, WSR and BMI were higher than the normal cut-offs of Asia Pacific Classification. The prevalence of overweight and obesity was high (74.8%) in the women (Table 4.17). On comparing the values between pre and post-menopausal women it was found that mean values of WC ($p<0.001$), WHR ($p<0.05$) and WSR ($p<0.001$) were significantly higher in post-menopausal women than pre-menopausal ones. Also significant higher mean value of body fat ($p<0.0001$) was observed in post-menopausal women. Abdominal obesity was assessed through three major indices (WC, WHR and WSR), and a staggeringly high prevalence was observed. However no difference between the prevalence of abdominal obesity was observed between pre

TABLE 4.15: FAMILY HISTORY AND SELF-REPORTED DISEASE PROFILE OF THE ENROLLED WOMEN (n, %)

Disease	Family History (n=131)	Self-reported Disease History			OR (95% CI)
		Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	
Diabetes	51 (39.7)	14 (10.7)	2 (3.1)	12 (18.2)	7.0* (1.4-66.3)
Hypertension	69 (52.5)	28 (21.4)	6 (9.2)	22 (33.3)	4.9* (1.8-13.1)
CHD	36 (27.5)	2 (1.5)	0 (0)	2 (3)	--
Hyperlipidemia	15 (11.4)	5 (3.8)	1 (1.5)	4 (6.1)	4.1 (0.4-206.5)
Hypo/ Hyper-thyroidism	13 (10)	8 (6.1)	2 (3.1)	6 (9.1)	3.1 (0.6-16.2)
Asthma	19 (14.4)	0(0)	0 (0)	0 (0)	--
Cancer	21 (16.1)	0 (0)	0 (0)	0 (0)	--

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

and post-menopausal women. When looked at the categories of obesity (Figure 4.6), it was found that higher per cent of pre-menopausal women were under normal and overweight category whereas post-menopausal women had high prevalence of obesity and morbid obesity.The post-menopausal women were having 6.5 times higher odds (95% CI 1.3-62.3) of having high body fat per cent (Figure 4.7).

4.2.3. Prevalence of hypertension

As depicted in Table 4.16 mean systolic blood pressure of the women was 129.4±19 mmHg which fall under the pre-hypertension category. A significantly higher mean value of SBP ($p<0.001$) was found in post-menopausal women than pre-menopausal ones. The frequency distribution of various categories of hypertension (Figure 4.8) revealed that in pre-menopausal women 9.2% of the women reported having history of hypertension however 15.4% were newly diagnosed as hypertensives. The scenario seemed worse in case of post-menopausal women in which 33.3% of the women reported having hypertension and about 26% were identified with hypertension. About 23% of pre-menopausal and 28% of post-menopausal women had pre-hypertension. Limitation of this data was that blood pressure was measured only once however other precautions to measure blood pressure were followed adequately. The univariate analysis (Table 4.17) of effect on menopause on hypertension revealed that menopause posed 4.4 times higher odds of developing hypertension in the women (Figure 4.9).

4.2.4. Prevalence of dyslipidemia

The mean values of TC, HDL-C, TG, TC/HDL, LDL/HDL, TG/HDL were optimal in the women (Table 4.18). The mean LDL-C levels and AIP were slightly higher than the normal cut-off for women. Post-menopausal women had significantly higher mean values of HDL-C levels ($p<0.05$). The mean levels of TC and LDL-C also showed similar trend although not statistically significant. The prevalence of high LDL-C levels was very high (72.2%). Around 45% had high TC levels. These aberrations were higher in the post-menopausal women (Figure 4.10), however the presence of post menopause was not found to bring any significant risk in women (Table 4.19). Hypertriglyceridemia was seen in 12.2% of the women. Low HDL-C levels were seen to be more prevalent in pre-menopausal women. The

TABLE 4.16: MEAN VALUES OF ANTHROPOMETRIC AND BIOPHYSICAL MEASUREMENTS OF THE WOMEN (Mean±SD)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	Students' 't' Value
Weight (Kg)	60.02±10.7	59.54±11.3	60.5±10.2	0.527
Height (cm)	152.5±5.99	153.5±6.1	151.2±5.8	1.82
Waist Circumference (cm)	93.93±10.5	91.43±10.1	96.4±10.3	2.78**
Hip Circumference (cm)	101.26±9.9	100.01±10.1	102.5±9.7	1.44
Waist Hip Ratio	0.93±0.07	0.92±0.07	0.94±0.06	2.19*
Waist Stature Ratio	0.62±0.07	0.6±0.07	0.64±0.07	3.34***
Body Mass Index (BMI)	25.82±4.45	25.3±4.66	26.3±4.2	1.36
Body fat (%)	36.49±5.76	34.7±5.6	38.2±5.4	3.64***
Systolic Blood Pressure (mmHg)	129.4±19.0	123.14±17.2	135.6±18.8	3.97***
Diastolic Blood Pressure (mmHg)	79.5±9.8	78.3±9.6	80.8±9.8	1.46

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

TABLE 4.17: PREVALENCE OF OVERWEIGHT/OBESITY AND HYPERTENSION IN THE WOMEN (n, %)

Parameters	Total (n=131)	Pre Menopause (n=65)	Post Menopause (n=66)	OR (95% CI)
BMI ≥23	98 (74.8)	47 (72.3)	51(77.3)	1.3 (0.6-2.9)
WC >80cm	122 (93.1)	59 (90.8)	63 (95.5)	2.1 (0.4-13.7)
WHR ≥0.85	116 (88.5)	56 (86.2)	60 (90.9)	1.6 (0.5-4.8)
WSR >0.50	127 (96.9)	62 (95.4)	65 (98.5)	3.1 (0.2-167.6)
Body Fat >30%	118 (90.1)	54 (83.1)	64 (97)	6.5* (1.3-62.3)
Confirmed and Newly Diagnosed Hypertension	55 (42)	16 (24.6)	39 (59.1)	4.4* (2.1-9.3)

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

FIGURE 4.6: PERCENT PREVALENCE OF VARIOUS CATEGORIES OF OBESITY IN WOMEN

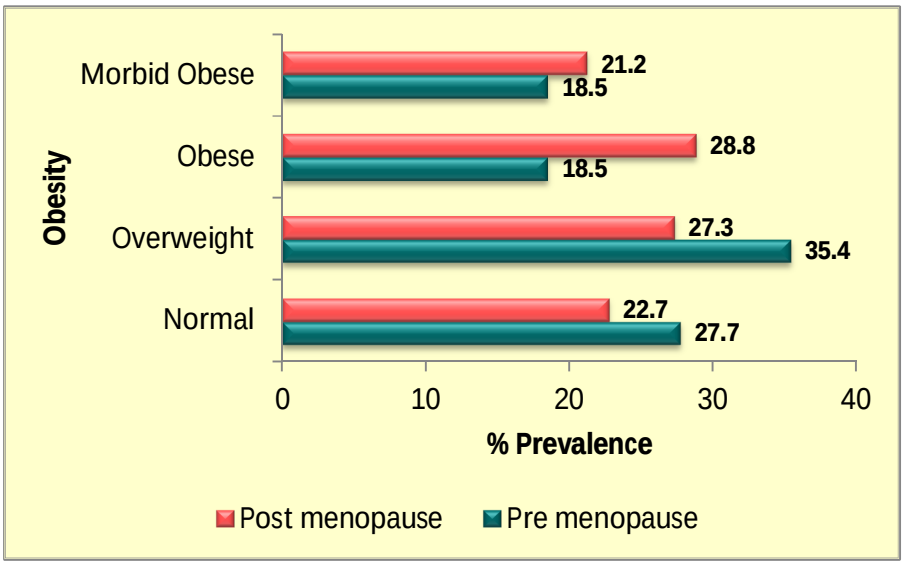


FIGURE 4.7: MENOPUASE AS A RISK FACTOR FOR HIGH BODY FAT PERCENT THROUGH UNIVARIATE ANALYSIS

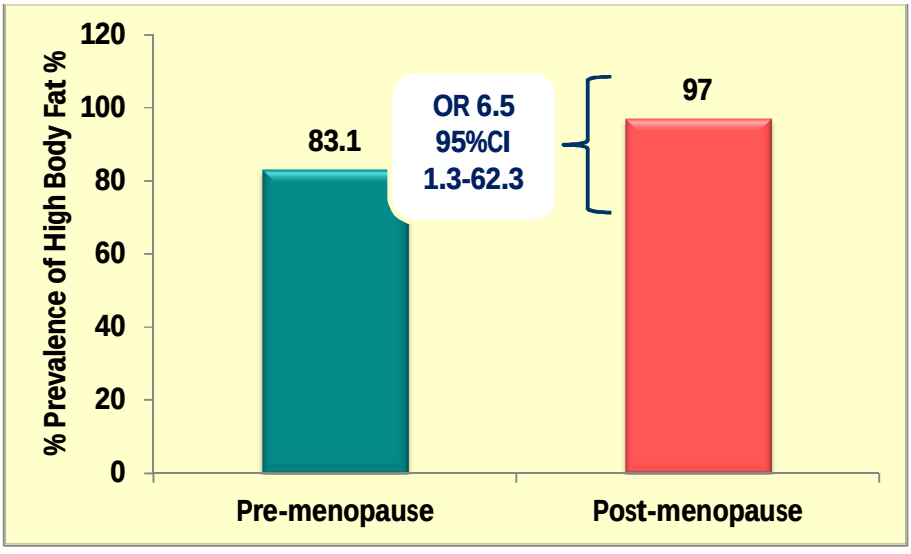


FIGURE 4.8: PERCENT PREVALENCE OF VARIOUS CATEGORIES OF HYPERTENSION IN WOMEN

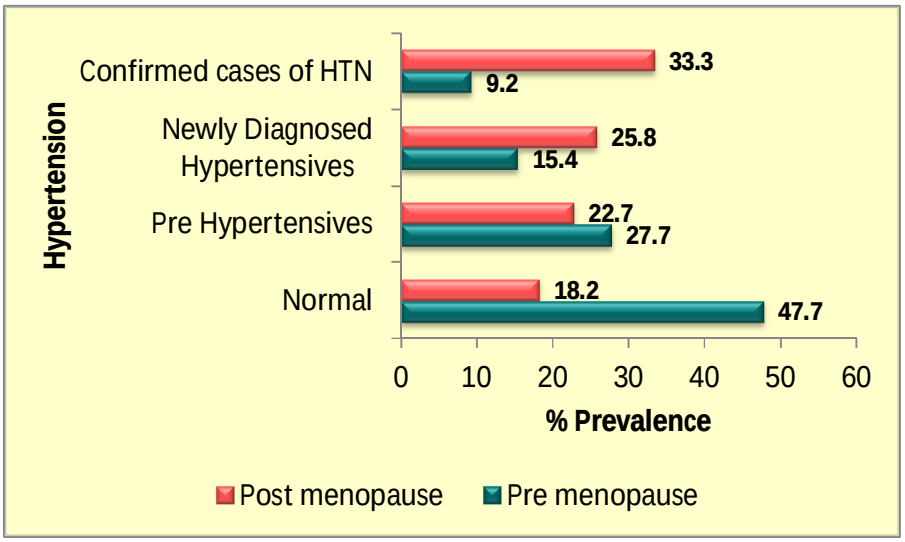


FIGURE 4.9: MENOPUASE AS A RISK FACTOR FOR HYPERTENSION THROUGH UNIVARIATE ANALYSIS

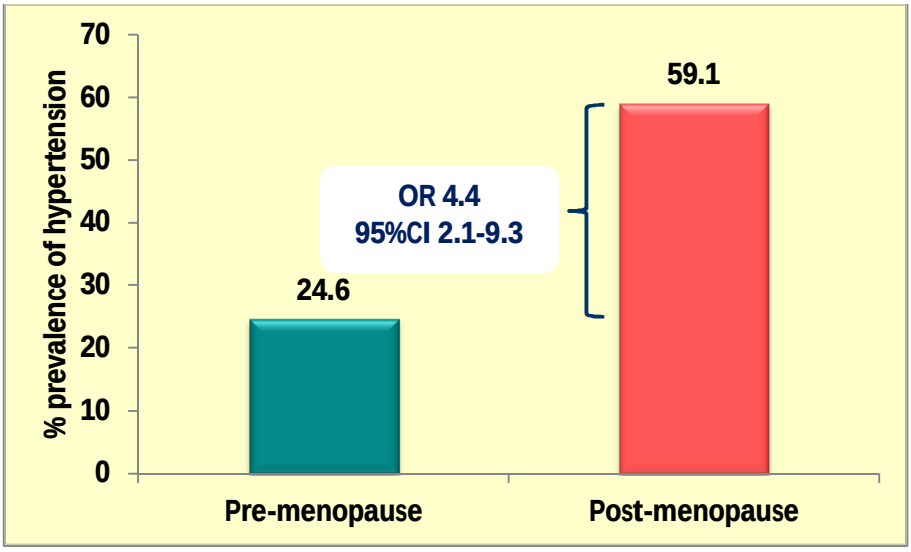


TABLE 4.18: LIPID PROFILE OF THE WOMEN (Mean±SD)

Parameters	Total (N=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value
TC (mg/dl)	193.93±36.75	188.72±33.56	199.14±37.57	1.35
HDL-C (mg/dl)	55.55±12.28	52.92±11.26	58.12±12.84	2.02*
LDL-C (mg/dl)	115.63±31.2	112.8±31.85	118.46±30.63	0.86
TG (mg/dl)	113.75±48.62	114.83±56.9	112.67±39.26	0.21
VLDL-C (mg/dl)	22.75±9.73	22.97±11.38	22.53±7.86	0.21
TC/HDL-C	3.59±0.76	3.67±0.81	3.51±0.71	0.98
LDL/HDL-C	2.4±0.6	2.18±0.62	2.1±0.58	0.69
TG/HDL-C	2.26±1.57	2.44±1.89	2.08±1.16	1.07
AIP	0.29±0.23	0.30±0.26	0.27±0.19	0.54

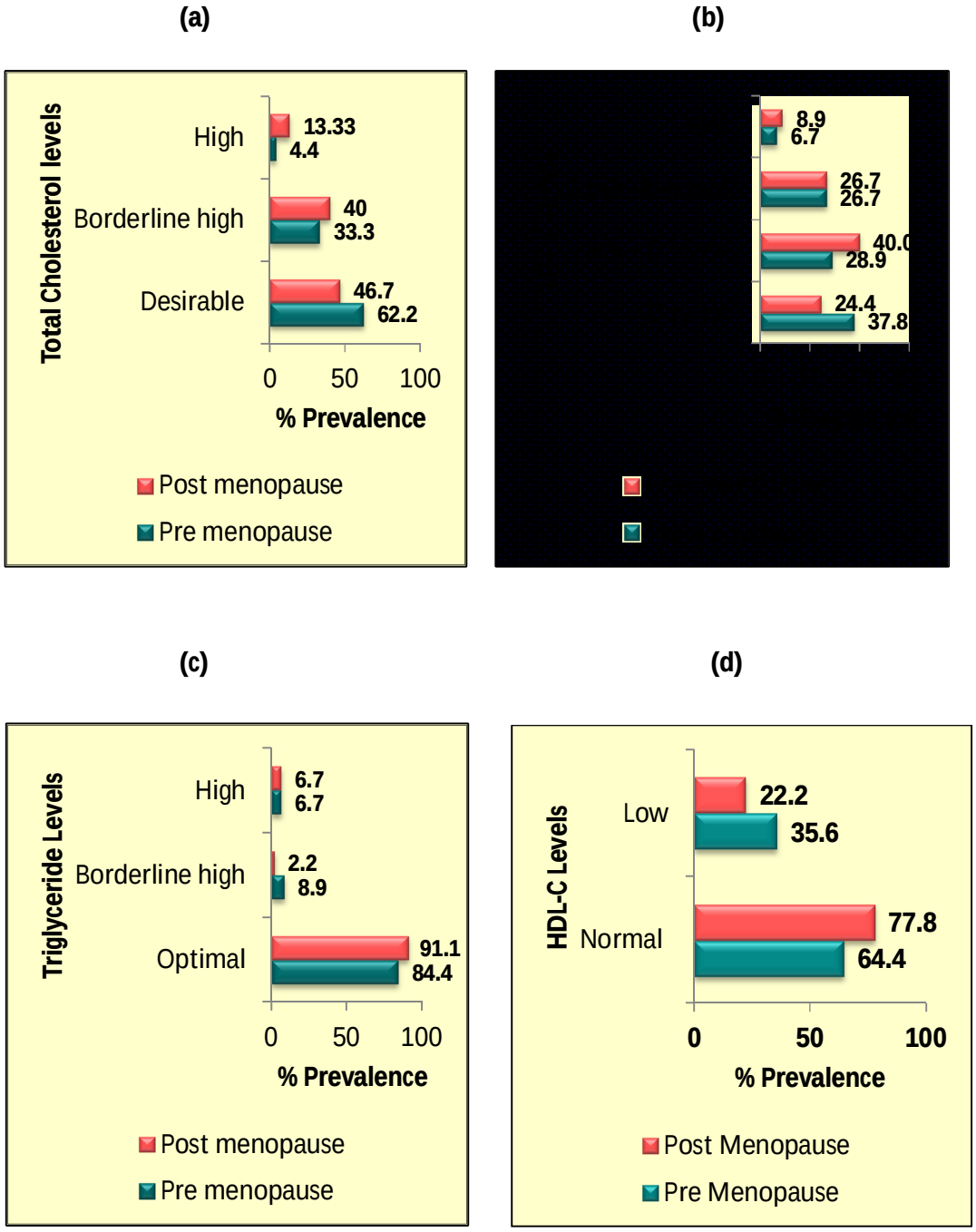
Two tailed significance*p<0.05

TABLE 4.19: PREVALENCE OF LIPEMIC ABERRATIONS AND ATHEROGENIC RISK IN THE WOMEN (n, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
TC (≥200mg/dl)	41 (45.6)	17 (37.8)	24 (53.3)	1.88 (0.81-4.36)
HDL-C (<50mg/dl)	26 (28.9)	16 (35.6)	10 (22.2)	0.52 (0.20-1.3)
LDL-C (≥100mg/dl)	62 (72.2)	28 (62.2)	34 (75.6)	1.88 (0.76-4.65)
TG (≥150mg/dl)	11 (12.2)	7 (15.6)	4 (8.9)	0.53 (0.14-1.95)
TC/HDL (>5)	5 (5.6)	3 (6.7)	2 (4.4)	0.67 (0.10-4.19)
LDL/HDL (>3.5)	2 (2.2)	1 (2.2)	1 (2.2)	1.0 (0.06-16.5)
TG/HDL (>3)	13 (14.4)	9 (20)	4 (8.9)	0.39 (0.11-1.38)
AIP (>0.21)	58 (64.4)	29 (64.4)	29 (64.4)	1.0 (0.42-2.37)

Values in parenthesis indicate percentages

FIGURE 4.10: PERCENT PREVALENCE OF DYSLIPIDEMIA IN THE SUBJECTS



prevalence of atherogenicity was equal in both the groups as indicated by atherogenic index of plasma.

4.2.5. Prevalence of Diabetes

The mean values of various glycemic indices depicted in Table 4.20 reveal that post-menopausal women had higher mean values of FBS, HbA1C, Insulin, HOMA IR and ABG while none of them were significantly different. The prevalence of diabetes was estimated using self-reported history of diabetes and FBS value >126mg/dl. Around 12% of the women were found to be diabetic according to these criteria. A considerable number of the women had high HbA1C levels (26.7%). As indicated in Table 4.21, post-menopausal women had 5.37 times higher odds of developing diabetes and 3.3 times higher odds of having high HbA1C levels as compared to pre-menopausal women (Figure 4.11 and 4.12 respectively).

4.2.6. Prevalence of Inflammation

Inflammation in the women was assessed using HsCRP as an indicator in the women. The mean HsCRP levels were found to be slightly higher in post-menopausal women (Table 4.20). About 64.4% had high HsCRP levels. Frequency distribution of HsCRP risk categories (Figure 4.13) showed that a higher per cent of post-menopausal women were under high risk category. Equal per cent of both groups had normal HsCRP levels, rest having average risk of inflammation. Menopause was not found to be a significant contributor for development of inflammation as indicated by univariate odds ratio analysis (Table 4.21).

4.2.7 Prevalence of metabolic syndrome

Metabolic syndrome, which is a cluster of three or more metabolic derangements, was prevalent in one fourth of the female population (Table 4.21). Nevertheless menopause was not found to pose any significant risk of developing metabolic syndrome in the women (OR 1.12; 95% CI 0.43-2.9).

TABLE 4.20: GLYCEMIC AND INFLAMMATORY PROFILE OF THE WOMEN (Mean±SD)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value
FBS (mg/dl)	91.04±32.78	87.36±32.4	94.73±33.10	1.07
HbA1C (%)	6.07±1.3	5.90±1.39	6.23±1.19	1.21
Insulin (µIU/ml)	11.22±7.24	10.92±5.53	13.36±14.82	1.04
%B	135.69±59.95	142.74±52.21	128.64±66.64	1.12
%S	87.76±39.54	86.42±33.86	89.09±44.85	0.32
HOMA IR	1.44±0.91	1.39±0.69	1.48±1.1	0.5
ABG (mg/dl) (n=89)	116.10±43.4	110.6±46.35	121.7±39.91	1.21
HsCRP (mg/dl)	0.23±0.25	0.20±0.24	0.25±0.26	1.00

TABLE 4.21: PREVALENCE OF GLYCEMIC AND INFLAMMATORY ABERRATIONS IN THE WOMEN (n, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
Diabetes Present	11 (12.2)	2 (4.4)	9 (20)	5.37* (1.09-26.49)
HbA1c (>6%)	24 (26.7)	7 (15.6)	17 (37.8)	3.3* (1.20-9.02)
HsCRP (>0.1mg/dl)	58 (64.4)	29 (64.4)	29 (64.4)	1.0 (0.42-2.37)
MS (IDF, 2005)	23 (25.6)	11 (24.4)	12 (26.7)	1.12 (0.43-2.9)

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

FIGURE 4.11: MENOPUASE AS A RISK FACTOR FOR DIABETES THROUGH UNIVARIATE ANALYSIS

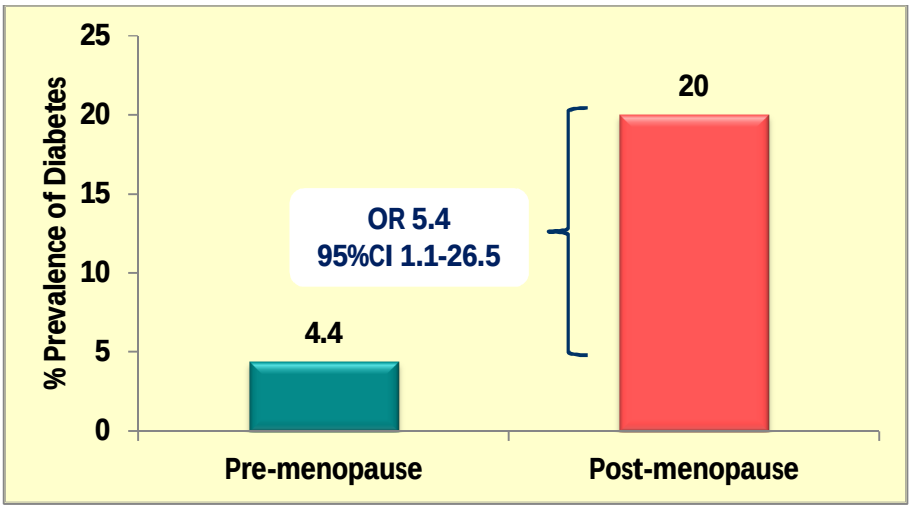


FIGURE 4.12: MENOPUASE AS A RISK FACTOR FOR HIGH HbA1c LEVELS THROUGH UNIVARIATE ANALYSIS

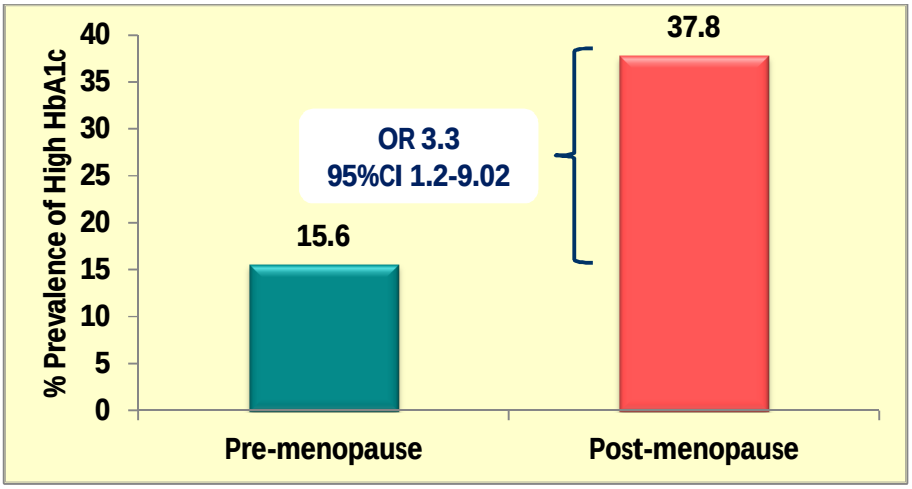
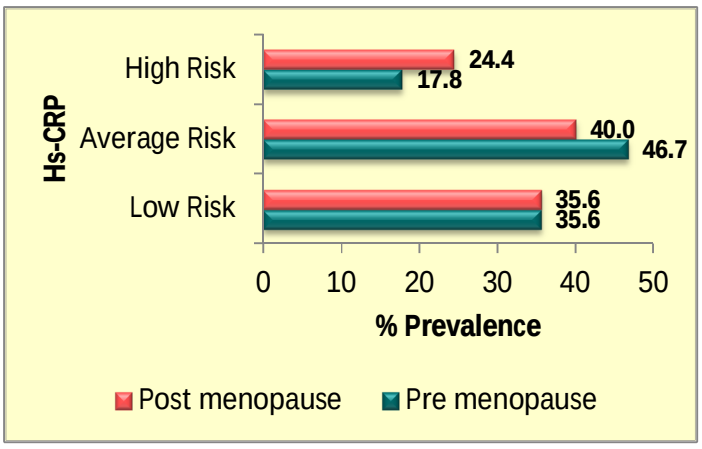


FIGURE 4.13: PERCENT PREVALENCE OF VARIOUS CATEGORIES OF Hs-CRP LEVELS



4.2.8 Prevalence of hypothyroidism

As illustrated in Table 4.22, the mean values of three major indicators of hypothyroidism i.e. TSH, Total T3 and Total T4 were under the normal range**.** No new case of hypothyroidism was diagnosed in the study. Nonetheless the subclinical hypothyroidism (High TSH levels) was present in around 29% of the women (Table 4.23). No significant difference between the prevalence of subclinical hypothyroidism was found between pre and post-menopausal women.

4.2.9 Haematological indices and prevalence of nutritional anaemia:

Iron deficiency anaemia has been a public health problem in India for years. In the recent days the prevalence of vitamin B12 and folic acid deficiency is coming into bigger picture as they can lead to other forms of nutritional anaemia. The mean values of haematological indices (Table 4.24) were under reference range except for MCHC which was on the lower side of the reference range and RDW CV and ESR which were slightly higher than the normal values. Mean Vitamin B12 values also fell under marginal deficiency category indicating suboptimal vitamin B12 levels in the women. The comparison of mean values between pre and post-menopausal women depicted that mean neutrophils ($p<0.05$), basophils ($p<0.05$) and TIBC ($p<0.05$) were significantly higher whereas ferritin ($p<0.05$) values were significantly lower in pre-menopausal women. Leucopenia which is an indicator of low WBC levels was found to be prevalent in only 1.1% of the women (Table 4.25). A minute fraction (2.2%) of the study population was suffering from thrombocytopenia (Low platelet levels).

The prevalence data of various indicators of nutritional anaemia illustrated that around 49% of the women were having iron deficiency anaemia diagnosed by low haemoglobin levels. The other indicators of iron deficiency anaemia were low haematocrit levels, microcytosis, low serum iron levels, high total iron binding capacity, low per cent transferrin saturation and low ferritin levels with varied prevalence of 21.1%, 14.4%, 26.4%, 13.8%, 34.5 and 21.1% respectively. The prevalence of macrocytic anaemia was low in the subject (2.2%). However, the deficiency of one major nutrient responsible for macrocytosis (Vitamin B12) was very high (71.1%). Prevalence of Marginal vitamin B12 deficiency was comparatively

TABLE 4.22: THYROID PROFILE OF THE WOMEN (Mean±SD)

Parameters	Total (n=90)	Pre-menopause (n=45)	Post-menopause (n=45)	Students' 't' Value
TSH (µIU/ml)	3.83±3.75	3.51±3.3	4.14±4.17	0.8
T3 (ng/dl)	117.99±20.48	116.84±18.25	119.13±22.65	0.53
T4 (µg/dl)	3.83±3.75	8.68±1.27	8.67±1.47	0.04

TABLE 4.23: PREVALENCE OF HYPOTHYROIDISM IN THE WOMEN (N, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
TSH (>4µIU/ml)	26 (28.9)	13 (24.4)	15 (33.3)	1.55 (0.62-3.88)
T3 (200ng/dl)	0 (0)	0 (0)	0 (0)	--
T4 (12µg/dl)	0 (0)	0 (0)	0 (0)	--

Values in parenthesis indicate percentages

higher in pre-menopausal women (33%) than post-menopausal ones (20%). In contrast vitamin B12 deficient cases were high in post-menopausal women (51.1%)

(Figure 4.14). Only a small per cent of the women (5.6%) had folic acid deficiency with on major difference between two groups (Figure 4.15).

Figure 4.16 demonstrates the prevalence of anaemia in pre and post-menopausal women. Moderate iron deficiency anaemia was equally present in both the groups though the prevalence of mild IDA was slightly higher in post-menopausal women. Menopause did not seem to affect the prevalence of various indicators of anaemia in the women (Table 4.25).

4.2.10 Prevalence of liver function abnormalities

Liver functions are assessed through a cluster of tests which can be categorized under three broad categories i.e. bilirubin levels, liver enzymes and serum proteins. The mean values of indicators under all three categories were between the reference ranges (Table 4.26). Mean alkaline phosphatase levels were significantly higher in post-menopausal women ($p<0.001$). Around one fourth of the women had high prevalence of atypical alkaline phosphatase values (Table 4.27) and post-menopausal had 3.6 times higher odds (CI 1.33-9.9) of having higher alkaline phosphatase levels (Figure 4.17). The prevalence of high levels of other liver enzymes was 12.2%, 21.1% and 10% for SGOT, SGPT and GGT respectively. High direct bilirubin levels were prevalent in 16.7% of the women. Occasional case (1.1% for albumin) of low serum proteins was observed in the women. Post-menopause was not found to have any significant influence on the liver function indicators except for alkaline phosphatase.

4.2.11. Prevalence of kidney function abnormalities

As illustrated in Table 4.28, the mean levels of various kidney function indicators were between normal ranges in the women. None of the women had high BUN levels; nevertheless 3.3% had high creatinine levels. Around 9% of the women had high uric acid level which is an indicator of Gout (Table 4.29). Menopausal status was not found to pose risk for developing kidney function abnormalities.

TABLE 4.24: HAEMATOLOGICAL INDICES OF THE WOMEN (Mean±SD)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value	Reference Range
TLC ($\times 10^3$ /µl)	7.34±1.7	7.61±1.56	7.06±1.81	1.54	4.4-11
Neutrophils (%)	53.9±8.15	55.82±7.49	51.98±8.41	2.29*	40-80
Lymphocyte (%)	35.61±6.77	34.32±6.03	36.9±7.28	1.82	20-40
Monocyte (%)	5.66±1.86	5.55±1.67	5.77±2.05	0.59	0-10
Eosinophils (%)	4.43±2.53	3.94±2.28	4.91±2.70	1.85	0-6
Basophils (%)	0.40±0.19	0.44±0.19	0.18±0.18	2.06*	0-1
Total RBC ($\times 10^6$ /µl)	4.95±4.92	4.46±0.36	5.44±6.96	0.94	3.5-5
Hematocrit (%)	37.98±3.44	38.19±3.47	37.76±3.44	0.59	>36
MCV (fL)	86.72±8.11	85.86±7.5	87.58±8.68	1.0	80-100
MCH (pg)	27.18±2.77	26.73±2.51	27.63±2.96	1.56	34.9-46.9
MCHC (%)	31.27±1.21	30.06±1.17	31.48±1.22	1.64	33.4-37
RDW CV (%)	15.18±1.32	15.17±1.25	15.19±1.39	0.07	11.5-14.5
Platelet Count ($\times 10^3$ /µl)	297.36±89.89	310.29±91.86	284.4±86.96	1.37	150-400
PDW (%)	15.96±0.53	15.98±0.60	15.95±0.45	0.24	>10
MPV (%)	9.07±1.18	9.19±1.4	8.96±0.90	0.94	6.5-12
ESR (mm/hr)	21.48±15.28	20.14±15.4	22.81±15.24	0.8	0-20

Two tailed significance*$p<0.05$

TABLE 4.24: HAEMATOLOGICAL INDICES OF THE WOMEN (Mean±SD) contd..

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value	Reference Range
Hb (gm/dl)	11.88±1.05	11.87±1.08	11.89±1.02	0.08	>12
Iron (µg/dl) (n=87)	70.04±28.32	72.63±31.59	67.52±24.83	0.84	>50
TIBC (µg/dl) (n=87)	382.59±59.79	395.87±62.71	369.62±54.4	2.09*	<450
% Transferrin Saturation (%) (n=87)	18.89±8.25	19.1±9.13	18.7±7.39	0.22	>15
Ferritin (ng/ml)	42.21±53.17	29.12±30.33	55.3±66.69	2.4*	>15
Folic Acid (ng/ml)	11.43±6.23	10.5±5.48	12.36±6.84	1.43	>4
Vit B12 (pg/ml)	280.54±242.72 (Range 67-1835)	285.79±277.4	275.3±205.3	0.20	>300

Two tailed significance*p<0.05

TABLE 4.25: PREVALENCE OF VARIOUS INDICATORS OF NUTRITIONAL ANEMIA IN THE WOMEN (n, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
Leucopenia (WBC<3.5 /mcL)	1 (1.1)	0 (0)	1 (2.2)	--
Mild and Moderate Anemia	49 (54.4)	22 (48.9)	27 (60)	1.56 (0.68-3.62)
Hematocrit (<36%)	19 (21.1)	9 (20)	10 (22.2)	1.14 (0.42-3.15)
Microcytosis (MCV<80)	13 (14.4)	8 (17.8)	5 (11.1)	0.58 (0.17-1.96)
Macrocytosis (MCV>100)	2 (2.2)	1 (2.2)	1 (2.2)	1.0 (0.06-16.5)
Thrombocytopenia (Platelet<150×103 /mcl)	2 (2.2)	1 (2.2)	1 (2.2)	1.0 (0.06-16.5)
Folic Acid (<4ng/ml)	5 (5.6)	3 (6.7)	2 (4.4)	0.65 (0.10-4.1)
Iron (<50µg/dl)	23 (26.4)	10 (23.3)	13 (29.5)	1.63 (0.63-4.19)
TIBC (>450)	12 (13.8)	9 (20.9)	3 (6.8)	0.29 (0.07-1.14)
Transferrin Saturation (<15%)	30 (34.5)	15 (34.9)	15 (34.1)	1.0 (0.42-2.4)
Ferritin (<15µg/l)	19 (21.1)	13 (28.9)	6 (13.3)	0.38 (0.13-1.11)
Vitamin B12 Deficiency (<300pg/ml)	64 (71.1)	32 (71.1)	32 (71.1)	1.0 (0.40-2.49)

Values in parenthesis indicate percentages

FIGURE4.14: PERCENT PREVALENCE OF VITAMIN B12 DEFICIENCY IN WOMEN

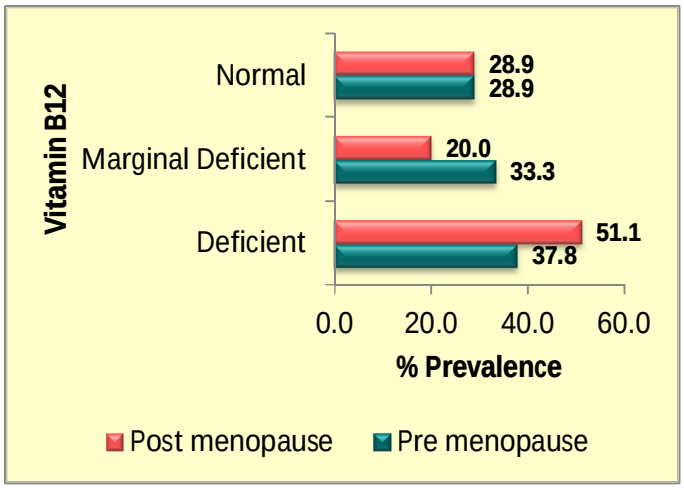


FIGURE 4.15: PERCENT PREVALENCE OF FOLIC ACID DEFICIENCY IN WOMEN

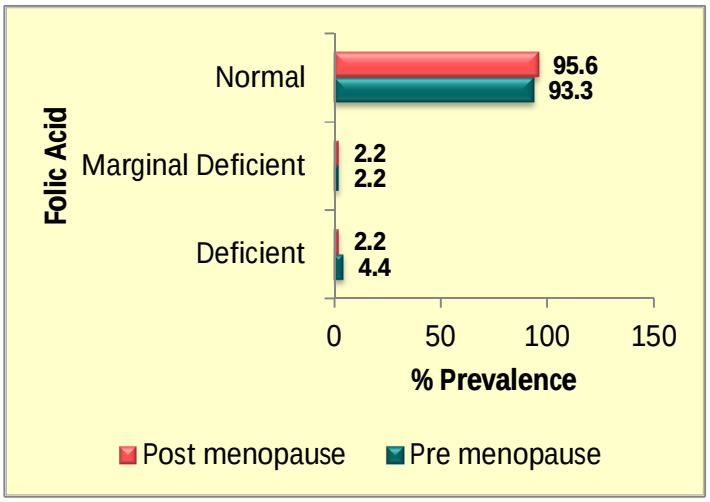


FIGURE 4.16: PERCENT PREVALENCE OF IRON DEFICIENCY ANEMIA IN WOMEN

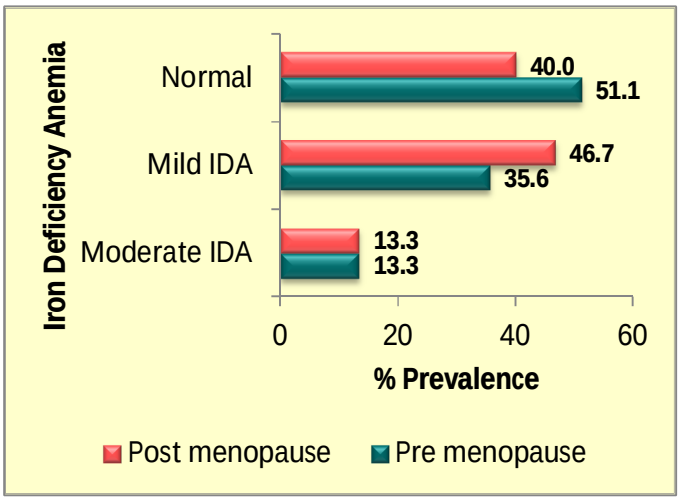


TABLE 4.26: LIVER FUNCTION TESTS OF THE WOMEN (Mean±SD)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value	Reference Range
Alkeline Phosphatase (U/L)	85.77±23.05	77.56±20.11	93.98±23.1	3.6***	42-98
Total Bilirubin (mg/dl)	0.54±0.16	0.54±0.16	0.54±0.15	0.16	0-1.2
Indirect Bilirubin (mg/dl)	0.38±0.12	0.39±0.14	0.38±0.11	0.35	0-0.3
Direct Bilirubin (mg/dl)	0.16±0.04	0.16±0.04	0.16±0.05	0.44	0-0.9
SGOT (U/L)	23.86±12.08	24.4±15.03	23.33±8.29	0.42	0-31
SGPT (U/L)	23.57±15.57	26.01±20.65	21.13±7.21	1.5	0-31
GGT (U/L)	21.31±11.29	19.63±7.23	22.99±14.13	1.42	0-30
Serum Albumin (gm/dl)	4.27±0.27	4.29±0.23	4.24±0.3	0.91	3.2-5
Total Protein (gm/dl)	7.61±0.32	7.65±0.30	7.57±0.34	1.11	6-8.3
Serum Albumin/ Globulin Ratio	1.3±0.19	1.3±0.17	1.3±0.21	0.08	0.9-2

Two tailed significance***p<0.001

TABLE 4.27: PREVALENCE OF LIVER FUNCTION ABNORMALITIES IN THE WOMEN (n, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
Alkaline Phosphatase (>98 U/L)	25 (27.8)	7 (15.6)	18 (40)	3.62* (1.33-9.9)
Total Bilirubin (>1.20mg/dl)	0 (0)	0 (0)	0 (0)	--
Indirect Bilirubin (>0.9mg/dl)	1 (1.1)	1 (2.2)	0 (0)	--
Direct Bilirubin (>0.20mg/dl)	15 (16.7)	6 (13.3)	9 (20)	1.63 (0.53-5.02)
SGOT (>31 U/L)	11 (12.2)	7 (15.6)	4 (8.9)	0.53 (0.14-1.95)
SGPT (>28 U/L)	19 (21.1)	10 (22.2)	9 (20)	0.88 (0.32-2.41)
GGT (> 38 U/L)	9 (10)	2 (4.4)	7 (15.6)	3.96 (0.78-20.2)
Serum Albumin (<3.5gm/dl)	1 (1.1)	0 (0)	1 (2.2)	--
Total Protein (<6.6gm/dl)	0 (0)	0 (0)	0 (0)	--

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

FIGURE 4.17: MENOPAUASE AS A RISK FACTOR FOR HIGH ALKALINE PHOSPHATASE LEVELS THROUGH UNIVARIATE ANALYSIS

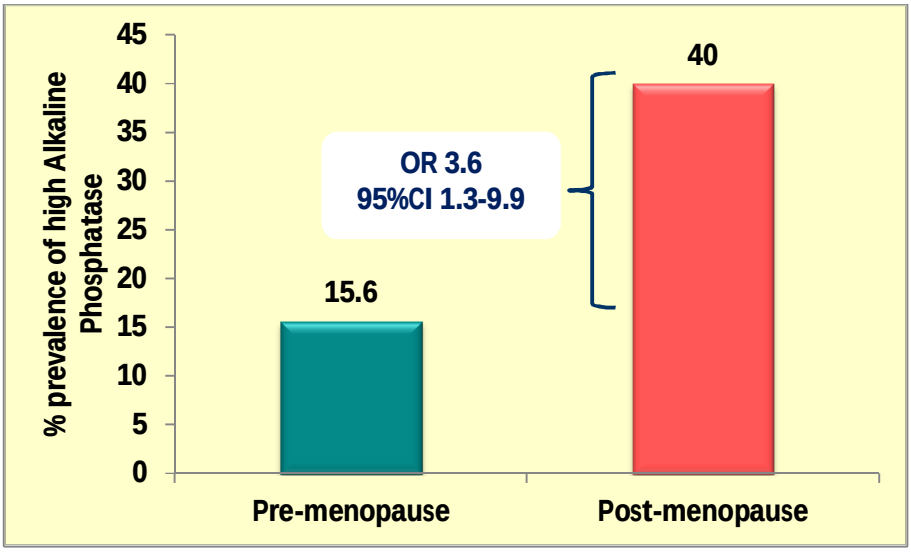


TABLE 4.28: KIDNEY FUNCTION TESTS OF THE WOMEN (Mean±SD)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	Students' 't' Value	Reference Range
BUN (mg/dl)	9.67±2.73	9.38±2.32	9.95±3.09	0.98	9-23
Creatinine (mg/dl)	0.58±0.09	0.59±0.09	0.57±0.1	0.91	0.5-0.8
Uric Acid (mg/dl)	4.43±0.98	4.42±1.07	4.44±0.9	0.1	2.3-6.1
BUN/ Sr. Creatinine ratio	16.93±4.8	17.44±4.71	16.42±5.06	0.1	9:1-23.1
Calcium (mg/dl)	9.46±0.43	9.46±0.49	9.46±0.37	0.09	8.8-10.6

TABLE 4.29: PREVALENCE OF KIDNEY FUNCTION ABNORMALITIES IN THE WOMEN (n, %)

Parameters	Total (n=90)	Pre Menopause (n=45)	Post Menopause (n=45)	OR (95% CI)
BUN (>20mg/dl)	0 (0)	0 (0)	0 (0)	--
Creatinine (>0.8mg/dl)	3 (3.3)	1 (2.2)	2 (4.4)	2.05 (0.18-32.41)
Uric Acid (>6mg/dl)	8 (8.9)	6 (13.3)	2 (4.4)	0.30 (0.06-1.59)
Calcium (<8.8mg/dl)	1 (4.4)	0(0)	1 (2.2)	

Values in parenthesis indicate percentages

Table 4.30: HIGHLIGHTS SHOWING ASSOCIATION OF MENOPAUSE STATUS ON VARIOUS PHYSIOLOGICAL AND METABOLIC ABERRATIONS

Variables	Significantly high mean values in post-menopausal women	Significantly high Odds ratio in post-menopausal women
Waist Circumference	√	NS
Waist Hip Ratio	√	NS
Waist Stature Ratio	√	NS
Body Fat %	√	√
Hypertension	√	√
HDL-C	√	NS
Diabetes	NS	√
HbA1c	NS	√
Neutrophils	√	NS
Basophils	√	NS
Ferritin	√	NS
Alkaline Phosphatase	√	√

Discussion

Unhealthy diet and behavioural practices followed for a long period of time may pose higher risk of physiological and metabolic derangements in the body. Obesity is very first step towards evolution of non-communicable diseases. According to WHO estimate (2011), nearly 300 million women are obese globally, in comparison to 200 million men. It clearly shows the prevalence of obesity in women is far more than men. In the present study around three fourth of the women were overweight /obese. The prevalence of generalized overweight/obesity in women in two different large cross sectional studies i.e. CURES-47 (Deepa et al, 2009) and Five city study group (Singh et al, 2007) was 51.6% and 50.2% respectively, which is lower than the results of present study. The above mentioned studies are half decade old and the prevalence of obesity is on a rise in urban women according to the Jaipur heart watch studies (Gupta et al, 2008) from 15.7% in JHW 1 (1993) to 57.7in JHW 4% (2008). Overweight/obesity prevalence was around 84% in a departmental study performed on urban women of Vadodara (Elayath and Iyer, 2013).

Prevalence of high abdominal obesity indices WC (93.1%), WHR (88.5%) was also observed in the present study in comparison to data available from various regions of the country and the regional data (Elayath and Iyer, 2013; Rao et al, 2013; Khokhar et al, 2010; MMH et al, 2011; Yadav et al, 2008). Waist stature ratio, the upcoming indices to assess CVD risk also showed high prevalence of risk (96.9%) in the subjects. As the cases of under-nutrition were removed in experimental design of the study, it might lead to slightly skewed prevalence of obesity towards higher side.

Menopause poses a higher risk of increase in fat mass and fat redistribution in the body as demonstrated in various studies (Atapattu, 2015; Davis et al, 2012; Ho et al, 2010). A longitudinal study of 30 months on 348 women showed that menopause transition has an independent effect on increase in fat mass ($p<0.001$), and abdominal obesity ($p<0.001$) (Ho et al, 2010). Whether it affects the overall weight gain and generalized obesity, is still unclear (Davis et al, 2012; Dubnov-Raz et al, 2007). The mechanism behind the same is thought to be estrogen deficiency

(Atapattu, 2015). Estrogen is hypothesized to affect fat metabolism, appetite and satiety control, energy regulation and adipokine secretion through different experimental models (Lizcano and Guzman, 2014). Estrogen-only or estrogen–progestin therapy can ameliorate accumulation of abdominal fat and increase in fat mass (Davis et al, 2012). The results of present study were in line with the literature available as mean waist circumference ($p<0.01$), waist hip ratio ($p<0.05$), waist stature ratio ($p<0.001$) and body fat percent ($p<0.001$) were significantly higher in post-menopausal women despite no major difference in mean body weight and BMI in comparison to pre-menopausal women.

A concern regarding premenopausal women's health arouse from the results as mean of all anthropometric indices were higher than normal cut off in those women. Khokhar et al (2010) also depicted similar trends showing a high prevalence of general and abdominal obesity in pre-menopausal women in a study conducted in Punjab. Obesity at the time of menopausal transition can significantly affect hormonal levels (Freeman et al, 2010) and increase the prevalence of menopausal symptoms (Thurston and Joffe, 2011). Therefore weight control should be focused in pre-menopause only to maintain the quality of life during peri and post menopause (Davis et al, 2012).

Hypertension is a known risk factor for cardio vascular diseases and in diabetes a risk for developing macro and microvascular complications (Long and Dagogojack, 2011). Many times hypertension is asymptomatic and underdiagnosed (Levy and Cline, 2009). In the present study out of 42% of the hypertensive subjects only half were aware about their hypertensive status. More than half of the subjects were having family history of hypertension. Various Indian studies have shown diverse prevalence of hypertension in the female population ranging from 23%-40% (Rao et al, 2013; MMH et al, 2011; Yadav et al, 2008) which are in line with the present study. Only one day measurement of the blood pressure is a major limitation of the present study, as it can over/underestimate the prevalence of the hypertension.

Estrogen hormone is hypothesized to maintain the homeostasis of blood pressure through increase in angiotensinogen levels, decrease in renin levels (Fischer et al,

2002) and vasodilation effect by the α- and β-receptors in the vessel wall (Mendelsohn and Karas, 1999). However conflicting results have been seen regarding the role of menopause in the development of hypertension (Coylewright et al, 2008). In the present study mean systolic blood pressure was significantly higher ($p<0.001$) and odds of having hypertension was 4.4 times higher (96%CI 2.1-9.3) in post-menopausal women. The results were supported by different studies performed at north and west regions of India (Tyagi et al, 2015; Doshi et al, 2014; Gupta et al, 2014; Mahajan et al, 2012) showing significant differences in prevalence or mean blood pressure levels between pre and post-menopausal women.

Abnormal lipid levels, another major risk factor for development of cardio vascular diseases were studied in the present study. The prevalence of LDL-C (72.2%), TC (45.6%) and atherogenic index of plasma (64.4%) was quite high in the women. Comparatively lower prevalence (28.9%) of low HDL-C levels was observed. A previous departmental study on women showed similar trends of abnormal lipid levels (LDL-C 65%, TC 33.8%) except for low HDL-C levels (47.3%) (Elayath and Iyer, 2013). According to ICMR INDIAB study the prevalence of dyslipidemia is very high all across the country despite variations from region to region (Joshi et al, 2014). The most common lipid abnormality found in ICMR INDIAB study was low HDL-C levels (73%). Prevalence of high TC and low HDL-C were reported as 32.7% and 55.3% respectively in women in Jaipur Heart Watch Study 5 (Gupta et al, 2012). A series of studies done by Jaipur Heart Watch study group over two decade demonstrated rising trend of lipid abnormalities ($p<0.001$). In a study performed by Sekhari et al (2014) which included 1966 women government employee from 20 cities of India depicted prevalence of high TC and low HDL-C levels as 27.6% and 76% respectively.

Menopause can bring about adverse changes in lipid levels showing a peak during late peri and early post-menopause according to 7 years SWAN cohort on 2,659 women (Derby et al, 2009). Several studies explored the abnormal lipid levels as an effect of menopause, most of them reporting significantly high levels/prevalence of LDL-C and low levels/prevalence of HDL-C in post-menopausal women in comparison to their pre-menopausal counterparts. However some inconsistencies were seen regarding TG and TC. (Dosi et al, 2014; Bade et al, 2014; Kanwar et al, 2014;

Deshpande et al, 2012; Igwah et al, 2005). In the present study mean TC and LDL-C levels as well as prevalence of abnormal levels of these factors were high in postmenopausal women, though not statistically significant. Surprisingly the mean HDL-C levels were significantly high ($p<0.05$) in post-menopausal women. A recent study performed by Shenoy and Vernekar (2015) supported the trend in which mean HDL-C levels were slightly higher in post-menopausal women (55.84±22.11) than pre-menopause ones (52.15±14.98), though not statistically significant. Though obesity is associated with abnormal lipid levels, thinner women may experience the highest increases in low density lipoprotein cholesterol during the menopause transition due to hormonal effects (Derby, 2009).

India is having the highest number of diabetics around the world and its prevalence is rising as an epidemic (Mohan et al, 2007). The prevalence of diabetes (Self-reported and diagnosed under study) and high HbA1c levels was 12.2 % and 26.7% respectively in the present study. The initial trends of largest ongoing research on diabetes in India (ICMR-INDIAB) the prevalence of diabetes (both known and newly diagnosed) was 10.4% 8.4%, 5.3%, and 13.6% in Tamilnadu, Maharashtra Jharkhand and Chandigarh respectively (Anjana et al, 2011). The CURES-47 study, which included 1254 women, depicted prevalence of diabetes as 13.4% in women (Deepa et al, 2009). Mean HbA1c levels were 5.97±1.3, which are quite comparable with the present study. Elayath and Iyer (2013) reported the prevalence of diabetes to be 8.5% in the urban female population of Vadodara. The prevalence of diabetes in women was estimated to be 10.8% according to latest Jaipur heart Watch study trends (Gupta et al, 2012).

In the present study, mean FBS, HbA1c, HOMA IR, and average blood glucose was higher in postmenopausal women although not statistically significant. Post-menopausal women had higher odds of developing diabetes (5.37, 95%CI 1.09-26.49) and high HbA1c (3.3, 95%CI 1.20-9.02) levels. The results are supported by the study performed by Dasgupta et al (2012) on 316 pre and post-menopausal females. In a study performed by Jesmin et al, 2013 in rural Bangladesh (n=1802) depicted significantly higher fasting glucose levels (5.77 ± 2.12 v/s 6.91 ± 3.35 mmol/L, $p<0.001$) and higher odds of having high fasting plasma glucose (2.84, 95%CI 2.32-

3.47) in post-menopausal females. Low levels of sex hormone binding globulin (SHBG) have been identified as a strong predictor of development of type 2 diabetes in a case-control study performed on 359 newly diagnosed type 2 diabetic and 359 control women (Ding et al, 2009). Estrogen deficiency has also been projected as a fundamental step in the development of diabetes (Rossi et al, 2004).

Prevalence of metabolic syndrome, a cluster of various risk factors, is rapidly increasing in developing countries and many studies have reported higher prevalence of metabolic syndrome in women (Mishra and Khurana, 2008). Around 24% American women were found suffering from metabolic syndrome under Women Health study (8 years cohort) performed on 14719 women (Ridker et al, 2003). Ramchandran et al (2003) reported a high prevalence of metabolic syndrome (ATP III criteria) in Indians (41.1%) with women having significantly higher prevalence (46.5% v/s 34.6% χ^2=4.6, *P*=0.03) than men. In a study performed by Pandey et al (2010) including 398 urban women, the prevalence of metabolic syndrome using IDF criteria was 56.6%. However, 26% of the women were found to have metabolic syndrome in the present study. The difference is age group studied and regional variation may be a major reason behind this disparity.

The effect of menopause on prevalence of metabolic syndrome (MS) was assessed by Pandey et al (2010) on 498 urban females and found than post-menopausal women had significantly high prevalence of metabolic syndrome but the significance was lost after adjusting for age. A study performed by Shah et al (2010) depicted prevalence of metabolic syndrome using NCEP III criteria as 22.2% and 32.4% in pre and post-menopausal women respectively. A rural study in Bangladesh revealed significantly higher prevalence of metabolic syndrome in post-menopausal women (39.3%) than pre-menopausal counterparts (16.8%) of age above 15 years (Jesmin et al, 2013). In the present study no significant difference in prevalence of MS was found between these two groups. Inclusion of younger population and urban-rural difference may justify the differences.

The prevalence of mild to moderate anemia using hemoglobin as an indicator was 54.4% in women, which is in line with that reported in NFHS-3 (NFHS, 2005-06).

Mean levels of ferritin, which is an indicator of iron deficiency anemia were significantly lower in pre-menopausal women (29.12±30.33 v/s 55.3±66.69, $p<0.05$). Mean TIBC was found to be significantly higher (395.87±62.71 v/s 369.62±54.4 $p<0.05$) in these subjects showing greater chances of iron deficiency anemia in pre-menopausal women.

The deficiency of folic acid was found to be low (5.6%) in the present study. Khanduri et al (2005) reported 8% of the females having folate deficiency in a cross sectional study. None of the female adolescents were found to have folate deficiency in a study performed at northern Himalayan state of India (Bhardwaj et al, 2013). According to Caramel et al (2002) none of the Asian Indian adolescent subjects (n=60) based in US were having folate deficiency. A high prevalence (22.5, 40.4, and 52.2% for HIG, MIG and LIG) of folate deficiency was observed in Delhi based adolescents (n=347) of various socio economic groups (Kapil and Bhadoria, 2014).

A high prevalence of vitamin B12 deficiency (71.1%) was found in the subjects with pre and post-menopausal women having similar prevalence. Around 46% if the females were found to be having low cobalamin levels in a study performed by Khanduri et al (2005) on 50 females of New Delhi. The cut off use by Khanduri et al (2005) was higher than that used in the present study (434pg/ml). Pandey et al (2006) reported the prevalence of vitamin B12 deficiency as 30.2% in 116 pre and post-menopausal women studied. Another study be Refsum et al (2001), indicated 47% of men and women as Vitamin B12 deficient (n=204). Bhatia et al (2012) reported 33.8% of the subjects having B12 deficiency in a hospital based study at Bangalore. About 50% of the subjects were found Vitamin B12 deficient in a cohort from eastern Indian states (Sukla et al, 2014). The higher prevalence of vitamin B12 deficiency in the present study may be because of relatively higher percentage of vegetarian women in comparison to other studies.

Macrocytosis (MCV>100) is an indicator of megaloblastic anemia which occurs due to folate and/or vitamin B12 deficiency. In the present study only 2.2% of the subjects were having macrocytosis in comparison to a high prevalence of vitamin B12 deficiency. This shows macrocytosis is not a good indicator to identify suboptimal levels of B12 deficiency. Similar trends were found in other studies

(Bhatia et al, 2012; Khanduri et al, 2005). According to Metz (2008), just the subclinical deficiency of vitamin B12 of folate cannot be translated to nutritional anemia, and there is lack of specificity of tests to identify the true deficiency to be resulted into anemia. Thrombocytopenia, which can occur due to Vitamin B12 deficiency (Oh et al, 2003), was found to be only in 2.2% subjects. According to Wadia et al (2000) vitamin B12 and folate deficiency may be present without any hematological abnormality.

Pre-menopause did not seem to have significant risk of development of nutritional anemia in women in this study as depicted by odds ratio analysis. According to WHI Observational Study, nutritional anemia in post-menopausal women is mainly the effect of low dietary intake of red meat, iron, folate and B12 deficiency in the diet (Thompson et al, 2011).

Increase in age and hormonal changes can lead to development and progression of liver disease in postmenopausal women (Brady, 2015). A study performed by Kumari et al (2010) reported that postmenopausal women had significantly higher mean values of AST ($p<0.001$), Total bilirubin ($p<0.01$) and direct bilirubin ($p<0.001$). In contrast significantly lower levels of serum albumin ($p<0.0001$) and calcium ($p<0.0001$) were observed. This indicated higher chances of altered liver functions, liver damage and increased risk of osteoporosis in post-menopausal women (Kumari et al, 2010). In the current study no significant difference of mean values and prevalence of abnormal liver function markers between pre and post-menopausal women except for alkaline phosphatase.

The mean values of serum alkaline phosphatase were significantly higher in post-menopausal women in the present study. Post-menopausal women also had 3.6 times higher odds (95%CI 1.33-9.9) of having high levels of alkaline phosphatase. Various studies have shown that increased alkaline phosphatase levels in post-menopausal women are associated with higher bone turnover which can accelerate bone mass reduction (Mukaiyama et al, 2015; Bhattara et al, 2014; Crilly et al, 1980).

SUMMARY

- The prevalence of obesity and abdominal obesity was very high in the women with mean values of abdominal indices and body fat per cent significantly higher in post-menopausal women.
- Around 20.6% of the women were found as newly diagnosed cases of hypertension in both the groups. Post-menopausal women had significantly higher odds of developing hypertension.
- About half of the women were diagnosed with iron deficiency anaemia using Haemoglobin as a tool.
- The prevalence of macrocytosis was very low although a considerable number of women were suffering from vitamin B12 deficiency.
- Dyslipidemia and risk of atherogenesis were found to be astonishingly high in the women. However menopause was not found to be associated with dyslipidemia.
- Around one eighth of the women were suffering from diabetes with a significantly high prevalence in post-menopausal women.
- Inflammation as measure by HsCRP was found to present in 64.4% of the women and it seemed not to be altered by menopausal status.
- About one fourth of the women were diagnosed with metabolic syndrome and menopause was not associated with its prevalence.
- The subclinical hypothyroidism was present in 29 of women with on significant difference between pre and post-menopausal women.
- Nearly one fourth of the women had high alkaline phosphatase levels with menopause eliciting significant risk of developing high alkaline phosphatase levels. Post menopause was not found to have impact on any other liver function indicator. Menopause was not found to pose any risk of developing kidney function abnormalities.
- In univariate analyses the major variables associated with menopause were high body fat percent, hypertension, diabetes, high HbA1C levels and high alkaline phosphatase levels.

Conclusion

A trend of higher prevalence of anthropometric and metabolic aberrations in post-menopausal women than pre-menopausal women was observed, though some of the differences were not considerably distinct. The major reason behind this was high presence of these metabolic alterations in pre-menopausal women also. Metabolic aberrations in post-menopausal can increase the risk of NCD's and presence of obesity and deranged metabolic profile in pre-menopause can adversely affect the age of menopause, menopausal symptoms and development of non-communicable diseases vice versa. Therefore a healthy life-style should be focused from the early stages of life with extra caution during and post menopause.

4.3. EFFECT OF AGE AND OBESITY ON THE ASSOCIATION OF MENOPAUSE WITH METABOLIC PROFILE

Menopause is a physiological condition which can bring about various changes in metabolism and endocrinology of the body. In the present study a 2*2 factorial design based on menopausal status and BMI was used to draw the sample. The effect of menopause on metabolic derangements in the women has been demonstrated in aforementioned section. Nevertheless the effect of obesity on these alterations is not indicated. Age is an important variable which can have confounding effect on the relationship of various metabolic aberrations with menopause. Therefore further analyses were performed to assess the independent association of menopause with metabolic alterations irrespective of age and obesity status of the women.

For this purpose initially age and obesity adjusted ANCOVA was performed on the variables whose mean values were significantly different between pre and post-menopausal women in the earlier analyses. The results indicated that mean WC ($p<0.05$), WSR ($p<0.05$), Body Fat percent ($p<0.001$), HDL-C ($p<0.01$) and alkaline phosphatase ($p<0.01$) levels were significantly higher in post-menopausal women even after adjustment for age as well as obesity (Table 4.31).

A logistic regression analysis was performed to get the true picture of metabolic risks linked with menopause after adjustment for age and obesity. The analysis was performed on variables which showed significant association with menopause during univariate odds ratio analysis. Out of five variables analyzed, only body fat was found to be significantly associated with menopause after adjustment for age and obesity ($p<0.05$) (Table 4.32).

TABLE 4.31: COMPARISON OF MEAN VALUES OF VARIOUS METABOLIC FACTORS AFTER ADJUSTING FOR AGE AND OBESITY (Mean±SD)

Variables	Pre-Menopause (n=45)	Post Menopause (n=45)	Age and obesity adjusted ANCOVA 'F' value
WC (cm)	91.43±10.1	96.4±10.3	5.4*
WHR (cm)	0.92±0.07	0.94±0.06	2.6
WSR	0.6±0.07	0.64±0.07	5.7*
Body Fat (%)	34.7±5.6	38.2±5.4	23.2***
SBP (mmHg)	123.14±17.2	135.6±18.8	0.26
HDL-C (mg/dl)	52.92±11.26	58.12±12.84	9.6**
Neutrophils (%)	55.82±7.49	51.98±8.41	3.5
Basophils (%)	0.44±0.19	0.18±0.18	2.6
Ferritin (μg/l)	29.12±30.33	55.3±66.69	2.02
Alkaline Phosphatase (U/L)	77.56±20.11	93.98±23.1	7.3**

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

TABLE 4.32: AGE AND OBESITY ADJUSTED ANALYSIS OF EFFECT OF MENOPAUSE ON RISK OF DEVELOPING METABOLIC ABBERATIONS USING FORWARD LOGISTIC REGRESSION

Variables	Age adjusted logistic regression	
	Exp (B)	95% CI
High Body Fat %	1.01E4*	1.5-6.06E8
Hypertension	1.01	0.22-4.6
Diabetes	1.57	0.11-22.0
High HbA1c levels	0.68	0.11-4.01
High Alkaline Phosphatase levels	1.1	0.21-5.97

Table 4.33: HIGHLIGHTS SHOWING EFFECT OF OBESITY ON PHYSIOLOGICAL AND METABOLIC ABERRATIONS IN PRE AND POST-MENOPAUSAL WOMEN FOLLOWED BY AGE ADJUSTED MULTIVARIATE ANALYSES

Association of menopause on various metabolic factors using ANCOVA and Logistic regression analysis adjusted for age and obesity		
Variables	**ANCOVA**	**Logistic Regression**
WC	√	--
WSR	√	--
Body fat %	√	√
HDL-C	√	--
Alkaline Phosphatase	√	--

Discussion

Menopause brings a robust change in every aspect of health in a woman's life, be it psychological, physical or social health. These changes can, directly or indirectly have an impact on risks of developing chronic diseases. But in recent years there is a debate going on whether it's menopausal transition which leads to higher risk of NCD's or merely an effect of increase in age.

In the present study high prevalence of risk factors for developing non-communicable diseases was seen in both pre and post-menopausal women. Mean values of WC, WSR, body fat percent, HDL-C and alkaline phosphatase were significantly higher in post-menopausal women after adjusting for age and obesity. Logistic regression adjusted for age and obesity showed that menopause independently affected the body fat percent of women regardless of age and BMI.

The effect of age and menopause on body composition has been studied since long. A study performed in late nineties on 373 early post-menopausal women using DEXA suggested that postmenopausal changes in body fat and fat distribution are more dependent on age than on menopause. However the authors accepted the fact that effect of menopause cannot be separated completely from age (Wang et al, 1994). Another study during the similar decade using DEXA for body composition analysis (n=407) concluded that total body and abdominal fat can increase in the years preceeding menopause independent of age (Svendsen et al, 1995). Tremollieres et al (1996) also depicted that changes in fat distribution are more related with menopause than age. This change in fat distribution was thought to increase the risk of cardiovascular diseases post menopause (Tremollieres el al, 1996). In all three studies, variable 'years after menopause' was used for analysis rather than a clear division between pre and post-menopausal women. Central fat distribution is directly related to the all mortality rate in the middle aged women (Milewicz et al, 2001).

A study performed by Dasgupta et al (2012) on 169 pre and 147 post-menopausal women indicated that menopause was independently associated with metabolic aberrations as mean WC, fat percentage, blood pressure, TG, TC, LDL-C levels were

found to be significantly higher in post-menopausal women using age and BMI adjusted ANCOVA. Contradictory results were depicted by Pandey et al (2010), who studied prevalence of metabolic syndrome and its factors in 498 urban pre and post-menopausal women. Metabolic syndrome was found to be significantly higher in post-menopausal women in this study; however significance was lost after adjustment for age using logistic regression.

In a 30 month longitudinal study from China (n=438), menopause emerged as a significant and independent predictor of the decrease in lean mass and the increase in percent of body fat, TFM and trunk–leg fat mass ratio through multivariate linear regression analysis (Ho et al, 2010). Eshtiaghi et al (2010) studied 940 Iranian women to explore the role of menopause as a risk factor for CVD's. The age-adjusted odds ratio (OR) analysis depicted that post-menopausal women had 2.85 times higher risk of development of metabolic syndrome (95%CI: 1.31-6.20) (P<0.008).

SUMMARY

- Mean WC, WSR, body fat, HDL-C and alkaline phosphatase levels were significantly higher in post-menopausal women after adjustment of age and obesity.
- Through logistic regression analysis menopause was found to be independently associated with body fat percent irrespective of age and obesity status of women.

Conclusions

To conclude, association of menopause with body composition alterations and tendency of central fat deposition is established through various studies and supported by the present study. Menopause can indirectly affect risk of cardio vascular disease through these fat distribution changes. However, still larger longitudinal studies are required to establish menopause as a primary risk factor for non-communicable diseases, as various other factors like age, decreased physical activity etc. can act as confounders.

4.4. CAUSAL FACTORS OF VITAMIN B12 AND FOLIC ACID DEFICIENCY AND THEIR RELATIONSHIP WITH RISK FACTORS OF NON-COMMUNICABLE DISEASES IN WOMEN

Vitamin B12 and folic acid are two major nutrients which contribute to metabolism of homocystine in body. Deficiency of these two nutrients can lead to hyperhomocystinemia which is known as independent risk factor for cardio vascular diseases. As the major sources of vitamin B12 are non-vegetarian food items, there is a high risk of developing vitamin B12 deficiency in the vegetarians. In this study the prevalence of vitamin B12 and folic acid deficiencies, the causal factors and their association with risk factors of CVDs was studied. The results for the same are portrayed in this section.

4.4.1 Prevalence of vitamin B12 and folic acid deficiency

As shown in Figure 4.18, around 44% of the women had vitamin B12 deficiency and 27% were suffering from marginal deficiency. A stark disparity in the prevalence of folic acid deficiency was seen with only 3.3% and 2.2% women under deficient and marginal deficient categories (Figure 4.19).

4.4.2. Average dietary intake of folic acid and frequency of consumption of folic acid rich food

The average folic acid intake of the subjects obtained through 24 hour dietary recall was 167.5±82.6 µg/day which met about 84% of daily requirement as per RDA (Table 4.9). Most of the subjects consumed green leafy vegetables with high or moderate frequency. The consumption of legumes in the subjects was low (Table 4.34).

4.4.3. Average dietary intake of vitamin B12 and frequency of consumption of vitamin B12 rich food

The mean dietary intake as described in the earlier section was 0.35±0.3 µg/day (Table 4.9) which met only 35% of the daily requirement as per RDA. On comparing the mean values between subjects with low serum vitamin B12 levels and normal ones (Figure 4.20), it indicated that subjects with normal B12 levels had significantly

FIGURE 4.18: PREVALENCE OF VITAMIN B12 DEFICIENCY IN THE WOMEN

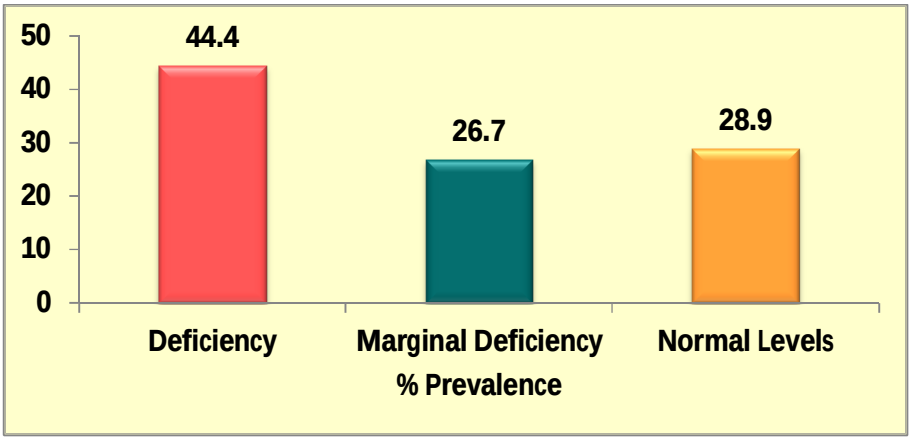


FIGURE 4.19: PREVALENCE OF FOLIC ACID DEFICIENCY IN THE WOMEN

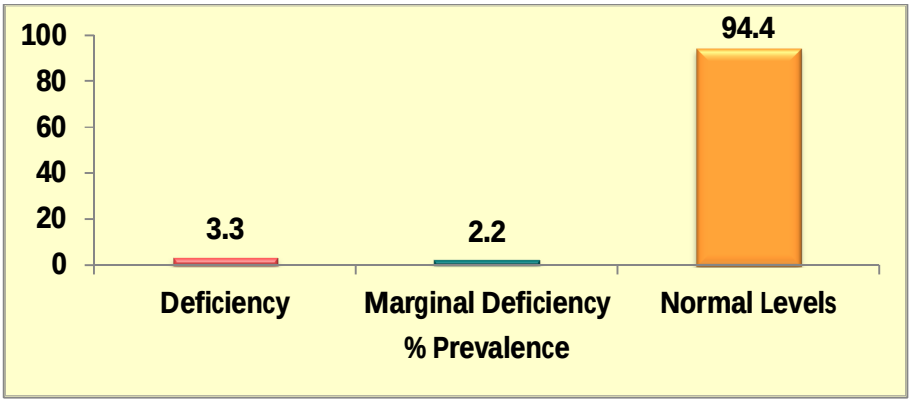


FIGURE 4.20: COMPARISON OF MEAN VALUE OF DIETARY INTAKE OF VITAMIN B12 BETWEEN SUBJECTS WITH LOW SERUM VITAMIN B12 AND NORMAL LEVELS

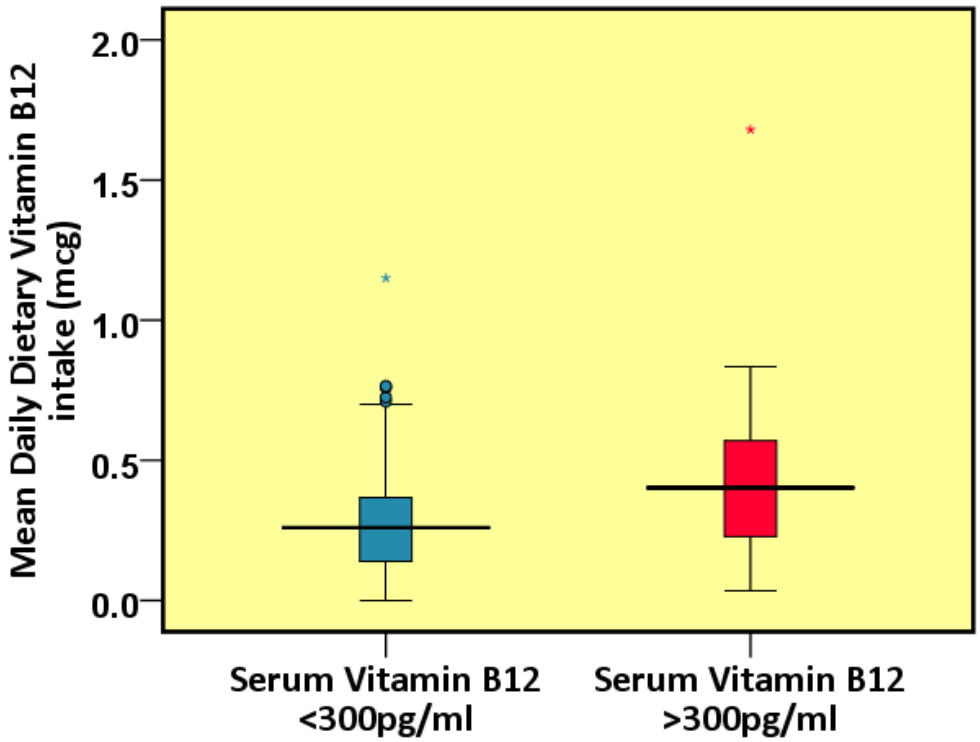


TABLE 4.34: FREQUENCY OF CONSUMPTION OF VITAMIN B12 AND FOLIC ACID RICH FOOD ITEMS (n=90) (n, %)

Food Item	High Frequency	Moderate Frequency	Low Frequency	Average Amount Consumed per Serving (g)(Mean±SD)
Green Leafy Vegetables	58 (44.3)	70 (53.4)	3 (2.3)	118.7±50.3
Legumes	10 (7.6)	11 (8.4)	110 (83.97)	16.5±11.97
Nuts	54 (41.2)	14 (10.7)	63 (48.1)	12.3±11.6
Liver	0 (0)	0 (0)	131 (100)	--
Kidney	0 (0)	0 (0)	131 (100)	--
Mutton	0 (0)	1 (0.8)	130 (99.2)	96.7±54.3
Chicken	2 (1.5)	3 (2.3)	126 (96.2)	103.3±57.5
Fish	0 (0)	4 (3.1)	127 (96.9)	78.6±79.04
Egg	3 (2.3)	8 (6.1)	120 (91.6)	94.3±38.8

Values in parenthesis indicate percentages

higher mean dietary intake of vitamin B12 ($p<0.01$). The frequency of consumption of vitamin B12 rich food items was very low (Table 4.34) as most of the subjects studied were vegetarians. Even in the non-vegetarians none of the subjects fell under high frequency consumption pattern.

Further cross tabulations were performed only for vitamin B12 deficiency due to insufficient numbers in folic acid deficiency categories for cross tabulations. For this purpose the cut off used was 300pg/ml i.e. the marginal deficiency cut off of vitamin B12 for the women.

4.4.4. Various Causal Factors of Vitamin B12 Deficiency

There are various factors which can affect the serum vitamin B12 levels. Major factors which did not involve any invasive procedure were included in the study. The univariate analysis (Table 4.35) of various causal factors revealed that age, vegetarianism, general gastro-intestinal ailments and use of tea did not have any significant risk for development of vitamin B12 deficiency. The major causal factors identified were: very low (<0.3µg/day) dietary intake of vitamin B12 ($p<0.01$), no use of supplements ($p<0.05$) and no regular health check-up ($p<0.001$). The results are depicted in Figure 4.21, 4.22, 4.23 respectively for above mentioned factors.

These three identified factors were entered into a forward logistic regression model and adjusted for age and vegetarianism (Table 4.36). The results showed that two factors emerged as independent casual factors affecting vitamin B12 deficiency in women. These independent factor i.e. no regular health check-up ($p<0.01$) and very low dietary intake of vitamin B12 ($p<0.01$) explained 20.9%-29.9% variability in vitamin B12 levels as depicted by cox & snell (0.209) and Nagelkerke (0.299) R square.

4.4.5. Association of Vitamin B12 deficiency with risk factors of non-communicable diseases

A comparison on mean values of various risk factors of NCDs was made according to vitamin B12 deficiency categories (Table 4.37). Mean SBP ($p<0.05$), TC/HDL-C ratio ($p<0.05$), LDL/HDL-C ratio ($p<0.05$) and HsCRP levels ($p<0.01$) were significantly

TABLE 4.35: PREVALENCE OF VARIOUS CAUSAL FACTORS OF VITAMIN B12 DEFICIENCY IN THE WOMEN (n, %)

Factors	Vitamin B12 Marginal Deficient and Deficient Cases (<300pg/ml) (n=64)	Normal Vitamin B12 levels (>300pg/ml) (n=26)	χ^2 Value
Age			
30-40y	20 (31.2)	9 (34.6)	1.74
41-50y	21 (32.8)	5 (19.2)	
51-60y	23 (35.9)	12 (46.2)	
Diet			
Vegetarian	54 (84.4)	19 (73.1)	3.47
Ovo-lactarian	4 (6.2)	5 (19.2)	
Non-Vegetarian	6 (9.4)	2 (7.7)	
Use of Water Purifier			
No Purifier	27 (42.2)	8 (30.8)	1.80
Reverse Osmosis	16 (25)	10 (38.5)	
Other Purifier	21 (32.8)	8 (30.8)	
Regular Health Check-up			
No	56 (87.5)	14 (53.8)	12.12***
Yes	8 (12.5)	12 (46.2)	
Use of Any Supplement			
No	51 (79.7)	14 (53.8)	6.15*
Yes	13 (20.3)	12 (46.2)	
Any Gastro-intestinal Ailment Present			
No	44 (68.8)	18 (69.2)	0.002
Yes	20 (31.2)	8 (30.8)	
Dietary B12 Levels			
Dietary intake <0.3 µg	43 (67.2)	8 (30.8)	9.88**
Dietary intake >0.3 µg	21 (32.8)	18 (69.2)	

Values in parenthesis indicate percentages

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

higher in women with low vitamin B12 levels. The significance was maintained for all four variables even after adjustment for age and BMI using ANCOVA. A univariate risk analysis (Table 4.38) showed that vitamin B12 deficient women had 2.9 times higher odds of developing hypertension (95% CI 1.04-8.1) (Figure 4.24) and 4.3 times higher odds of having low HDL levels (95% CI 1.2-15.9) (Figure 4.25). As hypertension and low HDL-C levels can be fairly affected by the age and BMI of a person, logistic regression was performed to study the association of B12 deficiency with these two variables after adjustment for age and obesity. The results depicted that hypertension was significantly associated ($p<0.05$) with vitamin B12 deficiency in women irrespective of their age and BMI (Table 4.39).

FIGURE 4.21: VERY LOW DIETARY INTAKE OF VITAMIN B12 AS A CAUSAL FACTOR FOR VITAMIN B12 DEFICIENCY THROUGH UNIVARIATE ANALYSIS

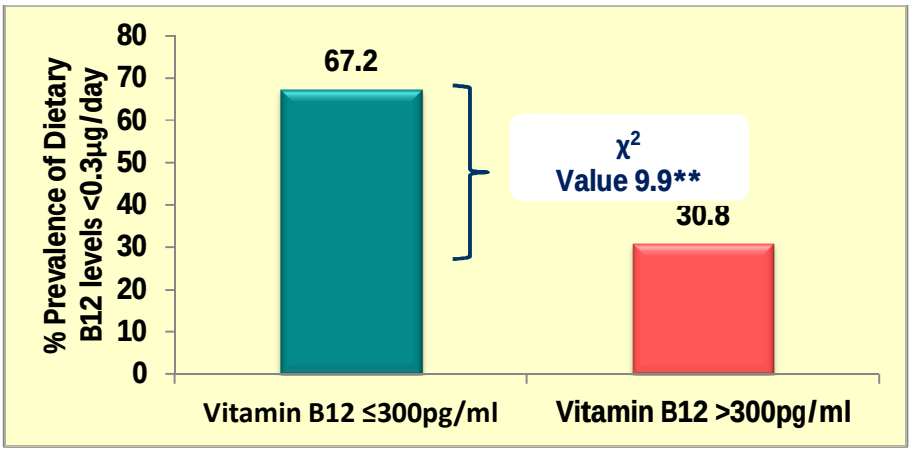


FIGURE 4.22: NON USE OF HEALTH SUPPLEMENTS AS A CAUSAL FACTOR FOR VITAMIN B12 DEFICIENCY THROUGH UNIVARIATE ANALYSIS

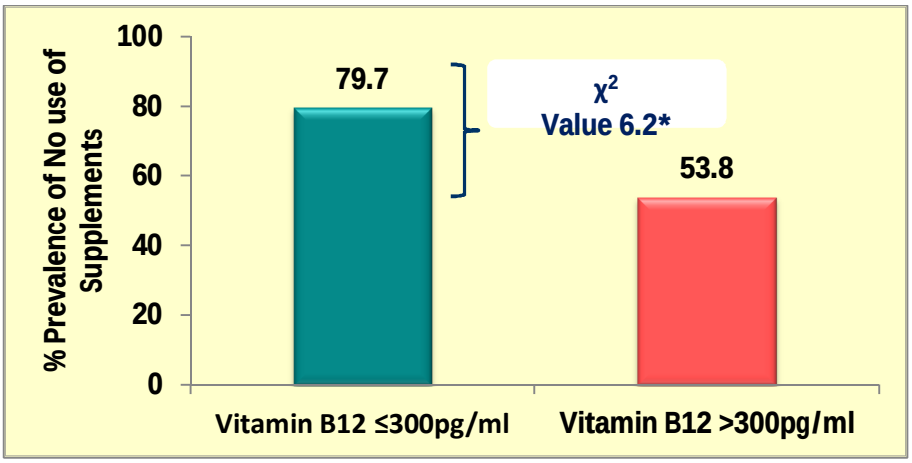


FIGURE 4.23: NO REGULAR HEALTH CHECK-UP AS A CAUSAL FACTOR FOR VITAMIN B12 DEFICIENCY THROUGH UNIVARIATE ANALYSIS

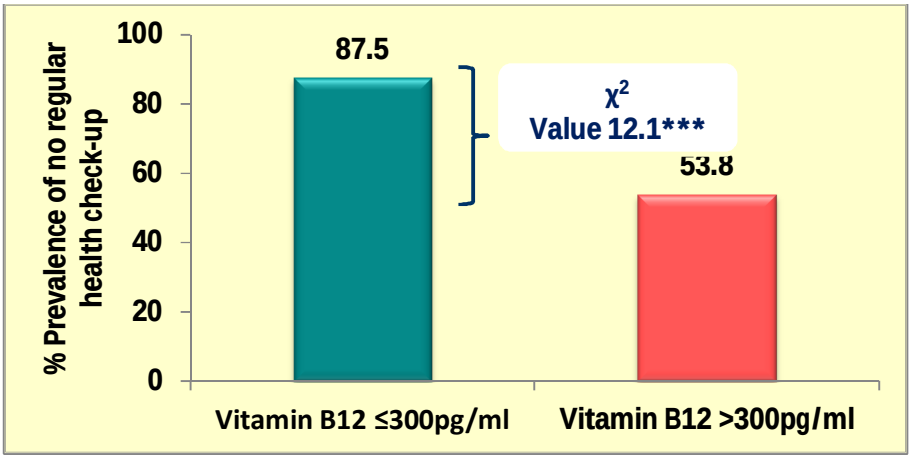


TABLE 4.36: INDEPENDENT CAUSAL FACTORS OF VITAMIN B12 DEFICIENCY IN THE WOMEN

	Variables	Significance	Exp(B)	95% CI of Exp(B)	R Square
Variables in the model	Dietary Intake of Vitamin B12	0.003	5.2**	1.8-15.3	**0.209-0.299**
	No Health Check-up	0.001	6.8**	2.2-22.1	
Variables excluded from the model	No use of any nutritional Supplement	0.19	--	--	--
	Age	0.93	--	--	
	Vegetarianism	0.44	--	--	

TABLE 4.37: COMPARISON OF MEAN VALUES OF VARIOUS RISK PARAMETERS OF NCDS BETWEEN VITAMIN B12 DEFICIENT AND NORMAL WOMEN (Mean±SD)

Parameters	Vitamin B12 Levels <300pg/ml (n=64)	Vitamin B12 Levels >300pg/ml (n=26)	Students' 't' Value	Age and obesity adjusted 'F' value
BMI	2.9±4.8	25.5±4.1	0.4	--
WC (cm)	94.29±10.6	91.34±10.2	1.2	--
WHR	0.93±0.1	0.91±0.1	1.3	--
SBP (mmHg)	131.7±18.1	122.2±16.2	2.3*	6.8*
DBP (mmHg)	80.2±10.3	77.5±8.3	1.2	--
FBS (mg/dl)	90.03±29.8	93.5±39.7	0.45	--
HbA1C (%)	6.1±1.3	6.0±1.4	0.3	--
Insulin (μIU/ml)	10.98±5.5	11.8±10.8	0.48	--
TC (mg%)	195.2±37.3	190.8±35.9	0.52	--
HDL (mg%)	54.2±13	58.8±9.6	1.62	--
LDL (mg%)	117.5±30	111±33.1	0.9	--
TG (mg%)	117.4±50.1	104.8±43.1	-1.1	--
TC/HDL	3.7±0.8	3.3±0.7	2.4*	5.02*
LDL/HDL	2.2±0.6	1.9±0.6	2.13*	4.27*
VLDL (mg%)	23.5±10.1	20.9±8.6	1.1	--
TG/HDL	2.4±1.8	1.8±0.9	1.6	--
HsCRP (mg/dl)	0.23±0.3	0.14±0.1	2.86**	4.57*

Two tailed significance*p<0.05, **p<0.01

TABLE 4.38: PREVALENCE OF VARIOUS RISK FACTORS OF NCDS IN RELATION TO VITAMIN B12 STATUS OF THE WOMEN (n, %)

Risk Factors	Vitamin B12 Marginal Deficient and Deficient Cases (<300pg/ml) (n=64)	Normal Vitamin B12 Levels (>300pg/ml) (n=26)	Odds Ratio (95% CI)
WC (>80cm)	60 (93.7)	24 (92.3)	1.2 (0.2-7.3)
WHR (≥0.85)	58 (90.6)	21(80.8)	2.3 (0.6-8.3)
BMI (≥23)	45 (70.3)	20 (83.3)	0.7 (0.2-2.05)
Hypertension	30 (46.9)	6 (25)	2.94*(1.04-8.1)
Diabetes	6 (9.4)	5 (20.8)	0.4 (0.1-1.6)
HbA1c (>6%)	19 (29.7)	5 (20.8)	1.8 (0.6-5.4)
TC (>200mg%)	31 (48.4)	10 (41.7)	1.5 (0.6-3.8)
HDL (<50mg%)	23 (35.9)	3 (11.5)	4.3* (1.2-15.9)
LDL (>100mg%)	46 (71.9)	16 (66.7)	1.6 (0.6-4.2)
TG (>150mg%)	9 (14.1)	2 (8.3)	1.96 (0.4-9.8)
HsCRP (>0.1mg%)	17 (26.6)	2 (8.3)	4.34(0.9-20.4)
Metabolic Syndrome	18 (28.1)	5 (19.2)	1.6 (0.54-5.02)

Values in parenthesis indicate percentages
Two tailed significance*p<0.05

TABLE 4.39: OBESITY AND AGE ADJUSTED ASSOCIATION OF VITAMIN B12 DEFICIENCY WITH RISK FACTORS OF CVDS USING LOGISTIC REGRESSION

Variables	Significance	Exp(B)	95% CI of Exp(B)
Hypertension	0.031	3.4*	1.12-10.3
Low HDL-C	0.73	0.67	0.07-6.9

Two tailed significance*p<0.05

FIGURE 4.24: ASSOCIATION OF VITAMIN B12 DEFICIENCY WITH HYPERTENSION THROUGH UNIVARIATE ANALYSIS

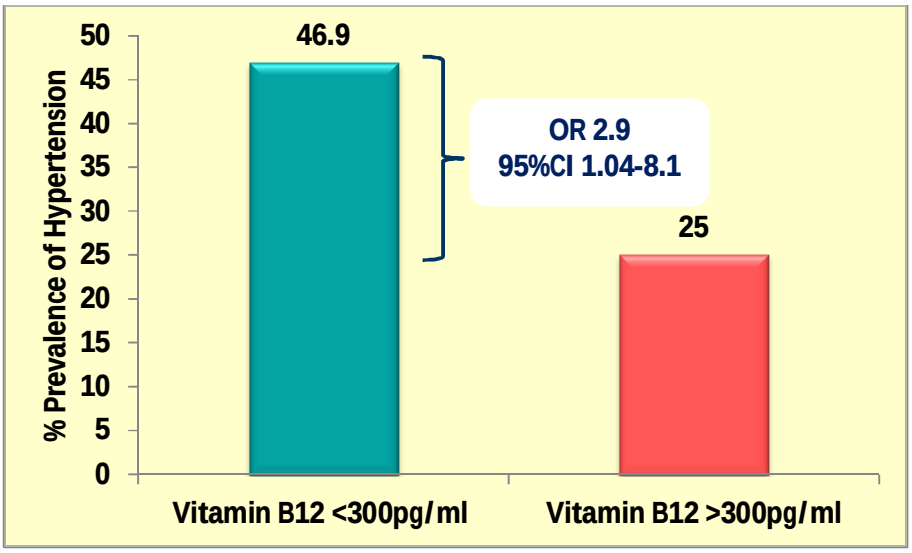


FIGURE 4.25: ASSOCIATION OF VITAMIN B12 DEFICIENCY WITH LOW HDL-C LEVELS THROUGH UNIVARIATE ANALYSIS

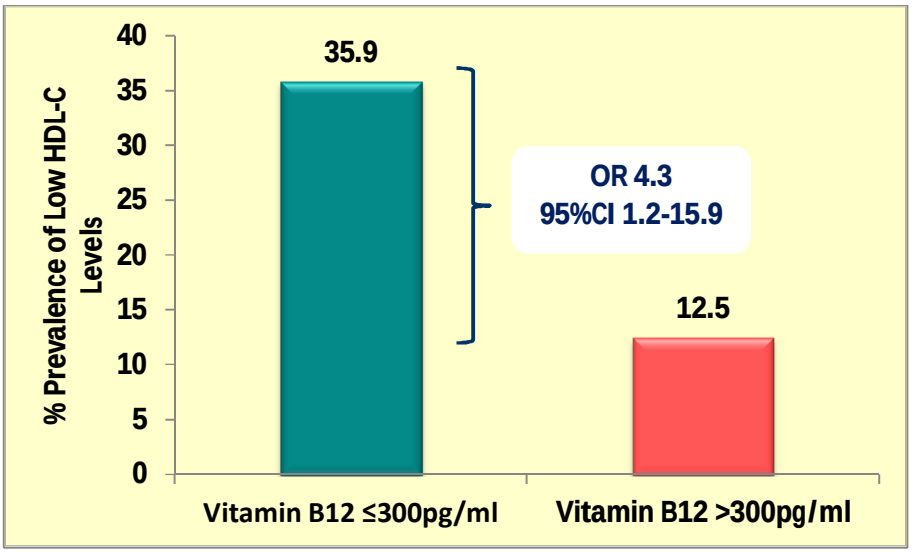


Table 4.40: HIGHLIGHTS SHOWING MAJOR CAUSAL FACTORS AND METABOLIC ABERRATIONS ASSOCIATED WITH VITAMIN B12 DEFICIENCY

Causal Factors		
Variables	**Significant Univariate analysis**	**Significant Multivariate Analysis**
No Regular Health Check-up	√	√
Use of Any Kind of supplement	√	NS
Dietary Vitamin B12 levels <0.3µg/day	√	√
Metabolic Aberrations		
Variables	**Significantly High Mean Values**	**Significantly High Mean Values after adjusting for obesity and age**
SBP	√	√
High HsCRP	√	√
High TC/HDL-C	√	√
High LDL/HDL-C	√	√
Variables	**Significantly High Odds**	**Significantly High Odds after adjusting for obesity and age**
Hypertension	√	√
Low HDL-C	√	NS

Discussion

Vitamin B12 and folate deficiency can lead to various adverse health conditions including increased risk of CVD's through rise in homocysteine levels (Klee, 2000). Homocysteine has been identified as an independent risk factor for cardio vascular diseases. It is produced during metabolism of methionine which requires vitamin B12 and folate for remethylation into methionine (Parikh and Kapoor, 2009). The high prevalence of vitamin B12 and folate deficiency is seen worldwide without geographical differences indicating it as public health problem. The prevalence of vitamin B12 deficiency was quite high (71.1%) in the present study, in contrast to low folic acid deficiency (4.4%). These results were discussed in the earlier section. The causes of vitamin B12 and folate deficiency and their relation with CVD's are discussed in this section.

The main causal factors of folic acid deficiency are low dietary intake, loss during cooking, bioavailability (50% from food), alcoholism and certain medications. (Allen, 2008). In the present study the mean folate intake of the subjects was 167.5±82.6 which is about 84% of the RDA. This shows a fair intake of folic acid in the population.

Conventional causes of vitamin B12 deficiency are low intake (vegetarianism or Lacto-ovo-vegetarianism), malabsorption due to certain gastric diseases, pernicious anemia, infections like H. Pylori infection, certain medications etc. (Appold, 2012; Allen, 2008). Increase in age is also thought to cause vitamin B12 deficiency through poor absorption (Appold, 2012; Baik and Russell, 1999). In the present study vitamin B12 deficiency was not related to age and presence of gastric ailments. The reason behind may be higher use of multivitamin nutritional supplements by older population. No significant difference in prevalence of vitamin B12 deficiency between vegetarian and non-vegetarian subjects was found. These results can be explained by the low frequency of eating non-vegetarian food in specific subjects. The very low daily dietary intake leads to significantly higher prevalence of vitamin B12 deficiency in both univariate and multivariate analysis. H pylori infection was not

considered under the study. The presence of pernicious anemia was very low as assessed by macrocytosis (2.2%).

Other unconventional causes which may affect vitamin B12 deficiency were also studied. One of them is use of reverse osmosis technology for purification of water. There is lot of ongoing discussion that use of reverse osmosis water can lead to vitamin B12 deficiency however no scientific evidence for the same is there (Devarhubli and Jha, 2011). A recent cross sectional study performed by Gupta el al (2016) reported that 50.6% of the subjects using RO water were Vitamin B12 deficient against 17.5% of those using other sources (χ^2 $p<0.001$). Logistic regression analysis also showed an independent effect of use of RO purifier on vitamin B12 deficiency. In the present study, RO purifier was not found to be significantly associated with vitamin B12 deficiency. The study performed by Gupta et al (2016) followed a strict inclusion and exclusion criteria regarding vitamin B12 supplementation and other etiological factors. The present results in this section are subpart of the study therefore it was not possible to follow such stringent inclusion exclusion criteria.

Further analysis in present study was performed to explore whether use of supplements and regular health check-up can affect the B12 deficiency status, as these factors could act as confounding variables for effect of age, vegetarianism and use of RO water. The results indicated that subjects going for regular health check-up ($p<0.001$) and using nutritional supplements ($p<0.05$) showed significant higher percent of normal B12 levels. A logistic regression analysis showed that low dietary vitamin B12 intake and no regular health check-up are independently associated with vitamin B12 deficiency and explained 15-20% variability in the B12 levels.

According to Sadeghian et al (2006) Vitamin B12 deficiency is a preventable cause of hyperhomocystinemia. In the recent years various studies are studying the independent effect of vitamin B12 on risk of CVD's. Vitamin B12 levels were significantly lower in CAD patients (368) than controls (448) in a study performed by Kumar et al (2009). Surprisingly homocysteine levels were not associated with CAD in this study and cysteine levels were significantly higher in CAD patients ($p<0.01$). The

authors hypothesized that during vitamin B12 deficiency, homocysteine is rapidly metabolized via the transsulfuration pathway leading to increased cysteine levels.

A study performed by Mahalle et al (2013) on 300 CAD patients showed that vitamin B12 levels were significantly lower in subjects with dyslipidemia, DM, hypertension. Vitamin B12 levels were inversely associated with TG ($p<0.05$), VLDL ($p<0.05$), and positively associated with HDL-C levels ($p<0.05$) in multiple linear regression.

Results from a comparative study on association of vitamin B12 levels with CAD risk between European (n=342) and Indian (n=321) type 2 DM patients depicted that vitamin B12 levels were independently associated with triglycerides in both the populations and TC/HDL-C ratio in Indians (Adaikalakoteswari et al, 2014). Maiti and Das (2015) compared vitamin B12 levels between metabolic syndrome (n=100) and age-sex match controls (n=100) and observed that vitamin B12 levels were significantly lower in MS syndrome patients (161.0 ± 97.3 pg/ml v/s 312.88 ± 119.7 pg/ml, $p<0.001$). Vitamin B12 deficiency is hypothesized to increase cholesterol levels through reduced s-adenosylmethionine (AdoMet) to s-adenosylhomocystine (AdoHcy) ratio using human adipocytes model (Adaikalakoteswari et al, 2015). In the present study Vitamin B12 deficiency was significantly associated with low HDL-C, High blood pressure ($p<0.05$), TC/HDL ($p<0.05$), LDL/HDL ratio ($p<0.05$) and HsCRP ($p<0.05$) even after adjusting for age and BMI. A study performed by Gammon et al (2012) on South Asian females (n=135) showed no correlation between vitamin B12 and HOMA IR levels. Similar trends were seen in the present study.

A study performed by McMahon et al (2007) showed that vitamin B12, B6 and folate supplementation significantly reduced homocysteine levels however could bring only 1 mmHg difference in blood pressure levels in 246 elderly participants. High dietary intake of Vitamin B12 levels were significantly associated with lower blood pressure levels in a study performed by Tamai et al (2011) on pre-school children (n=418). Karatela and Sainani (2009) indicated that BMI can significantly affect the vitamin B12 levels in the hypertensive subjects (n=130), however the association was found to be independent of BMI in the present study.

SUMMARY

- The prevalence of vitamin B12 deficiency was quite high (71%) in the women.
- Only 5.5% of the women studied had folic acid deficiency.
- The frequency of consumption of vitamin B12 rich food items was low in women.
- Two key causal factors identified through multivariate analysis for vitamin B12 deficiency were: Very low dietary intake of vitamin B12 (<0.3µg/day) and no regular health check-up.
- High body fat percent, hypertension, low HDL-C levels and high TC levels were three metabolic alterations associated with vitamin B12 deficiency as predicted by forward logistic regression.
- Mean levels of SBP, TC/HDL-C and LDL/HDL-C ratio and HsCRP were significantly higher in vitamin B12 deficient subjects as depicted by independent 't' test results. The significance was maintained for all even after adjustment of age and BMI using ANCOVA.
- Vitamin B12 deficiency posed significant higher risk for development of hypertension and low HDL-C levels through univariate analysis. After adjustment for age and BMI using logistic regression B12 deficiency emerged as independent predictor for development of hypertension among women.

Conclusions

The prevalence of vitamin B12 deficiency was found quite high in the present study in comparison to available literature. Dietary Intake and lack of health check-up have come up as two major causal factors for vitamin B12 deficiency. Regular health check-up can lead to early detection and treatment of vitamin B12 deficiency. The independent role of vitamin B12 deficiency as a risk for NCD's is still debatable, however in the present study the strongest association of vitamin B12 deficiency appeared with hypertension. The area needs to be further explored considering the very high prevalence of Vitamin B12 deficiency in the population.

4.5. ASSOCIATION OF INFLAMMATION WITH NCD RISK FACTORS IN WOMEN

Inflammation is known to increase the risk of CVDs. Hs-CRP is a non-specific inflammatory marker which was used in the study to evaluate inflammation in the women.

As depicted in Figure 4.26 about 64% of the women fell under at risk category. Out of which 43% had average risk and 21% had high risk of inflammation. A correlation analysis (Table 4.41) of Hs-CRP values with various risk factors of NCDs was performed and the results revealed that WC ($p<0.000$), WSR ($p<0.000$), BMI ($p<0.000$), SBP ($p<0.05$), AIP ($p<0.05$) and HbA1C ($p<0.001$) values were positively correlated whereas HDL-C ($p<0.05$) values were negatively correlated with Hs-CRP.

The mean values of various risk factors of NCDs were stratified according to Hs-CRP levels. Table 4.42 shows that mean values of WC ($p<0.05$), WSR ($p<0.05$), BMI ($p<0.01$), body fat per cent ($p<0.01$), AIP ($p<0.05$) and HbA1C ($p<0.05$) were significantly higher in women with at risk category. The univariate risk analysis of variables revealed that women with high Hs-CRP levels had significantly higher risk of developing low HDL-C levels (OR 4.3 95%CI 1.3-13.8) (Figure 4.27) high AIP levels (OR 3.25; 95% CI 1.31-8.1) (Figure 4.28) and metabolic syndrome (OR 8.5; 95%CI 1.85-32.45) (Figure 4.29). The prevalence of hypertension, high TG, high HbA1C levels and diabetes was also high in women with high Hs-CRP levels although not statistically significant (Table 4.43).

The variables which were found to be associated with Hs-CRP levels in univariate odds ratio analysis were entered into forward logistic regression model, which is illustrated in Table 4.44. Out of all variables, only metabolic syndrome was found to be independently associated with Hs-CRP levels ($p<0.01$). Presence of metabolic syndrome explained 16.7%-22.9% variability in the HsCRP levels as depicted by cox & snell (0.167) and Nagelkerke (0.229) R square.

FIGURE 4.26: PREVALENCE OF HIGH HsCRP LEVELS IN THE WOMEN

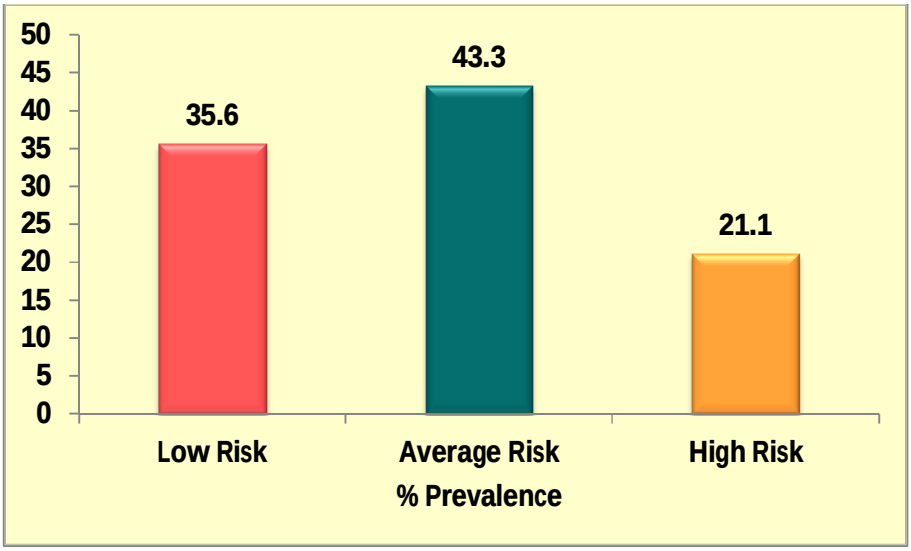


TABLE 4.41: CORRELATION OF HsCRP VALUES WITH VARIOUS VARIABLES IN WOMEN

Variables	Correlation Coefficient	p value
Age (y)	0.11	0.32
WC (cm)	0.44***	0.000
WHR	0.1	0.36
WSR	0.45***	0.000
BMI	0.48***	0.000
Systolic BP (mmHg)	0.24*	0.025
TC (mg %)	-0.17	0.11
HDL (mg%)	-0.25*	0.017
LDL (mg%)	-0.14	0.19
TG (mg%)	0.11	0.29
AIP	0.21*	0.047
HbA1C (%)	0.33***	0.001
HOMA IR	0.13	0.21

Two tailed significance*p<0.05, ***p<0.001

TABLE 4.42: COMPARISON OF MEAN VALUES OF THE WOMEN WITH AT RISK AND NORMAL HsCRP LEVELS (Mean±SD)

Variables	HsCRP >0.1mg/dl (n=58)	HsCRP <0.1mg/dl (n=32)	Students' 't' value
Age (y)	46±10	46.3±9.6	0.13
WC (cm)	97.18±11.01	90.28±8.9	2.16*
WHR	0.93±0.1	0.92±0.1	0.1
WSR	0.63±0.1	0.6±0.1	2.45*
BMI	26.8±5.04	24.1±3.13	3.1**
Body fat (%)	37.7±5.9	34.3±5.2	2.76**
Systolic BP (mmHg)	130.4±19.6	126.4±14.9	1.09
Diastolic BP (mmHg)	80.43±9.8	77.56±9.64	1.34
TC (mg %)	190.1±36.3	200.9±37.1	1.35
HDL (mg%)	53.6±11.6	59.1±12.9	2.08*
LDL (mg%)	112.7±30.6	120.9±32.1	1.19
TG (mg%)	118.7±47.1	104.9±50.8	1.29
VLDL (mg%)	23.7±9.4	20.97±10.2	1.29
AIP	0.32±0.2	0.22±0.2	2.11*
HbA1C (%)	6.3±1.5	5.7±0.5	2.51*
HOMA IR	1.47±0.71	1.38±1.2	0.43
Carbohydrate (g)	203.1±58.4	206±45.6	0.24
Energy (Kcal)	1528±445	1601±386	0.78
Fat (g)	55.8±21.7	62.8±22	1.47
Total Dietary Fiber (g)	14.6±5.9	16.5±8.5	1.24

Two tailed significance*p<0.05, **p<0.01

TABLE 4.43: PREVALENCE OF VARIOUS CONDITIONS IN RELATION TO HsCRP VALUES (N, %)

Variables	HsCRP >0.1mg/dl (n=58)	HsCRP <0.1mg/dl (n=32)	OR (95% CI)
WC >80cm	53 (91.4)	31 (96.9)	0..34 (0.04-3.06)
WHR ≥0.85	49 (84.5)	30 (93.8)	0.36 (0.07-1.79)
WSR >0.5	52 (89.7)	28 (87.5)	1.24 (0.32-4.76)
BMI ≥25	43 (74.1)	21 (65.6)	1.5 (0.59-3.83)
Body fat (>30%)	54 (93.1)	28 (87.5)	1.93 (0.44-8.3)
Hypertension	27 (46.6)	9 (28.1)	2.23 (0.88-5.63)
Post Menopause	29 (50)	16 (50)	1 (0.42-2.37)
TC (≥200mg %)	24 (41.4)	17 (53.1)	0.62 (0.26-1.48)
HDL (<50mg%)	22 (37.9)	4 (12.5)	4.3* (1.3-13.8)
LDL (≥100mg%)	38 (65.5)	24 (75)	0.66 (0.24-1.66)
TG (≥150mg%)	9 (15.5)	2 (6.3)	2.75 (0.56-13.62)
AIP (>0.21)	43 (74.1)	15 (46.9)	3.25* (1.31-8.1)
HbA1c (>6%)	19 (32.8)	5 (15.6)	2.63 (0.87-7.91)
Diabetes	9 (15.5)	2 (6.3)	2.75 (0.56-13.62)
MS (IDF)	21 (36.2)	2 (6.2)	8.51* (1.85-32.45)
Reuse of Oil for Frying	12 (20.7)	5 (15.6)	1.41 (0.45-4.43)
Total Dietary Fiber <20g	45 (77.6)	24 (75)	1.15 (0.42-3.17)
Energy >1900Kcal	11 (19)	7 (21.9)	0.84 (0.29-2.42)
Use of Cotton Seed Oil	25 (43.1)	13 (40.6)	1.11 (0.42-3.17)

Values in parenthesis indicate percentages

Two tailed significance*p<0.05

TABLE 4.44 FORWARD LOGISTIC REGRESSION ANALYSIS FOR PREDICTOR VARIABLES OF HsCRP

	Variables	Significance	Exp(B)	95% CI of Exp(B)	R square
Variable in the model	Metabolic Syndrome	0.005	18.94**	2.4-148.8	**0.167-0.229**
Variable excluded from the model	High AIP	0.28	--	--	--
	Low HDL-C	0.54	--	--	

FIGURE 4.27: ASSOCIATION OF INFLAMMATION WITH LOW HDL-C LEVELS THROUGH UNIVARIATE ANALYSIS

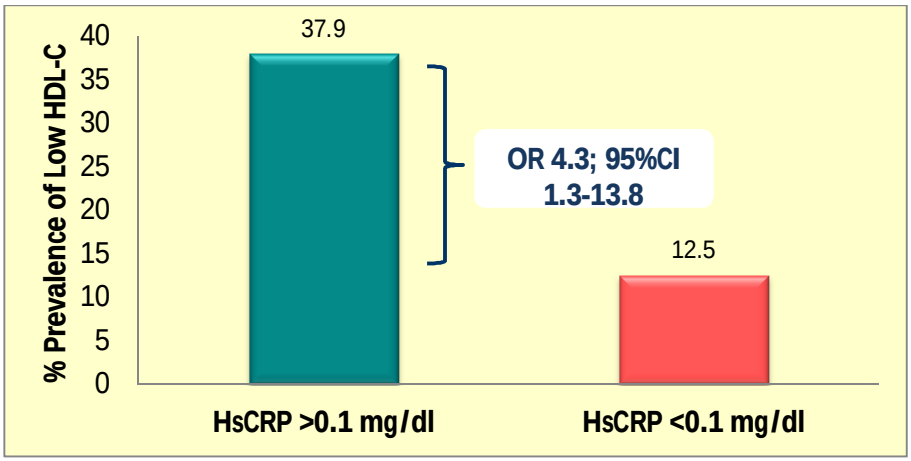


FIGURE 4.28: ASSOCIATION OF INFLAMMATION WITH HIGH AIP LEVELS THROUGH UNIVARIATE ANALYSIS

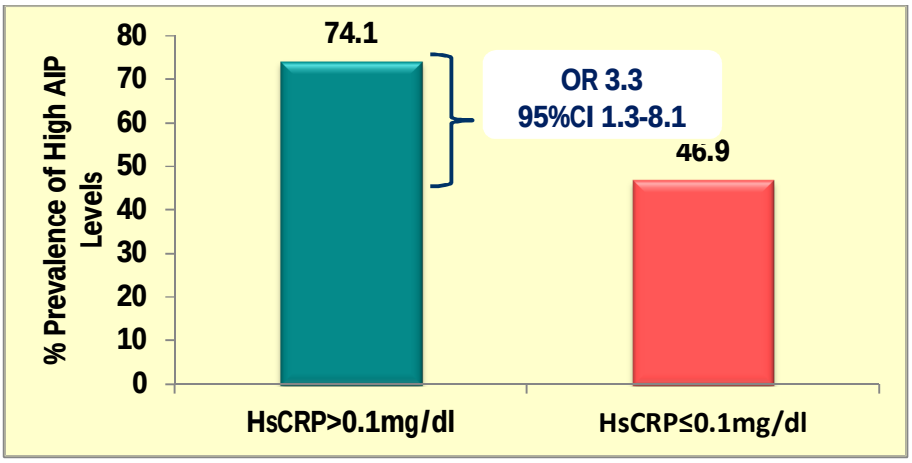


FIGURE 4.29: ASSOCIATION OF INFLAMMATION WITH METABOLIC SYNDROME THROUGH UNIVARIATE ANALYSIS

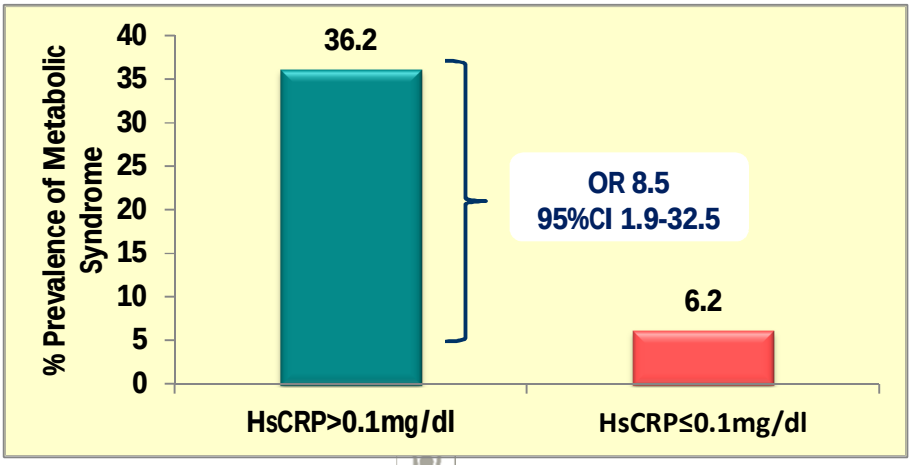


Table 4.45: HIGHLIGHTS OF ASSOCIATION OF HsCRP WITH VARIOUS RISK FACTORS OF NCDs

Variables	Significantly High Mean Values	Significant Univariate analysis	Significant Multivariate Analysis
Waist Circumference	√	NS	--
Waist Stature ratio	√	NS	--
BMI	√	NS	--
Body Fat %	√	NS	--
HDL-C	√	√	NS
AIP	√	√	NS
HbA1c	√	NS	--
Metabolic Syndrome	--	√	√

Discussion

C-reactive protein, an acute phase protein is a good indicator of inflammatory activity and tissue damage (van Leeuwen and van Rijswijk, 1994). According to Centers for Disease Control and Prevention and the American Heart Association, C-reactive protein can be used as an adjunct to the established risk factors in order to assess the risk of coronary heart disease (Pearson et al, 2003).

In the present study total 64% of the women had high HsCRP levels (43% and 21% under average and high risk category respectively). A study performed by Chandorkar et al (2011) including 146 females working at university revealed that 37% of the females were having average risk and 33% having high risk of developing CVDs.

HsCRP levels were significantly associated with various risk factors of CVDs like WC, WSR, BMI, Body fat%, HDL-C, AIP, HbA1c and SBP in the present study. A study performed by Jeemon et al (2011) indicated that BMI and abdominal obesity can surrogate the high HsCRP levels. The multivariate model showed that odds ratio for elevated CRP (CRP ≥ 2.6 mg/dl) was significantly higher in females in comparison to males (1.63, 95% CI; 1.02-2.58).

Chmabers et al (2001) compared the HsCRP levels and CVD associated risk between Asian Indians residing in UK (n=518) and European whites (n=507). They concluded that HsCRP levels were significantly associated with abdominal obesity and insulin resistance. The mean HsCRP levels were significantly higher in Indians even after adjustment for other CVD risk factors. Obesity was found to be associated with pre-inflammatory changes in normotensive, normoglycemic women as depicted by Dev and Marcus (2012) in a hospital based study.

Various Indian studies have found strong association between high HsCRP levels and type 2 diabetes as well as cardio vascular diseases. A cross sectional study on 2520 urban subjects (n=1410 T2DM; 1110 non diabetic) indicated that HsCRP levels were significantly higher ($p<0.0001$) in diabetic subjects and posed higher risk of development of CVDs in diabetics. HbA1c levels were significantly associated with

elevated HsCRP levels irrespective of age, sex and BMI ($p<0.05$) (Mahajan et al, 2009). Hs-CRP showed a strong association with CAD and diabetes, even after adjusting for age and gender in Chennai Urban Rural Epidemiology Study (CURES-6) (Mohan et al, 2005).

Various studies across the world have shown association of HsCRP levels with metabolic syndrome and its risk factors. A study performed on Taiwanese population (n=1305) on association between MS and inflammation revealed that elevated concentrations of Hs-CRP showed a strong association with metabolic syndrome after adjustment for age and lifestyle factors including smoking, and alcohol intake, especially in women (Lai et al, 2010). Another study on 146 non diabetic Cuban subjects showed that subjects with metabolic syndrome had about 4 times higher odds of having elevated HsCRP levels than in those without it (Huffman et al, 2009).

Mahajan et al (2012) performed a large cross sectional study (n=9517) on urban Indian using ATP III criteria and concluded that subjects with high risk hsCRP levels (>3mg/l) were at high risk of MetS (OR 1.65; 95%CI 1.41-1.92), independent of obesity and insulin resistance. The results from the present study are following the same trend depicting a very strong association between MS and HsCRP levels using both univariate and multivariate analysis. HsCRP is being considered as an added clinical criterion for metabolic syndrome and for the creation of an hsCRP-modified coronary risk score useful for global risk prediction (Ridker et al, 2004).

SUMMARY

- The prevalence of high Hs-CRP levels was around 64%.
- Various anthropometric indices and lipemic aberrations were significantly associated with high Hs-CRP levels in univariate analysis however on entering variables into multivariate regression model clustering risk factor i.e. Metabolic Syndrome was found to be the key variable associated with Hs-CRP.

Conclusions

Metabolic syndrome and its risk factors were well associated with inflammation measured by HsCRP in the present study. As various studies have shown higher HsCRP levels in women than men, HsCRP can be used as suggestive tool while assessing metabolic syndrome as well as diabetes and CVD risk in women.

4.6. ASSOCIATION OF INSULIN RESISTANCE WITH NCD RISK FACTORS IN NON-DIABETIC WOMEN

Early detection of insulin resistance can help in prediction of development of diabetes and by life style interventions we can delay the onset of diabetes in the high risk population. HOMA IR is an upcoming index to assess the insulin resistance and β cell functioning in the population. As type II diabetic women typically suffer from insulin resistance, all the analyses in this section were performed on non-diabetic women (n=79) of the study population. The prevalence of insulin resistance according to HOMA-IR was found to be 21.5% (Figure 4.30) which was calculated using upper quartile of the HOMA IR values of the study population as the cut-off. Table 4.46 shows the correlation coefficient values of various variables with HOMA IR. HOMA-IR values were significantly correlated with waist circumference ($p<0.01$), waist stature ratio ($p<0.05$), body mass index ($p<0.001$), very low density lipoproteins ($p<0.01$), log triglycerides ($p<0.01$), atherogenic index of plasma (AIP) ($p<0.01$), carbohydrate intake ($p<0.001$) and energy intake ($p<0.01$).

A comparison of mean values of CVD risk factors made between insulin resistant and non-insulin resistant women is shown in Table 4.47. It was found that mean values of WC ($p<0.01$), WSR ($p<0.01$), BMI ($p<0.001$) and body fat ($p<0.01$) present were significantly higher in insulin resistant women. Similar trends were seen in case of TG ($p<0.01$), VLDL-C ($p<0.01$), AIP ($p<0.001$), HbA1c ($p<0.05$) and Hs-CRP ($p<0.05$), energy intake ($p<0.05$) and CHO intake ($p<0.001$). Mean HDL-C levels ($p<0.01$) were significantly lower in insulin resistant women.

A univariate risk analysis (Table 4.48) depicted that women with high BMI (Figure 4.31), adverse triglyceride, AIP, HbA1c levels, low HDL-C levels (Figure 4.32) and high energy intake (Figure 4.33) had 6.8 (95%CI 1.97-23.6), 7.9 (95%CI 1.9-32.7), 6.6 (95% CI 1.4-31.3), 4.7 (95% CI 1.4-15.98), 9.5* (95% CI 2.9-31.8) and 3.7 (95% CI 1.06-12.74) times higher odds of developing insulin resistance respectively. Insulin resistance was also associated with higher risk of having Metabolic Syndrome (OR 6.98; 95%CI 2.03-24.02) in the women. Family history of diabetes also posed significant risk of developing insulin resistance (OR 4.15; 95% CI 1.34-12.87).

FIGURE 4.30: QUARTILES OF HOMA IR VALUES

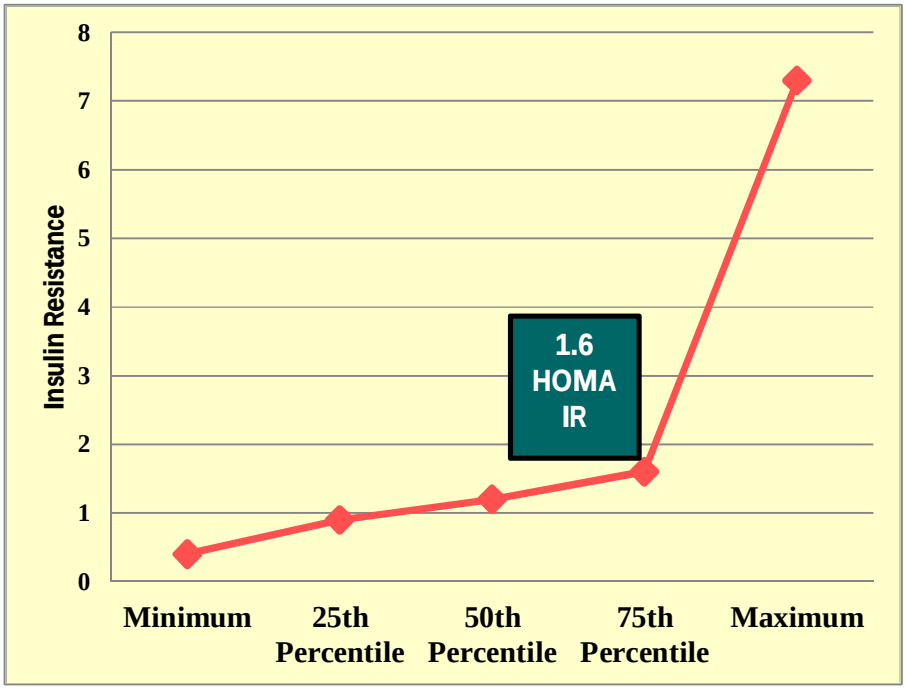


TABLE 4.46: CORRELATION OF HOMA IR VALUES WITH VARIOUS VARIABLES IN NON-DIABETIC WOMEN (n=79)

Variables	Correlation Coefficient	p value
Age (y)	0.000	0.998
WC (cm)	0.344**	0.002
WHR	0.095	0.404
WSR	0.257*	0.022
BMI	0.379**	0.001
Systolic BP (mmHg)	-0.086	0.453
TC (mg %)	-0.129	0.256
HDL (mg%)	-0.215	0.057
LDL (mg%)	-0.165	0.146
TG (mg%)	0.302**	0.007
AIP	0.331**	0.003
HbA1C (%)	0.331**	0.003
HsCRP (mg/dl)	0.187	0.100
Carbohydrate Intake	0.386***	0.000
Energy Intake	0.331**	0.003
Total Fat Intake	0.193	0.088
Total Dietary Fiber Intake	0.155	0.17

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

Further attempts were made to explore the independent predictor variables for development of insulin resistance in the women. Results of the same are depicted in Table 4.49, in which the forward logistic regression model revealed that Low HDL-C levels ($p<0.01$), high BMI ($p<0.05$) and high energy intake ($p<0.05$) were the independent variables associated with insulin resistance in the women. These three variables explained 28.5%-44% variability in the HOMA-IR levels as depicted by cox & snell (0.285) and Nagelkerke (0.44) R square.

TABLE 4.47: COMPARISON OF MEAN VALUES OF VARIABLES STRATIFIED BY INSULIN RESISTANCE (Mean±SD)

Variables	HOMA IR >1.6 (n=17)	HOMA IR ≤1.6 (n=62)	Students' 't' Value
Age (y)	44.41±10.9	45.13±9.2	0.3
WC (cm)	100.05±11.7	91.24±9.5	3.2**
WHR	0.93±0.1	0.92±0.1	0.3
WSR	0.65±0.1	0.6±0.1	2.8**
BMI	28.90±4.8	24.52±3.9	3.9***
Body fat (%)	39.54±5.8	34.92±5.5	3**
Systolic BP (mmHg)	127.65±13.8	126.60±17.3	0.2
Diastolic BP (mmHg)	80.82±11.5	78.77±9.5	0.8
TC (mg %)	185.91±30.8	197.69±37.5	1.2
HDL (mg%)	48.29±11.3	58.38±12.2	3.1**
LDL (mg%)	109±24.6	118.53±32.5	1.1
TG (mg%)	143.6±56.4	103.98±45.1	3.04**
AIP	0.45±0.2	0.23±0.2	3.8***
HbA1C (%)	6.37±1.2	5.59±0.5	2.6*
HsCRP (mg/dl)	0.31±0.3	0.18±0.2	2.1*
Carbohydrate (g)	241.7±65.3	192.4±51.2	3.31***
Energy (Kcal)	1721±427	1436±425	2.44*
Fat (g)	60.8±22.4	53.4±22.3	1.22
Total Dietary Fiber (g)	15.8±5.5	14.3±6.5	0.37

Two tailed significance*p<0.05, **p<0.01, ***p<0.001

TABLE 4.48: PREVALENCE OF VARIOUS CONDITIONS IN RELATION TO INSULIN RESISTANCE (n, %)

Variables	HOMA IR >1.6 (n=17)	HOMA IR ≤1.6 (n=62)	OR (95% CI)
Family History of Diabetes	11 (64.7)	19 (30.7)	4.15* (1.34-12.87)
WC >80cm	17 (100)	57 (87.7)	--
WHR ≥0.85	16 (94.1)	54 (87.1)	2.4 (0.3-20.4)
WSR >0.5	16 (94.1)	53 (85.5)	2.7 (0.3-23.1)
BMI ≥25	13 (76.5)	20 (32.3)	6.8* (1.97-23.6)
Body fat (>30%)	16 (94.1)	55 (88.7)	2.04 (0.2-17.8)
Hypertension	8 (47.1)	21 (33.9)	1.7 (0.6-5.2)
Post Menopause	6 (35.3)	36 (58.1)	0.6 (0.2-1.8)
TC (≥200mg %)	5 (29.4)	33 (53.2)	0.4 (0.1-1.2)
HDL (<50mg%)	11 (64.7)	10 (16.1)	9.5* (2.9-31.8)
LDL (≥100mg%)	12 (70.6)	44 (70.97)	4.2 (0.8-23.1)
TG (≥150mg%)	6 (35.3)	4 (6.5)	7.91* (1.9-32.7)
AIP (>0.21)	15 (88.2)	33 (53.2)	6.6* (1.4-31.3)
HbA1c (>6%)	7 (41.2)	8 (12.9)	4.7* (1.4-15.98)
HsCRP (>0.1mg%)	13 (76.5)	36 (58.1)	2.4 (0.7-8.02)
MS (IDF)	8 (47.1)	7 (11.3)	6.98* (2.03-24.02)
Reuse of Oil for Frying	6 (35.3)	10 (16.1)	2.84 (0.85-9.45)
Total Dietary Fiber <20g	4 (23.5)	12 (19.4)	1.28 (0.35-4.64)
Energy >1900Kcal	6 (35.3)	8 (12.9)	3.7* (1.06-12.74)
CHO intake >60% of RDA for energy	5 (29.4)	3 (4.8)	8.2* (1.6-44.9)
Use of Cotton Seed Oil	10 (58.8)	23 (37.1)	2.42 (0.81-7.24)

Values in parenthesis indicate percentages

Two tailed significance*p<0.05

FIGURE 4.31: ASSOCIATION OF INSULIN RESISTANCE WITH OBESITY THROUGH UNIVARIATE ANALYSIS

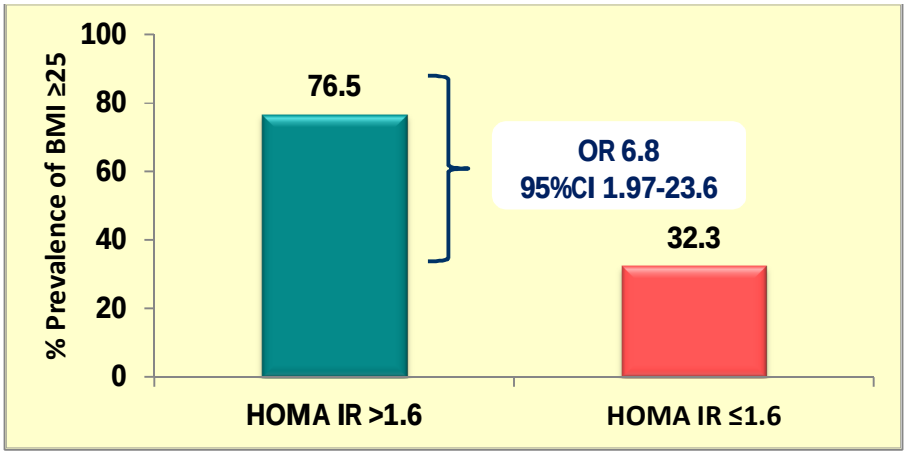


FIGURE 4.32: ASSOCIATION OF INSULIN RESISTANCE WITH LOW HDL-C LEVELS THROUGH UNIVARIATE ANALYSIS

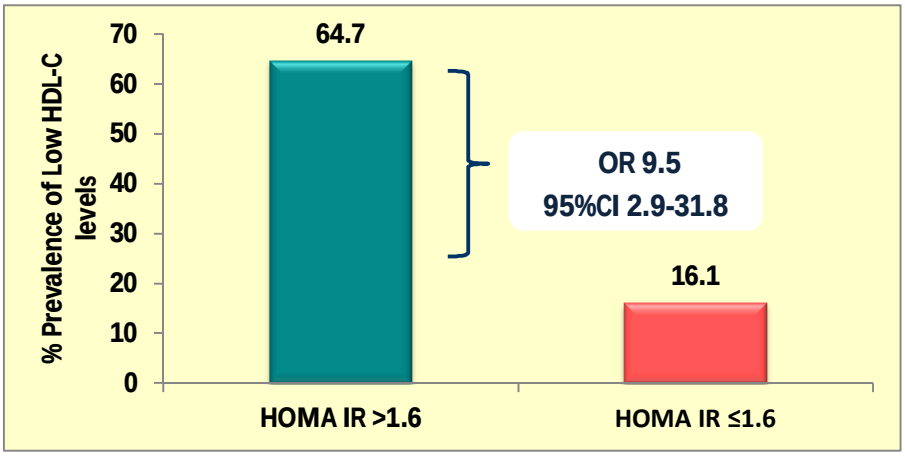


FIGURE 4.33: ASSOCIATION OF INSULIN RESISTANCE WITH HIGH ENERGY INTAKE THROUGH UNIVARIATE ANALYSIS

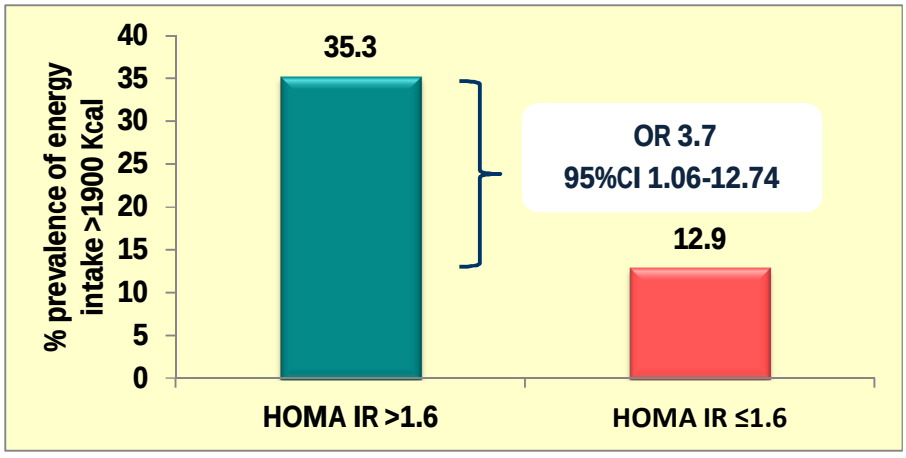


TABLE 4.49: FORWARD LOGISTIC LINEAR REGRESSION FOR PREDICTOR VARIABLES OF HOMA IR

	Variables	Significance	Exp(B)	95% CI of Exp(B)	R square
Variables in the Model	Low HDL-C	0.002	8.5**	2.1-33.9	**0.285-0.440**
	BMI	0.014	2.3*	1.2-4.5	
	High energy intake	0.02	6.6*	1.3-33.4	
Variables excluded from the model	TG levels	0.64	--	--	--
	AIP levels	0.64	--	--	
	HbA1c levels	0.42	--	--	
	Metabolic Syndrome	0.99	--	--	
	High CHO intake	0.49	--	--	
	FH of diabetes	0.07	--	--	

Table 4.50: HIGHLIGHTS OF ASSOCIATION OF HOMA IR WITH VARIOUS RISK FACTORS OF NCDs

Variables	Significantly High Mean Values	Significant Univariate analysis	Significant Multivariate Analysis
Family History of Diabetes	--	√	NS
Waist Circumference	√	NS	--
Waist Stature ratio	√	NS	--
BMI	√	√	√
Body Fat %	√	NS	--
Low HDL-C	√	√	√
TG	√	√	NS
AIP	√	√	NS
HbA1c	√	√	NS
HsCRP	√	NS	--
Metabolic Syndrome	--	√	NS
Carbohydrate Intake	√	√	NS
Energy Intake	√	√	√

Discussion

Insulin resistance is a principal component for the development of type 2 diabetes mellitus and can be managed through lifestyle modifications. HOMA IR is a reliable tool and can be used as alternate to other sophisticated techniques to detect insulin resistance (Bonora et al, 2002). It can also serve as independent predictor of CVD in type 2 diabetes mellitus patients (Bonora et al, 2002). However there is a lack of data on HOMA IR values of different sex, age and ethnic groups and lack of standardized reference values have limited its clinical application (Qu et al, 2011).

In the present study HOMA IR levels were significantly correlated with anthropometric indices in the female subjects. BMI emerged as independent predictor for insulin resistance. The results were in line with various Indian studies. In a study performed by Vikram et al (2006) on males, fasting insulin correlated significantly with BMI, waist circumference, and triceps and subscapular skinfold thickness. Subjects with generalized obesity ($p < 0.001$) and abdominal obesity ($p < 0.001$) had significantly higher HOMA-IR even after adjusting for age and gender in a study performed by Sandeep et al (2011) on south Indian population (CURES-66). Body fat per cent was also found to be higher in subjects with insulin resistance similar to the findings of Vikram et al (2006) in which fasting insulin was correlated with body fat per cent in north Indian male subjects. Percentage body fat explained approximately two thirds of the difference in HOMA-IR between rural and urban middle-class men of western India (Yajnik et al, 2008).

Hypertension was not associated with insulin resistance in the present study. The studies from other parts of India have shown mixed results on this relationship (Sandeep et al, 2011; Snehalatha et al, 2000) which needs to be further explored.

Various theories and models have supported the impact of inflammation on insulin resistance however in present study Hs-CRP levels were not found to be significantly associated with HOMA IR. Vikram et al (2006) also elucidated similar results in northern male population. As Hs-CRP can be affected by various other physiological factors, studies with more specific markers can be carried out to draw a clear picture.

Studies from different parts of India have shown hypercholesterolemia and hypertriglyceridemia as independent factors associated with insulin resistance (Sandeep et al, 2011; Vikram et al, 2006; Snehalatha et al, 2000) in different age and sex groups. In the present results also it was closely associated with high triglyceride levels (OR 7.91; 95%CI 1.9-32.7) and atherogenic indiex of plasma (OR 6.6; 95%CI 1.4-31.3). Insulin resistance has been known to be an integral factor for development of metabolic syndrome. The present study (OR 6.98; 95%CI 2.03-24.02) and the literature available for Indian population supports the fact (Sandeep et al, 2011; Gupta et al, 2010). Another independent predictor of HOMA IR in the study was low HDL-C. The results from CURES-66 study showed the similar trends. HDL cholesterol ($p<0.001$) was negatively correlated with HOMA IR even after adjusting age, gender and BMI (Sandeep et al, 2011). There is dearth of studies specific for female population on insulin resistance. This section was an attempt towards building the women specific database; however had the limitation of small sample size.

The association of HOMA IR with dietary factors like fiber, energy, carbohydrate and energy intake was studied in the present study. High carbohydrate intake posed significant higher risk for development of insulin resistance (OR 3.7; 95%CI 1.06-12.74). High energy intake was found to be an independent predictor ($p<0.05$) for development of insulin resistance. Dietary glycemic load was found to be directly associated with risk of CHD after adjustment for age, smoking status, total energy intake, and other coronary disease risk factors in a 10 year cohort performed on 75521 women (Liu et al, 1999). Another cohort (Barcley et al, 2007) on relatively smaller group of females (n=1833) using FFQ on subjects under 70 years of age, linked high-GI carbohydrate diet to increased risk of diabetes. Vegetable fiber was independently associated with reduced risk of diabetes. A review performed by Bessesen (2001) concluded that although the association between total carbohydrates and carbohydrate subtypes in the diet and insulin sensitivity have been explored in various studies, the area still remain controversial.

SUMMARY

- About 21.5% of the non-diabetic women were suffering from insulin resistance.
- Various anthropomeric indices and metabolic aberrations were significantly associated with high HOMA IR values.
- The dietary factors posing high risk for development of insulin resistance were high energy and carbohydrate intake.
- Women with family history of diabetes were at higher risk of developing insulin resistance.
- The major determinants of insulin resistance predicted through logistic regression model were low HDL-C, high BMI and high energy intake.

Conclusions

As elucidated in this section, majority of the metabolic and dietary risk factors of CVDs were associated with insulin resistance in the middle aged female population of Vadodara. Therefore life style interventions and dietary changes with a focus of weight management should be promoted to reduce the risk of insulin resistance in females.

Phase II

IDENTIFICATION OF FLAXSEED VARIETY FOR SUPPLEMENTATION AND ESTIMATION OF ITS NUTRITIVE PROFILE

With NCDs emerging as the biggest killer of human race in the recent years, the concept of functional foods has bought a gleam of hope for prevention as well as control of such diseases. Functional foods are natural food or their processed form which contain certain bioactive compounds that exert beneficial effects on various NCDs. The exquisiteness of functional foods which makes them stand unique is their natural form. The efficacy of phytochemicals and other protective compounds is believed to be more through consumption of these foods than the extracted bioactive compounds. Even though one consumes the purified form of bio active compounds, one has to devour a large amount than that present in natural form in food to achieve desired results.

Flaxseed has been recognized as a functional food. It provides fair amount of alpha linolenic acid (ALA) which is a plant source of n-3 fatty acid. However, the ALA content of the flaxseeds may vary from variety to variety. In the present research a supplementation study of flaxseeds was planned and the PKV NL-260 variety was selected for supplementation. Analysis of flaxseeds was performed to ascertain the fatty acid profile of this particular variety and other bioactive compounds present in flaxseeds which may prove beneficial for metabolic disorders.

For this purpose, sample was drawn following a sequential procedure (Figure). The sample was sent to Indian Institute of Crop processing Technology, Thanjavur for analysis. The flaxseeds were subjected to analysis for micronutrient, crude fibre, sodium, potassium, calcium and iron. Fatty acid profile was analysed using GC-MS. The total polyphenol and flavonoid content of flaxseeds was determined. Antioxidant capacity was quantified using DPPH RSA and FRAP methods by Prof. Vinayak Patel at PG studies of Home Science, S.P. University, Vidhyanagar.

4.7. Macronutrient content of the flaxseeds

As shown in Table 4.51 the PKV NL-260 variety of flaxseeds contained good amount of fat (26.6%) and protein (25.83%). A fair amount of carbohydrates (17.7%) was also present in flaxseeds. The crude fibre content of flaxseeds was 6.67%.

4.8. Mineral content of the flaxseeds

The major minerals which were analysed using ICP-OES, was found to be very low in the flaxseeds. The sodium and potassium content of the flaxseeds was 5.5mg/kg and 9.55mg/kg respectively (Table 4.52). The iron content was estimated to be 0.56mg/kg and calcium content was 8.87mg/kg.

4.10. Phenolic content and antioxidant capacity of the flaxseeds

The total phenols and flavonoid content of the flaxseeds was found to be 365.8±18.2 mgGAE/100g and 148.6±4.0 mgRE/100g respectively (Table 4.53). The antioxidant capacity was analysed using two methods i.e. FRAP and DPPH RSA method. The FRAP method measured antioxidant capacity as 1776.6±80.1 μmolTE/100g whereas DPPH RSA method quantified it to be 643.7±2.6 μmolTE/100g.

4.11. Fatty Acid profile of the flaxseeds

The fatty acid profile was performed using GC-MS. Various fatty acids were identified at different retention time (Figure 4.34) from the flaxseed extract and quantified using the peak area per cent and the total fat content of the flaxseeds. The results revealed that the major fatty acids found in the flax seeds was n-3 fatty acids. Two major n-3 fatty acids were identified i.e Linolenic acid (20.8g/100g of flaxseed) and Dihomo-gamma-linolenic acid (0.3g/100g of flaxseed) (Table 4.54). Saturated fatty acids were found to second highest fatty acid present in the flaxseeds. A variety of saturated fatty acids were identified with varying amounts. The highest amount of palmitic acid (4.3g/100g of flaxseeds) was found to be there among all saturated fatty acids. A very small fraction of n-6 (0.5g/100g of flaxseed) and n-7 (0.06g/100g of flaxseed) fatty acids was also present in the flaxseeds.

TABLE 4.51: MACRONUTRIENT CONTENT OF THE FLAXSEEDS (PKV NL-260)

Parameter	% Amount (wet basis)
Protein (%)	25.83
Carbohydrate (%)	17.77
Fat (%)	26.60
Crude Fiber (%)	6.67

TABLE 4.52: MINERAL CONTENT OF THE FLAXSEEDS (PKV NL-260) USING ICP-OES

Minerals	Wavelength	Amount (mg/ kg)
Sodium (Na)	589.592	5.507
Iron (Fe)	238.204	0.562
Calcium (Ca)	317.933	8.867
Potassium (K)	213.617	9.548

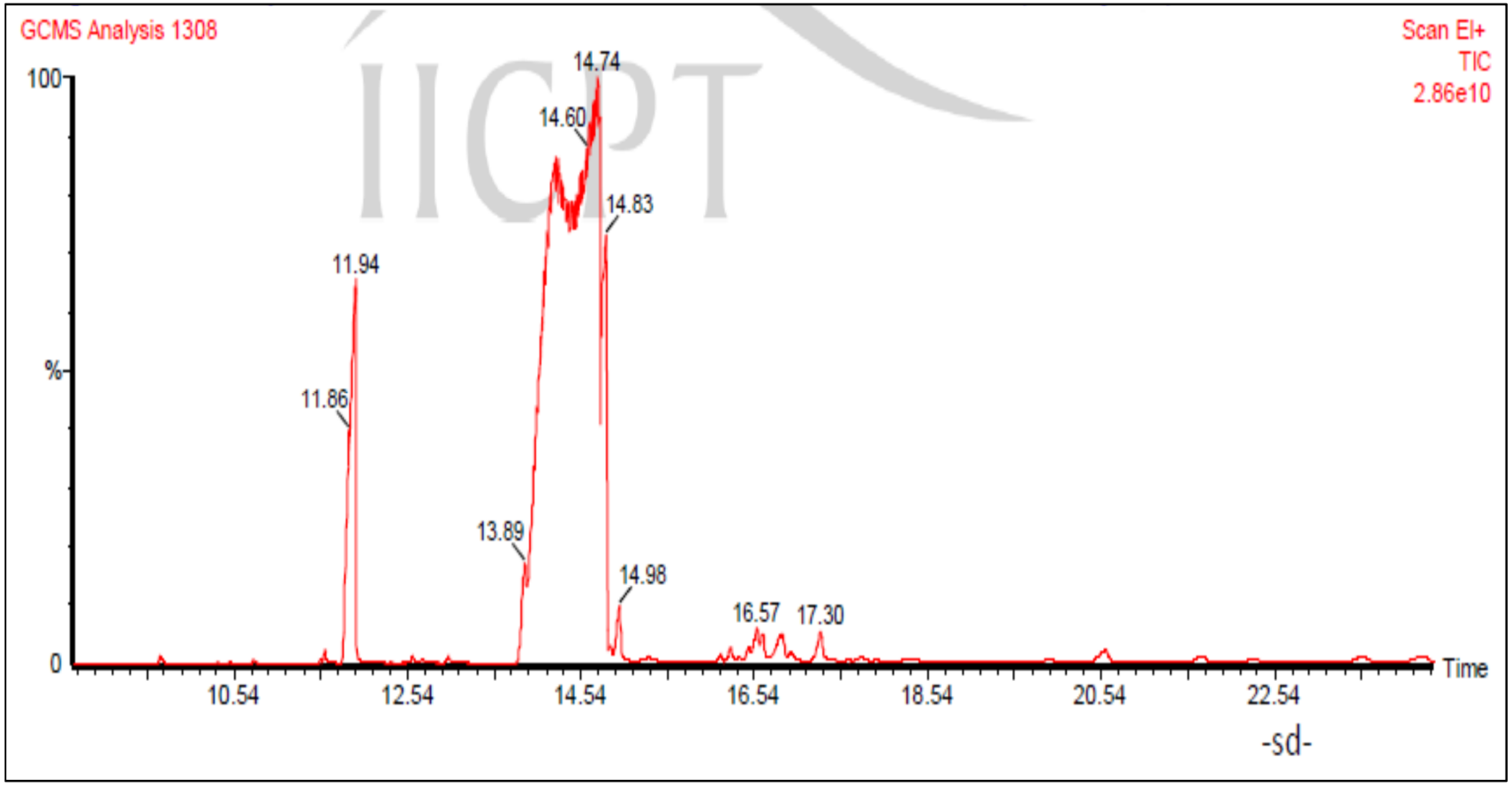
TABLE 4.53: PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF FLAXSEEDS (PKV NL-260)

Parameters	Amount
Total Phenol (mgGAE/100g)	365.8±18.2
Flavonoid (mgRE/100g)	148.6±4.0
FRAP (µmolTE/100g)	1776.6±80.1
DPPH RSA (µmolTE/100g)	643.7±2.6

TABLE 4.54: FATTY ACID PROFILE OF PKV NL-260 VARIETY OF FLAXSEEDS USING GC-MS

Name of the compound (IUPAC Name)	Common name	Type of Lipid	RT	Molecular Formula	MW	Peak Area %	Amount in grams in 100g flaxseed sample
Decanoic acid, methyl ester	Capric acid methyl ester	SFA	9.69	$C_{11}H_{22}O_2$	186	0.11	0.03
Pentadecanoic acid, methyl ester	Methyl pentadecanoate	PUFA	10.76	$C_{16}H_{32}O_2$	256	0.05	0.01
9-Hexadecenoic acid, methyl ester, (Z)-	Palmitoleic acid, methyl ester	PUFA (n-7)	11.59	$C_{17}H_{32}O_2$	268	0.21	0.06
Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	SFA	11.94	$C_{17}H_{34}O_2$	270	16.01	4.3
Hexadecanoic acid, ethyl ester	Palmitic acid, ethyl ester	SFA	12.60	$C_{18}H_{36}O_2$	284	0.09	0.02
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	Cyclopropaneoctanoic acid	SFA	12.71	$C_{18}H_{34}O_2$	282	0.06	0.02
Heptadecanoic acid, methyl ester	Margaric acid methyl ester	SFA	13.00	$C_{18}H_{36}O_2$	284	0.19	0.05
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Linolenic acid, methyl ester	PUFA (n-3)	14.74	$C_{19}H_{32}O_2$	292	78.04	20.8
8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	Dihomo-gamma-linolenic acid	PUFA (n-3)	14.98	$C_{20}H_{34}O_2$	306	1.14	0.3
Octadecanoic acid, ethyl ester	Stearic acid, ethyl ester	SFA	15.33	$C_{20}H_{40}O_2$	312	0.12	0.03
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	Arachidonic acid	PUFA (n-6)	16.57	$C_{21}H_{34}O_2$	318	0.79	0.2
8,11,14-Docosatrienoic acid, methyl ester	Docosatrienoic acid	PUFA (n-6)	16.87	$C_{23}H_{40}O_2$	348	1.04	0.3
Eicosanoic acid, methyl ester	Arachidic acid methyl ester	SFA	17.30	$C_{21}H_{42}O_2$	326	1.00	0.3
Docosanoic acid, methyl ester	Behenic acid, methyl ester	SFA	20.59	$C_{23}H_{46}O_2$	354	0.70	0.2
Tetracosanoic acid, methyl ester	Lignoceric acid methyl ester	SFA	24.25	$C_{25}H_{50}O_2$	382	0.44	0.1

FIGURE 4.34: CHROMATOGRAM OF FATTY ACID PROFILE OF FLAXSEEDS (PKV NL-260) USING GC-MS



Discussion

Flaxseeds have the potential to exert many health benefits through various bioactive compounds like α-linolenic acid, lignans and phytochemicals present in it. In the present study macronutrient content, mineral content, fatty acid profile and antioxidant capacity of PKV NL-260 variety of flaxseeds were analyzed. The protein, carbohydrate, fat and crude fiber content of the flaxseeds were 25.8%, 17.8%, 26.6% and 6.7% respectively.

The details of the nutritive value provided by supplier of PKV NL-260 variety indicated flaxseeds containing 20g of protein, 41.7g of fats, and 27.7g of total fiber per 100g (Ensign Diet Care Pvt Ltd.). PKV NL-260 variety was developed under "All India Crop Improvement Project" and is reported to contain 38% oil by the developers (NAIP Report, 2014). The oil content ranged from 33.97% to 42.27% among 48 different varieties of flaxseeds, determined by wide line nuclear magnetic resonance (NMR) (Pali and Mehta, 2014). Deepika (42.27%) variety was having highest oil content where as Neela contained lowest percent (33.97%). Chemical analysis of Neelam variety of flaxseeds indicated 42.4% of oil content on 6.99% dry basis in a study performed by Singh et al (2012). Hiremath et al (2014) reported fat content of three different varieties of flaxseeds ranging from 33.4 to 35.3g/100 g. Morris (2007) described average fat content of Canadian flaxseeds as 41%.

On comparing with the literature available, fat content of PKV NL-260 variety was found to be very low in the present study. The oil and moisture content of the flaxseeds can be affected by the level of maturity of seed. The oil content increases whereas moisture content decreases with increase in maturity of seed (Herchi et al, 2014). Although the moisture content is not directly analysed in the study, it appears to be quite high (18-20%) after accounting for total carbohydrates, protein, fats and ash content of the flaxseeds. Presence of lower percent of fats and higher percent of moisture indicates toward probability of harvesting of flaxseeds before proper maturation.

Protein content of flaxseeds was slightly higher in the present study on comparing with Canadian flaxseeds (20%) and Indian flaxseeds (18.6%) (IFCT, 2017; Morris,

2007). The protein content of the seed decreases as the oil content increases as reported by Morris (2007). Presence of lower fat content can partly explain the higher content of protein in present analysis. Crude fiber (6.7%) was higher than that reported by National Institute of Nutrition (4%) (Gopalan et al, 2012). The iron content (5.6mg/100g) was equivalent (5.4mg/100g) whereas calcium (88.7mg/100g) was lower in comparison to IFCT, 2017 (257mg/100g).

Flaxseeds are rich in various phytochemical compounds especially phenolics such as lignans, phenolic acids and flavonoids (Kasote, 2013). Oomah et al (1995) reported that phenolic acids ranged from 8-10 g/kg among eight flaxseeds cultivars of Canada, containing about 5 g/kg of esterified phenolic acids and 3-5 g/kg of etherified phenolic acids (Oomah et al, 1995). The phenolic acid content of five cultivars from Egypt and Europe ranged from 162-360mgGAE/100g (El-Beltagi et al, 2007). A study performed by Monica and Joseph (2012) reported phenolic acid content of commercially available flaxseed on an South Indian organic store as 340mgGAE/100g which is comparable to the results of present study.

The range of flavonoids content of different flaxseeds varieties was found to be 35-71mg/100g as depicted by Oomah et al (1996). El-Beltagi et al (2007) analysed five flax seed varieties and their flavonoid content ranged from 16.1-20mgRE/100g. In the present study flavonoid content was found to be 148.6±4.0mgRE/g in PKV NL-60 variety of flaxseeds.

Another important bioactive phenolic in flaxseeds is Secoisolariciresinoldiglucoside oligomers (lignan). Flaxseeds are the richest source of lignans. Beejmohun et al (2007) reported the mean SDG content of flaxseeds as 16.1+/-0.4 mg/g. According to Fuentealba et al (2015) the SDG content in different varieties of flaxseed ranged from 10.8 to 17.9 mg g^{-1} in defatted flaxseed flour and from 6.0 to 10.9 mg g^{-1} in whole flaxseed. Lignan analysis was not performed in the present study.

The antioxidant capacity of the flaxseeds was analyzed using DPPH (1776.6±80.1 µmolTE/100g) and FRAP (643.7±2.6 µmolTE/100g) method in the present study. Amin and Thakur (2014) reported the IC^{50} value of flaxseed ethanol extract for DPPH free radical as 256.313µg/ml. The ability of defatted flaxseed extracts and non-

defatted flaxseed extracts to scavenge the DPPH radical ranged from 19.7 to 76.1 % and 25.7 to 76.3 % respectively according to Brodowska et al (2014). Dharshini et al (2013) studied the antioxidant capacity of raw, roasted, soaked and pressure cooked flaxseeds and reported that antioxidant capacity of roasted flaxseeds was quite high (76%) than the raw ones (35%) using DPPH radical scavenging activity method. In the present study roasted flaxseeds were used for supplementation having high antioxidant capacity.

The fatty acid profile of flaxseeds has been widely studied by various researchers all over the world. According to Morris (2007) Canadian flax contains about 9% of SFA, 18% of MUFA, 16% of linoleic and 57% of alpha linolenic fatty acids. Fatty acid analysis of the 48 flax varieties in a study performed by Pali and Mehta (2014) reported mean value of linoleic, oleic, stearic and palmitic acid as 15.88%, 27.76%, 6.26% and 6.07%, respectively. The variety RLC-92 (54.82%) exhibited the highest whereas variety Kiran (33.14%) exhibited the lowest linolenic acid content. Hiremath et al (2014) demonstrated the range of stearic acid, pamitic acid, oleic acid, linoleic acid and linolenic acid as 6.97-7.69%, 6.79-9.96%, 29.76-32.43%, 11.47-12.9% and 38.65-43.65% of total fats in three different Indian varieties of flaxseeds. Singh et al (2012) reported the linolenic acid content of Neelam variety of flaxseeds as 54.66% of total fats.

Surprisingly in the present study the SFA content was found to be 18.77%, n-6 fatty acid as 1.83% and n-3 fatty acid as 79.18%. Thus the fatty acid profile of PKV NL-260 variety was quite different than that reported in literature. As reported by the marketing company, the linolenic acid content of the PKV NL-260 variety was 0.234g/g of flaxseeds. The calculated amount of linolenic acid came to be 0.208g/g of flaxseeds. This shows although the total fat content of the PKV NL-260 variety was substantially low, the linolenic acid content was not much compromised. PKV NL-260 variety is a breed of R-552 and RLC-6 variety. The purpose of this breeding was to generate a variety which can mature faster and resistant to infestation. In the present study its fat content and fatty acid profile has been found to be quite different than the traditional varieties. The fat content and fatty acid profile were

analyzed in a lab certified by NABL. No authentic scientific literature is available for this variety for comparison.

Parameshwari and Nazni (2012) identified 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) as the major component of roasted flaxseed fatty acid with peak area of 93.7% in a recent study. The common name of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) is linolenic acid. This shows that about 93.7% of the fatty acids were linolenic acid, however neither total fat percent of flaxseeds was analysed in that study nor the variety of flaxseeds was mentioned. Authors denoted such differences in the fatty acid profile as changes due to roasting. In the present study also roasted flaxseeds were used and percent of linolenic acid was found quite high (79.18%) of total fat content. Therefore the probability of improvement in fatty acid profile due to roasted cannot be denied. Contradictory results were found in a study performed by Moknatjou et al (2014) which studied the effect of roasting on fatty acid profile of brown flaxseeds. It depicted that the linolenic acid content decreased with increase in roasting temperature (54.63±0.03% and 52.77±0.31% of total fat in unroasted and roasted at 350˚c respectively). Schorno et al (2003) also reported that linolenic acid content can be decreased on subjecting flaxseeds to higher temperature and longer processing time. Further studies are required to clarify the effect of roasting on fatty acid profile of flaxseeds.

Roasting can have various other beneficial impacts on nutritive value and storage quality of flaxseeds. Yang et al (2004) studied the different processing methods on cynogenic glycosides of flaxseeds and concluded that microwave roasting reduced the highest percent of HCN (82%) in comparison to autoclaving (27%) and one time solvent extraction (52%). Similar study performed by Feng et al (2003) reported initial HCN content of human grade flaxseeds as 139 $mg/kg^{-1.}$ Microwave roasting and autoclave significantly reduced ($p<0.05$) the HCN content by 83.3% and 29.7% respectively. According to Schorno et al (2003) processing of flaxseeds for 4 and 8 minutes at 140 and 160˚c improves quality of flaxseeds. Roasting can increase the shelf life as peroxide value and free fatty acid content reduced after roasting of flaxseeds.

SUMMARY

- The macronutrient analysis of the flaxseeds (PKV NL-260) showed that fat constituted around 26.6% of the flaxseeds on wet basis.
- Sodium and potassium content of the flaxseeds was 5.5mg/kg and 9.6mg/kg respectively.
- Flaxseeds contained 365.8±18.2 mgGAE/100g of total polyphenols and 148.6±4.0 mgRE/100g of flavonoids.
- The antioxidant capacity of the flaxseeds using DPPH RSA and FRAP methods was 1776.6±80.1 mmolTE/100g and 643.7±2.6 mmolTE/100g respectively.
- The fatty acid profile showed the highest percent (78.04%) of n-3 fatty acid in the PKV NL-260 variety of flaxseeds.
- Various forms of saturated fatty acids were present in the flaxseeds in small quantities.

Conclusions

Though the total fat content of the flaxseeds (PKV NL-260 variety) analysed was lower than the average percent reported in various studies, the linolenic acid content of the same was not compromised and provided a fair amount of ALA as required to be supplemented in the study. The presence of fair amount of polyphenols, flavonoids and antioxidant capacity along with ALA made the flaxseeds (PKV NL-260) ideal to exert beneficial impact on cardio vascular risk factors.

PHASE III:

METABOLIC AND INFLAMMATORY RESPONSE TO SUPPLEMENTATION OF WHOLE ROASTED FLAXSEEDS IN PRE-MENOPAUSAL OVERWEIGHT/OBESE FEMALE SUBJECTS

Persistent metabolic aberrations among apparently healthy subjects may lead to development of non-communicable diseases. Minor changes in the diet like addition of functional foods can hinder the metabolic anomalies thus providing better quality of life for a longer period of life. A number of functional foods have been identified till now which contain numerous amounts of different phytochemical, flavonoids, phytosterols etc. However, the individualized quantities for each and every functional food to exert beneficial effect have not been established yet.

Flaxseed is a rich source of ALA and lignans. Various studies have demonstrated its favourable impact in averting and controlling lipid abnormalities, inflammation, and oncogenic events. However most of the studies have used a high amount of flaxseeds which is not feasible to consume on daily basis. Furthermore if we compare the total ALA content of doses used in various studies it seems to be quite high than the recommended levels. Therefore a supplementation study was planned in effort to come up with a recommended dose of daily consumption of flaxseeds which can be consumed with ease and helps in maintenance of metabolic status.

As the research was primarily focussed on females, in the supplementation study also only female subjects were recruited. The results of phase I elucidated a high prevalence of metabolic aberrations among pre-menopausal subjects. Furthermore a lot of work has been done on the effect of flaxseeds on post menopause. Therefore, for the present supplementation study pre-menopausal females were specifically selected.

A screening of females from 30-50 years of age was performed and the data regarding medical history, life style habits, anthropometry and blood pressure was collected. The subjects were scrutinized using inclusion and exclusion criteria and those who fit into the criteria were contacted to take consent for participation in the supplementation study. Ninety pre-menopausal overweight/obese females were

identified and were further divided into three groups i.e. control group, supplementation group I and group II. The supplementation continued for a period 8 weeks in which supplementation group I and II were asked to consume 5g and 10g of flaxseeds respectively and control group advised to continue with their normal dietary habits. Anthropometric and biophysical measurements and biochemical estimations were performed twice: before and after the intervention for all three groups. The results of the study are as follows:

Phase III (a)

4.12. Screening and baseline characteristics of the subjects

4.12.1. Characteristics of screened subjects

About 400 women aged between 30-50y were screened for the supplementation study (Table 4.55). The results revealed that around 17.2% of the women were normal according to Asia-Pacific classification of BMI. The prevalence of underweight and obesity was around 2.5% and 17% respectively. Nearly 40.8% and 20.5% women were found to be suffering from obesity and morbid obesity respectively. When looked at the menopausal status of the women, it reflected that majority of the women fell under pre-menopause (68.5) category. About 8.5% of women were going through peri menopausal state, 20% were under post menopause category and 3% were hysteractomized.

The data on medical history of the women showed that 10.5% of them were suffering from hypertension. About 3.8% had history of diabetes and 3.3% had hypothyroidism. Only 1% of the women reported having CHD and none of them were suffering from cancer. The data on blood pressure measurement taken at the time of screening depicted that about 22.2% of the women fell under pre-hypertension category and 24% were diagnosed as having high blood pressure. Out of 400 screened women only 291 turned up for fasting blood glucose testing through glucometer and among them 7.2% were detected to have FBS >126mg/dl.

The life style pattern of the women showed that none of them had habit of smoking or alcohol intake. <1% of the women chewed tobacco and only 1.5% were doing any kind of vigorous physical activity.

TABLE 4.55: CHARACTERISTICS OF SCREENED SUBJECTS (N, %)

Variables	N (%)
Age (y)	
30-35	94 (23.5)
36-40	106 (26.5)
41-45	97 (24.3)
45-50	103 (25.7)
Prevalence of Obesity	
Underweight	10 (2.5)
Normal	69 (17.2)
Overweight	76 (19)
Obese	163 (40.8)
Morbid Obese	82 (20.5)
Menopausal Status	
Pre Menopause	274 (68.5)
Peri Menopause	34 (8.5)
Post Menopause	80 (20)
Hysteractomy	12 (3)
Disease Profile	
Diabetes (n= 291)	
History of Diabetes	15 (3.8)
FBS >126 mg/dl by Glucometer	29 (7.2)
Hypertension	
History of Hypertension	42 (10.5)
Pre Hypertension	89 (22.2)
Newly Diagnosed cases of Hypertension	96 (24)
CHD	4 (1.0)
Hypothyroidism	13 (3.3)
Cancer	0 (0)
Life Style Habits	
Smoking	0 (0)
Tobacco Chewing	3 (0.8)
Alcohol Intake	0 (0)
Vigorous Physical Activity	6 (1.5)

Values in parenthesis indicate percentages

4.12.2. Baseline clinical characteristics of the study subjects

The 90 subjects, identified for supplementation study based on inclusion-exclusion criteria and consent of the participants were divided randomly into three groups and the Baseline clinical characteristics of the study subjects in three groups were compared. Almost similar prevalence of obesity, abdominal obesity and high body fat per cent was there among the subjects of three groups (Table 4.56). The prevalence of pre-hypertension was lower in control group than other two groups although not statistically significant. Similarly prevalence of stage I hypertension found to be slightly higher in 5g supplementation group.

As described in Table 4.57, the prevalence of insulin resistance and inflammation was comparable among the three groups. Table 4.58 depicts the results of comparison of prevalence of hyperlipidemia among three groups. It shows that prevalence of dyslipidemia was comparable in all three groups except for HDL-C. The prevalence of low HDL-C levels was found to be significantly higher ($p<0.05$) in 5g flaxseeds group (64.3%) than the other two groups (31% and 37.9% in control and 10g flaxseed group respectively). No case of high triglycerides and TC/HDL-C was noted in 10g flaxseeds group.

The prevalence of anemia (Hb<12g/dl) was significantly lower ($p<0.01$) in control group in comparison to other two groups (Table 4.59). Low prevalence (non-significant) of other abnormal haematological indices among control subjects was also observed.

The prevalence of abnormal thyroid function tests (Table 4.60), liver function tests (Table 4.61) and kidney function tests (Table 4.62) was comparable in all the three groups.

Table 4.63 depicted the daily nutrient intake of the subjects in all three groups. Comparison of mean values of major macro and micronutrients showed no significant differences among control, 5g and 10g flaxseed group.

TABLE 4.56: ANTHROPOMETRIC AND BIOPHYSICAL PROFILE OF THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
Age (y)				
30-40	7 (24.1)	12 (42.9)	11 (37.9)	2.4
41-50	22 (75.9)	16 (57.1)	18 (62.1)	
Obesity				
BMI ≥25	18 (62.1)	19 (67.9)	18 (62.1)	0.27
BMI <25	11 (37.9)	9 (32.1)	11 (37.9)	
Abdominal Obesity				
WC (>80cm)	29 (100)	28 (100)	29 (100)	--
WHR (≥0.85)	27 (93.1)	27 (96.4)	29 (100)	--
WSR(>0.5)	28 (96.6)	28 (100)	29 (100)	--
Body Fat (>30%)	29 (100)	27 (96.4)	29 (100)	--
Blood Pressure				
Pre Hypertension	3 (10.3)	9 (32.1)	9 (31)	9.3
Stage I Hypertension	4 (13.8)	7 (25)	2 (6.9)	

Values in parenthesis indicate percentages

TABLE 4.57: PREVALENCE OF GLYCEMIC AND INFLAMMATORY ABERRATIONS AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
HOMA IR (>1.2)	9 (31)	7 (25)	4 (13.8)	2.5
Hs-CRP (>0.1mg/dl)	22 (75.9)	19 (67.9)	17 (58.6)	1.97

Values in parenthesis indicate percentages

TABLE 4.58: PREVALENCE OF LIPEMIC ABERRATIONS AND ATHEROGENIC RISK AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
TC (≥200mg/dl)	5 (17.2)	4 (14.3)	4 (13.8)	0.16
HDL-C (<50mg/dl)	9 (31)	18 (64.3)	11 (37.9)	**7.08***
LDL-C (≥100mg/dl)	15 (51.7)	14 (50)	16 (55.2)	0.16
TG (≥150mg/dl)	5 (17.2)	7 (25)	0 (0)	**--**
AIP (>0.21)	16 (55.2)	16 (57.1)	14 (48.3)	0.50

Two tailed significance *p<0.05

Values in parenthesis indicate percentages

TABLE 4.59: PREVALENCE OF VARIOUS INDICATORS OF IRON DEFICIENCY ANEMIA AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
Iron (<50µg/dl)	8 (27.6)	12(42.9)	14 (48.3)	2.79
TIBC (>450)	0 (0)	2 (7.1)	1 (3.4)	2.16
Transferrin Saturation (<15%)	11(37.9)	12 (42.9)	17 (58.6)	2.72
Hb <12g/dl	7 (24.1)	19 (67.9)	15 (51.7)	**11.2****

Two tailed significance **p<0.01
Values in parenthesis indicate percentages

TABLE 4.60: PREVALENCE OF HYPOTHYROIDISM AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
TSH (>4µIU/ml)	7(24.1)	9 (32.1)	3 (10.3)	4.04
T3 (200ng/dl)	0 (0)	0 (0)	0 (0)	--
T4 (12µg/dl)	0 (0)	0 (0)	0 (0)	--

Values in parenthesis indicate percentages

TABLE 4.61: PREVALENCE OF LIVER FUNCTION ABNORMALITIES AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
Alkeline Phosphatase (>98 U/L)	1 (3.4)	1 (14.3)	4 (13.8)	2.3
Total Bilirubin (>1.20mg/dl)	0 (0)	0 (0)	0 (0)	--
Indirect Bilirubin (>0.9mg/dl)	0 (0)	0 (0)	0 (0)	--
Direct Bilirubin (>0.20mg/dl)	4 (13.8)	4 (14.3)	3 (10.3)	0.24
SGOT (>31 U/L)	1 (3.1)	1 (3.6)	0 (0)	1.04
SGPT (>28 U/L)	4 (13.8)	2 (7.1)	1 (3.4)	2.13
GGT (> 38 U/L)	1 (3.4)	1 (3.6)	1 (3.4)	0.001
Serum Albumin (<3.5gm/dl)	0 (0)	0 (0)	0 (0)	--
Total Protein (<6.6gm/dl)	4 (13.8)	1 (3.6)	4 (13.8)	2.1

Values in parenthesis indicate percentages

TABLE 4.62: PREVALENCE OF KIDNEY FUNCTION ABNORMALITIES AMONG THE STUDY SUBJECTS (N, %)

Parameters	Control Group	5g Flaxseeds	10g Flaxseeds	χ^2 Value
Ca (8.4-10.6mg/dl)	0 (0)	0 (0)	0 (0)	--
BUN (>20mg/dl)	0 (0)	0 (0)	0 (0)	--
Creatinine (0.8mg/dl)	2 (6.9)	0 (0)	0 (0)	--
Uric Acid (>6mg/dl)	2 (6.9)	3 (10.7)	0 (0)	3.1

Values in parenthesis indicate percentages

TABLE 4.63: MEAN VALUES OF VARIOUS NUTRIENT INTAKE AMONG THE STUDY SUBJECTS (MEAN±SD)

Nutrients	Control Group	5g Flaxseeds	10g Flaxseeds	Anova 'F' Value
Energy (Kcal)	1547±488	1575±330	1465±362	0.59
Protein (g)	46±16	43±12	41±10	0.81
Total Fat (g)	57±25	61±17	51±21	1.54
Calcium (mg)	783.1±121.7	701.2±412.1	621.4±343.9	0.79
Iron (mg)	11.15±4.4	12.23±5.7	13.17±6.8	0.91
Ascorbic Acid (mg)	88.96±75.6	93.3±51.2	87.3±58.7	0.07
Total Dietary Fiber (g)	14.5±6.9	16.6±7.8	14.9±5.4	0.74

Phase III (b)

Randomised control trial to study the effect of whole roasted flaxseeds on lipid profile, insulin resistance and inflammation in pre-menopausal overweight/obese females

4.13. Impact on the lipid profile of the study subjects

As shown in Table 4.64 and 4.65, there was no significant difference in mean values of baseline lipid parameters and atherogenic indices among control, 5g and 10g flaxseed group except for HDL-C ($p<0.05$) and AIP levels ($p<0.05$). The LSD post hoc test indicated that mean HDL-C levels were significantly lower ($p<0.05$) and mean AIP levels were significantly higher ($p<0.05$) in 5g flaxseed group than the 10g group (Table 4.66) before supplementation.

5g flaxseed supplementation showed a non-significant positive impact on HDL-C, TG, VLDL-C, TC/HDL-C, LDL/HDL-C and AIP levels. TC and LDL-C levels were slightly increased in 5g supplementation group. 10g flaxseed supplementation could lead to only minute improvement in HDL-C levels (Table 4.65).

TC, LDL-C, TC/HDL-C, LDL/HDL-C levels were found to be significantly increased ($p<0.001$) in control group after supplementation period. Levels of other variables of lipid profile i.e. HDL-C, VLDL-C, TG and AIP also showed adverse but non-significant trend in control group (Table 4.65).

Table 4.67 depicts the changes in prevalence of dyslipidemia post supplementation. No significant changes in prevalence of various lipid aberrations were found in the three groups. Further attempt was made to perform analysis based on initial levels of anthropometric and biochemical variables like BMI, LDL-C, AIP, HsCRP and HOMA IR to explore whether initial levels of these variables have any substantial influence on the magnitude of effect of flaxseed supplementation.

4.13.1. Impact on the lipid profile of the study subjects based on initial BMI

The trends of changes in lipid profile in subjects with BMI >25 were similar to the results of total subjects for all the groups; however no significant impact was noticed. Beneficial impact of 5g flaxseed supplementation on lipid profile of

overweight subjects (BMI<25) was comparatively minimal to obese subjects. HDL-C, TG and AIP levels showed greater improvement in overweight subjects than obese in 10g supplementation group (Table 4.68).

4.13.2. Impact on the lipid profile of the study subjects based on initial LDL levels

As depicted in the Table 4.69 subjects with initial high LDL-C levels showed a non-significant improvement in TC, LDL-C, TC/HDL-C and LDL/HDL-C levels in comparison to those with normal ones after 5g flaxseed supplementation. Whereas subject with normal LDL-C levels were found to have better HDL-C, TG and AIP levels post 5g flaxseed supplementation. 10g flaxseed supplementation was noted to have more beneficial impact on almost all lipid parameters in high LDL-C group. Significant adverse effect on TC, LDL-C, TC/HDL-C and LDL/HDL-C levels were observed in control group subjects with both normal and high LDL-C levels.

4.13.3. Impact on the lipid profile of the study subjects based on initial AIP levels

Analysis based on initial AIP levels revealed that 5g supplementation showed a better improvement in TC, LDL-C, HDL-C, TG, VLDL-C TC/HDL-C, LDL/HDL-C and AIP levels in subjects with high AIP. In 10g supplementation group HDL-C, TG, VLDL-C and AIP were marginally improved in high AIP subjects whereas no such effect was observed in subjects with normal AIP. In control subjects, significant increase in TC, LDL-C TC/HDL-C and LDL/HDL-C levels were seen in both normal and high AIP groups (Table 4.70).

4.13.4. Impact on the lipid profile of the study subjects based on initial HsCRP levels

When subjects were segregated and analysed based on their initial HsCRP levels, it was found that all the lipid parameters were maintained in high HsCRP group post 5g flaxseed supplementation. However no beneficial impact of 5g flaxseed supplementation was seen on lipid profile of normal HsCRP subjects (Table 4.71).

Similarly 10g flaxseed supplementation could not bring any desirable changes in both normal and high HsCRP group except for HDL-C levels. HDL-C levels showed a

non-significant increase in subjects with normal HsCRP levels after 10g flaxseed supplementation. In control group a distinct adverse levels of TC, HDL-C, LDL-C, TC/HDL-C, LDL/HDL-C was observed in subjects with high HsCRP levels. Similar but non-significant trend was seen in normal HsCRP control group.

4.13.5. Impact on the lipid profile of the study subjects based on initial insulin resistance

5g flaxseed supplementation showed positive impact on the lipid profile of the subjects with HOMA IR >1.2, except for HDL-C levels, which showed a slight reduction post supplementation. NO beneficial impact of 5g flaxseed supplementation on lipid profile was observed in subjects with normal HOMA IR levels. 10g flaxseed supplementation could not bring any reduction in lipid profile of the subjects with or without insulin resistance. Adverse trends of changes in lipid profile in control subjects were observed, with trend being more pronounced in normal HOMA IR subjects for TC, LDL-C, TC/HDL-C, LDL/HDL-C (Table 4.72).

4.14. Impact on the glycemic and inflammatory profile of the study subjects

As shown in the table 4.76 mean values of insulin, % insulin sensitivity, HOMA IR and HsCRP were comparable in all three groups at the initiation of supplementation. Fasting glucose levels were significantly higher in 10g flaxseed group than 5g flaxseed ($p<0.05$) and control group ($p<0.05$) (Table 4.77). Post supplementation period mean FBS levels significantly increased in all three groups however the values were within the normal range. Insulin and HOMA IR levels decreased and % insulin sensitivity increased in both the supplementation group though not statistically significant. After 8 week of supplementation mean insulin and HOMA IR levels were significantly lower in 5g ($p<0.05$ for both) and 10g ($p<0.01$ for both) flaxseed group in comparison to control group. % insulin sensitivity was found to be significantly higher in 5g ($p<0.01$) and 10g ($p<0.05$) flaxseed group in comparison to controls at the end of supplementation (Table 4.77). Mean HsCRP levels showed a non-significant decrease in all three groups (Table 4.76). Prevalence of insulin resistance decreased in both supplementation group and increased in control group post

TABLE 4.64: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
TC (mg/dl)	Pre	175.28±24.05	176.71±34.51	176.69±28.67	0.02
	Post	186.38±29.39	180.25±39.66	180.76±31.05	0.3
	Paired 't' test Value	**3.76*** **	0.84	1.13	
	% Difference	↑ 6.33	↑ 2.0	↑ 2.3	
HDL (mg/dl)	Pre	53.07±11.37	48.18±10.81	55.45±11.03	**3.18***
	Post	50.66±11.65	49.21±12.03	56.21±12.40	2.7
	Paired 't' test Value	2	0.85	0.73	
	% Difference	↓ 4.55	↑ 2.15	↑ 1.37	
LDL (mg/dl)	Pre	102.78±25.22	102.51±30.19	103.86±23.72	0.02
	Post	115.22±27.37	105.38±29.06	106.62±25.07	1.12
	Paired 't' test Value	**4.26*** **	0.80	1.07	
	% Difference	↑ 12.10	↑ 2.79	↑ 2.66	
TG (mg/dl)	Pre	97.14±41.11	130.11±130.23	86.90±28.07	2.28
	Post	102.52±41.72	128.29±140.43	89.66±27.65	1.51
	Paired 't' test Value	1.03	0.22	0.62	
	% Difference	↑ 5.54	↓ 1.4	↑ 3.17	
VLDL (mg/dl)	Pre	19.08±8.54	26.00±26.05	17.38±5.62	2.31
	Post	20.5±8.36	25.65±28.09	17.92±5.53	1.52
	Paired 't' test Value	1.24	0.21	0.61	
	% Difference	↑ 7.46	↓ 1.34	↑ 3.08	

Two tailed significance *p<0.05, ***p<0.001

TABLE 4.65: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
TC/HDL	Pre	3.49±1.04	3.88±1.35	3.28±0.62	2.44
	Post	3.85±1.01	3.86±1.37	3.30±0.61	2.7
	Paired 't' test Value	**3.87*** **	0.19	0.49	
	% Difference	↑ 10.26	↓ 0.47	↑ 0.76	
LDL/HDL	Pre	2.09±0.88	2.26±0.88	1.93±0.51	1.26
	Post	2.41±0.85	2.26±0.81	1.96±0.50	2.82
	Paired 't' test Value	**4.28*** **	0.01	0.58	
	% Difference	↑ 15.4	↓ 0.03	↑ 1.25	
AIP	Pre	0.24±0.24	0.39±0.31	0.18±0.19	**3.3***
	Post	0.28±0.24	0.33±0.3	0.2±0.17	2.3
	Paired 't' test Value	1.53	0.58	0.66	
	% Difference	↑ 18.71	↓ 4.94	↑ 8.12	

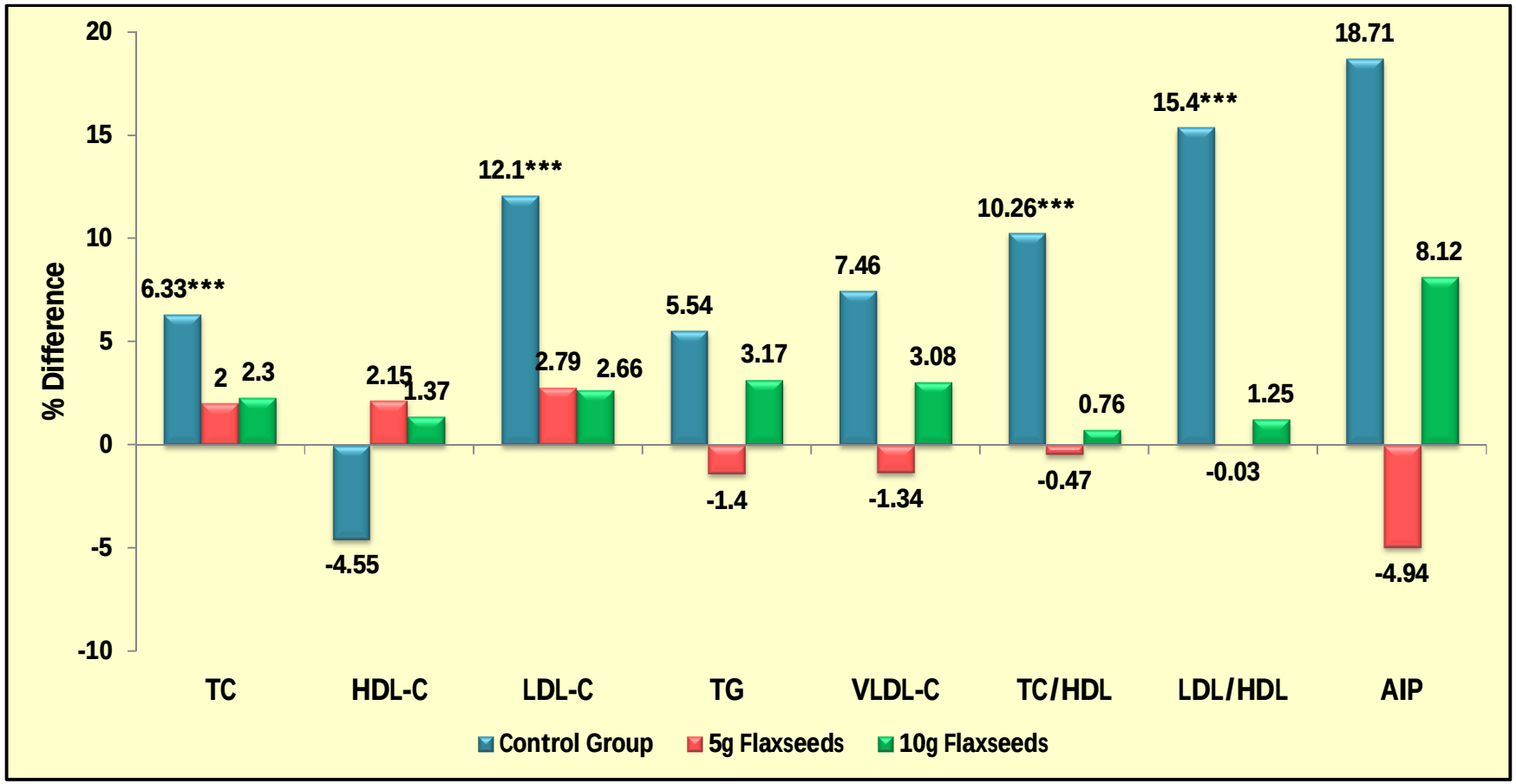
Two tailed significance *p<0.05, ***p<0.001

TABLE 4.66: POST HOC TEST LIPID PROFILE AND ATHEROGENIC INDICES

Parameters	Control v/s 5g Flaxseed 'p' Value	Control v/s 10g Flaxseeds 'p' Value	5g v/s 10g Flaxseeds 'p' Value
Pre HDL-C	0.099	0.416	0.015*
Pre AIP	0.097	0.393	0.013*

Two tailed significance *p<0.05

FIGURE 4.35: PERCENT CHANGE IN LIPID PROFILE AND ATHEROGENIC INDICES OF THE SUBJECTS POST SUPPLEMENTATION



Two tailed significance ***p<0.001

TABLE 4.67: CHANGE IN PER CENT PREVALENCE OF LIPID ABNORMALITIES AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
TC (≥200mg/dl)	5(17.2)	9 (31)	1.51	4 (14.3)	6 (21.4)	0.49	4 (13.8)	6 (20.7)	0.48
HDL-C (<50mg/dl)	9 (31)	16 (55)	3.44	18 (64.3)	16 (57.1)	0.3	11 (37.9)	9 (31)	0.31
LDL-C (≥100mg/dl)	15 (51.7)	19 (65.5)	1.14	14 (50)	15 (53.6)	0.07	16 (55.2)	19 (65.5)	0.49
TG (≥150mg/dl)	5 (17.2)	5 (17.2)	0.0	7 (25)	5 (17.9)	0.42	0 (0)	3 (10.3)	3.2
TC/HDL (>5)	2 (6.9)	3 (10.3)	0.22	4 (14.3)	3 (10.7)	0.16	0 (0)	0 (0)	--
LDL/HDL (>3.5)	1 (3.4)	2 (6.9)	0.35	0 (0)	1 (3.6)	1.02	0 (0)	0 (0)	--
AIP (>0.21)	16 (55.2)	16 (55.2)	0.00	16 (57.1)	18 (64.3)	0.3	14(48.3)	10 (34.5)	1.14

Values in parenthesis indicate percentages

TABLE 4.68: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE AND ATHEROGENIC INDICES OF THE STUDY SUBJECTS BASED ON BMI (MEAN±SD, mg/dl)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
BMI ≥25	**n= 18**			**n= 19**			**n= 18**		
TC (mg/dl)	173.2±25.1	186±28.9	**3.41****	181.4±39.8	185.4±46.8	0.67	177.9±33.2	180.3±38.8	0.46
HDL (mg/dl)	53.4±12.4	52.3±12.8	0.75	46.7±11.8	48.1±13.6	0.83	56.6±12.3	56.6±14.6	0.00
LDL (mg/dl)	101.7±27.8	114.8±27.95	**3.5****	105.6±34.04	109.2±32.9	0.75	103.8±27.6	104.9±31.04	0.31
TG (mg/dl)	90.5±39.6	94.2±40.2	0.50	145.8±154.9	140.4±167.8	0.49	87.9±28.2	94.4±32.1	1.34
VLDL (mg/dl)	18.1±7.9	18.8±8.1	0.51	29.1±30.99	28.1±33.6	0.48	17.6±5.6	18.9±6.4	1.33
TC/HDL	3.5±1.2	3.8±1.1	**2.21***	4.2±1.54	4.1±1.57	0.35	3.3±0.69	3.3±0.72	0.89
LDL/HDL	2.1±0.99	2.4±0.9	**2.66***	2.4±0.98	2.4±0.89	0.09	1.9±0.6	1.93±0.58	0.57
AIP	0.21±0.26	0.23±0.25	0.73	0.39±0.35	0.36±0.34	0.96	0.18±0.19	0.22±0.20	1.56
BMI <25	**n= 11**			**n= 9**			**n= 11**		
TC (mg/dl)	178.6±23.1	187±31.6	1.7	166.8±16.95	169.4±13.5	0.55	174.6±20.6	181.5±11.7	1.53
HDL (mg/dl)	52.5±9.9	47.9±9.4	**2.3***	51.3±8.03	51.6±8.1	0.17	53.6±8.9	55.6±8.4	1.45
LDL (mg/dl)	104.6±21.5	115.9±27.7	**2.3***	96.1±20.01	97.4±17.6	0.27	103.96±16.8	109.5±10.4	1.47
TG (mg/dl)	108±43.03	116.2±42.3	1.1	96.9±37.4	102.7±45.7	0.49	85.2±29.2	81.8±16.7	0.39
VLDL (mg/dl)	20.7±9.6	23.2±8.5	1.4	19.4±7.5	20.5±9.1	0.48	17.1±5.9	16.4±3.3	0.40
TC/HDL	3.5±0.85	4.01±0.94	**3.8****	3.31±0.54	3.4±0.61	0.72	3.3±0.52	3.3±0.41	0.13
LDL/HDL	2.1±0.71	2.5±0.80	**3.7****	1.9±0.53	1.94±0.53	0.28	1.99±0.42	2.01±0.37	0.28
AIP	0.29±0.21	0.36±0.20	1.53	0.25±0.19	0.28±0.19	0.56	0.18±0.2	0.16±0.11	0.34

Two tailed significance *p<0.05, **p<0.01

TABLE 4.69: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE AND ATHEROGENIC INDICES OF THE STUDY SUBJECTS BASED ON INITIAL LDL LEVELS (MEAN±SD, mg/dl)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
LDL ≥100 mg/dl	**n= 15**			**n= 14**			**n= 16**		
TC (mg/dl)	191.3±18.1	202.6±19.5	**2.97****	194.8±21.97	191.5±32.4	0.47	192.8±27.4	192.6±30.3	0.03
HDL (mg/dl)	48.1±8.3	46.7±8.3	0.78	46.4±9.7	47±10.6	0.40	55.5±9.4	56.6±10.1	0.84
LDL (mg/dl)	122.1±17.6	133.9±15.7	**3.14****	125.1±21.5	121.1±28.6	0.72	118.9±20.8	119.2±23.7	0.09
TG (mg/dl)	106.1±41.6	110.1±39.5	0.51	116.4±48.1	117.2±52.6	0.07	91.6±28.4	83.8±22.1	1.48
VLDL (mg/dl)	20.6±9.1	22.03±7.9	0.82	23.3±9.6	23.4±10.5	0.07	18.3±5.7	16.8±4.4	1.49
TC/HDL	4.1±0.96	4.5±0.83	**2.19***	4.4±0.9	4.2±0.9	1.01	3.5±0.5	3.5±0.5	1.17
LDL/HDL	2.7±0.8	2.96±0.7	**2.6***	2.8±0.7	2.7±0.8	1.02	2.2±0.42	2.15±0.47	0.50
AIP	0.32±0.22	0.35±0.2	0.76	0.37±0.26	0.38±0.19	0.15	0.20±0.16	0.17±0.11	1.43
LDL <100 mg/dl	**n= 14**			**n= 14**			**n= 13**		
TC (mg/dl)	158.1±16.7	169±28.7	**2.31***	158.6±35.9	169±44.1	**2.4***	156.9±15.6	166.1±26.2	1.6
HDL (mg/dl)	58.4±12.1	54.9±13.5	2.1	49.9±11.9	51.4±13.3	0.73	55.4±13.2	55.7±15.2	0.18
LDL (mg/dl)	82.1±12.2	95.3±22.8	**2.78***	79.96±18.4	89.7±20.2	**2.5***	85.3±9.9	91.1±17.1	1.4
TG (mg/dl)	87.6±39.8	94.4±43.95	0.95	143.8±180.3	139.4±194.7	0.36	81.1±27.6	96.9±32.8	**2.7***
VLDL (mg/dl)	17.5±7.99	18.9±8.8	0.94	28.7±36.1	27.9±38.95	0.34	16.2±5.5	19.3±6.6	**2.6***
TC/HDL	2.8±0.62	3.2±0.74	**3.61****	3.4±1.6	3.5±1.7	1.15	2.97±0.6	3.1±0.67	**2.7***
LDL/HDL	1.5±0.5	1.8±0.6	**3.67****	1.7±0.64	1.8±0.6	1.13	1.6±0.46	1.7±0.45	2.1
AIP	0.15±0.26	0.21±0.26	1.39	0.33±0.36	0.29±0.38	1.16	0.16±0.21	0.24±0.23	**2.5***

Two tailed significance *p<0.05, **p<0.01

TABLE 4.70: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE AND ATHEROGENIC INDICES OF THE STUDY SUBJECTS BASED ON INITIAL AIP LEVELS (MEAN±SD, MG/DL)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
AIP >0.21	**n= 16**			**n= 16**			**n= 14**		
TC (mg/dl)	186.4±25.3	198.1±29.6	**2.74***	179.3±38.5	177.4±46.2	0.29	182.4±29.6	186.1±33.1	0.64
HDL (mg/dl)	45.8±8.2	44.1±6.6	1.08	44.3±10.4	44.5±9.99	0.14	50.3±8.4	51.4±10.5	0.81
LDL (mg/dl)	115.9±23.96	128.7±25.2	**2.82***	99.4±30.2	99.7±25.1	0.05	110.06±24.7	114.1±26.8	0.95
TG (mg/dl)	123.8±36.8	126.6±38.3	0.35	177.9±157.2	166.4±178	0.83	110.4±19.9	103.4±30.8	0.98
VLDL (mg/dl)	24.1±8.4	25.4±7.7	0.65	35.6±31.5	33.3±35.6	0.82	22.1±3.99	20.7±6.2	0.98
TC/HDL	4.2±0.9	4.5±0.7	**2.38***	4.3±1.5	4.2±1.5	0.91	3.7±0.54	3.69±0.55	0.15
LDL/HDL	2.6±0.80	2.96±0.7	**2.7***	2.4±0.95	2.35±0.71	0.57	2.2±0.46	2.26±0.44	0.56
AIP	0.42±0.14	0.44±0.13	0.51	0.54±0.27	0.47±0.31	1.75	0.34±0.11	0.3±0.17	1.35
AIP ≤0.21	**n= 13**			**n= 12**			**n= 15**		
TC (mg/dl)	161.6±13.5	172±22.7	**2.5***	173.3±29.7	184±30.4	**2.35***	171.3±27.7	175.7±29.2	0.95
HDL (mg/dl)	62.1±7.7	58.8±11.6	1.7	53.4±9.4	55.5±11.99	1.25	60.3±11.3	60.7±12.7	0.29
LDL (mg/dl)	86.7±16.02	98.7±20.5	**3.34***	106.6±31.03	113±33.2	1.34	98.1±22.1	99.6±22.0	0.5
TG (mg/dl)	64.3±11.6	72.7±21.95	1.43	66.4±12.9	77.5±16.5	2.1	64.9±11.7	76.9±16.9	**2.79***
VLDL (mg/dl)	12.8±2.34	14.5±4.41	1.43	13.3±2.6	15.5±±3.3	2.13	12.98±2.3	15.34±3.34	**2.81***
TC/HDL	2.65±0.42	2.99±0.52	**4.04****	3.3±0.82	3.5±1.05	1.4	2.9±0.4	2.9±0.42	0.70
LDL/HDL	1.43±0.37	1.73±0.44	**4.33*****	2.08±0.8	2.2±0.97	1.13	1.7±0.42	1.7±0.4	0.18
AIP	0.01±0.11	0.09±0.18	1.81	0.09±0.08	0.14±0.15	1.35	0.03±0.09	0.10±0.12	**2.89***

Two tailed significance *p<0.05, **p<0.01, ***p<0.001

TABLE 4.71: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE AND ATHEROGENIC INDICES OF THE STUDY SUBJECTS BASED ON INITIAL Hs-CRP LEVELS (MEAN±SD, mg/dl)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
HsCRP >0.1mg/dl	**n= 22**			**n= 19**			**n= 17**		
TC (mg/dl)	178.1±24.9	188.2±27.9	**3.25****	177.9±36.9	177.4±43	0.1	174.4±29.34	175.24±31.1	0.17
HDL (mg/dl)	52.45±10.8	49.86±11.45	**2.35***	46.9±11.1	47.2±12.5	0.19	55.9±12.54	55.5±14.1	0.31
LDL (mg/dl)	106.36±26.3	117.55±26.15	**3.91*****	100.85±28.6	101.9±24.9	0.22	100.89±24.4	102±23.11	0.3
TG (mg/dl)	96.14±37.69	104.1±44.1	1.43	150.74±153.2	141.6±167.4	0.82	87.9±28.3	88.5±27.4	0.13
VLDL (mg/dl)	18.77±7.98	20.82±8.84	1.63	30.13±30.6	28.31±33.5	0.82	17.58±5.68	17.68±5.48	0.11
TC/HDL	3.57±1.03	3.95±1.03	**4.18*****	4.04±1.52	3.99±1.51	0.42	3.2±0.6	3.3±0.6	0.49
LDL/HDL	2.2±0.9	2.5±0.87	**4.3*****	2.3±0.91	2.3±0.75	0.04	1.9±0.5	1.9±0.5	0.48
AIP	0.24±0.22	0.29±0.24	1.74	0.41±0.34	0.37±0.33	1.23	0.19±0.2	0.20±0.2	0.54
HsCRP <0.1mg/dl	**n= 7**			**n= 9**			**n= 12**		
TC (mg/dl)	166.57±20.3	180.57±35.30	1.82	174.2±30.9	186.3±33.1	2.17	179.9±28.6	188.6±30.6	1.62
HDL (mg/dl)	55±13.74	53.14±12.85	0.48	50.9±10.4	53.6±10.2	1.04	54.8±8.97	57.2±10.1	1.50
LDL (mg/dl)	91.51±18.77	107.91±31.96	1.93	106.02±35	112.8±36.9	1.35	108.1±23.1	113.2±27.3	1.53
TG (mg/dl)	100.29±53.9	97.57±35.77	0.20	86.56±36.2	100.1±47.1	1.29	85.5±28.9	91.3±29.2	0.70
VLDL (mg/dl)	20.03±10.77	19.5±7.16	0.20	17.3±7.2	20.04±9.4	1.32	17.1±5.8	18.3±5.8	0.70
TC/HDL	3.24±1.1	3.53±0.91	1.06	3.5±0.9	3.6±1.03	0.44	3.4±0.62	3.4±0.65	0.12
LDL/HDL	1.83±0.82	2.13±0.76	1.43	2.2±0.87	2.2±0.98	0.08	2.02±0.51	2.03±0.57	0.32
AIP	0.22±032	0.25±0.25	0.32	0.21±0.16	0.25±0.21	0.75	0.18±0.17	0.19±0.15	0.38

Two tailed significance *p<0.05, **p<0.01, ***p<0.001

TABLE 4.72: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE LIPID PROFILE AND ATHEROGENIC INDICES OF THE STUDY SUBJECTS BASED ON INSULIN RESISTANCE (MEAN±SD, mg/dl)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
HOMA IR >1.2	**n= 9**			**n= 7**			**n= 4**		
TC (mg/dl)	183.6±30.2	190.2±38.7	0.97	179.9±31.2	173±22.5	0.75	180±39.6	185.3±38.1	0.38
HDL (mg/dl)	47.6±8.4	46.1±8.1	0.74	48.7±10.9	47.3±8.1	0.96	60.3±11.7	57.5±10.8	1.04
LDL (mg/dl)	111.7±28.4	120.1±30.7	1.2	104.97±29.3	102.3±12.9	0.25	103.6±34.5	110.8±35.4	0.81
TG (mg/dl)	121.6±42.2	120.2±43	0.12	130.9±46.6	116.86±68.2	0.69	81±32.98	85±29.01	0.21
VLDL (mg/dl)	23.2±10.1	24.1±8.6	0.31	26.1±9.4	23.3±13.7	0.87	16.2±6.6	16.97±5.9	0.21
TC/HDL	3.9±0.8	4.2±0.8	1.15	3.9±1.3	3.7±0.67	0.75	3.1±0.83	3.3±0.86	1.95
LDL/HDL	2.4±0.7	2.6±0.69	1.3	2.3±1.02	2.23±0.50	0.41	1.8±0.7	1.98±0.74	2.2
AIP	0.4±0.16	0.4±0.19	0.01	0.41±0.26	0.35±0.23	0.80	0.11±0.22	0.16±0.19	0.65
HOMA IR ≤1.2	**n= 20**			**n= 21**			**n= 25**		
TC (mg/dl)	171.6±20.5	184.7±25.1	**4.35*** **	175.7±36.2	182.7±44.1	1.51	176.2±27.6	180.04±30.7	1.05
HDL (mg/dl)	55.6±11.8	52.7±12.6	1.85	48±11.04	49.9±13.2	1.21	54.7±10.98	56±12.8	1.19
LDL (mg/dl)	98.8±23.3	113.04±26.3	**4.95*** **	101.7±31.1	106.4±32.95	1.41	103.9±22.5	105.96±23.9	0.76
TG (mg/dl)	86.15±36.5	94.6±39.6	1.46	129.9±149.1	132.1±158.6	0.25	87.8±27.9	90.4±27.97	0.58
VLDL (mg/dl)	17.2±7.3	18.9±7.9	1.45	25.96±29.8	26.4±31.7	0.26	17.6±5.6	18.1±5.6	0.56
TC/HDL	3.3±1.1	3.7±1.1	**4.33*** **	3.9±1.4	3.9±1.5	0.47	3.3±0.59	3.3±0.59	0.05
LDL/HDL	1.9±0.9	2.3±0.9	**4.71*** **	2.24±0.86	2.3±0.9	0.40	1.96±0.5	1.96±0.47	0.06
AIP	0.17±0.25	0.23±0.25	1.89	0.33±0.32	0.32±0.32	0.12	0.19±0.18	0.20±0.17	0.41

Two tailed significance ***p<0.001

TABLE 4.73: SUMMARY TABLE FOR EFFECT OF 5g FLAXSEEDS SUPPLEMENTATION ON THE LIPID PROFILE OF THE STUDY SUBJECTS (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
TC	↑2.0	↑2.2	↓1.7	↓1.1	↓0.3	↓3.8
HDL-C	↑2.15	↑3.0	↑1.3	↑0.5	↑0.5	↓2.9
LDL-C	↑2.79	↑3.4	↓3.2	↑0.3	↑1.0	↓2.5
TG	↓1.4	↓3.7	↑0.7	↓6.5	↓6.1	↓10.7
VLDL	↓1.34	↓3.4	↑0.4	↓6.5	↓6.0	↓10.7
TC/HDL	↓0.47	↓2.4	↓4.5	↓2.3	↓1.2	↓5.1
LDL/HDL	↓0.03	0	↓3.6	↓2.1	0	↓3.0
AIP	↓4.94	↓7.7	↑2.7	↓13.0	↓9.8	↓14.6

TABLE 4.74: SUMMARY TABLE FOR EFFECT OF 10g FLAXSEEDS SUPPLEMENTATION ON THE LIPID PROFILE OF THE STUDY SUBJECTS (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
TC	↑2.3	↑1.3	↓0.1	↑2.0	↑0.5	↑2.9
HDL-C	↑1.37	0	↑2.0	↑2.2	↓0.7	↓4.6
LDL-C	↑2.66	↑1.1	↑0.3	↑3.7	↑1.1	↑6.9
TG	↑3.17	↑7.4	↓8.5	↓6.3	↑0.7	↑4.9
VLDL	↑3.08	↑7.4	↓8.2	↓6.3	↑0.6	↑4.8
TC/HDL	↑0.76	0	0	↓0.3	↑3.1	↑6.5
LDL/HDL	↑1.25	↑1.6	↓2.3	↑2.7	0	↑10.0
AIP	↑8.12	↑22.2	↓15.0	↓11.8	↑5.3	↑45.5

TABLE 4.75: SUMMARY TABLE FOR CHANGES IN LIPID PROFILE OF THE STUDY SUBJECTS UNDER CONTROL GROUP (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
TC	↑6.33***	↑7.4**	↑5.9**	↑6.3*	↑5.7**	↑3.6
HDL-C	↓4.55	↓2.1	↓2.9	↓3.7	↓4.9*	↓3.2
LDL-C	↑12.10***	↑12.9**	↑9.7**	↑11*	↑10.5***	↑7.5
TG	↑5.54	↑4.1	↑3.8	↑2.3	↑8.3	↓1.2
VLDL	↑7.46	↑3.9	↑6.9	↑5.4	↑10.9	↑3.9
TC/HDL	↑10.26***	↑8.6*	↑9.8*	↑7.1*	↑10.6***	↑7.7
LDL/HDL	↑15.4***	↑14.3*	↑9.6*	↑13.8*	↑13.6***	↑8.3
AIP	↑18.71	↑9.5	↑9.4	↑4.8	↑20.8	0

Two tailed significance *p<0.05, *p<0.01, ***p<0.001

supplementation but not enough to be statistically significant. Prevalence of high HsCRP levels remained constant in all three groups at the end of study (Table 4.78).

4.14.1. Impact on the glycemic and inflammatory profile of the study subjects based on initial BMI

As depicted in table 4.79 subjects with BMI <25 showed better improvement in mean insulin, % insulin sensitivity and HOMA IR levels after 5g and 10g flaxseed supplementation in comparison to obese subjects (BMI≥25). However, none of these improvements were statistically significant except for % insulin sensitivity, which significantly increased in overweight subjects ($p<0.05$) post 5g flaxseed supplementation. HsCRP levels also showed better reduction in overweight subjects in all three groups compared to obese ones.

4.14.2. Impact on the glycemic and inflammatory profile of the study subjects based on initial LDL levels

The impact of 5g flaxseed supplementation on insulin levels, % insulin sensitivity and HOMA IR was almost similar in subjects with initial high or low LDL-C levels. However % insulin sensitivity and HOMA IR showed beneficial trends in subjects with high LDL-C levels after 10g flaxseed supplementation. HOMA IR levels significantly increased in control subjects ($p<0.05$) with high initial LDL-C levels at the end of the study (Table 4.80).

HsCRP levels fairly reduced in the subjects with normal LDL-C levels after 5g and 10g flaxseed supplementation in comparison to high LDL-C group, though it was not statistically significant.

4.14.3. Impact on the glycemic and inflammatory profile of the study subjects based on initial AIP levels

Both groups with initial high or low AIP levels showed non-significant beneficial trend for insulin, % insulin sensitivity and HOMA IR levels. However 10g flaxseed supplementation was able to bring reduction in insulin, HOMA IR and improvement in % insulin sensitivity only in subjects with initial high AIP levels (Table 4.81).

HsCRP levels were found to have a non-significant decrease in normal AIP subjects after 5g flaxseed supplementation. 10g flaxseed supplementation brought significant reduction ($p<0.05$) in HsCRP levels of subject with normal AIP.

4.14.4. Impact on the glycemic and inflammatory profile of the study subjects based on initial HsCRP levels

As depicted in table 4.82 5g flaxseed supplementation was found to reduce insulin and HOMA IR levels; simultaneously increasing % insulin sensitivity in subjects with both high and normal HsCRP levels. % insulin sensitivity was significantly increased in high HsCRP group post 5g flaxseed supplementation ($p<0.05$). No difference in impact of 10g flaxseed supplementation on insulin, % insulin sensitivity and HOMA IR levels was found between normal and high HsCRP groups. In control subjects with high initial HsCRP, insulin ($p<0.05$) and HOMA IR ($p<0.05$) levels significantly increased at the end of study period. Subjects with high initial HsCRP levels were found to have better reduction in mean HsCRP levels post 5g and 10g flaxseed supplementation. Surprisingly a significant reduction in mean HsCRP levels ($p<0.05$) was noted in control subjects with high initial HsCRP levels post supplementation period; nullifying the effect of flaxseed supplementation.

4.14.5. Impact on the glycemic and inflammatory profile of the study subjects based on initial insulin resistance

5g flaxseed supplementation showed greater reduction in mean insulin and HOMA IR levels as well as increase in % insulin sensitivity in insulin resistant subjects, though not statistically significant. Similar trends were observed for 10g flaxseed supplementation. Mean insulin and HOMA IR levels significantly decreased and % insulin sensitivity significantly increased in insulin resistant subjects post 10g flaxseed supplementation. Insulin resistance significantly increased normal control subjects at the end of supplementation period. Other indices also showed non-significant adverse trends in control group (normal and insulin resistant) after 8 weeks of supplementation (Table 4.83). Reduction in HsCRP levels post supplementation was not greatly affected by the insulin resistant status of the subjects in all three groups.

TABLE 4.76: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
FBS (mg/dl)	Pre	79.55±6.83	78.07±7.3	74.48±5.25	**4.65***
	Post	82.45±9.17	82.64±7.9	82.86±5.84	0.46
	Paired 't' test Value	**3.15****	**2.54***	**6.78*****	
	% Difference	↑ 6.16	↑ 5.86	↑ 11.25	
Insulin (µIU/ml)	Pre	9.41±5.88	11.36±12.07	7.47±3.7	1.69
	Post	10.87±5.93	7.63±5.19	7.07±2.6	**5.30****
	Paired 't' test Value	1.65	1.57	0.59	
	% Difference	↑ 15.53	↓ 32.76	↓ 5.37	
%S	Pre	113.09±64.55	108.75±62.46	132.03±60.75	1.12
	Post	94.8±49.66	130.43±52.22	124.32±44.90	**4.35***
	Paired 't' test Value	1.64	1.69	0.58	
	% Difference	↓ 16.17	↑ 19.94	↓ 5.84	
HOMA IR	Pre	1.17±0.72	1.39±1.42	0.92±0.46	1.71
	Post	1.38±0.74	0.97±0.65	0.9±0.32	**5.5****
	Paired 't' test Value	1.86	1.47	0.29	
	% Difference	↑ 17.65	↓ 29.9	↓ 2.62	
HsCRP (mg/dl)	Pre	0.30±0.26	0.26±0.37	0.25±0.22	0.25
	Post	0.26±0.27	0.23±0.25	0.23±0.20	0.13
	Paired 't' test Value	1.36	0.46	0.93	
	% Difference	↓ 14.79	↓ 11.28	↓ 9.79	

Two tailed significance *p<0.05, **p<0.01, ***p<0.001

TABLE 4.77: POST HOC TEST FOR GLYCEMIC PROFILE

Parameters	Control v/s 5g Flaxseed 'p' Value	Control v/s 10g Flaxseeds 'p' Value	5g v/s 10g Flaxseeds 'p' Value
Pre FBS	0.393	0.004*	0.040*
Post Insulin	0.013*	0.003**	0.658
Post %S	0.007**	0.024*	0.639
Post HOMA-IR	0.011*	0.003**	0.636

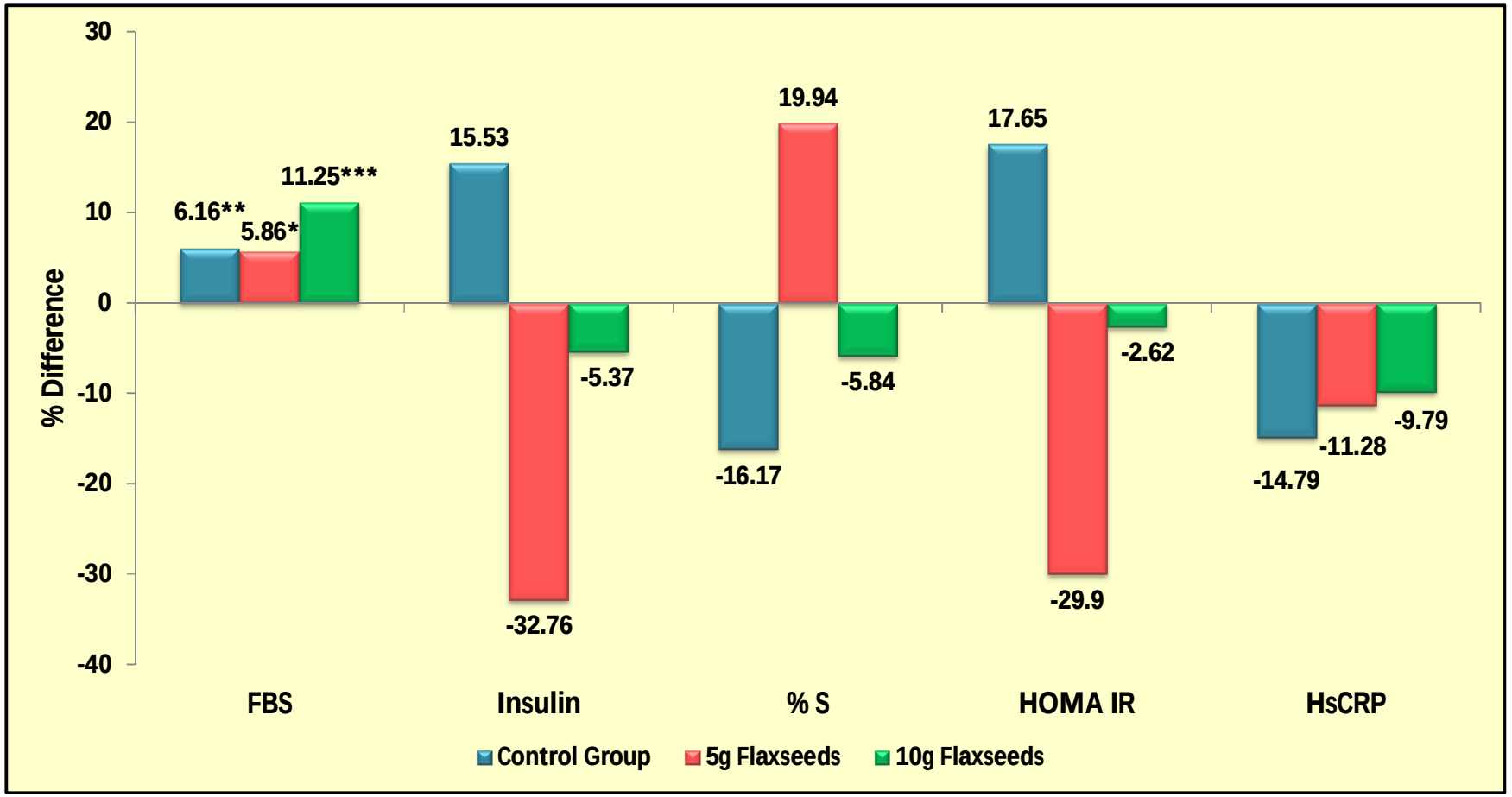
Two tailed significance *p<0.05, **p<0.01

TABLE 4.78: CHANGE IN PER CENT PREVALENCE OF INSULIN RESISTANCE AND INFLAMMATION AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
HOMA IR (>1.2)	9 (31)	15 (51.7)	2.6	7 (25)	4 (14.3)	1.02	4 (13.8)	3 (10.3)	0.16
Hs-CRP (>0.1mg/dl)	22 (75.9)	22 (75.9)	0.0	19 (67.9)	18 (64.3)	0.08	17 (58.6)	17 (58.6)	0.0

Values in parenthesis indicate percentages

FIGURE 4.36: PERCENT CHANGE IN GLYCEMIC AND INFLAMMATORY PROFILE OF THE SUBJECTS POST SUPPLEMENTATION



Two tailed significance *p<0.05, **p<0.01, ***p<0.001

TABLE 4.79: IMPACT OF FLAXSEED SUPPLEMENTATION ON INSULIN RESISTANCE AND INFLAMMATION OF THE STUDY SUBJECTS BASED ON BMI (MEAN±SD)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**
BMI ≥25	**n= 18**			**n= 19**			**n= 18**		
Insulin (μIU/ml)	10.4±6.8	11.3±6.5	0.72	9.2±5.9	8.9±5.8	0.28	7.8±3.7	7.7±2.7	0.06
%S	107.3±66.5	94.4±54.9	0.92	115.5±60.99	111.01±44.95	0.42	124.1±56.1	112.1±31.3	0.92
HOMA-IR	1.3±0.85	1.4±0.80	0.89	1.14±0.71	1.13±0.74	0.08	0.96±0.5	0.97±0.34	0.11
Hs-CRP (mg/dl)	0.35±0.31	0.30±0.33	1.06	0.22±0.23	0.23±0.22	0.53	0.28±0.24	0.28±0.24	0.14
BMI<25	**n= 11**			**n= 9**			**n= 11**		
Insulin (μIU/ml)	7.7±3.7	10.2±5.1	1.99	15.9±19.5	4.9±1.3	1.64	7.0±3.8	6.03±2.2	0.81
%S	122.5±63.2	95.4±42.2	1.4	94.6±66.8	171.4±43.2	**3.06***	145.1±68.4	144.4±57.2	0.02
HOMA-IR	0.97±0.4	1.3±0.64	2.2	1.9±2.3	0.63±0.19	1.62	0.86±0.48	0.78±0.24	0.54
Hs-CRP (mg/dl)	0.22±0.16	0.18±0.11	0.84	0.35±0.6	0.23±0.34	0.63	0.2±0.17	0.14±0.15	2.10

Two tailed significance *p<0.05

TABLE 4.80: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS BASED ON INITIAL LDL LEVELS (MEAN±SD)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**
LDL ≥100 mg/dl	**n= 15**			**n= 14**			**n= 16**		
Insulin (µIU/ml)	8.9±3.2	10.5±4.4	1.95	11.6±11.4	7.98±6.45	1.2	7.5±3.1	7.6±11.3	1.00
%S	106.9±59.5	89.7±42.5	1.71	122.99±76.7	130.5±52.5	0.39	122.4±42.5	134.1±53.5	0.65
HOMA-IR	1.11±0.4	1.35±0.57	**2.21***	1.45±1.4	1.01±0.80	1.56	0.92±0.39	0.84±0.28	0.75
Hs-CRP (mg/dl)	0.36±0.24	0.29±0.27	2.06	0.25±0.27	0.28±0.31	0.58	0.22±0.25	0.20±0.19	0.57
LDL <100 mg/dl	**n= 14**			**n= 14**			**n= 13**		
Insulin (µIU/ml)	9.9±7.9	11.3±7.4	0.82	11.0±13.2	7.3±3.8	1.9	7.5±4.5	7.7±2.8	0.14
%S	119.7±71.2	100.3±57.5	0.93	94.5±42.2	130.4±53.95	2.13	143.95±77.95	112.3±29.2	1.73
HOMA-IR	1.24±0.96	1.4±0.9	0.84	1.32±1.5	0.93±0.49	0.90	0.92±0.55	0.96±0.36	0.27
Hs-CRP (mg/dl)	0.24±0.28	0.23±0.27	0.23	0.27±0.46	0.18±0.17	0.74	0.29±0.18	0.25±0.22	0.71

Two tailed significance *p<0.05

TABLE 4.81: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS BASED ON INITIAL AIP LEVELS (MEAN±SD)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value	Pre	Post	Paired 't' test Value
AIP >0.21	**n= 16**			**n= 16**			**n= 14**		
Insulin (µIU/ml)	11.9±6.3	13.4±6.1	1.32	12.7±13.1	8.7±6.4	1.15	8.1±0.38	7.28±0.3	0.75
%S	80.5±29.2	71.4±35.9	1.2	100.7±51.3	119.2±52	1.21	115.9±52.1	126.2±55.7	0.53
HOMA-IR	1.5±0.79	1.7±0.73	1.56	1.5±1.5	1.1±0.8	1.03	1.0±0.41	0.94±0.41	0.51
Hs-CRP (mg/dl)	0.30±0.26	0.26±0.28	1.37	0.24±0.26	0.27±0.30	0.6	0.24±0.19	0.26±0.22	0.47
AIP ≤0.21	**n= 13**			**n= 12**			**n= 15**		
Insulin (µIU/ml)	6.41±3.61	7.73±4.05	0.97	9.5±10.8	6.2±2.5	1.04	6.9±4.0	6.9±1.8	0.04
%S	153.3±74.1	123.6±50.2	1.27	119.5±75.96	145.4±50.8	1.15	147.1±66.02	122.6±33.8	1.36
HOMA-IR	0.82±0.42	0.98±0.52	0.99	1.2±1.4	0.78±0.30	1.03	0.85±0.5	0.86±0.2	0.12
Hs-CRP (mg/dl)	0.30±0.28	0.26±0.27	0.7	0.3±0.49	0.19±0.18	0.77	0.26±0.25	0.19±.0.19	**2.47***

Two tailed significance *p<0.05

TABLE 4.82: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS BASED ON INITIAL HS-CRP LEVELS (MEAN±SD)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**
Hs-CRP >0.1mg/dl	**n= 22**			**n= 19**			**n= 17**		
Insulin (µIU/ml)	9.65±5.67	11.72±6.31	**2.19***	12.03±12.1	8.4±6.1	1.24	7.19±3.86	6.9±2.91	0.28
%S	107.4±65.1	88.26±49.68	1.5	94.14±52.28	125.87±59.63	**2.26***	142.1±71.8	128.95±48.9	0.63
HOMA-IR	1.2±0.7	1.5±0.8	**2.44***	1.46±1.38	1.07±0.77	1.16	0.89±0.48	0.88±0.36	0.09
Hs-CRP (mg/dl)	0.38±0.26	0.3±0.3	**2.15***	0.36±0.41	0.32±0.27	0.50	0.38±0.2	0.32±0.21	1.61
Hs-CRP <0.1mg/dl	**n= 7**			**n= 9**			**n= 12**		
Insulin (µIU/ml)	8.65±6.91	8.19±3.73	0.22	9.92±12.57	5.97±1.58	0.91	7.88±3.6	7.3±2.2	0.67
%S	130.9±64.4	115.34±47.1	0.63	139.6±73.8	140.06±32.4	0.02	117.8±38.95	117.8±39.61	0.004
HOMA-IR	1.07±0.9	1.03±0.48	0.16	1.23±1.6	0.77±0.2	0.86	0.96±0.46	0.92±0.27	0.40
Hs-CRP (mg/dl)	0.05±0.02	0.13±0.1	1.82	0.05±0.03	0.06±0.05	0.69	0.06±0.02	0.1±0.1	1.24

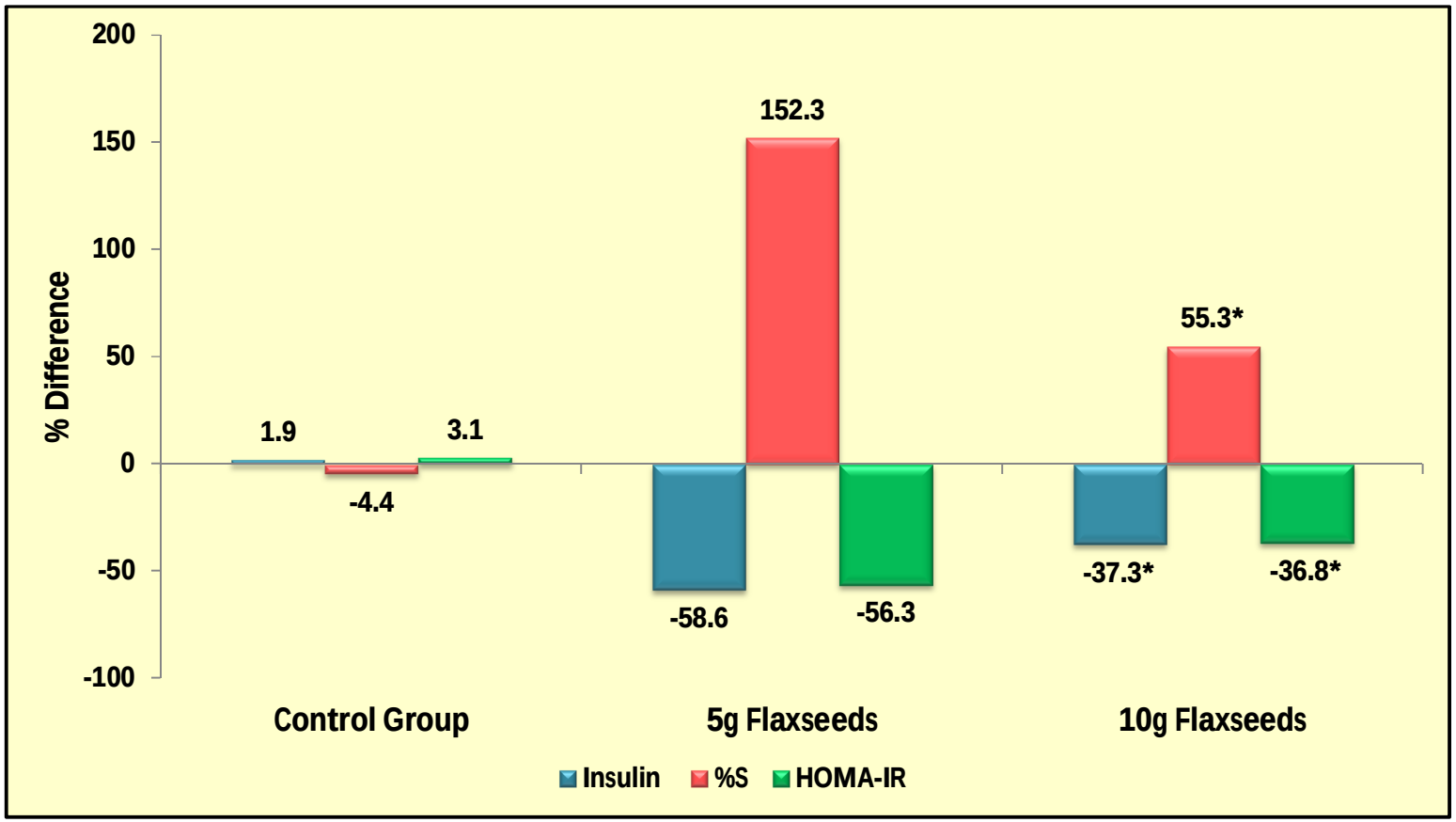
Two tailed significance *p<0.05

TABLE 4.83: IMPACT OF FLAXSEED SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS BASED ON INSULIN RESISTANCE (MEAN±SD)

	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**	**Pre**	**Post**	**Paired 't' test Value**
HOMA IR >1.2	**n= 9**			**n= 7**			**n= 4**		
Insulin (µIU/ml)	15.8±6.4	16.1±6.1	0.16	26.3±17.3	10.9±8.6	1.9	15.3±2.7	9.6±0.92	**4.32***
%S	56.5±16.4	54±16.3	0.37	43.2±23.5	109.01±62.8	2.2	53.9±10.95	83.7±9.3	**4.6***
HOMA-IR	1.96±0.8	2.02±0.7	0.27	3.2±1.99	1.4±1.1	1.87	1.9±0.36	1.2±0.14	**4.04***
Hs-CRP (mg/dl)	0.28±0.23	0.23±0.19	1.77	0.20±0.14	0.22±0.22	0.26	0.24±0.21	0.19±0.18	1.19
HOMA IR ≤1.2	**n= 20**			**n= 21**			**n= 25**		
Insulin (µIU/ml)	6.5±2.4	8.5±4.2	2.05	6.4±1.9	6.6±3.02	0.29	6.2±1.8	6.7±2.6	0.7
%S	138.6±61.9	113.2±48.8	1.61	130.6±55.7	137.6±47.8	0.55	144.5±55.8	130.8±44.96	0.90
HOMA-IR	0.82±0.26	1.1±0.54	**2.24***	0.79±0.23	0.84±0.41	0.66	0.76±0.22	0.85±0.31	1.14
Hs-CRP (mg/dl)	0.31±0.28	0.27±0.30	0.94	0.28±0.42	0.24±0.27	0.54	0.25±0.23	0.23±0.21	0.66

Two tailed significance *p<0.05

FIGURE 4.37: PERCENT CHANGE IN GLYCEMIC PROFILE OF THE SUBJECTS WITH HIGH HOMA IR LEVELS (>1.2) POST SUPPLEMENTATION



Two tailed significance *p<0.05

TABLE 4.84: SUMMARY TABLE FOR EFFECT OF 5g FLAXSEEDS SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
Insulin	↓ 32.76	↓3.3	↓31.2	↓31.5	↓30.2	↓58.6
%S	↑ 19.94	↓3.9	↑ 6.1	↑ 18.4	↑ 33.3*	↑152.3
HOMA-IR	↓ 29.9	↓0.9	↓30.3	↓26.7	↓26.7	↓56.3
Hs-CRP	↓ 11.28	↑4.5	↑12	↑12.5	↓11.1	↑10

Two tailed significance *p<0.05

TABLE 4.85: SUMMARY TABLE FOR EFFECT OF 10g FLAXSEEDS SUPPLEMENTATION ON THE GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
Insulin	↓ 5.37	↓1.3	↑1.3	↓10.1	↓4.0	↓37.3*
%S	↓5.84	↓9.7	↑9.6	↑8.9	↓9.3	↑55.3*
HOMA-IR	↓2.62	↑1.0	↓8.7	↓6.0	↓1.1	↓36.8*
Hs-CRP	↓9.79	0	↓9.1	↑8.3	↓15.8	↓20.8

Two tailed significance *p<0.05

TABLE 4.86: SUMMARY TABLE FOR CHANGES IN GLYCEMIC AND INFLAMMATORY PROFILE OF THE STUDY SUBJECTS UNDER CONTROL GROUP (% Difference)

Variables	Overall	Initial BMI >25	Initial LDL ≥100 mg/dl	Initial AIP >0.21	Initial HsCRP >0.1mg/dl	Initial HOMA IR >1.2
Insulin	↑15.53	↑8.7	↑18	↑12.6	↑21.5*	↑1.9
%S	↓16.17	↓12.0	↓16.1	↓11.3	↓17.8	↓4.4
HOMA-IR	↑17.65	↑7.7	↑21.6*	↑13.3	↑25*	↑3.1
Hs-CRP	↓14.79	↓14.3	↓19.4	↓13.3	↓21.1*	↓17.9

Two tailed significance *p<0.05

4.15. Impact on the anthropometric indices and biophysical measurements of the study subjects

The mean levels of anthropometric indices (Table 4.87), body fat percent and blood pressure (Table 4.88) were comparable among all three groups before initiation of the supplementation and remained unchanged in control group throughout the study period. Supplementation of 5g and 10g flaxseeds had no significant impact on the anthropometric indices. 5g flaxseed intake led to significant reduction in body fat percent ($p<0.05$) and blood pressure ($p<0.05$) post supplementation. However 10g flaxseed supplementation failed to bring such impact on body fat. Blood pressure showed a non-significant reduction in 10g flaxseed group post supplementation.

As depicted in Table 4.89, both 5g and 10g supplementation significantly reduced ($p<0.05$) the prevalence of pre and stage I hypertension in studied subjects.

4.16. Impact on the haematological profile of the study subjects

Table 4.90 displays the effect of supplementation on mean levels of haematological indices. Mean Hb levels were significantly higher in control group than 5g ($p<0.001$) and 10g ($p<0.05$) flaxseed group before supplementation using LSD post hoc test (Table 4.91) after finding significant difference between three groups. Supplementation of flaxseeds did not alter haematological indices significantly except for haemoglobin. Mean Hb levels increased significantly post supplementation in both supplementation groups ($p<0.001$ and $p<0.001$ for 5g and 10g supplementation group respectively). It cannot be marked as true effect of supplementation because a significant increase in mean Hb levels of control group subjects was also noted. There was no significant decrease in prevalence of iron deficiency anemia in subjects post supplementation as depicted in Table 4.92.

4.17. Impact on the thyroid profile of the study subjects

The mean T3 and T4 levels were almost equal before supplementation. Post supplementation, a slight increase in all three groups was seen which was statistically non-significant and fell under the normal reference range (Table 4.93). The mean TSH levels were found to be high in 5g flaxseed group before

TABLE 4.87: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE ANTHROPOMETRIC INDICES OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
BMI	Pre	26.24±2.27	26.29±2.20	26.14±2.0	0.03
	Post	26.33±2.18	26.27±2.26	26±2.15	0.19
	Paired 't' test Value	1.42	0.10	0.74	
	% Difference	↑ 0.34	↓ 0.08	↓ 0.54	
WC (cm)	Pre	94.05±6.88	92.10±5.96	93.29±5.57	0.73
	Post	94.0±6.91	91.98±5.93	92.71±5.4	0.86
	Paired 't' test Value	1.03	2.01	1.22	
	% Difference	↓ 0.05	↓ 0.13	↓ 0.62	
WHR	Pre	0.94±0.05	0.93±0.04	0.93±0.04	0.97
	Post	0.94±0.05	0.93±0.041	0.93±0.04	0.82
	Paired 't' test Value	1.36	0.36	1.39	
	% Difference	0	0	0	
WSR	Pre	0.60±0.05	0.604±0.04	0.60±0.03	0.28
	Post	0.61±0.05	0.603±0.04	0.60±0.03	0.36
	Paired 't' test Value	1	2.01	1.75	
	% Difference	↑ 1.7	↓0.1	0	

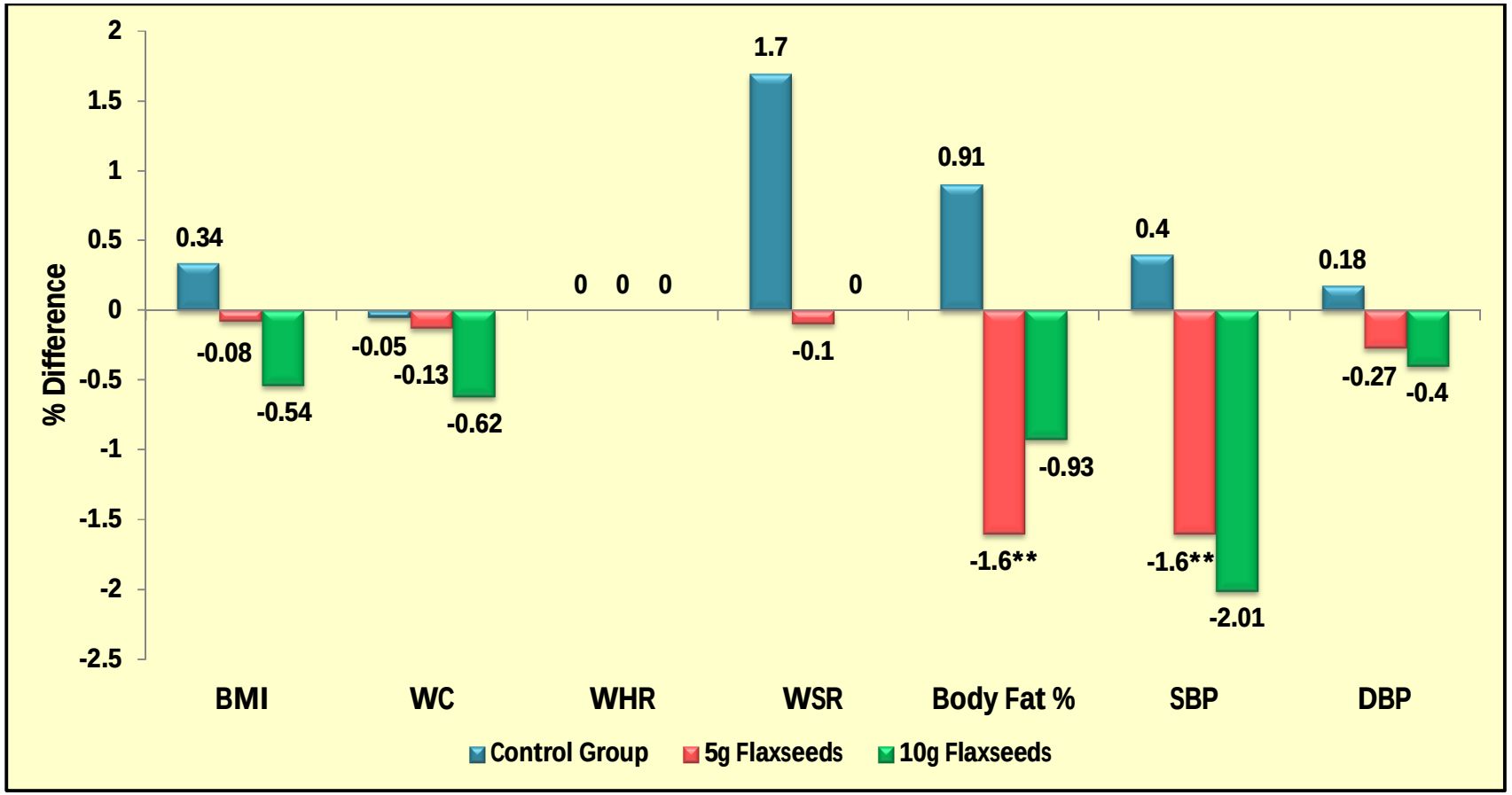
Two tailed significance *p<0.05, ***p<0.001

TABLE 4.88: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE BIOPHYSICAL MEASUREMENTS OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
Body Fat %	Pre	37.07±3.21	36.22±3.68	36.83±2.82	0.52
	Post	37.41±2.93	35.64±3.92	36.49±2.87	2.08
	Paired 't' test Value	1.17	**3.19****	1.21	
	% Difference	↑ 0.91	↓1.6	↓ 0.93	
SBP (mmHg)	Pre	119.28±9.68	124.68±12.47	119.90±9.58	2.19
	Post	119.76±8.94	122.68±10.55	117.48±6.31	2.52
	Paired 't' test Value	1.24	**3.23****	1.63	
	% Difference	↑ 0.40	↓ 1.6	↓ 2.01	
DBP (mmHg)		78.55±7.0	80.54±6.85	78.10±6.58	1.02
		78.69±6.66	80.75±6.24	77.79±5.78	1.68
	Paired 't' test Value	0.57	5.56	0.43	
	% Difference	↑ 0.18	↑ 0.27	↓ 0.40	

Two tailed significance **p<0.01

FIGURE 4.38: PERCENT CHANGE IN ANTHROPOMETRIC AND BIOPHYSICAL PARAMETERS OF THE SUBJECTS POST SUPPLEMENTATION



Two tailed significance **p<0.01

TABLE 4.89: CHANGE IN PER CENT PREVALENCE OF OBESITY AND HYPERTENSION AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
BMI (≥25)	18 (62.1)	20 (69)	0.31	19 (67.9)	18 (64.3)	0.08	18 (62.1)	16 (55.2)	0.28
WC (>80cm)	100 (100)	100 (100)	--	100 (100)	100 (100)	--	100 (100)	100 (100)	--
WHR (≥0.85)	27 (93.1)	27 (93.1)	0.0	27 (96.4)	27 (96.4)	0.0	29 (100)	29 (100)	--
WSR(>0.5)	28 (96.6)	28 (96.6)	0.0	28 (100)	28 (100)	--	29 (100)	29 (100)	--
Body Fat (>30%)	29 (100)	29 (100)	--	27 (96.4)	25 (89.3)	1.1	29 (100)	29 (100)	--
Pre and Stage I Hypertension	7 (24.1)	4 (13.8)	1.01	16 (57.1)	8 (28.6)	**4.7***	11 (37.9)	4 (13.8)	**4.4***

Values in parenthesis indicate percentages
Two tailed significance *p<0.05

FIGURE 4.39: CHANGE IN PER CENT PREVALENCE OF HYPERTENSION AMONG THE STUDY SUBJECTS

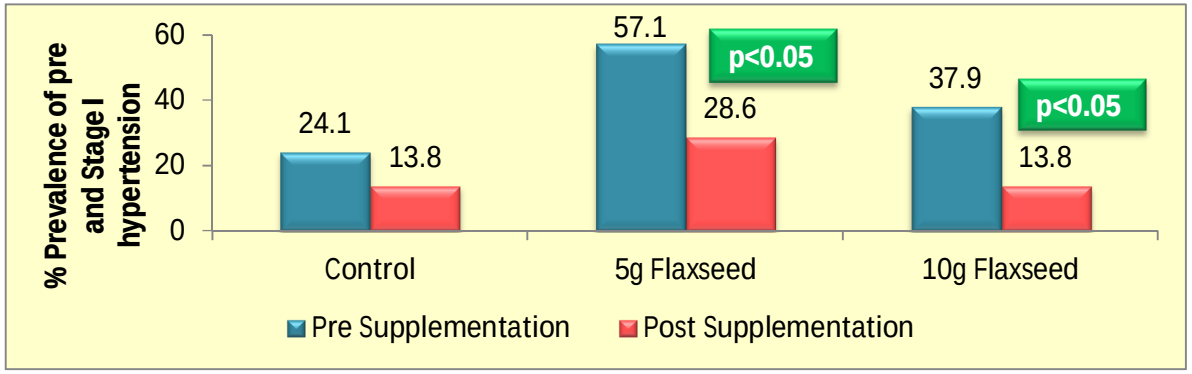


TABLE 4.90: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE HAEMATOLOGICAL INDICES OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
Iron (mg/dl)	Pre	68.86±33.81	55.6±25.13	54.05 ±28.12	2.23
	Post	70.32±28.38	56.65±27.98	59.12±33.99	1.66
	Paired 't' test Value	0.27	0.246	0.83	
	% Difference	↑ 2.13	↑ 1.89	↑ 9.39	
TIBC (µg/dl)	Pre	353.98±48.96	365.90±49.31	361.45±44.4	0.46
	Post	366.93±43.61	373.76±53.26	371.27±44.59	0.15
	Paired 't' test Value	**3.9*** **	1.69	1.95	
	% Difference	↑ 3.66	↑ 2.15	↑ 2.72	
Per cent TS	Pre	20.15±9.67	15.75±7.71	15.29±8.4	2.78
	Post	19.58±8.15	15.71±8.32	16.37±10.3	1.52
	Paired 't' test Value	0.39	0.03	1.82	
	% Difference	↓2.85	↓ 0.23	↑ 7.06	
Hb (g/dl)	Pre	12.48±1.28	11.18±1.24	11.62±1.39	**7.42*** **
	Post	12.75±1.19	11.79±0.96	12.34±1.0	**6.03** **
	Paired 't' test Value	**2.79** **	**4.36*** **	**3.64*** **	
	% Difference	↑ 2.15	↑ 5.43	↑ 6.17	

Two tailed significance **p<0.01, ***p<0.001

TABLE 4.91: POST HOC TEST FOR HEMOGLOBIN

Parameters	Control v/s 5g Flaxseed 'p' Value	Control v/s 10g Flaxseeds 'p' Value	5g v/s 10g Flaxseeds 'p' Value
Pre Hb	0.000***	0.014*	0.204
Post Hb	0.001**	0.138	0.051

Two tailed significance *p<0.05, **p<0.01, ***p<0.001

TABLE 4.92: CHANGE IN PER CENT PREVALENCE OF IRON DEFICIENCY AENEMIA AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
Iron (<50µg/dl)	8 (27.6)	7 (24.1)	0.09	12 (42.9)	14 (50)	0.29	14 (48.3)	17 (58.6)	0.62
TIBC (>450)	0 (0)	1 (3.4)	1.02	2 (7.1)	1 (3.6)	0.35	1 (3.4)	1 (3.4)	0.00
Transferrin Saturation (<15%)	11 (37.9)	10 (34.5)	0.08	12 (42.9)	14 (50)	0.29	17 (58.6)	18 (62.1)	0.07
Hb <12g/dl	7 (24.1)	3 (10.3)	1.93	19 (67.9)	14 (50)	1.85	15 (51.7)	9 (31)	2.6

Values in parenthesis indicate percentages

supplementation however due to a wider standard deviation; this difference from other two groups was not statistically significant using ANOVA. A slight increase in the TSH values of all three groups was found post supplementation, however the paired 't' test values showed no statistically significant rise. As depicted in the Table 4.94 the prevalence of subclinical hypothyroidism was also not found to be significantly altered post supplementation.

4.18. Impact on the liver function tests of the study subjects

The mean values for all the liver functions tests showed no significant difference among the three groups before supplementation (Table 4.95 a&b). The total bilirubin, direct bilirubin and indirect bilirubin values were significantly reduced among control subjects whereas SGPT and GGT enzymes showed a significant rise among control subjects post supplementation. However these changes were not much of physiological significance as the values were within the normal reference ranges for the same. No difference was seen in the mean values of liver function tests of 5g and 10g flaxseeds group post supplementation, except for SGOT value (increase $p<0.05$) in 5g flaxseeds group. Nevertheless it was again under normal reference range. The prevalence of liver function abnormalities was not found to be different between pre and post supplementation for all groups (Table 4.96).

4.19. Impact on the kidney function tests of the study subjects

The mean values of kidney function tests were under normal reference range for all three groups before and after supplementation (Table 4.97). BUN values were significantly higher in 10g flaxseed group than control and 5g supplementation before supplementation, however within the reference range (Table 4.98). The uric acids values were found to be significantly lower in both 5g ($p<0.0$) and 10g ($p<0.01$) flaxseeds group post supplementation with no significant decrease in control group. As shown in Table 4.99, no difference in the prevalence of kidney function abnormalities post supplementation was found among three groups.

TABLE 4.93: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE THYROID PROFILE OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	One way ANOVA Value
T3 (ng/dl)	Pre	103.28±16.7	101.96±18.27	104.21±14.88	0.13
	Post	104.48±17.45	106.21±22.05	106.93±21.47	0.11
	Paired 't' test Value	0.58	1.57	0.72	
	% Difference	↑ 1.17	↑ 4.17	↑ 2.61	
T4 (µg/dl)	Pre	8.65±1.65	8.51±2.38	8.87±1.16	0.29
	Post	8.66±2.07	8.67±2.60	9.19±1.96	0.53
	Paired 't' test Value	0.07	0.54	1.19	
	% Difference	↑ 0.20	↑ 1.85	↑ 3.54	
TSH (µIU/ml)	Pre	4.43±4.43	9.25±20.87	3.19±2.97	1.93
	Post	4.56±4.14	10.44±26.69	3.53±3.78	1.62
	Paired 't' test Value	0.31	0.62	1.6	
	% Difference	↑ 2.99	↑ 9.62	↑ 10.95	

TABLE 4.94: CHANGE IN PER CENT PREVALENCE OF THYROID FUNCTION ABNORMALITIES AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
TSH (>4µIU/ml)	7 (24.1)	11 (37.9)	1.3	9 (32.1)	14 (50)	1.85	3(10.3)	5 (17.2)	0.58
T3 (200ng/dl)	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	az1.02
T4 (12µg/dl)	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--

Values in parenthesis indicate percentages

TABLE 4.95 (a): EFFECT OF FLAXSEED SUPPLEMENTATION ON THE LIVER FUNCTION TESTS OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	Reference range	One way ANOVA Value
Alkaline Phosphatase (U/L)	Pre	77.33±19.51	71.13±17.71	74.88±20.09	42-98	0.76
	Post	74.86±17.96	71.21±18.44	73.99±18.07		0.31
	Paired 't' test Value	1.78	0.042	0.48		
	% Difference	↑ 3.19	↑ 0.11	↓ 1.18		
Total Bilirubin (mg/dl)	Pre	0.55±0.16	0.54±0.21	0.46±0.15	0-1.2	1.95
	Post	0.47±0.13	0.54±0.24	0.44±0.11		2.44
	Paired 't' test Value	**3.01****	0.010	0.84		
	% Difference	↓ 14.75	↑ 0.07	↓ 4.9		
Direct Bilirubin (mg/dl)	Pre	0.17±0.04	0.16±0.05	0.14±0.05	0-0.3	1.48
	Post	0.15±0.04	0.16±0.06	0.14±0.04		2.22
	Paired 't' test Value	**3.01****	0.28	0.84		
	% Difference	↓ 11.85	↑ 1.77	↓ 4.98		
Indirect Bilirubin (mg/dl)	Pre	0.38±0.13	0.38±0.16	0.32±0.11	0-0.9	1.93
	Post	0.32±0.09	0.38±0.18	0.31±0.08		2.44
	Paired 't' test Value	**2.81****	0.09	0.83		
	% Difference	↓ 15.81	↑ 0.57	↓ 4.83		
SGOT (U/L)	Pre	19.64±5.5	18.33±5.35	17.46±3.89	0-31	1.41
	Post	20.47±5.4	21.08±5.75	19.1±6.05		0.89
	Paired 't' test Value	0.88	**2.59***	1.87		
	% Difference	↑ 4.22	↑ 14.96	↑ 9.36		

Two tailed significance *p<0.05, **p<0.01

TABLE 4.95 (b): EFFECT OF FLAXSEED SUPPLEMENTATION ON THE LIVER FUNCTION TESTS OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	Reference Range	One way ANOVA Value
SGPT (U/L)	Pre	18.32±±6.93	16.86±8.83	16.62±5.25	0-31	0.48
	Post	21.90±8.7	20.77±17.26	15.64±6.06		2.39
	Paired 't' test Value	**3.04****	1.24	1.06		
	% Difference	↑ 19.53	↑ 23.17	↓ 5.91		
GGT (U/L)	Pre	17.74±8.19	16.74±8.02	17.4±7.68	0-30	0.12
	Post	20.15±10.19	16.32±6.12	16.64±6.7		2.1
	Paired 't' test Value	**2.82****	0.54	1.47		
	% Difference	↑ 13.5	↓ 2.5	↓ 4.36		
Total Protein (g/dl)	Pre	7.36±1.35	7.34±0.41	7.26±0.45	6-8.3	0.11
	Post	7.25±0.45	7.33±0.33	7.18±0.35		0.99
	Paired 't' test Value	0.51	0.15	1.14		
	% Difference	↓ 1.53	↓ 0.18	↓ 1.09		
Albumin (g/dl)	Pre	4.19±0.68	4.15±0.25	4.15±0.24	3.2-5	0.06
	Post	4.16±0.25	4.12±0.25	4.06±0.30		0.98
	Paired 't' test Value	0.23	0.75	1.76		
	% Difference	↓ 0.64	↓ 0.89	↓ 2.17		
Albumin/globulin Ratio	Pre	1.44±0.51	1.32±0.18	1.35±0.17	0.9-2	1.09
	Post	1.37±0.21	1.3±0.17	1.3±0.15		1.47
	Paired 't' test Value	0.74	1.22	1.68		
	% Difference	↓ 4.83	↓ 1.73	↓ 3.06		

Two tailed significance **p<0.01

TABLE 4.96: CHANGE IN PER CENT PREVALENCE OF LIVER FUNCTION ABNORMALITIES AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
Alkeline Phosphatase (>98 U/L)	1 (3.4)	1 (3.4)	0.0	4 (14.3)	3 (10.7)	0.16	4 (13.8)	4 (13.8)	0.0
Total Bilirubin (>1.20mg/dl)	0 (0)	0 (0)	--	0 (0)	1 (3.6)	1.02	0 (0)	0 (0)	--
Indirect Bilirubin (>0.9mg/dl)	0 (0)	0 (0)	--	0 (0)	1 (3.6)	1.02	0 (0)	0 (0)	--
Direct Bilirubin (>0.20mg/dl)	4 (13.8)	3 (10.3)	0.16	4 (14.3)	7 (25)	1.02	3 (10.3)	3 (10.3)	0.0
SGOT (>31 U/L)	1 (3.4)	1 (3.4)	0.0	1 (3.6)	2 (7.1)	0.35	0 (0)	2 (6.9)	2.1
SGPT (>28 U/L)	4 (13.8)	8 (27.6)	1.7	2 (7.1)	4 (14.3)	0.75	1 (3.4)	1 (3.4)	0.0
GGT (> 38 U/L)	1 (3.4)	3 (10.3)	1.1	1 (3.6)	0 (0)	1.02	1 (3.4)	1(3.4)	0.0
Serum Albumin (<3.5gm/dl)	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	1 (3.4)	1.02
Total Protein (<6.6gm/dl)	4 (13.8)	1 (3.4)	1.97	1 (3.6)	1 (3.6)	0.0	4 (13.8)	2 (6.9)	0.74

Values in parenthesis indicate percentages

TABLE 4.97: EFFECT OF FLAXSEED SUPPLEMENTATION ON THE KIDNEY FUNCTION TESTS OF THE STUDY SUBJECTS

Parameters		Control Group	5g Flaxseeds	10g Flaxseeds	Reference Range	One way ANOVA Value
Ca (mg/dl)	Pre	9.20±0.37	9.19±0.35	9.27±0.35		0.43
	Post	9.18±8.15	9.09±0.2	9.13±0.34		0.66
	Paired 't' test Value	0.29	1.46	1.82		
	% Difference	↓ 0.24	↓ 1.12	↓ 1.45		
BUN (mg/dl)	Pre	8.42±2.07	8.23±1.73	9.50±1.86	9-23	**3.79***
	Post	8.50±2.42	8.83±2.01	9.97±3		2.72
	Paired 't' test Value	0.26	1.62	1.01		
	% Difference	↑ 1.02	↑ 7.32	↑ 4.91		
Creatinine (mg/dl)	Pre	0.6±0.09	0.56±0.07	0.61±0.07	0.5-0.8	2.66
	Post	0.61±0.07	0.56±0.06	0.6±0.07		4.44
	Paired 't' test Value	0.91	0.87	0.7		
	% Difference	↑1.62	↓ 1.39	↓ 1.48		
Uric Acid (mg/dl)	Pre	4.29±1.11	4.43±0.94	4.26±0.77	2.3-6.1	0.27
	Post	4.25±1.13	4.15±1.03	4.03±0.74		0.36
	Paired 't' test Value	0.39	**2.16***	**2.85****		
	% Difference	↓ 1.0	↓ 6.35	↓ 5.26		
BUN/Cr ratio	Pre	14.17±3.38	14.78±3.69	15.79±3.27	9:1-23.1	1.64
	Post	14.13±3.97	16.00±3.79	16.79±4.86		3.01
	Paired 't' test Value	0.06	1.65	1.23		
	% Difference	↓ 0.24	↑ 8.29	↑ 6.35		

Two tailed significance *p<0.05, **p<0.01

TABLE 4.98: POST HOC TEST FOR KIDNEY FUNCTION TESTS

Parameters	Control v/s 5g Flaxseed 'p' Value	Control v/s 10g Flaxseeds 'p' Value	5g v/s 10g Flaxseeds 'p' Value
Pre BUN	0.703	0.032*	0.013*

Two tailed significance *p<0.05

TABLE 4.99: CHANGE IN PER CENT PREVALENCE OF KIDNEY FUNCTION ABNORMALITIES AMONG THE STUDY SUBJECTS

Parameters	Control Group			5g Flaxseeds			10g Flaxseeds		
	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value	Pre	Post	χ^2 Value
Ca (8.4-10.6mg/dl)	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--
BUN (>20mg/dl)	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Creatinine (0.8mg/dl)	2 (6.9)	1 (3.4)	0.35	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Uric Acid (>6mg/dl)	2 (6.9)	1 (3.4)	0.35	3 (10.7)	1 (3.6)	1.1	0 (0)	0 (0)	--

Values in parenthesis indicate percentages

Table 4.100: HIGHLIGHTS OF IMPACT OF FLAXSEED SUPPLEMENTATION ON BIOPHYSICAL, METABOLIC AND INFLAMMATORY PROFILE OF WOMEN

Variables	5g supplementation group		10g supplementation group		Control Group	
	Changes in mean values	Changes in Prevalence	Changes in mean values	Changes in Prevalence	Changes in mean values	Changes in Prevalence
SBP/ Pre and Stage 1 Hypertension	*↓	*↓	NS	*↓	NS	NS
Insulin (in subjects with HOMA IR >1.2)	NS	--	*↓	--	NS	--
% S (in subjects with HOMA IR >1.2)	NS	--	*↑	--	NS	--
HOMA IR (in subjects with HOMA IR >1.2)	NS	--	*↓	--	NS	--
TC	NS	NS	NS	NS	***↑	NS
LDL-C	NS	NS	NS	NS	***↑	NS
TC/HDL-C	NS	NS	NS	NS	***↑	NS
LDL/HDL-C	NS	NS	NS	NS	***↑	NS
Body fat %	*↓	--	NS	--	NS	--

Two tailed significance*p<0.05, ***p<0.001, NS- Non significant

Discussion

Flaxseeds are richest source of alpha linolenic acid and lignans. Alpha linolenic acid is the principal precursor for n-3 fatty acids (eicosapentaenoic and docosahexaenoic acid) (Brenna, 2002) in our body. Intake of n-3 fatty acids is associated with improved cardiovascular functions (Swanson et al, 2012). Flaxseed lignin (SDG) has potential to exert preventive impact on development of various diseases like cancer, CVDs, diabetes etc. through its anti-inflammatory, antioxidant, anti-mutagenic, anti-microbial, anti-obesity and anti-hypolipidemic properties (Imran et al, 2015). Various studies across the world have studied the cardio protective effect of whole/ ground flaxseeds and their isolated bioactive compounds. In women, the studies have mainly focused on post-menopause. The present study chose pre-menopausal women as study population because in the phase I a high prevalence of metabolic aberrations among pre-menopausal women was observed. Whole/milled flaxseeds have been given the status of GRAS (generally regarded as safe). Only minor gastrointestinal disturbances can be caused by high intake of flaxseeds (Flax council of Canada, 2009). In the present study no subject reported any kind of side effect due to flaxseed intake during the supplementation.

Impact of flaxseed supplementation on lipid profile

A meta-analysis performed by Pan et al (2009) including 28 trials on whole, ground or defatted flaxseeds, with doses ranging from 20-50g revealed that these forms of flaxseeds significantly reduced TC and LDL-C levels by 0.21mmol/L (8.1mg/dl) and 0.16mmol/L (6.2mg/dl) respectively. The effect was found to be more pronounced in women than men with mean TC changes of 0.24 mmol/L (9.3mg/dl) and 0.09 mmol/L (5.03mg/dl). The impact of flaxseed supplementation was affected by initial cholesterol levels of the subjects. In the studies including subjects with high initial TC concentrations, a significant reduction of 0.17mmol/L (6.6mg/dl) was noted; but studies enrolling subjects with low initial concentrations of TC showed only small reduction of 0.03mmol/L (1.2mg/dl). Similarly a change of 0.13mmol/L (5.03mg/dl) and 0.00mmol/L in LDL-C was observed for the studies with high and low initial concentrations of LDL-C respectively (Pan et al, 2009).

Dodin et al (2005) performed a randomized, double-blind, wheat germ placebo-controlled clinical trial on 199 menopausal women to assess the long term impact of 40g flaxseed supplementation. 179 women completed the study and 15 women out of total, dropped out due to digestive problems (n= 10 and 5 in experimental and control arm respectively). The study showed that no significant reduction in LDL-C and TG levels was observed after 12 month supplementation of 40g flaxseeds. In wheat germ placebo group TC ($p<0.001$) and LDL-C ($p<0.01$) increased significantly after 12 months. The authors denoted it as the ceiling effect, which means it was almost impossible to observe a distinct effect of flaxseed due to the good baseline characteristics of participants.

The results of this long term RCT are quite comparable with the results of present study. In the present study also significant increase in TC and LDL-C levels were observed in control group post supplementation with maintenance of lipid levels and atherogenic indices in experimental groups. The effect of flaxseed supplementation was more pronounced in subjects with high LDL-C levels. Dodin et al (2005) did not perform any analysis based on initial lipid levels of the subjects. The present study achieved the similar results as a 12 month long trial in 8 weeks' time period only and one major plus point of the present study was that only 5-10g flaxseeds were used for supplementation which is quite low dose than 40g, used by Dodin et al (2005). 40g flaxseed imparted digestive disturbances in 10 study subjects where as in the present study no adverse effect was reported by the subjects due to flaxseed consumption. There are very less chances of arising any digestive problem of such a low dose of flaxseed even when consumed for a long period of time.

Wu et al (2010) studied the effect of combination of life style counselling and flaxseed supplementation (30g/day) on 236 subjects with metabolic syndrome and concluded that life style counselling along with flaxseed supplementation reduced the prevalence of MS (20.2%) greater that the life style counseling alone (16.9%). A comparison of HRT with flaxseed supplementation (40g crushed/day) was performed through a randomized cross over design on 16 hypercholesterolemic post-menopausal women by Lemay et al (2002) and it was revealed that flaxseeds failed

to bring significant favourable changes in lipid profile of the subjects after 2 month supplementation. Coulman et al (2009) through a randomized crossover trial on 16 post-menopausal women for 4 week supplementation of 25g flaxseeds showed significant increase in serum fatty acid ($p<0.05$) and lignan acid ($p<0.05$) content; however it failed to bring any substantial improvement in lipid profile.

A trial performed by Saxena and Katre (2014) on 50 dyslipidemic adult (40-60y) using 30g roasted flaxseed powder for 3 months significantly reduced TC ($p<0.001$), LDL-C ($p<0.001$), TG ($p<0.001$), VLDL-C ($p<0.001$) and increased HDL-C levels ($p<0.001$). Parameshwari and Nazni (2012) reported a significant reduction ($p<0.001$) in TC, LDL-C and TG with increase in HDL-C levels after 4 week supplementation of roasted flaxseeds in hyperlipidemic subjects where as raw flaxseeds did not show such impact. No control group was present in this study. Torkan et al (2015) in a recent RCT depicted that 30g raw flaxseed powder supplementation significantly reduced the serum TC, LDL-C and TG levels in hyperlipidemic subjects.

Lignan is the major bioactive compound of the flaxseeds which can impart cardio protective impact. Effect of isolated flaxseed lignans (360mg/day) was studied by Pan et al (2007) on type 2 diabetic subjects with mild hypercholesterolemia (n=73) for 12 weeks in a randomized placebo control experimental trial. The results of this study revealed no improvement in lipid profile of the subjects post supplementation. Another study by Fukumutsu et al (2010) concluded that 100g lignan supplementation per day significantly reduced LDL/HDL-C ratio in hypercholesterolemic men after 12 week supplementation. Comparatively higher doses were used by Zhang et al (2008) to explore the role of lignans in reducing lipid levels in hypercholosterolemic subjects in a placebo controlled RCT. The results of the study revealed that both 300 and 600mg SDG supplementation reduced the TC and LDL-C level significantly after 8 week supplementation. However the magnitude of effect was dose dependent.

Lignan supplement significantly reduced TC and LDL-C concentrations by 0.28 mmol/L (10.8mg/dl) and 0.16 mmol/L (6.2mg/dl) respectively as reported in a meta-analysis including 5 trials with doses from 200 to 600 mg for lignans (Pan et al, 2009).

Flaxseed oil is rich in ALA which is another beneficial constituent of flaxseeds. A meta-analysis of 13 studies on flaxseed oil (ranging from 1-38g) supplementation revealed that flaxseed oil supplementation could not significantly reduce the TC and LDL-C levels (Pan et al, 2009). A 12 week double blind placebo controlled supplementation trial supplementing 1g flaxseed oil in 86 healthy subjects did not show any significant difference in lipid profile post supplementation (Kaul et al, 2008). However, flaxseed oil supplementation in combination with MUFA rich Canola oil showed significant reduction in TC ($p<0.001$), LDL-C ($p<0.001$), HDL-C ($p<0.001$) and LDL/HDL-C ratio ($p<0.01$) among hypercholosterolemic subjects after 4 week supplementation (Gillingham et al, 2011).

Various animal studies have explored the mechanism behind beneficial impact of flaxseed supplementation of lipid levels. Lucas et al (2011) compared the effect of flaxseeds (15% wt/wt) and isolated flaxseed oil (amount equivalent to the oil contribution of whole flaxseed) in female ovariectomized golden Syrian Hamsters (n=48) and concluded that flaxseeds significantly reduced the TC levels of the hamsters (12%) than flaxseed oil (4%). However, HDL-C and TG levels showed no significant difference by both the supplementation. The authors hypothesized that increased bile acid synthesis is one of the major cholesterol-lowering mechanisms of flaxseed.

Mohammadi et al (2013) studied the mechanism of action of flaxseeds on the absorption of cholesterol in intestine through a diabetic rat model. He concluded that flaxseed supplementation significantly decreased Intestinal NPC1L1 transporter gene expression ($P<0.01$) which is a major transporter of cholesterol from intestinal lumen to erythrocytes. Gene expression of ABCG5 and G8 transporters which pump cholesterol from enterocytes back into intestinal lumen for excretion, was increased significantly ($p<0.001$) in rats supplemented with flaxseeds. A study performed by Tzang et al (2009) comparing effect of Butter, coconut and flaxseed oil based high fat diet showed that the flaxseed oil exerted hypocholesterolemic effect and this effect might be due to increases in LDL-receptor mRNA expression, and cholesterol catabolism/output.

Effect of ALA supplementation on cholesterol metabolism was explored by Morise et al (2004) by feeding flaxseed and butter based diets (12.5%) to hamsters for a 9 week period. Authors concluded that the favorable impact of flaxseed diet was imparted through modified activities of HDL receptor (SR-BI), HMG CoA reductase and cholesterol 7alpha-hydroxylase enzymes. A recent study on goats results that high ALA diet upregulates the expression of transcription factor PPAR-α which plays an important role in lipid metabolism (Ebrahimi et al, 2015).

Impact of flaxseed supplementation on inflammation

In the present HsCRP was used as a marker for measurement of inflammation. Both 5g and 10g supplementation failed to bring any positive impact on the HsCRP levels in comparison to control subjects.

Several mechanisms have been proposed by which ALA can modulate the inflammatory process in human. n-3 PUFAs are hypothesized to affect inflammation mainly through altered eicosanoid production, also through cell signaling and gene expression (Calder et al, 2011; Stulnig, 2003). n-3 PUFAs can impact the expression of the cytokine genes TNFα and IL-6, thus altering their production (Joffe et al, 2013).

Hutchins et al (2013) revealed in a crossover RCT on obese pre diabetic men and women that 13g and 26g of flaxseed supplementation for 12 weeks did not bring any desirable changes in inflammatory markers high sensitivity C-reactive protein, adiponectin, and high-sensitivity interleukin-6. A study performed by Dewell et al (2011) compared effect of low and high doses of both plant and marine n-3 fatty acid on the inflammatory markers of subjects with metabolic syndrome (n=100). After 8 week supplementation of plant n-3 fatty acid (2.2 and 6.6g/day for low and high group respectively) and marine n-3 oil ((1.2 and 3.6 g/d EPA/DHA for low and high group respectively) no significant difference was observed in three distinct inflammatory markers i.e IL-6, monocyte chemotactic protein-1 (MCP-1) and soluble intercellular adhesion molecule-1 (sICAM-1).

Flaxseeds imparted beneficial impact on inflammation while supplementing in conjunction with a weight loss in a study performed by Cassani et al (2015). A diet

with low carbohydrate (32% of total energy) and 60g flaxseeds for 42 days significantly ($p<0.05$) reduced HsCRP (25%) and TNFα levels (46%) among subjects in comparison to diet with 35% carbohydrate and 60g rice powder.

Fantuch et al (2011) assessed the impact of flaxseed powder supplementation on morbid obese candidates of bariatric surgery (n=16) with HsCRP levels >5mg/L. 60 g flaxseed powder containing 10g ALA did not significantly reduce HsCRP levels after 12 week supplementation in this double blind placebo RCT. However other inflammatory and coagulation markers like neutrophil count decreased and fibrinogen, complement C4, prothrombin time and carotid diameter remained stable post flaxseed supplementation. In placebo (cassava powder) these measurements increased further.

A recent systematic review and meta-analysis performed by Ren et al (2016) on the effect of flaxseed intervention (flaxseed/ flaxseed oil/ lignans) on inflammatory marker CRP concluded that flaxseed interventions had no effect on reduction of CRP (-0.13mg/L; 95% CI: −0.44 to 0.19; p = 0.428). The meta-analysis included 20 randomized control trials with doses ranging from 13-60g of whole/ground flaxseed, 1.2-11.6g of ALA or 21-640g of lignans per day. The results are in line with the present study.

The sub group analysis of the studies revealed that magnitude of obesity affected CRP levels post supplementation as studies with mean BMI of the subjects >30 showed significant reduction in CRP as they tend to have greater levels of initial CRP levels (Ren et al, 2016). In the present study only overweight and obese subject without morbid obesity were included. When data was analyzed based on initial HsCRP levels it showed that 5g supplementation did not have any added advantage on reduction of CRP levels in high HsCRP group post supplementation. 10g flaxseed supplementation presented a higher reduction in subjects with high HsCRP levels however the effect seemed to be nullified as in control group a significant reduction in subjects with high HsCRP levels ($p<0.05$) was observed.

Increase in age (>50y) also showed a non-significant trend towards greater reduction of CRP levels in the meta-analysis (Ren et al, 2016). Present study included pre-

menopausal women ranging from 30-50 years of age thus the probability of significant reduction of HsCRP levels further decreased.

The meta-analysis further revealed that whole flaxseed alone was found to have borderline significant reduction in CRP levels. However flaxseed oil did not show any beneficial impact on CRP levels. The analysis included studies with both healthy and diseased subjects like cancer, renal failure and authors specified that the magnitude of influence of flaxseed supplementation on inflammation may be different in healthy subjects than the diseased ones.

Although whole flaxseeds were used in the study, the apparently young, healthy and non-morbid obese population of the present study possibly would have led to minimal effect of flaxseed supplementation on the CRP levels of the subjects.

Impact of flaxseed supplementation on insulin resistance

In the present study insulin sensitivity was improved and insulin resistance decreased through both 5g and 10g flaxseed supplementation though not statistically significant. Controls showed adverse impact on insulin resistance post supplementation period. 10g flaxseed supplementation significantly reduced HOMA IR level ($p<0.05$) and increased % sensitivity ($p<0.05$) in subjects with HOMA IR levels >1.2. The results are in line with a crossover RCT performed by Hutchins et al (2013) on 25 obese pre-diabetic men and post-menopausal women. The results showed that 13g ground flaxseed supplementation for 12 weeks significantly reduced insulin and HOMA IR levels; however no impact was observed after 0g (control group) and 26g flaxseed supplementation.

A randomized cross over trial performed by Rhee and Brunt (2011) on 9 obese glucose intolerant subjects showed that 40g flaxseed supplementation for 12 weeks significantly reduced HOMA IR values ($p<0.05$). The reduction was hypothesized due to antioxidant activity of flaxseeds as thiobaribituric acid reactive substance (TBARS) levels also significantly reduced in the study ($p<0.05$) and found to be correlated with HOMA IR ($r=0.62$; $p<0.001$). Muramatsu et al (2010) studied the association of n-3 fatty acids with insulin resistance in a cross sectional study on 3383 Japanese

subjects (35-66y). The study revealed that the odds of having IR decreased across the quartiles of ALA intake (p= 0.01) in subjects with a BMI of < 25 kg/m^2 (*P* for interaction = 0.033). A meta-analysis performed by Akinkoulie et al (2011) including 11 RCT and 618 subjects resulted that n-3 PUFA significantly increased insulin sensitivity (SMD 0.30, CI 0.03–0.58) in the HOMA IR subgroup, when compared to placebo; though overall analysis found no effects of n-3 PUFA on insulin sensitivity (SMD 0.08, 95% CI –0.11–0.28). A study performed by Rasic-Milutinovic et al (2007) on chronic kidney failure patients on maintenance hemodialysis revealed that 8 week supplementation of EPA and DHA (2.4g/day) significantly reduced insulin resistance denoted by HOMA-IR and serum insulin levels (p<0.01).

n-3 fatty acids can improve the glucose metabolism through altering different mechanisms like increase in membrane fluidity, enhance the number of insulin receptors and insulin action, increase the number of GLUT-4 receptors, regulation of balance between pro- and anti-oxidants (Das, 2005; Lichtenstein and Schwab, 2000; Simopoulos, 1999). Increased percentage of AA, EPA and DHA in the skeletal muscle phospholipids is also hypothesized to reduce insulin resistance through improvement in action of insulin in muscles. n-3 fatty acids can also modulate the activity of PPAR-γ leading to increased insulin activity (Olalla et al, 2009). A study performed by Prasad (2002) resulted that the hypoglycemic effect of the SDG is possibly through suppressing expression of phosphoenolpyruvate carboxykinase (PEPCK) gene. PEPCK works as rate-limiting enzyme during gluconeogenesis and its suppression can lead to improved glucose metabolism. A review performed by Bhaswant et al, 2015 stated that different n- fatty acid from diet i.e. ALA, EPA and DHA can decrease insulin resistance through different mechanisms. ALA predominantly increases insulin sensitivity through its anti-obesity effect. Flax lignan have potential to improve insulin sensitivity through upregulation of GLUT-4 expression as indicated in a recent study (Wang et al, 2015) on obese mice.

Impact of flaxseed supplementation on obesity

Flaxseeds are believed to exert beneficial impact on obesity primarily through appetite control. Monteiro et al (2016) studied three different forms of flaxseeds on

the appetite and satiety of overweight and obese females (n=265). The results indicated that 30g supplementation of defatted brown flaxseed showed highest appetite reduction and increased satiety in comparison to control, whole brown flaxseed flour, golden flaxseed flour and guar gum fiber probably due to its high viscosity and protein content. Another single blinded crossover RCT performed by Ibrugger et al (2012) on 24 subjects revealed that flax drink containing 2.5g soluble fiber significantly increased satiety and sensation of fullness in comparison to control drink. Flax drink and flax tablet both showed decreased appetite in the subjects. In contrast the satiety and feeling of fullness as indicated by visual analog scale did not differ in women in late postoperative stage of bariatric surgery after consuming whole and defatted flaxseed enriched meal (Cohen et al, 2013).

A nutrigenomic study performed by McCullough et al (2011) explored the association of flaxseed supplementation with leptin levels and observed that 10% flaxseed diet significantly increased ALA levels of adipose tissues which were positively associated with leptin gene expression. However the adiponectin gene expression remained unaffected. SDG can attenuate obesity through increased in serum adiponectin level, decrease in gene expression of fatty acid synthase and sterol regulatory element-binding protein-1c in liver, promotion of gene expression enzymes and receptors linked to β-oxidation like acyl-CoA oxidase, carnitine palmitoyl transferase-1, and peroxisome proliferator-activated receptor α (Tominaga et al, 2012; Fukumitsu et al, 2008). LC n-3 PUFA can potentially increase in lean tissue mass and metabolic rate thus leading to body fat reduction (Buckley and Howe, 2010).

In the present study both 5g and 10g flaxseed supplementation showed no significant improvement on BMI and abdominal obesity of the women. Body fat percent was significantly reduced ($p<0.01$) in 5g flaxseed group post supplementation, but did not improve in 10g flaxseed group.

A study performed by Kapoor et al (2011) showed that 15g and 20g flaxseed supplementation in diabetic post-menopausal women reduced the BMI and weight after 2 month supplementation though not statistically significant. Gillingham et al

(2012) compared the effect of saturated, MUFA and ALA rich diet (37% fat from total energy; 72% of total fat coming from MUFA or ALA rich oil) on total energy expenditure and body composition of hypercholosterolemic subjects (n=34). The results revealed that 4 weeks supplementation of canola oil (MUFA rich) and flaxseed oil (ALA rich) neither increased the energy expenditure and substrate oxidation nor improved body composition. Effect of lifestyle counselling alone (LC) and its combination with either flaxseed (LCF, LC+30g flaxseed) or walnut (LCW, LC+30g walnut) on management of metabolic syndrome (n=283) was studied by Wu et al (2010) through a randomized controlled trial. After 12 week supplementation all three groups showed significant reduction in the prevalence of central obesity and the reversion rate was highest in the LCF (19.2%; P = 0.008) in comparison to LCW (16.0%; P = 0.04) and LC group (6.3%). Similar trial was performed (except the walnut group) in a study performed by Yari et al (2016) on metabolic syndrome subjects (n=44) and observed that weight, waist circumference, and body mass index decreased significantly in both groups. The reduction in flaxseed group was significantly higher in comparison with controls ($p < 0.05$).

A study performed by Machando et al (2015) depicted that 28g/day flaxseed supplementation (brown or golden) for 11 weeks did not significantly alter the anthropometric indices among overweight adolescent subjects in comparison to wheat bran (28g/day) controls. Barre et al (2012) studied the effect of SDG (600mg/day) on CVD risk factors in type 2 diabetic subjects (n=62) through a double-blind, randomized crossover placebo-controlled study design and elucidated that after 3 months supplementation of SDG mean waist circumference levels were found to be significantly different in SDG group than placebo after adjustment for multiple comparison adjustment for age, gender and order of treatment.

Impact of flaxseed supplementation on blood pressure

In the present study systolic blood pressure was significantly reduced after 5g flaxseed supplementation. Prevalence of pre and stage I hypertension was significantly reduced after 5g and 10g flaxseed supplementation ($p<0.05$ for both).

In the recent years two meta-analyses have been performed by different authors focusing exclusively on effect of flaxseed and its products on blood pressure. Khalesi et al (2015) performed a meta-analysis on 11 studies (14 trials) and resulted that flaxseed supplementation significantly reduced SBP (-1.77 mm Hg; 95% CI: -3.45, -0.09 mm Hg; P = 0.04) and DBP (-1.58mm Hg; 95% CI: -2.64, -0.52 mm Hg; P = 0.003). The sub group analysis showed that consumption of whole flaxseed and duration of supplementation (≥12 weeks) exerted more pronounced reduction in SBP and DBP. Higher baseline blood pressure (>130 mm Hg) did not influence the magnitude of reduction of blood pressure. The doses of whole/ground flaxseeds, flax oil, ALA and lignan were 30-50g/day, 1.2-3.6g flax oil, 2.2-6.6g/day and 360-600mg/day respectively in the studies included for meta-analysis.

Another meta-analysis executed by Ursoniu et al (2016) with 15 trials (comprising 19 treatment arms) with 1302 participants reported similar results. As indicated by Ursoniu et al (2016) supplementation of flaxseeds and its components significantly reduced SBP (-2.85 mmHg, 95%CI: -5.37 to -0.33, p = 0.027) and DBP (-2.39 mmHg, 95%CI: -3.78 to -0.99, p = 0.001). In sub group analysis flaxseed powder/ whole flaxseed were found to significantly reduce SBP levels ($p < 0.001$) but not oil ($p = 0.211$) and lignan extract ($p = 0.885$). Supplementation for longer duration (≥12 weeks) had greater impact on reduction in SBP and DBP. Thus both the meta-analyses performed during almost similar timeline concluded with comparable results. However none of them recommended any dose of flaxseeds and its components to exert desirable impact. The results of present study showed reduction in SBP using a lower dose in comparison to the studies included in meta-analyses.

Caliguiri et al (2014) explored the mechanism behind reduction in blood pressure through a randomized double-blinded, controlled clinical trial, in subjects with peripheral arterial disease (75% hypertensive). The study indicated that 30g milled flaxseed supplementation for 6 months brought significant reductions in systolic (−10 mm Hg) and diastolic (−7 mm Hg) blood pressure. Subjects with decreased soluble epoxide hydrolase–derived oxylipins, demonstrated a significant decrease in SBP in comparison to those with increased plasma soluble epoxide hydrolase–

derived oxylipins. Soluble epoxide hydrolase–derived oxylipins can lead to vascular relaxation and promote inflammation. Further analysis showed that increasing concentrations of α-linolenic acid was correlated with decreased soluble epoxide hydrolase activity although not statistically significant. The study concluded that inhibition of soluble epoxide hydrolase, through ALA would have altered oxylipin concentrations leading to decreased blood pressure in subjects with peripheral arterial disease. ALA is also projected to reduce blood pressure through increases of prostaglandin I_2 and nitric oxide through bradykinin stimulation in an animal study performed by Sekine et al (2007).

The role of SDG on blood pressure has also been explored through different animal studies. Prasad (2004) demonstrated that SDG can act as anti-hypertensive agent through inhibiting guanylate cyclase enzyme, which is a potential enzyme in regulating vasodilation (Buys and Sips, 2014). SDG is also emerging as a potent ACE inhibitor through its effect on angiotensin I induced rise in the arterial blood pressure (Prasad, 2013).

Impact of flaxseed supplementation on thyroid profile, hematological indices, liver and kidney functions

There is scarce of studies on effect of flaxseed supplementation on thyroid disorders. In the present study 5g and 10g flaxseed supplementation did not bring any significant difference in mean TSH, T3 and T4 hormone levels. A double-blind, placebo-controlled, randomized clinical trial performed by Simbalista et al (2010) showed that 25g of flaxseed supplementation for 12 weeks did not bring any significant change in mean TSH and free thyroxine levels in postmenopausal women.

Stuglin and Prasad (2005) studied the effect of 32.7g flaxseed supplementation on biochemical profile of healthy males (n=15) and concluded that after 4 weeks supplementation of flaxseeds mean hemoglobin levels remained unaltered. Babu et al (2000) depicted through an animal model that whole and defatted flaxseed flour did not bring any significant changes in hemoglobin levels of the rats after 8 weeks supplementation. Another animal study on rabbits using isolated SDG (40mg/kg

body weight) also reported maintenance of the hemoglobin levels of the rabbits (Prasad, 2005) after 2 months supplementation. In the present study Hb levels were significantly increased in all the three groups therefore the increase cannot be attributed to effect of flaxseed intake.

Liver and kidney function tests were performed in the present study to rule out any possible adverse effects of flaxseed supplementation on these systems. The liver or kidney functions remained unaltered or their increase/decrease had little physiological significance. A study performed by Stuglin and Prasad (2005) reported that 32.7g flaxseed supplementation for 4 weeks did not bring and deleterious effect on both renal and hepatic functions. Levels of uric acid, which is a risk factor for cardiovascular diseases reduced significantly in both 5g and 10g flaxseed group post supplementation in the present study, though the initial levels were within the normal range. Similar results were observed in an animal study in which uric acids levels decreased after 20% flaxseed meal supplementation in F344 rats. (Bhathena et al, 2002).

SUMMARY

- No significant difference in the mean values of lipid profile was observed in the two supplementation groups (5g and 10g) post supplementation.
- In the control group a significant rise of TC ($p<0.001$), LDL-C ($p<0.001$), TC/HDL-C ratio ($p<0.001$) and LDL-C/HDL-C ratio ($p<0.001$) was found.
- On segregating the data based on BMI, HsCRP and HOMA IR categorization, it was revealed that elevated levels of these parameters did not alter the magnitude of impact of flaxseeds supplementation on the lipid profile of the subjects.
- Both 5g and 10g flaxseed supplementation brought better impact on the lipid profile of the subjects with initially high levels of LDL-C or AIP, though not statistically significant.
- HOMA IR and insulin values indicated a non-significant decrease after supplementations of both 5g and 10g flaxseeds.

- % insulin sensitivity fairly increased after 5g flaxseed supplementation.
- Insulin, HOMA IR and % insulin sensitivity improved greater in subjects having initially high HOMA IR levels (>1.2) post 5g flaxseed supplementation.
- Insulin ($p<0.05$) and HOMA IR ($p<0.05$) levels significantly decreased and % insulin sensitivity ($p<0.05$) significantly increased in subject with high HOMA IR levels (>1.2) post 10g flaxseed supplementation.
- Inflammatory status of the subjects remained unchanged after flaxseed supplementation as indicated by mean Hs-CRP values which showed a non-significant decrease in all three groups.
- The mean values of BMI, WC, HC, WHR and WSR remained constant throughout the study.
- Mean body fat percent ($p<0.01$) and SBP ($p<0.01$) levels significantly reduced in 5g flaxseed group post supplementation. However 10g flaxseed supplementation showed only a non-significant decrease in these parameters.
- Prevalence of pre/stage I hypertension significantly reduced in both 5g ($p<0.05$) and 10g ($p<0.05$) supplementation group.
- After supplementation, Hb values of all three groups ($p<0.01$, $p<0.001$, $p<0.001$) increased significantly than baseline.
- No difference in the values of thyroid function test was observed post supplementation.
- A significant decrease in the total ($p<0.01$), indirect ($p<0.01$) and direct bilirubin ($p<0.01$) was seen among control groups. On the contrary SGPT ($p<0.01$) and GGT ($p<0.01$) values increased significantly in this group. However, all the values were within the normal physiological range.
- Uric acid values of two supplementation groups decreased significantly ($p<0.05$ and $p<0.01$ for 5g and 10g group respectively). No such change in control group was observed.

Conclusions

Flaxseed supplementation trial exhibited mixed results on the metabolic profile of the subjects. Flaxseeds exerted beneficial impact on blood pressure, insulin resistance (in subjects with HOMA IR >1.2) and body fat percent (5g flaxseed group), maintained lipid profile, anthropometric indices and failed to alter inflammation in the healthy overweight/obese subjects. Most of the studies available in literature on flaxseed supplementation have used high doses of flaxseeds and its components which is tedious to incorporate in the day to day life style of apparently healthy individuals. Therefore hassle free strategies like inclusion of 5-10g of roasted flaxseeds in the form of “mukhwas” in the daily diet can be adapted to reduce the risk of metabolic aberrations in population.