RESULTS AND DISCUSSION

The results of the present research are presented and discussed in this chapter under the following heads

Phase I: Prevalence of vitamin-D deficiency and determinants of vitamin-D status among free-living adult population of Vadodara city

Phase II: Impact of Vitamin-D supplementation on cardio-metabolic profile of subjects with type-II diabetes mellitus

- ***Phase II (a):*** Screening and collection of baseline data
- ***Phase II (b):*** Randomized control trial to study the impact of vitamin-D3 granules on serum 25(OH)D status and cardio-metabolic profile of subjects with T2DM
- ***Phase II (c)*** Washout effect of vitamin-D supplementation on serum 25(OH)D status of T2DM subjects

Phase III: Comparison between the free-living population (non-diabetic) and the diabetic population in respect to their vitamin-D status, biochemical parameters, biophysical measurements, physical activity pattern and nutrient intake.

Phase IV: Development of Nutrition health Education (NHE) material regarding vitamin-D and type-II diabetes mellitus.

PHASE I

PREVALENCE OF VITAMIN-D DEFICIENCY & DETERMINANTS OF VITAMIN-D STATUS AMONG FREE-LIVING ADULT POPULATION OF VADODARA CITY

Vitamin D is a group of fat-soluble prohormone. It is a well known fact that vitamin-D does the traditional function of maintaining calcium-phosphate homeostasis in the body. Vitamin-D is utilized by the body through various vitamin-D receptors (VDR) present on many tissues and organs in the body. These VDRs have now opened up new roles for vitamin-D in the pathogenesis of many auto-immune and non-communicable diseases. Vitamin-D deficiency (VDD) has assumed pandemic proportions world-wide, with the prevalence ranging from 31-98 % across the globe (Wacker & Holick, 2013). Studies in India too report widespread vitamin-D deficiency/ insufficiency among various socio-economic groups, different ages, on both genders and different race, as well as different disease states, such as primary hyperparathyroidism (Harinarayan & Joshi, 2009; Harinarayan et al., 1995). Though VDD is highly prevalent in India, there is paucity of data about the vitamin-D status of population residing in western India which receives ample sunlight round the year. Also there is need to

identify and study the various determinants of vitamin-D levels among the population and its effect of various health parameters and disease conditions. Thus keeping this in view, the present study was planned to map the prevalence of VDD among adult population of Vadodara, which is the 18th largest city of the country located in western part of India. Also an attempt was made to identify the determinants of vitamin-D status and relate them to various health conditions present among the population.

Experimental Design

For the study, the subjects were enrolled from the free-living population of urban Vadodara. The detailed methodology regarding selection of subjects is given in the methods and material chapter. In all, there were 129 subjects selected from five zones of Vadodara in the age group of 30 to 60 years.

The results of this section are discussed under the following broad heads:

1. Background information of the subjects
2. Bio-physical characteristics of the subjects
3. Lifestyle practices of the subjects
4. Non-invasive determinants for vitamin-D status
5. Prevalence of vitamin-D deficiency and other clinical conditions
6. Analysis by segregating the subjects based on their
    a. Vitamin-D status
    b. Body Mass Index (BMI)
    c. Vitamin-D quartiles

1) BACKGROUND INFORMATION OF THE SUBJECTS

Background information on the subjects was collected using a pre-tested questionnaire through a one-to-one interview and included information on religion, education, occupation and type of family and income. The background information of subjects enrolled for prevalence of vitamin-D deficiency and its risk factor analysis is given in Table 4.1.1. About 98.4% subjects were Hindus and 1.6% subjects were Jain. Information on educational background of the subjects depicted that all the subjects were literate. About 39.5% of the subjects were graduates and 21.7% of subjects had SSC or HSC education. Majority of the subjects were married (92.2%) and belonged to nuclear families (59.7%). Most of the women (84.1%) were housewives. Men were either engaged in service (63.8%) or had their own business (19.1%). Most of the subjects (44.9%) had per capita income between Rs 5000-10,000/-.

Table 4.1.1 BACKGROUND INFORMATION OF THE SUBJECTS (n, %)

Background Information	Females (n=82)	Males (n=47)	Total (n=129)
Religion			
Hindu	82 (100)	45 (95.7)	127 (98.4)
Jain	0	2 (4.3)	2 (1.6)
Education			
Primary	3 (3.6)	1 (2.1)	4 (3.1)
SSC	21 (25.6)	7 (14.9)	28 (21.7)
HSC	20 (24.4)	8 (17)	28 (21.7)
Graduate	28 (34.1)	23 (48.9)	51 (39.5)
Postgraduate	9 (10.9)	8 (17)	17 (13.2)
Others	1 (1.2)	0	1 (0.7)
Marital Status			
Unmarried	0	5 (10.6)	5 (3.9)
Married	77 (93.9)	42 (89.4)	119 (92.2)
Widow/widower	5 (6.1)	0	5 (3.9)
Occupation			
Unskilled Labour	2 (2.4)	1 (2.1)	3 (2.3)
Housewife	69 (84.1)	0	69 (53.5)
Service	8 (9.7)	30 (63.8)	38 (29.5)
Business	3 (3.6)	15 (31.9)	18 (13.9)
Retired	0	1 (2.1)	1 (0.7)
Type of family			
Nuclear	52 (63.4)	25 (53.2)	77 (59.7)
Joint	21 (25.6)	9 (19.1)	30 (23.3)
Extended	9 (10.9)	13 (27.6)	22 (17.1)
Per Capita Income (Rs) Range		1000 - 69,444	
<5000	25 (30.5)	16 (34)	41 (31.8)
5000-10,000	40 (48.8)	18 (38.3)	58 (44.9)
>10,000	17 (20.7)	13 (27.6)	30 (23.3)

Values in parenthesis indicate percent

Age-wise distribution of the subjects

The age-wise distribution of the subjects is given in Table 4.1.2. As observed, the mean age of subjects was 44.3 years. Men were slightly younger than women subjects (43.5 vs 45.1 years). The subjects were more or less equally distributed in the various age ranges of 30-40, 41-50 and 51-60 years, however majority (37.2%) were in 30-40 years group.

Family history of diseases among the subjects

The family history of diseases among the subjects is depicted in Table 4.1.3. The information revealed that the family history was highest for hypertension (53.5%) followed by diabetes mellitus (41.1%) and then cancer (17.8%).

Medical history of the subjects

The self reported medical history of the subjects is given in the Table 4.1.4. Information on medical history of the subjects showed that prevalence of hypertension was maximum (11.6%) followed by the prevalence of thyroidism (4.7%). Overall the prevalence of medical conditions was quite low among the subjects.

Nutritional supplements taken by the subjects

The information regarding the nutritional supplements taken by the subjects is given in Table 4.1.5. About 22.5% (29/129) of the subjects were taking nutritional supplements. The commonly consumed supplement was a combination of calcium, iron and folic acid (20.7%) followed by calcium alone (17.2%). Six subjects (4.6%) were taking vitamin-D supplements either in combination of calcium and iron or alone.

Dependency Syndrome among the subjects

The dependency syndrome present among the subjects is given in Table 4.1.6. It revealed that none of the female subjects were presently having any dependency habit. However 10.6% males were consuming gutka regularly and 6.4% alcohol occasionally. Men also had past history of pan, gutka consumption and smoking.

2) BIO-PHYSICAL CHARACTERISTICS OF THE SUBJECTS

The anthropometric profile and blood pressure measurements of the subjects are showed in Table 4.1.7. The BMI was found to be on the higher side with a mean value of 25.9 Kg/m^2. Similarly the mean for parameters of abdominal obesity was also found to be on the higher side with mean WC of 92.5 cm, mean WHR of 0.92 and mean WSR of 0.6.

Table 4.1.2 AGE WISE DISTRIBUTION OF THE SUBJECTS (n, %)

Age (Years)	**Females (n=82)**	**Males (n=47)**	**Total (n=129)**
30-40	29 (35.4)	19 (40.4)	48 (37.2)
41-50	26 (31.7)	16 (34)	42 (32.6)
51-60	27 (32.9)	12 (25.5)	39 (30.2)
Mean age (Mean±SD)	45.1 ± 9.5	43.5 ± 8.4	44.3 ± 9.1

Values in parenthesis indicate percent

TABLE 4.1.3 FAMILY HISTORY OF DISEASES AMONG SUBJECTS (n, %)

	Females (n=82)	Males (n=47)	Total (n=129)
Diabetes Mellitus			
History Present	33 (40.2)	20 (42.6)	53 (41.1)
Parents	22 (26.8)	15 (31.9)	37 (28.7)
Siblings	2 (2.4)	1 (2.1)	3 (2.3)
Both	6 (7.3)	0	6 (4.7)
Grandparents + family	3 (3.6)	4 (8.5)	7 (5.4)
Hypertension			
History present	47 (57.3)	22 (46.8)	69 (53.5)
Parents	31 (37.8)	19 (40.4)	50 (38.7)
Siblings	3 (3.6)	2 (4.3)	5 (3.9)
Both	12 (14.6)	0	12 (9.3)
Grandparents + family	1 (1.2)	1 (2.1)	2 (1.5)
Dyslipidemia/ CHD			
History Present	12 (14.6)	7 (14.9)	19 (14.7)
Parents	10 (12.2)	6 (12.8)	16 (12.4)
Siblings	1 (1.2)	0	1 (0.7)
Both	1 (1.2)	0	1 (0.7)
Grandparents + family	0	1 (2.1)	1 (0.7)
Asthma			
History Present	13 (15.8)	6 (12.8)	19 (14.7)
Parents	9 (10.9)	3 (6.4)	12 (9.3)
Siblings	2 (2.4)	1 (2.1)	3 (2.3)
Grandparents + family	2 (2.4)	2 (4.3)	4 (3.1)
Cancer			
History Present	16 (19.5)	7 (14.9)	23 (17.8)
Parents	10 (12.2)	5 (10.6)	15 (11.6)
Siblings	3 (3.6)	0	3 (2.3)
Grandparents + family	3 (3.6)	2 (4.3)	5 (3.9)
Thyroid			
History Present	10 (12.2)	7 (14.9)	17 (13.2)
Parents	5 (6.1)	6 (12.8)	11 (8.5)
Siblings	4 (4.9)	1 (2.1)	5 (3.9)
Grandparents + family	1 (1.2)	0	1 (0.7)

Values in parenthesis indicate percent

TABLE 4.1.4 MEDICAL HISTORY OF THE SUBJECTS (SELF REPORTED) (n, %)

Condition	Females (n=82)	Males (n=47)	Total (n=129)
Hypertension	12 (14.6)	3 (6.4)	15 (11.6)
CHD	1 (1.2)	0	1 (0.7)
Dyslipidemia	2 (2.4)	1 (2.1)	3 (2.3)
Thyroidism	5 (6.1)	1 (2.1)	6 (4.7)
Rheumatiod Arthritis	2 (2.4)	0	2 (1.6)

Values in parenthesis indicate percent

TABLE 4.1.5 NUTRITIONAL SUPPLEMENTS TAKEN BY THE SUBJECTS (n, %)

	Females (n=82)	Males (n=47)	Total (n=129)
Supplements Taken	21 (25.6)	8 (17.0)	29 (22.5)
Types of supplements			
Calcium	5 (23.8)	0	5 (17.2)
Vitamin D	0	1 (12.5)	1 (3.4)
Iron	1 (4.8)	1 (12.5)	2 (6.9)
Vitamin B12	1 (4.8)	2 (25)	3 (10.3)
Multivitamin	2 (9.5)	2 (25)	4 (13.8)
Protein supplement	2 (9.5)	1 (12.5)	3 (10.3)
Calcium + Vitamin-D	2 (9.5)	0	2 (6.9)
Calcium + Iron + Vitamin-D	3 (14.3)	0	3 (10.3)
Calcium+ Iron+ Folic acid	5 (23.8)	1 (12.5)	6 (20.7)

Values in parenthesis indicate percent

TABLE 4.1.6 DEPENDENCY SYNDROME AMONG THE SUBJECTS (n, %)

Habits	Females (n=82)		Males (n=47)		Total (n=129)	
	Past	Present	Past	Present	Past	Present
Pan	0	0	2 (4.3)	0	2 (1.6)	0
Padiki	0	0	1 (2.1)	0	1 (0.8)	0
Gutka	0	0	4 (8.5)	5 (10.6)	4 (3.1)	5 (3.9)
Cheekni	1 (1.2)	0	0	0	1 (0.8)	0
Cigarette	0	0	1 (2.1)	0	1 (0.8)	0
Alcohol	0	0	1 (2.1)	3 (6.4)	1 (0.8)	3 (2.3)

Values in parenthesis indicate percent

TABLE 4.1.7 BIOPHYSICAL MEASUREMENTS OF THE SUBJECTS (Mean ± SD)

Variables	Females (n=82)	Males (n=47)	Total (n=129)	t-test *p* value
Weight (Kg)	59.6 ± 11.4	74.4 ± 15.5	65 ± 14.8	**0.000*** **
Height (cm)	152.9 ± 6.3	167.2 ± 6.6	158.1 ± 9.4	**0.000*** **
BMI (Kg/m^2)	25.5 ± 4.7	26.5 ± 4.7	25.9 ± 4.7	0.286
WC (cm)	92.8 ± 10.9	91.7 ± 11.6	92.5 ± 11.1	0.590
HC (cm)	100.8 ± 10.2	98.4 ± 9.8	99.9 ± 10.1	0.187
WHR	0.92 ± 0.07	0.93 ± 0.05	0.92 ± 0.07	0.704
WSR	0.61 ± 0.07	0.5 ± 0.06	0.6 ± 0.07	**0.000*** **
% Body fat	35.9 ± 5.8	29.8 ± 5.6	33.7 ± 6.5	**0.000*** **
SBP (mmHg)	126.7 ± 16.8	132.2 ± 16.3	128.7 ± 16.7	0.073
DBP (mmHg)	79.2 ± 9.9	83.6 ± 10.6	80.8 ± 10.3	**0.024*** [EVNA]

p< 0.001***, <0.05* EVNA= equal variance not assumed

The mean systolic blood pressure was 128.7 mmHg whereas mean diastolic blood pressure was 80.8 mmHg. Comparisons between male and female subjects revealed that weight, height and DBP values were significantly higher in males while WSR and percent body fat was higher among females. The blood pressure values however were in physiologically normal range.

Prevalence of overweight and obesity among the subjects

The prevalence of overweight and obesity as classified by BMI is shown in Table 4.1.8. Majority of the subjects were falling in the category of obesity with a percent prevalence of 48.8% and only 30% of the subjects studied had normal BMI. The abdominal obesity as assessed by WC, WSR and WHR revealed that more than 80% of the subjects had higher measurements, increasing the risk for Cardio Vascular Disease. The prevalence based on WC and WSR was significantly higher among female subjects as compared to male subjects.

3) LIFESTYLE PRACTICES OF THE SUBJECTS

The lifestyle practices of the subjects were assessed based on their physical activity pattern and their nutrient intake. The frequency of eating vitamin-D rich foods was also assessed using a food frequency questionnaire.

Physical Activity pattern of the subjects

An overview of the self reported physical activity pattern of the subjects as assessed by International Physical Activity (Short) Questionnaire is given in Table 4.1.9. The subjects were categorized according to their physical activity under 3 heads – low, moderate and high physical activity level. About 24.8% of the subjects were physically less active, of which most of the subjects were males. 58.9% of the subjects were moderately active; and predominately they were women. The mean hours of sitting was found to be 4.5 ± 1.6 with females spending little longer time in sitting activities as compared to male subjects (4.6 vs 4.3 hours per day).

Nutrient Intake of the subjects

Information on nutrient intake was collected by 24 hour dietary recall method. The mean value for each of the nutrient as per National Institute of Nutrition's 2010 guidelines is given in Table 4.1.10(A). Intake of calcium and vitamin-C was significantly higher among female subjects. Females were able to meet about 78% of the RDA for energy and 75% for proteins while males could meet around 65% of RDA for both nutrients (Table 4.1.10 (B)).

Table 4.1.8 PREVALENCE OF OVERWEIGHT AND OBESITY AMONG THE SUBJECTS (n, %)

Category based on BMI[#]	Females (n=82)	Males (n=47)	Total (n=129)	χ^2 *p* value
Underweight (BMI<18.5)	0	1 (1.2)	1 (0.7)	0.63
Normal (BMI:18.5 – 22.9)	26 (31.7)	12 (25.5)	38 (29.5)	
Overweight (BMI:23 – 24.9)	22 (26.8)	5 (10.6)	27 (20.9)	
Obese (BMI: ≥25)	34 (41.5)	29 (61.7)	63 (48.8)	
Based on % Body fat				
Body fat (F>30%, M>20%)	72 (87.8)	44 (93.6)	116 (89.9)	0.291
Based on Anthropometric Indices				
WC (F≥80, M≥90 cm)	75 (91.5)	30 (63.8)	105 (81.4)	**0.000*** **
WSR (≥ 0.5)	78 (95.1)	38 (80.9)	116 (89.9)	**0.01***
WHR (F≥0.85, M≥0.9)	73 (89)	38 (80.9)	111 (86)	0.197

p< 0.001***, <0.05* Values in parenthesis indicate percent

[#] **BMI criteria: Asia Pacific Classification**

TABLE 4.1.9 LEVELS OF PHYSICAL ACTIVITY AMONG SUBJECTS (n, %)

Physical Activity Level	Females (n=82)	Males (n=47)	Total (n=129)
Low	4 (4.9)	28 (59.6)	32 (24.8)
Moderate	57 (69.5)	19 (40.4)	76 (58.9)
High	21 (25.6)	0	21 (16.3)
Hours spent in sitting (Mean±SD)	4.6 ± 1.8	4.3 ± 1.2	4.5 ± 1.6

Values in parenthesis indicate percent

TABLE 4.1.10(A) NUTRIENT INTAKE OF THE SUBJECTS (MEAN ± SD)

Nutrients	Females (n=82)	Males (n=47)	Total (n=129)	t-test *p* value
Energy (Kcal)	1481 ± 445	1511 ± 389	1492 ± 424	0.697
Protein (gm)	41 ± 13.2	39.2 ± 12.1	40.6 ± 12.8	0.347
Fat (gm)	54.5 ± 22.3	57.9 ± 19.5	55.7 ± 21.3	0.381
Carbohydrates (gm)	200.4 ± 59.6	201.8 ± 54.4	200.9 ± 57.5	0.898
Iron (mg)	11.9 ± 6.3	12.2 ± 4.8	12.0 ± 5.8	0.761
Calcium	644.6 ± 438.5	472.1 ± 180.9	581.8 ± 374.7	**0.002**^{**EVNA}
β carotene (µg)	1867 ± 3299	1300 ± 2168	1701 ± 3014	0.359
Vitamin C (mg)	96 ± 86.2	48.9 ± 40.4	78.9 ± 76.2	**0.000**$^{*** EVNA}$
Crude Fibre (gm)	6.1 ± 2.9	5.2 ± 1.7	5.8 ± 2.6	0.027*
Total Dietary Fibre	14.4 ± 6.4$^{\#}$	10.0 ± 5.6$^{\#}$	12.8 ± 6.5$^{\#}$	**0.000**$^{*** EVNA}$
Insoluble Dietary Fibre	10.7 ± 4.9$^{\#}$	7.5 ± 4.6$^{\#}$	9.5 ± 5.0$^{\#}$	**0.000*** **
Soluble Dietary Fibre	3.5 ± 1.7$^{\#}$	2.4 ± 1.3$^{\#}$	3.2 ± 1.6$^{\#}$	**0.000*** **

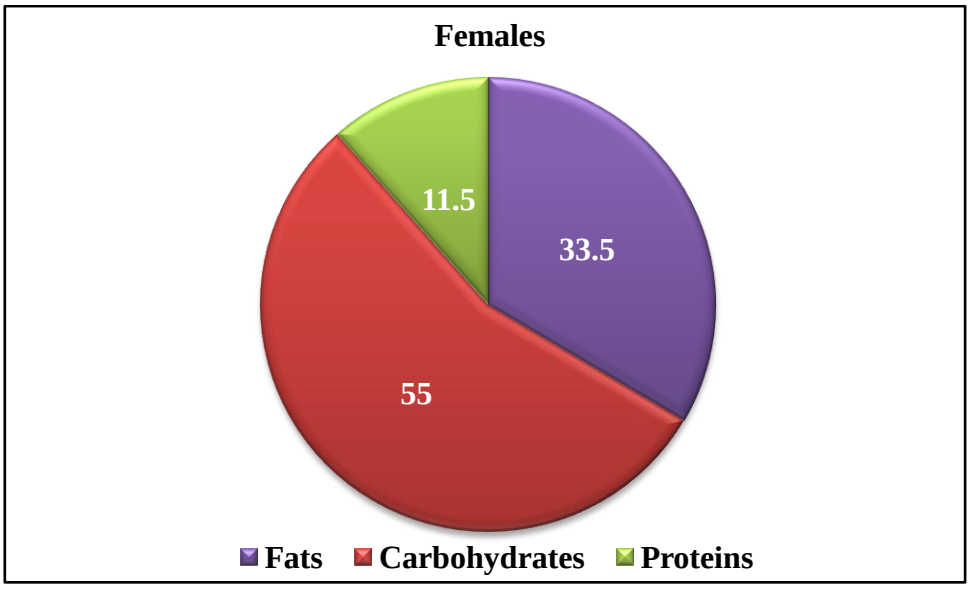
$^{\#}$as reported by NIN for listed foods

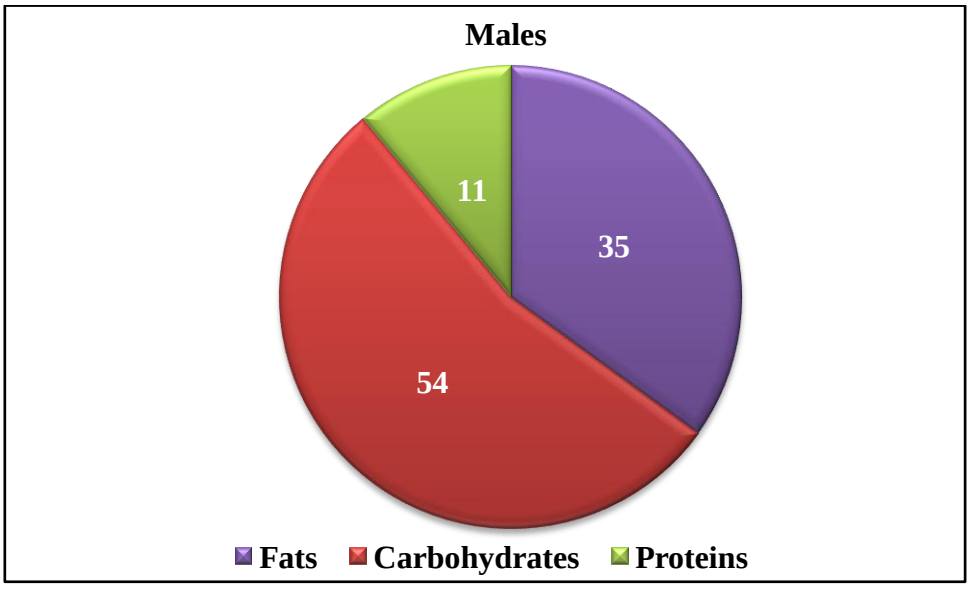
p< 0.001***, <0.01**, <0.05* EVNA= equal variance not assumed

TABLE 4.1.10(B) PERCENT RDA OF THE SUBJECTS

Nutrients	% RDA	
	Females (n=82)	Males (n=47)
Energy (Kcal)	77.9	65.1
Protein (gms)	74.5	65.3
Fat (gms)	272.5	231.6
Iron (mg)	56.7	71.8
Calcium (mg)	107.4	78.7
β carotene (µg)	38.9	27.1
Vitamin C (mg)	240	122.3

FIGURE 4.1.1 PERCENT ENERGY (KCAL) FROM MACRONUTRIENTS





Fat intake was very high among all the subjects. The β carotene intake was <50% of RDA for both female and male subjects. Similarly the fibre intake of the subjects was found to be low as per the recommended allowance of >20 gm/day. However, the subjects had more than adequate intake for vitamin C and for calcium in females. The percent energy from macronutrients revealed that fats contributed about 33.5% & 35% of the total calories in females and males respectively which clearly exceeded the recommended allowance of 20% energy. This resulted in lower percent of calories for females & males from carbohydrate (55% & 54% respectively) and very low from proteins (11.5% & 11% respectively) (Figure 4.1.1).

Dietary practices followed by the subjects

Information regarding a variety of dietary practices followed by the subjects was collected using a pretested questionnaire. The details are given in Table 4.1.11. Information revealed that majority of the subjects consumed vegetarian diets (68.2%) followed by ovo-vegetarians (16.3%). About 77.5% of the subjects consumed tea on regular basis and almost all the subjects (91.5%) consumed iodized salt. Regarding oil consumption practices only 14% subjects practiced rotation of oil and most of them did it at every three months (6.9%) or at two months (5.4%). About 34% of the subjects were discarding the left over oil after deep frying and only 14% were using it again for deep frying. Majority of the subjects (49.6%) were using the left over oil in sautéing vegetables or kneading dough for making bhakris, rotis or parathas. An attempt was made to study the amount of oil, salt and sugar consumed by the subjects per day. The data revealed that per day oil (42.8 ± 0.02 g/day) and salt (7.7 ± 0.005 g/day) consumption was very high among all the subjects. The per day salt consumption of females was significantly higher as compared to males. The mean per day sugar consumption was 32.7 ± 0.02 g/day, which was also on higher side.

Oil Consumption pattern of the subjects

The information on type of oil used for cooking purpose by the subjects was also collected through the questionnaire. As shown in the Table 4.1.12, almost 38% of the subjects reported the usage of cotton seed oil as compared to other oils. The other predominant oil used was ground nut oil (19.4%) followed by a wide variety and combinations in the type of oil used by the subjects. Only 11.6% subjects were doing blending of different oils and the most commonly used combination was Groundnut + Cotton seed + Corn oil (4.7%).

TABLE 4.1.11 DIETARY PRACTICES OF THE SUBJECTS (n, %)

Practices	Females (n=82)	Males (n=47)	Total (n=129)
Type of Diet			
Vegetarian	67 (81.7)	21 (44.7)	88 (68.2)
Non- vegetarian	8 (9.8)	12 (25.5)	20 (15.5)
Ovo- vegetarian	7 (8.5)	14 (29.8)	21 (16.3)
Hot beverage Consumption			
Tea	58 (70.7)	42 (89.4)	100 (77.5)
Coffee	7 (8.5)	5 (10.6)	12 (9.3)
Type of Salt consumed			
Iodized	73 (89)	45 (95.7)	118 (91.5)
Non-iodized	9 (11)	2 (4.3)	11 (8.5)
Same type of oil used whole year			
Yes	71 (86.6)	40 (85.1)	111 (86)
No	11 (13.4)	7 (14.9)	18 (14)
Duration of Oil rotation			
2 months	4 (4.9)	3 (6.4)	7 (5.4)
3 months	5 (6.1)	4 (8.5)	9 (6.9)
6 months	2 (2.4)	0	2 (1.5)
Usage of left over deep fried oil			
Deep Frying	14 (17.1)	4 (8.5)	18 (13.96)
Other Usage	44 (53.6)	20 (42.6)	64 (49.6)
Discard The Oil	23 (28)	21 (44.7)	44 (34.1)
Oil consumption per person per day (g/day)			
Mean ± SD	44.6 ± 0.02	39.6 ± 0.01	42.8 ± 0.02
't' test *p*-value	0.138		
Salt consumption per person per day (g/day)			
Mean ± SD	9.8 ± 0.005	3.9 ± 0.002	7.7 ± 0.005
't' test *p*-value	**0.000*** **		
Sugar consumption per person per day (g/day)			
Mean ± SD	31.6 ± 0.02	34.5 ± 0.02	32.7 ± 0.02
't' test *p*-value	0.410		

p< 0.001*** Values in parenthesis indicate percent

TABLE 4.1.12 TYPE OF OIL USED BY THE SUBJECTS

Type of oil	Females (n=82)	Males (n=47)	Total (n=129)
Groundnut (GN) oil	15 (18.3)	10 (21.3)	25 (19.4)
Cotton Seed oil	34 (41.5)	15 (31.9)	49 (37.9)
Corn oil	7 (8.5)	7 (14.9)	14 (10.8)
Sunflower oil	7 (8.5)	9 (19.1)	16 (12.4)
Mustard oil	3 (3.6)	0	3 (2.3)
Ricebran oil	4 (4.9)	3 (6.4)	7 (5.4)
Groundnut + Corn oil	1(1.2)	1 (2.1)	2 (1.5)
Groundnut + Sunflower	2 (2.4)	0	2 (1.5)
Corn + Sunflower + Soya	4 (4.9)	0	5 (3.9)
GN + Cotton seed + Corn	4 (4.9)	2 (4.3)	6 (4.7)

Values in parenthesis indicate percent

Type of milk consumed by the subjects

The milk consumption pattern of the subjects is given in Table 4.1.13. A variety of milk was consumed by the subjects with majority (34.1%) consuming the *Shakti* brand of Baroda Dairy followed by its full fat milk brand *Gold* (31.8%). About 18.6% and 11.6% subjects were consuming loose buffalo and cow's milk purchased from milk sellers respectively.

Frequency of consuming vitamin-D food sources

Major food sources of vitamin-D are milk and some milk products, eggs and certain varieties of fish for which frequency of consumption for the subjects was collected. The gender-wise consumption pattern is depicted in Table 4.1.14. As seen in the Figure 4.1.2, majority of the subjects being vegetarians, the consumption of eggs, fish and other non-vegetarian foods was very less. Highest consumption on daily basis was of ghee (81.3%) followed by milk (50.8%) and buttermilk (24.2%). Curd was consumed by maximum number of subjects three to five times a week (30.5%). Maximum consumption of paneer (43%), butter (39.8%) and cheese (39.1%) was on monthly basis. There were 29 (22.5%) subjects who did not consume milk at all and 24 (18.6%) subjects who did not consume curd at all. Therefore it was evident that consumption of vitamin-D rich food sources was not very frequent amongst the subjects

4) NON-INVASIVE DETERMINANTS FOR VITAMIN-D STATUS

The non-invasive determinants of vitamin-D levels such as sun exposure, skin type and type of clothing were studied among the subjects. Information on these determinants of vitamin-D revealed that almost 15% of the subjects had dark coloured skin, which is regarded as a risk factor for vitamin-D deficiency, while most of the subjects belonged to the wheatish skin colour category (54.3%). About 71% of the subjects were going out in the sun during day time between 10 am to 2 pm, with 73% of them having sun exposure for more than 20 minutes per day. The recommended level for sunlight exposure for appropriate vitamin-D synthesis is 15-20 minutes a day. However it was heartening to observe that the mean time of sun exposure was 36.5 minutes per day with maximum subjects (45.7%) going out on daily basis. Only 9% of the subjects reported the use of sunscreen who were all female and about 3% of females covered the skin while going out, which can lower the synthesis of vitamin-D because of less exposure (Table 4.1.15).

TABLE 4.1.13 TYPE OF MILK CONSUMED BY THE SUBJECTS (n, %)

Type of Milk	Females (n=82)	Males (n=47)	Total (n=129)
Cow	11 (13.4)	4 (8.5)	15 (11.6)
Buffalo	19 (23.2)	5 (10.6)	24 (18.6)
Skim milk	6 (7.3)	2 (4.3)	8 (6.2)
Shakti	23 (28.0)	21 (44.7)	44 (34.1)
Gold	26 (31.7)	15 (31.9)	41 (31.8)

Values in parenthesis indicate percent

TABLE 4.1.14 FREQUENCY OF CONSUMPTION OF VITAMIN-D RICH FOODS CROSS TABULATED BY GENDER (n, %)

Food Items	Females (n=82)					Males (n=47)				
	Daily	3-5 times a week	Weekly	Monthly	Never	Daily	3-5 times a week	Weekly	Monthly	Never
Milk	48 (59.3)	9 (11.1)	9 (11.1)	1 (1.2)	14 (17.3)	17 (36.2)	9 (19.1)	2 (4.3)	4 (8.5)	15 (31.9)
Curd	15 (18.5)	22 (27.2)	15 (18.5)	9 (11.1)	20 (24.7)	6 (12.8)	17 (36.2)	15 (31.9)	5 (10.6)	4 (8.5)
Cheese	0	2 (2.5)	13 (16.0)	34 (42.0)	32 (39.5)	2 (4.3)	3 (6.4)	8 (17.0)	16 (34.0)	18 (38.3)
Paneer	0	2 (2.5)	14 (17.3)	33 (40.7)	29 (35.8)	3 (6.4)	3 (6.4)	7 (14.9)	22 (46.8)	12 (25.5)
Buttermilk	22 (27.2)	21 (25.9)	10 (12.3)	5 (6.2)	23 (28.4)	9 (19.1)	10 (21.3)	17 (36.2)	7 (14.9)	4 (8.5)
Butter	1 (1.2)	5 (6.2)	13 (16.0)	33 (40.7)	29 (35.8)	0	5 (10.6)	8 (17.0)	18 (38.3)	16 (34.0)
Ghee	63 (77.8)	2 (2.5)	10 (12.3)	4 (4.9)	2 (2.5)	41 (87.2)	5 (10.6)	0	0	1 (2.1)
Egg	0	2 (2.5)	6 (7.4)	1 (1.2)	72 (88.9)	1 (2.1)	3 (6.4)	12 (25.5)	10 (21.3)	21 (44.7)
Fish	0	0	3 (3.7)	1 (1.2)	77 (95.1)	0	0	3 (3.7)	6 (12.8)	38 (80.9)
Mutton	1 (1.2)	1 (1.2)	0	3 (3.7)	76 (93.8)	0	0	1 (2.1)	4 (8.5)	42 (89.4)
Chicken	0	2 (2.5)	2 (2.5)	2 (2.5)	75 (92.6)	0	0	5 (10.6)	6 (12.8)	36 (76.6)

Values in parenthesis indicate percent

FIGURE 4.1.2 OVERALL CONSUMPTION PATTERN OF VITAMIN-D FOODS (n=129)

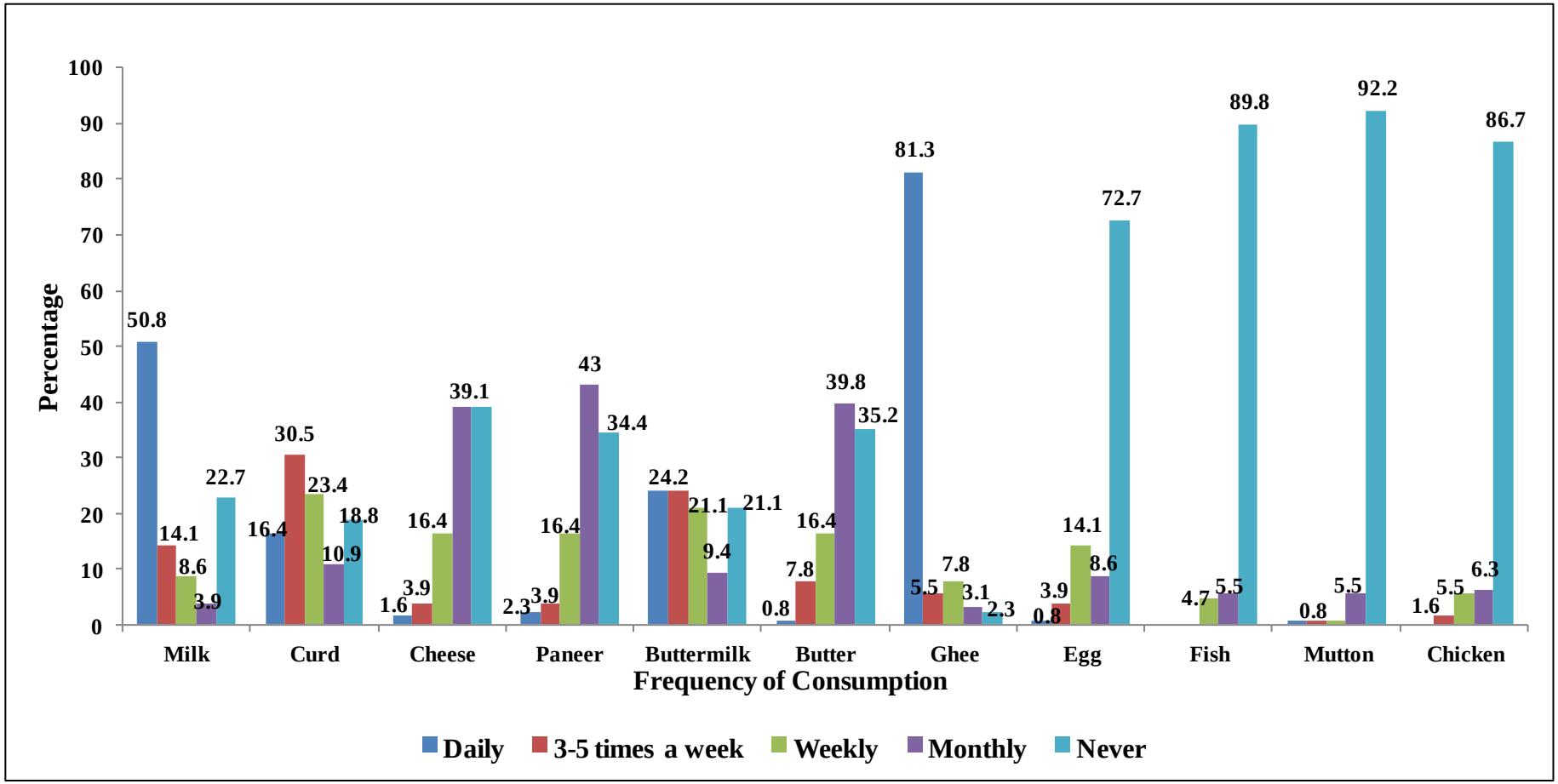


Table 4.1.15 NON-INVASIVE DETERMINANTS OF VITAMIN-D STATUS IN THE SUBJECTS (n, %)

Determinants	Females (n=82)	Males (n=47)	Total (n=129)
Skin type			
• Fair	16 (19.5)	24 (51.1)	40 (31.0)
• Wheatish	53 (64.6)	17 (36.2)	70 (54.3)
• Dark	13 (15.9)	6 (12.8)	19 (14.7)
Use of sunscreen	11 (13.6)	0	11 (8.6)
Going out in sun	55 (67.1)	37 (78.7)	92 (71.3)
Frequency of sun exposure			
• Daily	24 (43.6)	18 (48.6)	42 (45.7)
• 2-4 times a week	24 (43.6)	17 (45.9)	41 (44.6)
• Once a week	7 (12.7)	2 (5.4)	9 (9.8)
Minutes of sunlight exposure per day (Mean ± SD)	33.7 ± 90.9	40.5 ± 23.8	36.5 ± 28.4
Sunlight exposure ≥20 min/day	38 (69.1)	29 (78.4)	67 (72.8)
Type of clothing			
• Sari	28 (34.1)	0	28 (21.7)
• Salwar-Kameez	49 (59.8)	0	49 (38.0)
• Pant-Shirt	1 (1.2)	47 (100.0)	48 (37.2)
• Hand gloves+ Face covered	4 (4.9)	0	4 (3.1)

Values in parenthesis indicate percent

History of fractures among the subjects

Vitamin-D plays a vital role in bone health and so poor vitamin-D status many make the individual more prone to fractures. Hence information regarding history of fractures was also collected among the subjects. The prevalence is depicted in Table 4.1.16. Nearly 75.2% of the subjects did not have any fracture. Hand (31.3%), ankle (25%), legs (15.6%) and wrist (12.5%) were the type of fractures seen maximum among the subjects. The maximum prevalence of 29.2% was seen in the youngest age group which is quite astonishing, while the least prevalence was reported among the middle-age group (Figure 4.1.3).

5) PREVALENCE OF CLINICAL CONDITIONS

The primary objective of the study was to estimate the vitamin-D levels of the subjects and map the prevalence of vitamin-D deficiency. Along with serum vitamin-D other biochemical parameters were also estimated, the levels of which have been presented in the following tables.

Serum vitamin-D levels of the subjects

Serum 25-Hydroxy vitamin-D [25(OH)D] is considered as the 'gold standard' for the evaluation of vitamin-D status as it accounts for both the cutaneous production as well as the amount obtained through food. The mean value of serum 25(OH)D for the subjects was 13.7 ng/ml, which is much lower than the required optimum level of >30 ng/ml thus indicating a high level of sub-optimal vitamin-D status among the subjects. Female subjects had significantly lower level of vitamin-D as compared to males, thus suggesting a higher prevalence of deficiency among them (Table 4.1.17).

Gender-wise vitamin-D status of the subjects

The vitamin-D status of the subjects as categorised into deficiency, insufficiency and sufficiency groups based on their serum 25(OH)D levels is shown in Table 4.1.18. It was seen that almost 88% of the subjects were vitamin-D deficient with levels <20 ng/ml and only 4% were in the sufficiency range with levels >30 ng/ml. As expected from the mean serum 25(OH)D levels from the previous table the prevalence of deficiency was significantly higher among females as compared to male subjects (95% vs 77% respectively).

As a high number of subjects were identified as vitamin-D deficient, these were further classified into sub-category of mild, moderate and severe deficiency. As can be seen from Figure 4.1.4, about 53.5% subjects were falling under mild category with higher number of

TABLE 4.1.16 HISTORY OF FRACTURES AMONG THE SUBJECTS (n, %)

Site of Fracture	Females (n=82)	Males (n=47)	Total (n=129)
No fractures	63 (76.8)	34 (72.3)	97 (75.2)
Had fractures	19 (23.2)	13 (27.6)	32 (24.8)
• Shoulder	2 (10.5)	0	2 (6.3)
• Hand	4 (21.1)	6 (46.1)	10 (31.3)
• Legs	0	5 (38.5)	5 (15.6)
• Ankle	8 (42.1)	0	8 (25)
• Wrist	4 (21.1)	0	4 (12.5)
• Knee	1 (5.3)	0	1 (3.1)
• Hips	0	1 (7.7)	1 (3.1)
• Nose	0	1 (7.7)	1 (3.1)

Values in parenthesis indicate percent

FIGURE 4.1.3 PREVALENCE OF FRACTURES ACROSS AGE GROUPS (%)

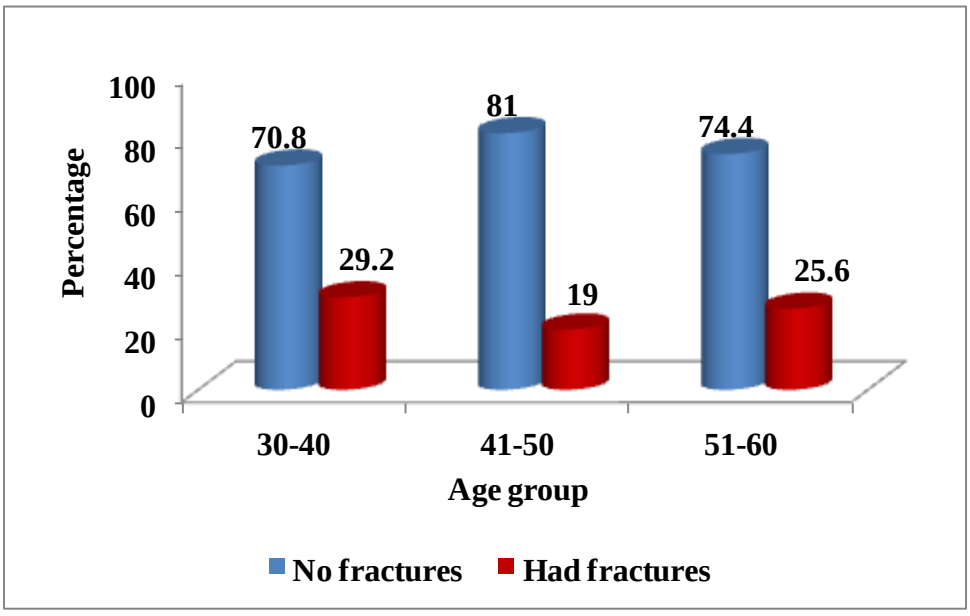


TABLE 4.1.17 VITAMIN-D LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameter	Females (n=82)	Males (n=47)	Total (n=129)	t-test *p* value
25Hydroxy Vitamin D	11.4 ± 7.1	17.6 ± 5.5	13.7 ± 7.2	**0.000*** **

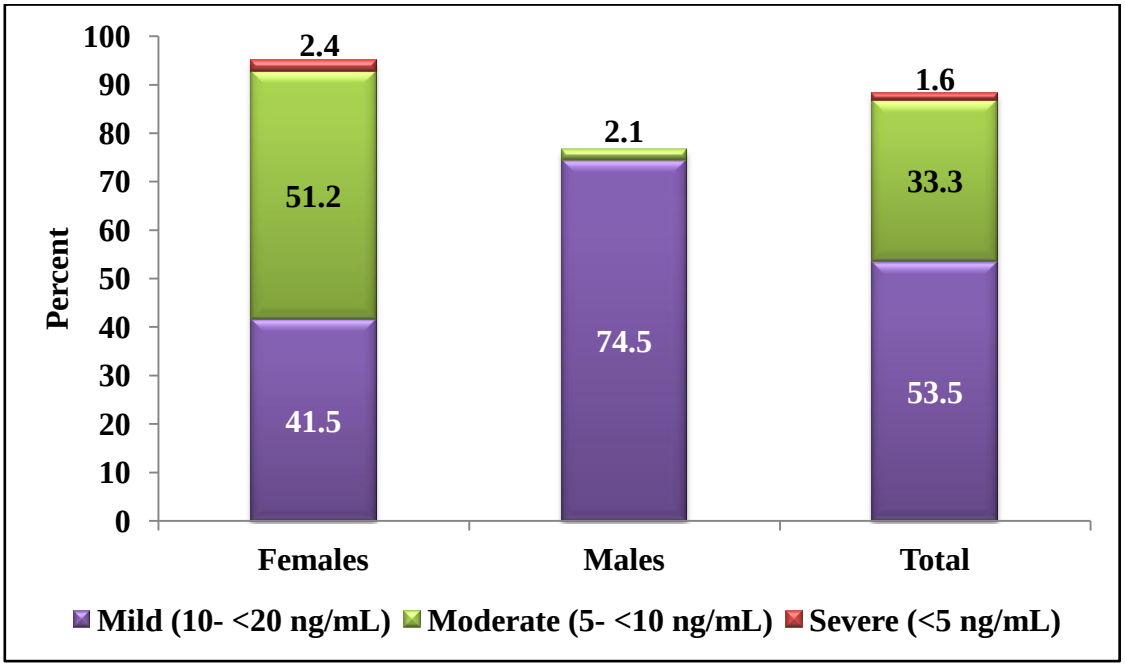
p< 0.001***

TABLE 4.1.18 GENDERWISE VITAMIN-D STATUS OF THE SUBJECTS (n, %)

Serum 25(OH)D ng/ml	Females (n=82)	Males (n=47)	Total (n=129)	χ^2 value
Deficiency (<20)	78 (95.1)	36 (76.6)	114 (88.4)	**10.55****
Insufficiency (20-≤ 30)	2 (2.4)	8 (17)	10 (7.7)	
Sufficiency (>30)	2 (2.4)	3 (6.4)	5 (3.8)	

p<0.01** Values in parenthesis indicate percent

FIGURE 4.1.4 GENDERWISE SUB-CLASSIFICATION OF VITAMIN-D DEFICIENCY (%)



males (74.5%) as compared to females (41.5%), while nearly half (51.2%) of the deficient female population showed moderate vitamin-D deficiency. It was heartening to see that only two of the females (2.4%) and none of the males had severe deficiency i.e. levels <5 ng/mL.

Age-wise vitamin-D status of the subjects

The age-wise vitamin-D status of the subjects is presented in Figure 4.1.5. It revealed that the highest prevalence of deficiency (93.8%) was observed in the youngest age group (30-40 yrs) and as the age increased the prevalence decreased. The 51-60 years age group had eight percent of vitamin-D deficient subjects while about 7.7% were in the sufficiency range.

Prevalence of anemia among the subjects

The iron status of the subjects is given in Table 4.1.19. The values for total iron, total iron binding capacity (TIBC) and percent transferrin saturation were in normal range for all the subjects. The mean haemoglobin of the subjects was 12.5 ± 1.4 g/dl. The mean value was found to be slightly lower than the normal values for females. Around 41% prevalence of anaemia was mapped among the subjects based on the haemoglobin values classified as per WHO 2001 guidelines. Of these, 31.8% had mild anaemia and 9.3% had moderate anaemia. However it was good to see that none of the subjects had severe anaemia. The overall prevalence of anaemia was significantly higher among females as compared to male subjects (57.3% vs 12.7%, $p<0.001$) as shown in Table 4.1.20.

Glycemic Profile of the subjects

The glycemic profile of the subjects is given in Table 4.1.21. The mean value of fasting blood sugar (FBS) was 84.5 mg/dl and glycoslated Hb (HbA1c) was 5.9% indicating that most of the subjects had normal fasting glucose according to ATP III guidelines. However the average blood glucose (ABG) values were significantly high for male subjects and little above the physiological normal range. Glucose control as seen by HbA1c, revealed that 31% subjects had levels greater than 6% and more than $1/4^{th}$ of the subjects had ABG values above 120 mg/dl indicating abnormal glycemic profile.

Lipid Profile of the subjects

The lipid profile of the subjects showed that values for total cholesterol, HDL-C and triglycerides and atherogenic fractions TC/H, TG/H and LDL/HDL ratio were all in normal range for both males and females. However, the mean values of LDL-C were on the higher side with significantly higher levels among female subjects. Further the mean values of AIP

FIGURE 4.1.5 AGEWISE VITAMIN-D STATUS OF THE SUBJECTS (%)

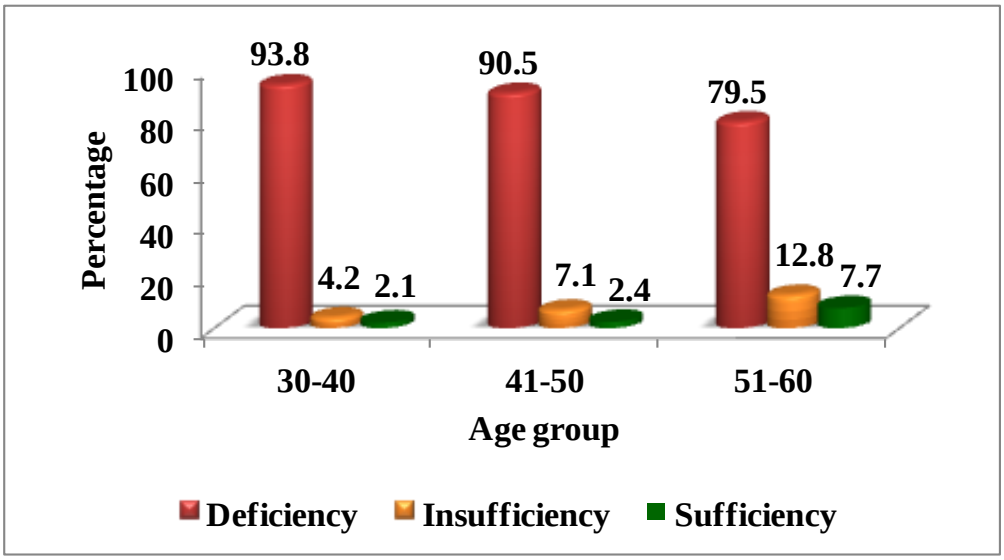


TABLE 4.1.19 IRON STATUS OF THE SUBJECTS (Mean ± SD)

Parameters	Normal Range	Females (n=82)	Males (n=47)	Total (n=129)
Hb (gm/dl)	Males >13 Females >12	11.8 ± 1.0	13.8 ± 0.9	12.5 ± 1.4
Iron (mcg/dl)	Male: 70-180 Female: 60-180	71.4 ± 29.8	85.2 ± 22.3	76.5 ± 27.9
TIBC (mcg/dl)	Male: 225-535 Female: 215-535	383.6 ± 60.9	350 ± 41.7	371.2 ± 56.8
% Transferrin Saturation	13-45	19.2 ± 8.6	24.5 ± 6.2	21.2 ± 8.2

TABLE 4.1.20 PREVALENCE OF IRON DEFICIENCY ANAEMIA AMONG SUBJECTS (n, %)

Classification based on Hb values	Females (n=82)	Males (n=47)	Total (n=129)	χ^2 value
Normal >12 (Females) >13 (Males)	35 (42.7)	41 (87.2)	76 (58.9)	
Mild (Females 11–11.9) (Males 11-12.9)	36 (43.9)	5 (10.6)	41 (31.8)	
Moderate (8 – 10.9)	11 (13.4)	1 (2.1)	12 (9.3)	
Severe (< 8)	0	0	0	
Total Anemic subjects	47 (57.3)	6 (12.7)	53 (41.1)	**24.55*** **

p<0.001*** Values in parenthesis indicate percent

TABLE 4.1.21 GLYCEMIC PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameters	Females (n=82)	Males (n=47)	Total (n=129)	*p* value
Fasting Blood Sugar (mg/dl)	84.2 ± 7.1	85.0 ± 12.4	84.5 ± 15.5	0.779
Average Blood Glucose (ABG)	108.2 ± 30.7	122.4 ± 17.7	113.4 ± 27.5	**0.001*** **
Glycoslated Hb (HbA1c) (%)	5.8 ± 0.9	5.9 ± 0.6	5.9 ± 0.8	0.573
Aberration in Glycemic profile (n, %)				
FBS ≥110 mg%	5 (6.1)	2 (4.3)	7 (5.4)	0.657
ABG ≥ 120 mg%	13 (15.9)	20 (42.6)	33 (25.6)	**0.001*** **
HbA1c > 6 %	19 (23.2)	16 (34.0)	40 (31.0)	0.181

p<0.001*** Values in parenthesis indicate percent

TABLE 4.1.22 LIPID PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameters	Females (n=82)	Males (n=47)	Total (n=129)	t-Test *p* value
Total Cholesterol	194.7 ± 36.6	186.8 ± 29.6	191.9 ± 34.3	0.211
Triglycerides	112.4 ± 49.4	137.7 ± 89.7	121.6 ± 67.7	0.079
LDL Cholesterol	116.2 ± 31.2	102.1 ± 21.6	111.1 ± 28.8	**0.003****
HDL Cholesterol	56.1 ± 12.5	45.2 ± 8.7	52.1 ± 12.4	**0.000*****
VLDL-C	22.5 ± 9.8	27.5 ± 17.9	24.3 ± 13.5	0.08
TC/HDL Ratio	3.6 ± 0.7	4.2 ± 0.9	3.8 ± 0.9	**0.000*****
LDL/HDL Ratio	2.1 ± 0.6	2.3 ± 0.5	2.2 ± 0.6	0.082
TAG/HDL Ratio	2.2 ± 1.3	3.3 ± 2.7	2.6 ± 2.2	**0.014***
AIP (log10 TG/H)	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.3	**0.002****
HsCRP	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.265

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

which indicates atherogenicity and HsCRP which signals inflammation were on the higher end among the subjects (Table 4.1.22).

Prevalence of Dyslipidemia among the subjects

When the data was segregated based on the cut off values, it was observed that around 41% had hypercholesterolemia with females having significant higher prevalence than male subjects (47.6% Vs 29.8%, $p< 0.05$). Almost 19% of the subjects had hyper-triglyceridemia with significant prevalence among males. An alarming observation was 64.3% had elevated LDL cholesterol levels with significantly higher levels in females and protective HDL lipoprotein was low in nearly 42% of the subjects which was significantly lower among males. TG/H ratio, which represents small dense lipoprotein was found to be high in 23.3% of the subjects (Table 4.1.23). Another indicator used was AIP. An improvisation of the indicator of small dense LDL, TAG/H, is its log transformed values, known as the Atherogenic Index of Plasma (AIP), is an efficient quantitative indicator of atherogenicity. It was found to be high in 69% of the subjects, while HsCRP was high in nearly 60% of the subjects. All these aberrations indicate great risk of CVDs among the study population and needs corrective preventive measures.

Levels of Thyroid hormones among the subjects

The thyroid hormones of the subjects were also assessed to examine the thyroid gland functioning. The mean values for Thyroid Stimulating Hormone (TSH) were found to be within the normal ranges. Similarly the values for Total Triiodothyronine (T_3) and Total Thyroxine (T_4) were within the normal ranges (Table 4.1.24).

Kidney Profile of the subjects

The kidney functioning tests assessed among the subjects is given in Table 4.1.25. The kidney profile of the subjects revealed that all the parameters i.e serum calcium, blood urea nitrogen (BUN), creatinine, uric acid and BUN-creatinine ratio were in normal range suggesting normal kidney functioning among the subjects. However the uric acid levels among the male subjects were on the higher side of the normal range. This was in line with the observation of high prevalence of aberrated uric acid levels among male and female subjects, wherein males had significantly higher levels as compare to their female counter parts (42.6% vs 8.5%, $p< 0.001$).

TABLE 4.1.23 PREVALENCE OF DYSLIPIDEMIA & INFLAMMATION AMONG THE SUBJECTS (n, %)

Parameter	Females (n=82)	Males (n=47)	Total (n=129)	χ^2 *p* value
TC ≥ 200 mg/dl	39 (47.6)	14 (29.8)	53 (41.1)	**0.048***
TG ≥ 150 mg/dl	10 (12.2)	15 (31.9)	25 (19.4)	**0.006****
LDL-C ≥ 100 mg/dl	58 (70.7)	25 (53.2)	83 (64.3)	**0.045***
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	22 (26.8)	32 (68.1)	54 (41.9)	**0.000*****
TC/HDL ≥ 5	4 (4.9)	8 (17.0)	12 (9.3)	**0.022***
TAG/HDL ≥ 3	11 (13.4)	19 (40.4)	30 (23.3)	**0.000*****
LDL/HDL ≥ 3.5	1 (1.2)	2 (4.3)	3 (2.3)	0.271
Atherogenic Index of Plasma (AIP)				
<0.11 (low risk)	19 (23.2)	5 (10.6)	24 (18.6)	0.155
0.11-0.21 (moderate risk)	11 (13.4)	5 (10.6)	16 (12.4)	
>0.21 (high risk)	52 (63.4)	37 (78.7)	89 (69.0)	
Inflammatory marker HsCRP				
≥ 0.1 mg/dl	46 (56.1)	34 (72.3)	80 (60.0)	0.067

p< 0.001***, <0.01**, <0.05* Values in parenthesis indicate percent

TABLE 4.1.24 THYROID HORMONES LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Females (n=82)	Males (n=47)	Total (n=129)
Thyroid Stimulating Hormone (TSH) µIU/ml	0.30-5.5	3.8 ± 3.9	2.8 ± 1.4	3.5 ± 3.2
Total Triiodothyronine (T_3) ng/dl	60-200	117.6 ± 20.2	111.2 ± 18.2	115.3 ± 19.7
Total Thyroxine (T_4) µg/dl	4.5-12.0	8.6 ± 1.4	8.6 ± 1.7	8.6 ± 1.5

Table 4.1.25 Kidney Profile of the Subjects (Mean ± SD)

Parameter	Normal Range	Females (n=82)	Males (n=47)	Total (n=129)
Calcium (mg/dl)	8.8-10.6	9.4 ± 0.4	9.5 ± 0.3	9.4 ± 0.4
BUN (mg/dl)	7.9-20	9.7 ± 2.7	9.8 ± 4.6	9.7 ± 3.5
Creatinine (mg%)	Male: 0.6-1.1 Female: 0.5-0.8	0.6 ± 0.1	0.8 ± 0.6	0.7 ± 0.4
Uric Acid (mg/dl)	Male: 3.5-7.2 Female: 2.6-6.0	4.4 ± 1.0	6.7 ± 1.5	5.2 ± 1.6
BUN/ Sr. Creatinine	9:1- 23:1	17.0 ± 4.8	12.3 ± 3.9	15.3 ± 5.1
Aberrations in parameters				
Uric Acid (mg/dl)		7 (8.5)	20 (42.6)	27 (20.9)
χ^2 *p*-value		**0.000*** **		

p <0.001*** Values in parenthesis indicate percent

Liver Functioning Tests among the subjects

The liver functioning tests were performed on the subjects to get an idea about levels of various liver parameters. All the liver parameters were in the physiological normal range suggesting healthy liver profile of the subjects as can be seen from Table 4.1.26.

6 (A) ANALYSIS BASED ON VITAMIN-D STATUS OF THE SUBJECTS

To get a clarity regarding the determinants responsible for low serum vitamin-D levels among subjects further analysis was done by segregating the subjects based on their serum 25(OH)D levels into two groups- less than 20 and more than or equal to 20 ng/ml. About 95.1% of the females and 76.6% of males had vitamin-D levels less than 20 ng/ml (Figure 4.1.6).

Biophysical measurements across the vitamin-D status of the subjects

The anthropometric and blood pressure measurements across the vitamin-D levels are shown in Table 4.1.27. It was observed that all the measurements except diastolic BP were higher for the low vitamin-D group. Significant higher values were observed for waist stature ratio and percent body fat in the low vitamin-D group when compared with the high vitamin-D group.

Non-invasive factors across the vitamin-D status of the subjects

It would be interesting to see if the non-invasive factors also influence the vitamin-D levels of the subjects, hence an attempt was made to study the factors which could act as risk factors for poor vitamin-D status among individuals. Table 4.1.28 depicts the risk factors for low vitamin-D levels among the subjects. It was seen that belonging to female gender and having high BMI, waist circumference and WSR had significantly higher odds for poor vitamin-D status. WHR also showed high odds for low vitamin-D levels though it was not statistically significant. Thus it signifies that high BMI and presence of abdominal obesity are the prominent risk factors for poor vitamin-D status.

Also as seen from the table factors like having hypertension or history of fractures, consuming vegetarian diet or no tea, low physical activity, belonging to fair skin type and having exposure to sun for less than 20 minutes per day were considered. It was observed that none of the factors had statistical significant relevance with the vitamin-D status of the subjects; however factors like presence of hypertension, consuming vegetarian diet and having sun exposure less than 20 minutes per day had higher odds for low vitamin-D levels

TABLE 4.1.26 LIVER PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Females (n=82)	Males (n=47)	Total (n=129)
Alkaline Phosphatase (U/L)	Male: 53-128 Female: 42-98	83.2 ± 20.6	81.6 ± 21.5	82.6 ± 20.9
Total Bilirubin (mg/dl)	0.3-1.20	0.53 ± 0.1	0.68 ± 0.3	0.6 ± 0.2
Direct Bilirubin (mg/dl)	0-0.2	0.16 ± 0.04	0.19 ± 0.07	0.17 ± 0.05
Indirect Bilirubin (mg/dl)	0-0.9	0.4 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
SGOT (U/L)	Male: 0-37 Female: 0-31	24.2 ± 12.4	24.2 ± 6.0	24.2 ± 10.5
SGPT (U/L)	Male: 13-40 Female: 10-28	23.5 ± 16.2	29.8 ± 15.7	25.8 ± 16.2
GGT (U/L)	Male: 0-55 Female: 0-38	20.7 ± 9.2	31.5 ± 18.9	24.6 ± 14.5
Serum Albumin (gm/dl)	3.5-5.2	4.3 ± 0.3	4.3 ± 0.2	4.3 ± 0.3
Total Protein (gm/dl)	6.6-8.3	7.6 ± 0.3	7.5 ± 0.3	7.6 ± 0.3
Serum Albumin/Globulin	0.9-2.0	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.2

FIGURE 4.1.6 DISTRIBUTION OF THE SUBJECTS BASED ON THEIR VITAMIN-D LEVELS (%)

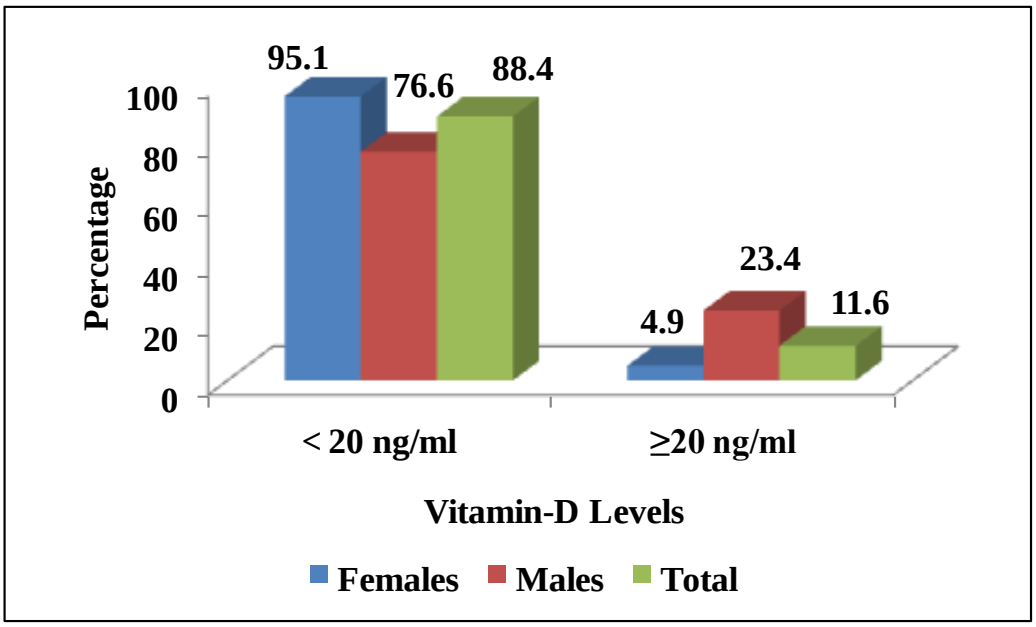


TABLE 4.1.27 BIOPHYSICAL MEASUREMENTS ACROSS VITAMIN-D STATUS OF THE SUBJECTS (MEAN ± SD)

Parameters	Vitamin D <20 ng/ml (n=114)	Vitamin D ≥20 ng/ml (n=15)	t-Test *p* value
Weight (kg)	65.1 ± 14.5	64.7 ± 17.4	0.921
Body Mass Index	26.11 ± 4.6	24.15 ± 5.3	0.130
Waist Circumference	92.95 ± 10.7	88.65 ± 13.7	0.159
Hip Circumference	100.5 ± 9.8	96.2 ± 11.3	0.118
WHR	0.93 ± 0.06	0.92 ± 0.08	0.763
WSR	0.59 ± 0.07	0.55 ± 0.09	**0.026***
% Body Fat	34.29 ± 6.1	29.53 ± 8.0	**0.007****
SBP	128.8 ± 16.7	128.4 ± 17.5	0.936
DBP	80.6 ± 10.4	82.4 ± 10.5	0.526

p<0.01**, <0.05* Values in parenthesis indicate percent

TABLE 4.1.28 NON-INVASIVE RISK FACTORS FOR LOW VITAMIN-D LEVELS AMONG THE SUBJECTS (n, %)

Parameters		Vitamin D <20 ng/ml (n=114)	Vitamin D ≥20 ng/ml (n=15)	Odds Ratio	χ^2 *p* value	95% CI
Gender	Females	78 (68.4)	4 (26.7)	5.95	**0.001****	1.77-19.99
	Males	36 (31.6)	11 (73.3)	--		
Age (≥ 40 years)		74 (64.9)	12 (80.0)	0.46	0.245	0.12-1.73
BMI (≥ 23)		83 (72.8)	7 (46.7)	3.05	**0.038***	1.02-9.14
WC (F≥80, M≥90 cm)		96 (84.2)	9 (60.0)	3.55	**0.024***	1.12-11.21
WSR (≥ 0.5)		105 (92.1)	11 (73.3)	4.24	**0.023***	1.12-16.06
WHR (F≥0.85, M≥0.9)		100 (87.7)	11 (73.3)	2.59	0.132	0.73-9.28
% BF (F>30, M>20)		106 (93.0)	14 (93.3)	0.9	0.960	0.11-8.14
SBP (≥ 120 mmHg)		75 (65.8)	12 (80.0)	0.48	0.269	0.12-1.8
DBP (≥ 80 mmHg)		61 (53.5)	9 (60.0)	0.76	0.63	0.25-2.29
HTN present		14 (12.3)	1 (6.7)	1.96	0.525	0.24-16.08
Having Fractures		27 (23.7)	5 (33.3)	0.62	0.417	0.19-1.97
Vegetarian diet		79 (69.3)	9 (60.0)	1.50	0.468	0.49-4.55
No Tea consumption		26 (23.0)	2 (13.3)	0.51	0.396	0.11-2.43
Low Physical activity		27 (23.7)	5 (33.3)	0.62	0.418	0.19-1.97
Sun exposure < 20 minutes/day		22 (26.8)	3 (20.0)	1.17	0.832	0.27-4.9
Fair skin type		35 (30.7)	5 (33.3)	0.88	0.836	0.28-2.78

p <0.01**, <0.05* Values in parenthesis indicate percent

among the subjects but was not statistically significant. This suggests that if the lifestyle is made active more so in sunlight then the medical conditions can be kept at bay and vitamin-D levels can be improved. Also consumption of vegetarian sources of vitamin-D should be encouraged like milk and milk products.

Lipid profile across vitamin-D status of the subjects

The lipid profile, athrogenic indices and inflammatory marker was also studied across the low and high vitamin-D groups (Table 4.1.29). None of the values were statistically significant between the two groups. The univariate analysis in the form of odds ratio was also studied for the prevalence of dyslipidemia across the vitamin-D groups (Table 4.1.30). It revealed that high levels of total cholesterol, triglycerides and atherogenic indices LDL-C, TAG/HDL-C and AIP showed high odds of having low vitamin-D levels thus identifying them as risk factors for poor vitamin-D status. However the odds ratio was statistically non-significant.

6 (B) ANALYSIS BASED ON BMI OF THE SUBJECTS

The mean biochemical parameters and nutrient intake were studied in relation to presence of obesity as defined by increased BMI as depicted in tables from 4.1.31 to 4.1.34.

Biochemical parameters across BMI of the subjects

Mean levels of lipid parameters were studied across various categories of BMI (Table 4.1.31). It was seen that the mean levels of HDL-C ($p=0.000$) AIP values ($p=0.005$) and HsCRP ($p=0.003$) was significantly different across the categories of BMI as assessed by one way analysis of variance. Similarly Table 4.1.32 shows the mean values for rest of the biochemical parameters. It was observed that haemoglobin, fasting and average blood glucose in glycemic profile, thyroid hormone TSH, liver enzymes SGPT & GGT and among kidney profile uric acid was significantly different across the categories of BMI.

A LSD post hoc test was applied to see the difference between each category of BMI among the subjects (Table 4.1.33). It revealed that triglycerides, VLDL-C, ratios TC/H & TAG/H, AIP levels, FBS, ABG & HbA1c, SGPT & GGT were all significant across normal vs obese category. HDL-C, HsCRP, haemoglobin and uric acid were significant across normal vs obese and overweight vs obese category while TSH was significantly different across normal vs overweight and overweight vs obese categories. This signifies that nearly fifteen biochemical parameters covering the whole body profile were elevated with increase in BMI i.e the obese category.

TABLE 4.1.29 LIPID PROFILE OF THE SUBJECTS ACROSS THEIR VITAMIN-D STATUS (MEAN ± SD)

Parameters	Vitamin D <20 ng/ml (n=114)	Vitamin D ≥20 ng/ml (n=15)	t-Test *p* value
Total Cholesterol	193.7±34.4	177.7±30.7	0.088
Triglycerides	125.6±69.3	91.2±44.4	0.064
LDL Cholesterol	112.6±29.1	99.9±25.1	0.110
HDL Cholesterol	52.2±12.6	51.6±10.7	0.872
VLDL Cholesterol	25.1 ± 13.9	18.2 ± 8.9	0.064
TC/HDL Ratio	3.8 ± 0.8	3.6 ± 0.8	0.226
LDL/HDL Ratio	2.2 ± 0.5	2.0 ± 0.6	0.163
TAG/HDL Ratio	2.7 ± 1.9	2.2 ± 1.3	0.192
AIP (log10 TG/H)	0.35 ± 0.3	0.22 ± 0.2	0.068
HsCRP	0.23 ± 0.2	0.26 ± 0.2	0.684

Values in parenthesis indicate percent

TABLE 4.1.30 PREVALENCE OF DYSLIPIDEMIA & INFLAMMATION ACROSS VITAMIN-D LEVELS OF THE SUBJECTS (n, %)

Parameter	Vitamin D <20 ng/ml (n=114)	Vitamin D ≥20 ng/ml (n=15)	OR	χ^2 *p* value	95% CI
TC ≥ 200 mg/dl	49 (42.9)	4 (26.7)	2.07	0.23	0.62-6.9
TG ≥ 150 mg/dl	24 (21.1)	1 (6.7)	3.73	0.186	0.46-29.8
LDL-C ≥ 100 mg/dl	75 (65.8)	8 (53.3)	1.68	0.345	0.56-4.98
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	48 (42.1)	6 (40.0)	0.91	0.877	0.3-2.74
TC/HDL ≥ 5	12 (10.5)	0	--	--	--
TAG/HDL ≥ 3	28 (24.5)	2 (13.3)	2.12	0.34	0.45-9.95
LDL/HDL ≥ 3.5	3 (2.6)	0	--	--	--
AIP ≥ 0.21	81 (71.1)	8 (53.3)	2.15	0.164	0.72-6.4
HsCRP ≥ 0.1 mg/dl	69 (60.5)	11 (73.3)	0.52	0.285	0.16-1.74

Values in parenthesis indicate percent

TABLE 4.1.31 LIPID PROFILE & HS-CRP LEVELS IN RELATION TO BMI OF THE SUBJECTS (MEAN ± SD)

Parameters	Normal BMI (n=39)	Overweight (n=27)	Obese (n=63)	ANOVA *p* value
Total Cholesterol	196.1 ± 40.4	198.8 ± 31.7	186.3 ± 30.7	0.188
Triglycerides	103.5± 45.5	121.3 ± 86.8	132.9 ± 68.6	0.104
LDL Cholesterol	113.3 ± 32.4	118.4 ± 31.8	106.6 ± 24.5	0.176
HDL Cholesterol	56.8 ± 15.0	55.6 ± 11.8	47.6 ± 9.1	**0.000*** **
VLDL Cholesterol	20.7 ± 9.1	24.3 ± 17.4	26.6 ± 13.7	0.102
TC/HDL Ratio	3.6 ± 1.1	3.7 ± 0.8	3.9 ± 0.7	0.103
LDL/HDL Ratio	2.1 ± 0.7	2.2 ± 0.6	2.3 ± 0.4	0.393
TAG/HDL Ratio	2.1 ± 1.7	2.6 ± 3.0	2.9 ± 1.9	0.117
AIP (log10 TG/H)	0.24 ± 0.2	0.29 ± 0.27	0.4 ± 0.24	**0.005** **
HsCRP	0.17 ± 0.2	0.15 ± 0.1	0.31 ± 0.28	**0.003** **

p <0.001***, <0.01**

Table 4.1.32 Biochemical Parameters in Relation to BMI of the Subjects (MEAN ± SD)

	Normal BMI (n=39)	Overweight (n=27)	Obese (n=63)	ANOVA *p* value
Serum 25(OH)D (ng/ml)	14.4 ± 7.1	11.8 ± 4.9	13.97 ± 8.0	0.307
Hb (gm/dl)	12.3 ± 1.1	11.8 ± 1.3	12.9 ± 1.4	**0.000*** **
FBS (mg /dl)	79.2 ± 7.3	83.4 ± 17.2	88.3 ± 17.4	**0.013***
ABG (mg/dl)	105.3 ± 27.3	11.2 ± 25.6	119.3 ± 27.4	**0.038***
HbA1c (%)	5.6 ± 0.8	5.9 ± 0.7	6.0 ± 0.8	0.067
TSH (µIU/ml)	2.9 ± 2.1	4.8 ± 5.9	3.2 ± 1.8	**0.05***
T3 (ng/dl)	118.1 ± 21.0	115.1 ± 19.8	113.6 ± 18.9	0.536
T4 (µg/dl)	8.5 ± 1.4	8.3 ± 1.3	8.8 ± 1.5	0.299
SGOT (U/L)	22.8 ± 12.8	22.6 ± 11.2	25.7 ± 8.3	0.264
SGPT (U/L)	20.8 ± 11.6	25.9 ± 21.7	28.9 ± 15.4	**0.046***
GGT (U/L)	20.4 ± 11.2	22.1 ± 10.5	28.3 ± 16.7	**0.015***
Calcium (mg/dl)	9.5 ± 0.3	9.5 ± 0.6	9.4 ± 0.3	0.652
BUN (mg/dl)	10.5 ± 5.1	9.5 ± 2.6	9.4 ± 2.4	0.293
Creatinine (mg %)	0.74 ± 0.7	0.6 ± 0.1	0.66 ± 0.1	0.342
Uric Acid (mg/dl)	4.7 ± 1.4	4.5 ± 1.4	5.9 ± 1.6	**0.000*** **

p <0.001***, <0.05*

TABLE 4.1.33 POST-HOC TEST FOR LIPID PROFILE AND BIOCHEMICAL PARAMETERS ACROSS VARIOUS GROUPS

Parameters	Normal vs Ow	Normal vs Ob	Ow vs Ob
TAG	0.291	**0.034***	0.456
HDL-C	0.690	**0.000*****	**0.004****
VLDL-C	0.288	**0.033***	0.455
TC/HDL-C	0.763	**0.048***	0.153
TAG/HDL-C	0.369	**0.039***	0.390
AIP	0.404	**0.002****	0.054
HsCRP	0.840	**0.005****	**0.006****
FBS	0.265	**0.003****	0.158
ABG	0.382	**0.012***	0.194
HbA1c	0.194	**0.02***	0.511
TSH	**0.026***	0.792	**0.028***
Hb	0.109	**0.017***	**0.000*****
SGPT	0.205	**0.013***	0.401
GGT	0.625	**0.007****	0.058
Uric Acid	0.742	**0.000*****	**0.000*****

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

Nutrient intake across BMI of the subjects

The nutrient intake across the categories of BMI is depicted in Table 4.1.34 (A). Among all the nutrients studied only calcium was significantly different across the groups. The values were significantly different across the normal vs overweight and overweight vs obese groups when assessed by a post hoc test (Table 4.1.34 (B)). This signifies that the intake of both macro and micro nutrients among the subjects didn't differ much as per their BMI.

6 (C) ANALYSIS BASED ON VITAMIN-D QUARTILES

To study the effect of vitamin-D levels on mean values of the parameters associated with anthropometry, lipid profile, iron status, glucose control, thyroid hormones and liver & kidney profile the data of all the above parameters was inspected across quartiles of the serum vitamin-D levels of the subjects which is depicted in the Tables 4.1.35 to 4.1.37. One way analysis of variance was applied for the difference among the quartiles and then a post hoc test was carried out to see how the groups are different from each other statistically. The gender-wise distribution of the subjects as per the vitamin-D quartiles showed that first quartiles had only females (40.2%), second quartile had 29.3% females while the third and fourth quartile had more of males- 36.2% and 46.8% respectively (Figure 4.1.7).

Anthropometric measurements across vitamin-D quartiles

The anthropometric measurements of the subjects across the vitamin-D quartiles are given in Table 4.1.35 (A) & (B). It was observed that of all the parameters studied only the percent body fat showed a significant decrease from 1^{st} to 4^{th} quartile. The post hoc analysis revealed that weight and WSR decreased significantly from 1^{st} to 4^{th} quartile while percent body fat was significantly different from 1^{st} to 3^{rd} and 1^{st} to 4^{th} quartile.

Lipid Profile levels across vitamin-D quartiles

The lipid profile values across the various quartiles of vitamin-D are shown in Table 4.1.36 (A). The bad cholesterol LDL and the protective cholesterol HDL both showed a significant declining trend from 1^{st} to 4^{th} quartile. The post hoc analysis (Table 4.1.36 (B)) revealed that HDL-C was significantly different for 1^{st} vs 3^{rd} and 2^{nd} vs 3^{rd} quartile while LDL-C for 1^{st} vs 4^{th} and 2^{nd} vs 4^{th} quartile. TC/H & TAG/H ratios and AIP levels were significantly different across 1^{st} and 3^{rd} quartile.

TABLE 4.1.34 (A) NUTRIENT INTAKE IN RELATION TO BMI OF THE SUBJECTS (MEAN ± SD)

Parameters	Normal BMI (n=39)	Overweight (n=27)	Obese (n=63)	ANOVA *p* value
Energy (Kcal)	1474 ± 477.1	1578 ± 491.5	1466 ± 356.1	0.495
Protein (gm)	40.6 ± 13.6	43.1 ± 14.7	39.7 ± 11.4	0.511
Fat (gm)	56.2 ± 23.8	60.8 ± 25.4	53.2 ± 17.3	0.293
Carbohydrates (g)	195.5 ± 62.5	204.4 ± 64.5	202.7 ± 51.7	0.780
Calcium	561.6 ± 392.8	747.6 ± 538.4	523.2 ± 241.1	**0.03***
Iron (mg)	12.2 ± 6.3	10.9 ± 5.7	12.3 ± 5.6	0.547
β carotene (µg)	1517 ± 3334	2084 ± 3488	1621 ± 2598	0.784
Vitamin C (mg)	87.7 ± 103.9	69.9 ± 45.6	77.3 ± 66.4	0.635
Crude Fibre (gm)	5.8 ± 2.9	5.7 ± 2.1	5.9 ± 2.6	0.885
Total Fibre	12.9 ± 7.1	13.1 ± 6.7	12.6 ± 6.0	0.942
Insoluble Fibre	9.8 ± 5.4	9.8 ± 5.3	9.2 ± 4.7	0.818
Soluble Fibre	3.1 ± 1.8	3.2 ± 1.6	3.2 ± 1.6	0.971

p<0.05*

TABLE 4.1.34 (B) POST-HOC TEST FOR NUTRIENT INTAKE ACROSS VARIOUS GROUPS

Parameters	Normal vs Ow	Normal vs Ob	Ow vs Ob
Calcium	**0.045***	0.609	**0.009****

p <0.01**

FIGURE 4.1.7 DISTRIBUTION OF THE SUBJECTS BASED ON VITAMIN-D QUARTILES (%)

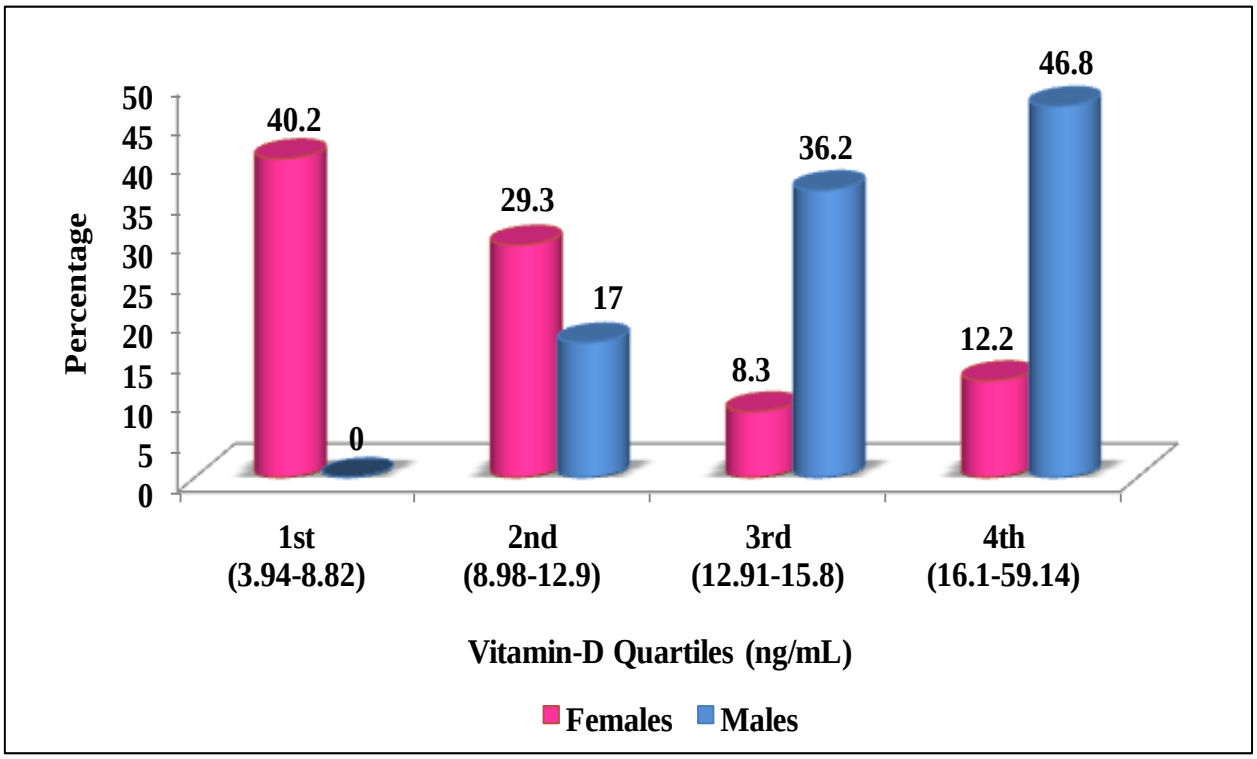


TABLE 4.1.35 (A) ANTHROPOMETRIC MEASUREMENTS OF THE SUBJECTS ACROSS VITAMIN-D QUARTILES (MEAN ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (3.94-8.82) (n=33)	2nd (8.98-12.9) (n=32)	3rd (12.91-15.8) (n=32)	4th (16.1-59.14) (n=32)	
Weight (Kg)	61.4 ± 13.3	62.9 ± 12.8	66.5 ± 15.8	69.3 ± 16.3	0.135
Height (cm)	152.5 ± 7.6	156.7 ± 7.6	160.2 ± 9.2	163.2 ± 9.6	--
WC (cm)	92.9 ± 12.2	92.9 ± 10.3	91.7 ± 10.1	92.1 ± 12.1	0.962
HC (cm)	101.5 ± 11.3	99.7 ± 8.6	98.5 ± 9.4	99.9 ± 10.9	0.685
WHR	0.92 ± 0.07	0.93 ± 0.07	0.93 ± 0.07	0.92 ± 0.07	0.703
WSR	0.61 ± 0.08	0.59 ± 0.06	0.57 ± 0.07	0.56 ± 0.08	0.075
BMI	26.5 ± 5.7	25.5 ± 3.8	25.7 ± 4.3	25.8 ± 4.9	0.850
Body fat (%)	37.1 ± 6.1	34.3 ± 4.7	31.7 ± 6.3	31.7 ± 7.2	**0.001****
Systolic BP (mmHg)	125.6 ± 15.8	130.3 ± 16.8	130.9 ± 19.1	128.1 ± 15.1	0.576
Diastolic BP (mmHg)	78.2±10.9	82.0 ± 7.9	81.1 ± 11.8	82.0 ± 10.2	0.382

p<0.01**

TABLE 4.1.35 (B) POST-HOC TEST FOR ANTHROPOMETRIC MEASUREMENTS ACROSS VITAMIN-D QUARTILES

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
Weight	0.685	0.162	**0.033***	0.323	0.085	0.459
WSR	0.345	0.052	**0.016****	0.316	0.143	0.642
% Body Fat	0.066	**0.001*****	**0.001*****	0.091	0.096	0.981

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

Table 4.1.36 (A) LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS ACROSS VITAMIN-D QUARTILES (MEAN ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (3.94-8.82) (n=33)	2nd (8.98-12.9) (n=32)	3rd (12.91-15.8) (n=32)	4th (16.1-59.14) (n=32)	
Total Cholesterol	195.5 ± 29.8	200.1 ± 41.6	190.5 ± 32.8	181.2 ± 30.5	0.150
Triglycerides	108.1 ± 34.9	124.3 ± 77.8	130.3 ± 57.2	124.1 ± 89.7	0.588
LDL Cholesterol	118.7 ± 24.7	118.5 ± 34.1	108.1 ± 27.6	98.9 ± 24.5	**0.013***
HDL Cholesterol	55.2 ± 8.1	55.2 ± 15.8	48.0 ± 12.2	49.8 ± 11.2	**0.032***
VLDL Cholesterol	21.6 ± 7.0	24.8 ± 15.6	26.1 ± 11.4	24.8 ± 17.9	0.590
TC/HDL Ratio	3.6 ± 0.6	3.8 ± 0.8	4.1 ± 0.9	3.8 ± 0.9	0.077
LDL/HDL Ratio	2.2 ± 0.5	2.2 ± 0.6	2.3 ± 0.6	2.0 ± 0.6	0.295
TAG/HDL Ratio	2.0 ± 0.7	2.5 ± 2.1	3.1 ± 2.2	2.8 ± 2.9	0.192
AIP (log10 TG/H)	0.27 ± 0.17	0.31 ± 0.28	0.41 ± 0.26	0.34 ± 0.29	0.183
HsCRP	0.24 ± 0.3	0.22 ± 0.25	0.23 ± 0.23	0.23 ± 0.19	0.522

p<0.05*

TABLE 4.1.36 (B) POST-HOC TEST FOR LIPID PROFILE ACROSS VITAMIN-D QUARTILES

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
HDL-C	0.998	**0.019****	0.074	**0.019****	0.075	0.565
LDL-C	0.981	0.127	**0.005****	0.136	**0.006****	0.191
TC/HDL-C	0.395	**0.011****	0.301	0.086	0.854	0.124
TAG/HDL-C	0.3	**0.038***	0.118	0.299	0.599	0.606
AIP	0.58	**0.035***	0.302	0.119	0.634	0.277

p <0.01**, <0.05*

Biochemical estimations across vitamin-D quartiles

The mean values of various biochemical parameters across the vitamin-D quartiles are presented in Table 4.1.37 (A). The pooled data showed that apart from vitamin-D levels, mean levels of haemoglobin significantly increased across the various quartiles. While TIBC, FBS and TSH values decreased significantly across the quartiles. The post hoc analysis revealed that haemoglobin increased from 1st to 4th quartile while TIBC, TSH and FBS decreased through the various quartiles (Table 4.1.37 (B)).

Nutrient intake of the subjects across vitamin-D quartiles

From the nutrient intake analysis it was observed that intake of all the macro and micro nutrients decreased from 1st to 3rd quartile but increased in 4th quartile except for vitamin-C and dietary fibre. The pooled data showed that means were significantly changed for calcium, vitamin-C and dietary fibre (Table 4.1.38 (A)). Post hoc analysis revealed that protein intake decreased significantly from 1st to 2nd and 1st to 3rd quartile. Intake of calcium, total, soluble & insoluble fibre showed significant decrease from 1st to 3rd and 1st to 4th quartile. It was only vitamin-c intake that was statistically different at three points i.e from 1st to 2nd, 1st to 3rd and 1st to 4th quartile (Table 4.1.38 (B)).

PREVALENCE OF METABOLIC SYNDROME AMONG THE SUBJECTS

The metabolic syndrome spells danger mainly because it represents a highly risky situation due to clustering of a number of interlinked risk factors that lead to different clinical conditions, namely obesity, hypertension, diabetes and dyslipidemia. According to IDF criteria (2005) the prevalence of metabolic syndrome was 31% with higher number of females having the condition than males. As per the ATP III (2001) guidelines about 32% subjects had metabolic syndrome, where the male subjects had significantly higher prevalence than their female counter parts. This is a matter of concern as it indicates the presence of multiple risk factors among the subjects (Figure 4.1.8 (A)). The prevalence of metabolic syndrome was looked across the vitamin-D status of the subjects using IDF classification as the ATP III criteria doesn't not consider waist circumference as a factor for MS. It revealed that the prevalence was more among the high vitamin-D group as shown in Figure 4.1.8 (B). When the prevalence was studied across the quartiles of vitamin-D it was seen that according to IDF classification it was highest for the 3rd quartile however no statistical significance was seen (Table 4.1.39).

TABLE 4.1.37 (A) BIOCHEMICAL PARAMETERS OF THE SUBJECTS ACROSS VITAMIN-D QUARTILES (MEAN ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (3.94-8.82) (n=33)	**2nd (8.98-12.9) (n=32)**	**3rd (12.91-15.8) (n=32)**	**4th (16.1-59.14) (n=32)**	
25(OH)D (ng/ml)	6.86 ± 1.3	10.97 ± 1.3	14.4 ± 0.79	22.6 ± 8.4	**0.000*** **
Hb (gm/dl)	11.8 ± 0.9	12.2 ± 1.5	12.8 ± 1.4	13.3 ± 1.2	**0.000*** **
TIBC (mcg/dl)	401.9 ± 77.0	379.4 ± 46.4	353.2 ± 35.7	350.5 ± 44.5	**0.000*** **
FBS (mg/dl)	89.9 ± 22.6	80.1 ± 11.3	86.1 ± 12.5	81.8 ± 10.7	**0.046***
HbA1c (%)	6.0 ± 1.2	5.7 ± 0.6	5.9 ± 0.9	5.8 ± 0.6	0.502
TSH (µIU/ml)	5.1 ± 5.4	2.8 ± 1.4	3.0 ± 2.2	2.8 ± 1.6	**0.007****

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

TABLE 4.1.37 (B) POST-HOC TEST FOR BIOCHEMICAL PARAMETERS ACROSS VITAMIN-D QUARTILES

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
Hb	0.319	**0.002****	**0.000*****	**0.04***	**0.001*****	0.175
TIBC	0.095	**0.000*****	**0.000*****	0.054	**0.034***	0.840
FBS	**0.01****	0.306	**0.034***	0.120	0.652	0.269
TSH	**0.004****	**0.008****	**0.004****	0.814	0.997	0.811

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

TABLE 4.1.38 (A) NUTRIENT INTAKE OF THE SUBJECTS ACROSS VITAMIN-D QUARTILES (Mean ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (3.94-8.82) (n=33)	2nd (8.98-12.9) (n=32)	3rd (12.91-15.8) (n=32)	4th (16.1-59.14) (n=32)	
Energy (Kcal)	1570 ± 411.9	1496 ± 389.6	1366 ± 413.5	1532 ± 470.2	0.240
Protein (gm)	45.1 ± 14.1	38.6 ± 9.2	37 ± 12.6	41.7 ± 13.6	0.052
Fat (gm)	57.4 ± 20.3	56.8 ± 20.6	50.5 ± 19.9	57.9 ± 24.2	0.465
Carbohydrates (g)	211 ± 54.8	201 ± 58.9	186.8 ± 55.4	204.7 ± 60.8	0.395
Calcium	751.1 ± 554.2	594.4 ± 370.7	460.8 ± 165.1	515.6 ± 218.8	**0.010****
Iron (mg)	11.9 ± 4.3	12.7 ± 7.6	10.6 ± 4.5	12.8 ± 6.2	0.382
β carotene (µg)	1928 ± 3304	1852 ± 3916	1437 ± 222.7	1520 ± 2153	0.906
Vitamin C (mg)	114.2 ± 115.1	77.2 ± 64.4	71.3 ± 47.8	51.9 ± 41.6	**0.008****
Crude Fibre (gm)	6.3 ± 2.6	6.1 ± 3.6	5.4 ± 1.5	5.5 ± 2.4	0.399
Total Fibre	15.1 ± 6.3	13.9 ± 7.3	11.0 ± 5.9	11.1 ± 5.5	**0.018****
Insoluble Fibre	11.3 ± 4.7	10.5 ± 5.5	8.2 ± 4.6	8.1 ± 4.6	**0.018****
Soluble Fibre	3.8 ± 1.7	3.4 ± 1.9	2.7 ± 1.4	2.6 ± 1.3	**0.009****

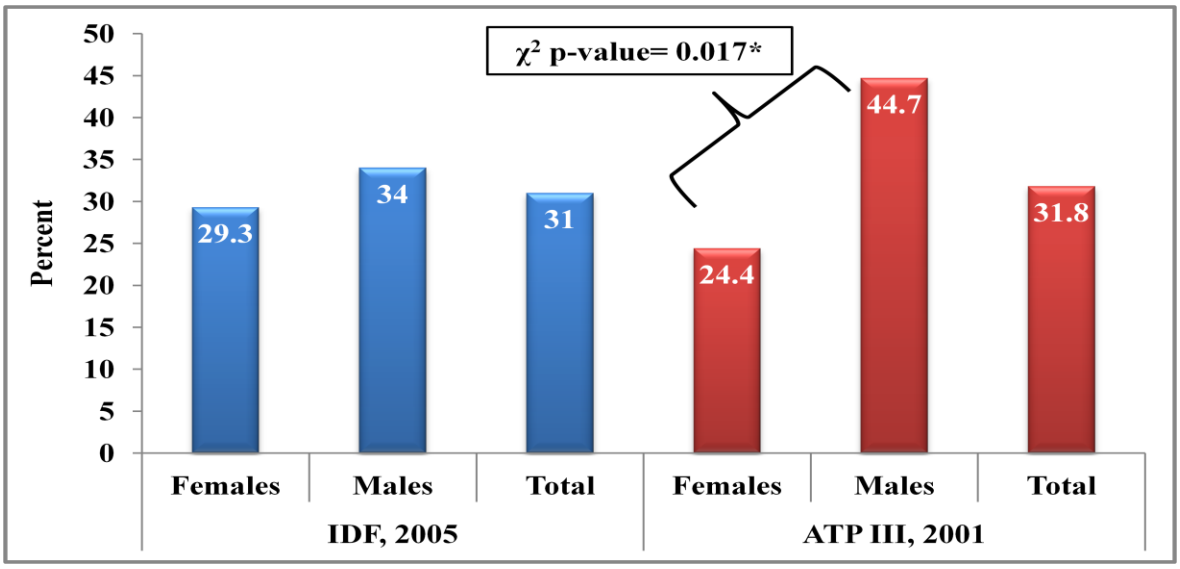
p<0.01**

TABLE 4.1.38 (B) POST-HOC TEST FOR NUTRIENT INTAKE ACROSS VITAMIN-D QUARTILES

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
Protein	**0.039***	**0.01****	0.28	0.614	0.321	0.136
Calcium	0.084	**0.002****	**0.01****	0.143	0.386	0.546
Vitamin-C	**0.045***	**0.02***	**0.001*****	0.747	0.173	0.297
Total Fibre	0.446	**0.01****	**0.011***	0.067	0.072	0.974
Insoluble Fibre	0.52	**0.013****	**0.01****	0.067	0.052	0.913
Soluble Fibre	0.295	**0.008****	**0.003****	0.104	0.053	0.753

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

FIGURE 4.1.8 (A) PREVALENCE OF METABOLIC SYNDROME AMONG THE SUBJECTS (n, %)



p<0.05*

FIGURE 4.1.8 (B) PREVALENCE OF METABOLIC SYNDROME ACROSS VITAMIN-D STATUS OF THE SUBJECTS (n, %)

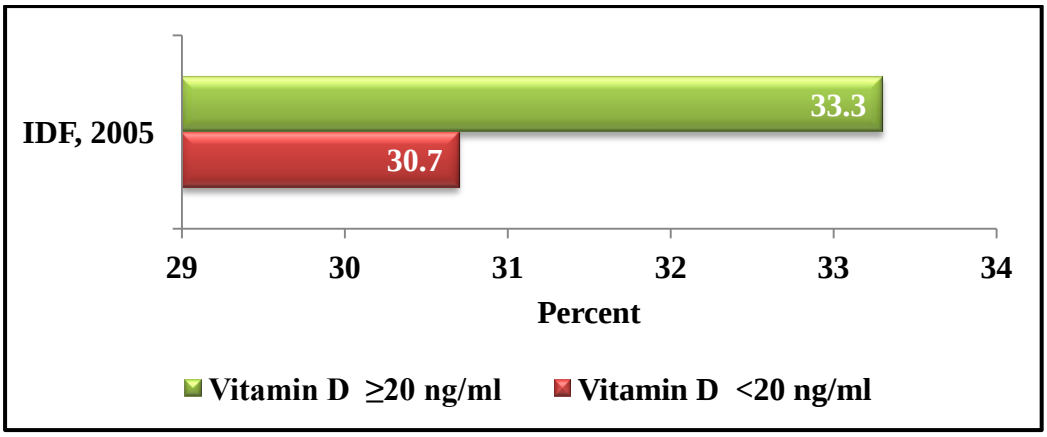


TABLE 4.1.39 PREVALENCE OF METABOLIC SYNDROME ACROSS VITAMIN-D QUARTILES OF THE SUBJECTS (n, %)

Parameters	Vitamin-D Quartiles (ng/ml)				χ^2 *p* value
	1st (3.94-8.82) (n=33)	2nd (8.98-12.9) (n=32)	3rd (12.91-15.8) (n=32)	4th (16.1-59.14) (n=32)	
IDF, 2005	10 (30.3)	7 (21.9)	14 (43.8)	9 (28.1)	0.283

Values in parenthesis indicate percent

CORRELATION BETWEEN VITAMIN-D STATUS AND RISK FACTORS

The previous analyses showed clearly that the aberrations in the vitamin-D levels could significantly affect many of the clinical parameters. However to test how linearly these levels are associated with parameters defining the clinical conditions, correlations between the vitamin-D levels of the subjects and various invasive and non-invasive parameters were assessed.

The correlations between vitamin-D levels and the non-invasive parameters are shown in Table 4.1.40. It was observed that among the anthropometric parameters, only percent body fat was negatively significantly correlated with the vitamin-D levels of the subjects i.e. as the body fat increased the vitamin-D levels decreased. When the correlations for various biochemical parameters were studied it was found that LDL cholesterol, total iron binding capacity and thyroid hormones- TSH & T3 had a significant negative correlation with the vitamin-D levels, while hemoglobin showed a significant positive correlation with vitamin-D levels meaning that with the increase in vitamin-D levels these parameters will also show a rise in their levels.

MULTIVARIATE PREDICTORS OF VITAMIN-D STATUS

To investigate how multiple variables in this study synergistically predicted variations in the vitamin-D status, multivariate analysis was carried out with serum 25(OH)D levels as the dependent variable (Table 4.1.41). The variables loaded in the equation were: age, WC, BMI, WHR, WSR, % body fat, TSH, T4, T3, haemoglobin, Total Cholesterol, HDL-C, LDL-C, TAG, Hs-CRP, FBS, HbA1c creatinine, blood urea, uric acid, serum calcium, SGOT, SGPT and GGT. It was observed that the multivariate model that explained maximum amount of variation consisted of seven variables: % body fat, age, TSH, haemoglobin, LDL cholesterol, T3 and FBS; with six predictors being statistically significant except percent body fat which just missed the significance. This particular model explained 27.9% of variation for the vitamin-D status among the subjects.

TABLE 4.1.40 CORRELATIONS OF SERUM VITAMIN-D LEVELS WITH ANTHROPOMETRIC MEASUREMENTS & BIOCHEMICAL PARAMETERS OF THE SUBJECTS

Variable	Pearson r value	*p* value
Anthropometric measurement		
Body fat (%)	-0.246	**0.005****
Biochemical Parameters		
LDL Cholesterol	-0.184	**0.036***
Hb (gm/dl)	0.314	**0.000*****
TIBC (mcg/dl)	-0.247	**0.005****
TSH	-0.223	**0.011***
T3	-0.180	**0.041***

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

TABLE 4.1.41 MULTIVARIATE PREDICTORS OF VITAMIN-D STATUS OF THE SUBJECTS (STEPWISE LINEAR REGRESSION)

Predictor Variables	Adjusted r^2	Standardized β coefficients	*p* value
% Body fat	0.279	-0.162	**0.057**^{NS}
Age		0.273	**0.001****
TSH		-0.267	**0.001****
Haemoglobin		0.292	**0.000*****
LDL cholesterol		-0.210	**0.008****
T3		-0.168	**0.031***
Fasting Blood Sugar		-0.169	**0.037***

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

DISCUSSION

India is a tropical country, wherein Gujarat state located in western region is blessed with sunlight almost throughout the year. Because of the strategic location, there appears very little doubt that the population residing in this state would have deficiency of the sunshine vitamin i.e. vitamin-D. To check on this hypothesis a study was carried out in Vadodara city; the third largest city of the State, located at 22.30°N 73.19°E in central Gujarat. Surprisingly a very high prevalence of vitamin-D deficiency (88.4%), defined as serum 25(OH)D levels <20ng/ml; was observed among apparently healthy adults (30-60 years) residing in an urban setting of the city, thus proving the hypothesis wrong. About 8% subjects had insufficient levels and only around 4% fell in the sufficiency range with levels >30 ng/mL. This finding falls true to the accepted notion that VDD is a pandemic condition. A recent review also reported such high prevalence in various countries throughout all continents, which ranged from 31% in Australia to 98% in Mongolia. The reported prevalence for Indian population was 75% (Wacker & Holiack, 2013).

The literature regarding vitamin-D status of population in Asian region includes few studies conducted among children, pregnant women or post-menopausal women and elderly population. Plasma 25(OH)D measured in a cross sectional sample of 1,443 men and 1,819 women aged 50–70 years from Beijing and Shanghai revealed that the geometric mean of plasma 25(OH)D was 40.4 nmol/l, and percentages of VDD [25(OH)D <50 nmol/l] and insufficiency [25(OH)D <75 nmol/l] were 69.2% and 24.4%, respectively among the middle-aged and elderly individuals (Lu et al., 2009).

In Korean population also vitamin-D deficiency (<20 ng/mL) was found in 56.9% of the subjects (47.3% males and 64.5% females) in the Korea National Health and Nutrition Examination Survey (KNHANES), whereas only 13.2% of male and 6.7% of female population had a serum 25(OH)D level of greater than 30 ng/mL (Choi et al., 2011).

As mentioned earlier, India has also not been spared of low serum vitamin-D levels. In a survey among hospital staff the mean serum levels were 30 nmol/L (Arya *et al*, 2004), while among pregnant women it was 35 nmol/L (Sachan et al., 2005) and 36 nmol/L for the post-menopausal women in India (Harinarayan, 2005). Marwaha et al. (2011), in a study of 1346 apparently healthy individuals above 50 years from north India reported VDD with serum 25(OH)D levels below 20 ng/ml in 91.2% of the population and vitamin-D insufficiency

(serum 25(OH)D 20-<30 ng/ml) in 6.8% of the population. Similar low vitamin-D levels (13.7ng/mL or 34.2 nmol/L) have been reported in our study also.

Gender and vitamin-D deficiency

The female subjects had significantly lower mean 25(OH)D levels than males (11.4 vs 17.6 ng/mL, $p<0.001$) in the present study. These results are similar to studies reported elsewhere. An analytical cross sectional study among 380 subjects, age 35 years and above in an existing Malay cohort in Kuala Lumpur reported that females had significantly lower mean Vitamin D levels (36.2 nmol/L; 95% CI: 34.5-38.0) compared to males (56.2 nmol/L; 95% CI: 53.2-59.2). Approximately 41% and 87% of males and females respectively had insufficient (< 50 nmol/L) levels of 25-hydroxyvitamin D ($p < 0.001$). Most of the participants were Muslims by religion with most of the females adapted to concealing dressing style such as veils (head scarves, face not covered), long sleeves, long skirts etc. Most of the employees were engaged in indoor work (Moy & Bulgiba, 2011).

Gaafar and Badr (2013) reported that among 365 participants, above the age of 18 years, randomly selected from five healthy care clinics in the state of Kuwait, about 25.2% were deficient and 36.2% were insufficient in vitamin D level. Among them 165 (45.2%) were males and 200 (54.8%) were females. Vitamin D deficiency and insufficiency were significantly more frequent among female and age group 60 years and more ($p<0.05$). A higher proportion of participants with vitamin D deficiency and insufficiency were involved in indoor work or were house wives or not working. The difference was statistically significant when compared with others involved in field (outdoor) work ($p<0.0001$).

Similar results have been reported by Indian authors also. A study carried out in Kashmir valley among 150 healthy volunteers, aged 18-40 years reported a prevalence of 83% among adults, with significant higher rates among females as compared to males (94.4 vs 76.6%, $p<0.001$) (Zargar et al., 2007). Another study reported VDD in women of reproductive age group (76%) and in post menopausal women (70%) with serum 25(OH)D levels <20ng/ml in south India (Harinarayan et al., 2011). The possible reasons contributing to this gender difference may be that non-working Indian women spend a large part of their day being engaged in indoor household work and so have poor sun exposure. In the present study also majority of the women (84.1%) were housewives and stayed at home most of the time. The clothing style of women, such as wearing long sleeves salwar-kameez, sari, or using hand gloves or sun-coat and covering the face with dupatta while going out reduces the total body

area exposed to sunlight and thus reducing the cutaneous production of vitamin-D. Also as majority of the population in this part of India is vegetarian, the vitamin-D intake from dietary sources would also be scarce due to fewer options available of vitamin-D rich foods.

To see the extent of deficiency among the population the subjects were further categorized in various stages of VDD as proposed by Lips (2001). The segregation of data showed that about 53.5% of the population fell in the mild category with serum 25(OH)D levels 10-<20 ng/mL. around 33.3% were in the moderate category (5-<10 ng/mL), of which a high percentage was of women as compared to men (51.2% vs 2.1%). However it was a relief to observe that only 1.6% of the population had levels <5ng/mL comprising the severe category and they were all females with such low levels. Similar analysis has also been reported by Marwaha et al., 2011 and Zargar et al., 2007 wherein the prevalence of severe deficiency (27.9% and 27.5% respectively) was much higher as compared to the present study.

Age and vitamin-D deficiency

Increasing age is also considered as a risk factor for poor vitamin-D status. It has been reported that aging can decrease by more than 2-fold the capacity of the skin to produce previtamin D3 in response to UV radiation (MacLaughlin and Holick, 1985). Unexpectedly, however, our results showed that the serum 25(OH)D level increases with age from 30-40 to 51–60 years. The highest prevalence of VDD (93.8%) was observed for age group 30-40 years followed by 90.5% among the 41-50 years old and the least (79.5%) among the oldest age group of 51-60 years. Age emerged as a positive predictor for vitamin-D levels in the multiple regression analysis.

Abiaka et al. (2013), also reported a significant age related increase of serum 25(OH)D levels among 206 healthy Omanis, aged 18-55 years. Women, as compared to men, had markedly lower concentrations of 25(OH)D (28.2±12.6 vs 36.8±16.7, p<0.0001) and the prevalence of VDD in the study population was found to be 87.5%.

In the KNHANES study also vitamin D insufficiency/deficiency was most prevalent in the age of 20–29, with a rate of 65% in males and 79.9% in females as compared to the older age groups (Choi et al., 2011). As explained by the authors, Korea's main industry has changed from agriculture and fishery to manufacturing and commerce and the younger generations have moved to and settled in urban areas and acquired indoor jobs, whereas older generations have stayed in rural areas, working in agriculture, forestry, or fishery. Thus the trend

observed would be probably due to the change in occupation and lifestyle pattern of the younger generation as compared to the older generations in Korea.

Rahnavard et al. (2010) in the Iranian Multi- Center Osteoporosis study among healthy male population; aged 19-83 years, pointed out that vitamin D levels varied significantly with age groups with mean values of 25 OHD levels being significantly lower in men younger than 50 year old rather than those between 50- 60 year old ($p< 0.05$) and older than 60 year old ($p< 0.05$).

A study conducted among 77 healthy women, age 19 to 66 years, working in nursing homes in Japan also reported low serum levels among the young age group. The mean serum 25(OH)D concentration in women younger than 30 years was 34.0±11.0 nmol/L and significantly lower than that in women 30 years and older (50.0± 14.4nmol/L). The proportion of subjects younger than 30 years who had serum 25(OH)D concentrations less than 30 nmol/L was 42.1% and was significantly higher ($p<0.001$) than the proportion of those 30 years and older (10.3%) (Nakamura et al.,2000).

The probable reasons for such findings maybe that the young people comprise of a working group with majority of their time spent in offices that too indoor, now-a-days in AC rooms and they also tend to have comparatively a sedentary lifestyle. This is an area of concern as age >65 years is considered a risk factor for VDD but in our population high prevalence was seen among young adults, thus suggesting that vitamin-D estimation should be regularly monitored from early age. In the present study the highest prevalence of vitamin-D sufficiency (7.7%) was observed among the subjects belonging to the oldest age group which is a favourable result as it may make them less prone to fractures and many of the non-communicable diseases.

Non-invasive determinants of vitamin-D status

As a high prevalence of VDD was observed in the young working age group and among the female subjects, many non-invasive determinants of vitamin-D status were also looked upon. But none of the factors showed a significant relevance with the vitamin-D status of the subjects. However, presence of hypertension (OR=1.96, 95% CI: 0.24-16.1), consuming vegetarian diet (OR=1.5, 95% CI: 0.49-4.55) and having sun exposure less than 20 minutes per day (OR=1.17, 95% CI: 0.27-4.9) had higher non-significant odds for low vitamin-D levels among the subjects and the levels of serum 25(OH)D also did not differ among the groups.

Sunlight is the most abundantly available natural source of vitamin D and exposure of atleast 20 minutes on arms and legs a day between 10am-3pm of day produces enough vitamin D in the body to attain adequacy (Holick, 2004). But due to urbanization, long indoor working hours and lack of physical activity, use of this natural source is to the minimum. This had been reported in many studies conducted both in Indian setting and globally.

The mean 25(OH)D concentrations varied among the 65 healthy subjects belonging to three different study groups and were related to direct sunlight exposure and skin pigmentation in a study conducted by Goswami et al. (2000). The highest mean 25(OH)D concentration was measured in the soldier group (47.17 ± 11.73 nmol/L). The physician and nurse group, which had significantly less sunlight exposure than did the soldier group, also had significantly lower 25(OH)D concentrations (7.98 ± 3.49, $p<0.05$). Interestingly, the depigmented group, for whom sunlight exposure was significantly lower than for the physician and nurse group, had significantly higher 25(OH)D values (18.2 ± 11.23, $p<0.05$) than in the physician and nurse group, presumably because of absent skin pigmentation. However, 25(OH)D concentrations in both the physician and nurse and depigmented groups were significantly lower than those in the soldier group ($p<0.05$).

Again in 2008,Goswami et al compared the serum vitamin-D of urban and rural population in North India and reported that the mean 25(OH)D values for subjects in rural area were much higher than the urban area population (36.4±22.5 vs 13.5±3.0 nmol/l). This could be explained by longer duration of sunshine exposure in the former.

In another study by Harinarayan et al (2008), in Andhra Pradesh the 25(OH)D levels (measured in ng/mL) of rural subjects were significantly higher ($p<0.001$) than that of urban subjects in both male (23.7 ±0.8 vs 18.5 ±0.8) and female (19±0.9 vs 15.5± 0.3) groups. This was probably due to occupation, dress code and duration of exposure to sunlight of the rural subjects, who are agricultural labourers working for about 8 hours a day in sunlight as cited by the researchers.

Vitamin-D deficiency is also associated with increased risk for fractures, however there are conflicting reports in the literature (Lips, 2001). In the present study around 32 subjects (24.8%) reported a history of fracture of which nearly 84.3% were having serum 25(OH)D levels <20ng/mL and only 15.6% of those with levels >20ng/mL had history of fractures. Thus the prevalence of having a fracture was higher among the subjects with low level of

vitamin D however the vitamin-D levels were not statistically significant between the 'had fracture' and 'no fracture' group (15.2±10.2 vs 13.2±5.6 ng/mL, *p*=0.166). These findings are in line with the study conducted among healthy Indian adults of age 50 years and above, where in also 27.8% subjects had history of fractures with no significant difference between vitamin-D levels of those with history of fractures (9.95±8.73 ng/ml) and those without (9.74±7.14 ng/ml, p=0.88) (Marwaha et al., 2011). Another study from UK also reported that patients with hip fracture had lower serum 25(OH)D levels compared with controls. This difference was not observed in case of fractures for other sites (Dixon et al., 2006).

Dietary habits and vitamin-D status

Dietary pattern or habits play an important role in preventing deficiency disorders and maintaining a healthy body. The population in present study were predominantly consuming a vegetarian diet (68.2%) and only around 4.6% of the subjects were taking vitamin-D supplements (alone or in combination). This trend makes them vulnerable to vitamin-D deficiency as practically very little would be available from food sources. However as mentioned earlier, being a vegetarian did not emerge as a significant determinant for poor vitamin-D status in our study. In the present study the diet of subjects constituted of approximately 1492 kcal/day which is low as compared to the RDA. Carbohydrates contributed around 55 per cent of the total energy intake, proteins 11 percent and fats a high of approximately 34 per cent. Thus the subjects were fairly consuming a balanced diet as per the Indian Council of Medical Research (ICMR) and Nutrition Society of India (NIN) 2011 guidelines, which give the calorie distribution as 50-60% from carbohydrates, preferably from complex carbohydrates, about 10-15% from proteins and 20-30% from both visible and invisible fats.

Calcium is closely related to vitamin-D. The calcium absorptive performance of the gut is a function of 25(OH)D status of an individual. When the 25(OH)D concentrations are low, the effective calcium absorption from the gut is reduced (Heaney et al., 2003; Heaney, 2003). In the present study calcium intake among the female subjects (645 mg/day) met the RDA requirements as given by the ICMR which is 600 mg/ day while males fell short by around 21 percent (472 mg/day). Across the vitamin-D quartiles, calcium intake decreased with the increase in serum vitamin-D levels till third quartile but showed a rise at the fourth quartile. However the calcium intake did not differ with the vitamin-D status of the subjects. The mean calcium intake in high serum 25(OH)D group (≥20 ng/mL) was 425.3±124.9 mg/day as

compared to 602.4±391.7 mg/day among the low vitamin- group (p=0.085). This trend would probably be because the low vitamin-D group consisted more of women subjects who as seen from the nutrient intake table had a higher calcium intake as compared to males.

Harinarayan et al., (2008) in his study reported that the diet of urban subjects constituted of 2200 kcal/day approximately. Carbohydrates contributed 55 per cent of the total energy intake, proteins 10 percent and fat 10 per cent. The daily dietary calcium intake of the urban (315 mg/day) population was low compared to that of recommended daily/dietary allowances (RDA) issued by (ICMR). In another study the dietary energy, carbohydrate, fat, and protein intakes of the physician and nurse group, the soldier group and the depigmented group were normal according to Indian normative data published by the ICMR. All the groups consumed predominantly vegetarian diets. The mean dietary calcium contents of the groups were also not significantly different and met the RDAs (Goswami et al., 2000). In both these studies along with ours the dietary calcium intake did not influence the vitamin-D levels of the subjects.

Studies have reported that intake of diet, rich in phytate (inositol hexaphosphate) retards the absorption of calcium from the gut (Panwar and Punia, 2000). Inositol hexaphosphate forms chelates with divalent cations calcium, and reduce the absorption of calcium from the gut and Indians tend to eat a diet rich in phyates. However in the present study the phytate intakes have not been analysed, which is a limitation of the research.

The food frequency data of probable good sources of vitamin-D for vegetarians revealed that ghee (81.3%) followed by milk (50.8%) and buttermilk (24.2%) were consumed on daily basis by the subjects. However as these products are not fortified with calcium or vitamin D in India, the amount of the nutrients available to the body is questionable. Also there is a lack of database about the vitamin-D content of various Indian foods hence the amount gained from the consumption of these products cannot be calculated or compared.

In an interesting study by Al-Othman et al. (2012), it was reported that vitamin D levels were significantly highest among those consuming 9–12 cups of tea/week in both genders ($p<0.01$ for boys and $p<0.05$ for girls) independent of age, gender, BMI, physical activity and sun exposure. This study was conducted among 330 randomly selected Saudi adolescents aged 11-14 years. Subjects who consumed 0–4 times of tea per week had significantly lower 25(OH)D levels as compared to those consuming 8–12 times/week (19.8 vs 24.2 nmol/L,

p=0.009). No significant difference in 25(OH)D was found when compared to coffee intake. As our population also predominantly consisted of tea drinkers (77.5%), the vitamin-D levels were studied across the number of cups consumed per day. However no significant difference was observed among the subjects consuming <3cups/day and ≥3 cups/day (12.8±6.4 vs 14.7±5.6, *p*=0.143), though vitamin-D levels were slightly higher among the high tea drinkers. The difference found in both the studies can be attributed to the method adopted for brewing and preparing the tea, also the variety of tea used and the amount of milk added would affect the caffeine content which is thought to interact with the TT genetic variant of vitamin-D receptor (Rapuri et al., 2001)

Overweight/obesity and vitamin-D deficiency

Vitamin-D deficiency has been linked to overweight/obesity and central adiposity (Young et al., 2009). Waist circumference (WC), waist-to-hip ratio (WHR) & waist-to-stature ratio (WSR) are the anthropometric indices widely used for the evaluation of central body fat distribution, while Body Mass Index (BMI) is widely used to classify the individuals as normal, overweight or obese. Based on BMI, a high prevalence of overweight and obesity (69.7%) was seen in our population with mean BMI of 25.9 ± 4.7; however no significant difference was observed between the genders. This may also be a probable reason for low vitamin-D levels among the subjects. As reported by Worstman et al. (2000), high BMI leads to a lower vitamin-D concentration because vitamin-D is a fat soluble vitamin that is not easily released from adipose tissue once absorbed among the obese individuals.

Abdominal obesity is also significantly associated with low vitamin-D levels as reported in many studies (Lu et al., 2009). This relationship was also observed in our study when various anthropometric indices were cross tabulated with the vitamin-D status of the subjects. WSR and % body fat were found to be significantly higher among the subjects with vitamin D levels <20ng/mL. Both these indices are related to poor physical activity pattern and hence direct towards maintaining a daily physical activity routine. Similar to our findings, modest but significant inverse relationships of vitamin D with weight (*p*=0.009), BMI (*p*= 0.005) and waist circumference (*p*=0.03) were reported in a study among 250 ambulant adults in Auckland, New Zealand, however no relationship was seen with fat % (McGill, Stewart, Lithander, Strik, & Poppitt, 2008).

In another study among healthy Omani population, serum concentrations of 25(OH)D were associated moderately (*p*=0.023) with BMI and markedly (*p*<0.0001) with WC, WHR, and

WHtR. Stepwise linear regression analysis revealed WHR as the main predictor (r=0.336; *p* <0.0001) of serum 25(OH)D concentrations (Abiaka et al., 2013).

The increased anthropometric measurements (BMI, WC & WSR) and being a woman showed to have high significant odds for poor vitamin-D status in our study. Moy & Bulgiba (2011) also reported age, female gender, BMI and waist circumference to be significantly associated with vitamin D insufficiency (<50 nmol/l). Further they reported that being a female (OR 8.68; 95% CI: 5.08-14.83) and having abdominal obesity defined by high WC (OR 2.57; 95% CI: 1.51-4.39) had significantly higher odds for insufficient Vitamin-D status. These results clearly emphasize the need of regular exercise to maintain desired anthropometric profile, more so if one belongs to the fairer sex.

Hypertension and vitamin-D

Li et al (2002) provided convincing support for vitamin D as a proximal inhibitor of the renin-angiotensin system (RAS) when they described a phenotype of excess plasma renin activity and hypertension in mice lacking the vitamin D receptor, which normalized after treatment with RAS antagonists. Their collective experiments indicated that vitamin D may inhibit the RAS by reducing renin gene expression (Li, 2003). However in our study the blood pressure levels were found to be in physiologically normal range and they did not differ when segregated across vitamin-D levels.

Hyperlipidemia and vitamin-D levels

Vitamin D has pleiotropic effects that may favorably influence cardiovascular health. Cross-sectional and retrospective case-control studies found deficient or insufficient levels of circulating 25-hydroxy-vitamin D in patients with established cardiovascular disease (Wang et al., 2012). In the present study hypercholesterolemia, hypertriglyceridemia, atherogenic indice TAG/HDL-C and the AIP levels all showed high odds for poor vitamin-D status, though none of them were statistically significant. However across the BMI, HDL-C decreased while AIP and inflammation increased from normal to obese. The overweight and obese subjects were seen to have non-significant low vitamin-D levels than the normals. Across the vitamin-D quartiles, the LDL-C decreased significantly and showed a significant negative correlation with vitamin-D levels. But disappointingly the good HDL-C went down with the increase in vitamin-D levels, while the inflammatory marker Hs-CRP remained constant. The multiple regression analysis result for serum vitamin D, showed total cholesterol as a significant suppressor. Thus very conflicting results were observed which

need further investigation. Misung et al (2013) reported some very favourable results in his study on 171 healthy Korean adults with no history of cardiovascular disease. The serum vitamin D levels showed a positive correlation with age ($p<0.05$), HDL-C ($p<0.05$), and the inflammatory marker adiponectin ($p<0.05$) levels. In the multiple regression analysis even after controlling for age, gender, BMI, smoking, and alcohol intake, serum vitamin D showed an effect on levels of triglyceride ($p<0.05$), HDL-cholesterol ($p<0.05$) and adiponectin ($p<0.05$).

Metabolic Syndrome and vitamin-D status

The relationship between metabolic syndrome and vitamin-D is still unclear. In our study, about one-third of the population (around 31%) had metabolic syndrome (atleast 3 of the 5 risk factors), but their vitamin-D status was not significantly related with the risk of developing it. Thus the results are in clear contradiction to the ones reported elsewhere in which vitamin-D insufficiency or deficiency was independently associated with increased risk of having metabolic syndrome (Moy & Bulgiba, 2011; Parkera et al., 2010; Kim et al., 2010; Ford et al., 2009).

Vitamin-D and biochemical parameters

To get a holistic picture of the body functioning apart from lipid profile, many other biochemical parameters such as iron status, glycemic profile, thyroid hormones and liver & kidney functioning tests were also carried out on the subjects. However the results revealed that almost all the parameters were in the normal physiological range.

The prevalence of anemia was seen to be 41.1% among the population with significantly higher among the females. This is an accepted fact that women are more prone to low haemoglobin (Hb) levels as compared to males, which was observed in our study also. However Hb levels increased across the vitamin-D quartiles showing a positive correlation. The population was a non-diabetic one and so the glycemic profile also didn't show any unfavourable alterations. Favourably fasting blood glucose (FBS) decreased across the vitamin-D quartiles but increased with an increase in BMI of the subjects. This is also a very well accepted result. The thyroid hormones TSH and T3 showed a significant negative correlation with vitamin-D levels and TSH significantly reduced across the vitamin-D quartiles. In the multiple linear regression thyroid hormones (TSH & T3) and FBS emerged as suppressor of vitamin-D while Hb was identified as a positive predictor. This signified that if these biochemical parameters are controlled, then vitamin-D deficiency can be prevented.

The liver enzyme-GGT and kidney parameter-uric acid increased with the increase in vitamin-D levels, showing a significant positive correlation. But more or less the levels of all the parameters remained unaltered. In a study among healthy Kashmiris also there were no differences observed in the results of blood counts and lipid concentrations and the indices of liver and kidney functions in subjects with and without VDD as reported by Zargar et al. (2007).

Salient Observations

Thus from the results obtained and the discussions made the following salient observations can be enlisted-

- Majority of the population studied was in its productive years with the mean age being around 44 years.
- Most of the subjects consumed vegetarian diets.
- A high prevalence of obesity and abdominal obesity was found among the subjects along with metabolic conditions like hyperlipidemia and inflammation and metabolic syndrome among the subjects.
- A high prevalence of vitamin-D deficiency was detected among the adult population with significantly higher number of females being deficient and vitamin-D deficiency decreased with increase in age.
- Percent body fat, age, TSH, hemoglobin, total cholesterol, T3 and FBS emerged as significant predictors for the vitamin-D status of the subjects.

Positives & negatives of the study

The study had both positive and negative aspects which need to be acknowledged and considered while interpreting the results. The short comings of our study include the cross-sectional design where the cause could not be attributed and the comparatively small sample size, which would have failed to identify the association of vitamin-D status with some of the risk factors. This study was based on a single measurement of serum 25(OH)D as an indicator of vitamin-D status and parathyroid hormone which is a functional index of vitamin-D status also could not be measured due to monetary constraints. The results cannot be generalised to entire nation as our participants belonged only to the urban setting of a city in western India.

However, to the best of our knowledge, this is the first study of its kind to map the prevalence of VDD in Gujarat and investigate the association of 25(OH)D levels with clinical conditions more so in a city which receives ample sunlight for almost nine months of the year. This study is also timely as literature reports a high prevalence of vitamin-D deficiency in India, which supports the need for a nationalized supplementation programme and will add to the knowledge of researchers and existing literature.

Brief conclusion

Thus from this cross sectional study it can be concluded that high vitamin-D deficiency was present in the apparently healthy adult population with women being at higher risk. The hypothesized determinants of poor vitamin-D status like sun exposure, vegetarian diet, history of fractures, skin complexion and presence of clinical conditions did not show much significant association. However indices of abdominal obesity and percent body fat emerged as strong determinants for low vitamin-D levels. Hemoglobin, thyroid hormones, total cholesterol and fasting blood glucose are the significant biochemical parameters to be considered for predicting the vitamin-D status of an individual. All the significantly emerged variables can ultimately be managed and maintained by adopting a healthy dietary pattern and an active lifestyle with adequate exposure to sunlight which is the message to be pondered from this research.

PHASE II

IMPACT OF VITAMIN-D SUPPLEMENTATION ON CARDIO-METABOLIC PROFILE OF SUBJECTS WITH TYPE-II DIABETES MELLITUS

Type-II diabetes mellitus (T2DM) accounts for more than 90% of all patients worldwide. In India, there were estimated 50 million diabetics in 2010; the number is projected to increase to nearly 87 million in 2030 (Shaw et al., 2010). If not timely treated T2DM can lead to multitude of chronic microvascular and macrovascular conditions such as retinopathy, nephropathy, neuropathy and cardiovascular disease (Champe et al., 2005). Vitamin-D is an essential nutrient having its classic function in calcium and phosphate metabolism by its actions on kidney, intestine and bones. Recent research has related vitamin-D to many other physiological conditions and diseases affecting energy metabolism, the immune system, cancers as well as the cardiovascular system (Vaidya and Forman, 2010; Bouillon et al., 2008). Studies on many animal models as well as humans have shown vitamin-D and calcium are clearly involved in glycemic homoeostasis, and that altered vitamin-D and calcium concentrations play a role in the development of diabetes. However the exact mechanism is not known yet. Vitamin-D supplementation in diabetic patients has shown improvements in their glycemic control and HbA1c levels. But still there lies a disparity about the exact supplementation dose to sustain the serum vitamin-D levels among the patients and also to restrict the various complications arising due to T2DM. This background and curiosity on the relationship between T2DM and vitamin-D led to the carrying out of the following study.

Experimental Design

This phase of the research was divided into three parts which is discussed in detail in the Methods & Material chapter. In all 209 T2DM subjects were enrolled from a diabetic clinic for the screening, of which 114 subjects consented for the blood test to estimate biochemical parameters. Ninety-four subjects who had serum vitamin-D levels <20ng/ml were chosen for the supplementation study. The washout effect of supplementation was observed on this group after eight weeks of post supplementation. The results of this phase are presented under the following heads.

Phase II (a): Screening and collection of baseline data

- Background information of the subjects
- Medical history and information on aspects related to T2DM
- Lifestyle and general dietary practices

- Anthropometric and blood pressure measurements

Detailed Risk factor Analysis (n=114)

- Physical activity pattern and nutrient intake
- Vitamin-D status and other biochemical estimations

Phase II (b): Randomised control trial to study the impact of vitamin-D3 granules on serum 25(OH)D status and cardio-metabolic profile of subjects with T2DM

- Impact of vitamin-D supplementation on serum 25(OH)D levels
- Impact on the biophysical measurements
- Impact of supplementation on iron status, HbA1c levels, lipid profile, thyroid hormones, kidney profile and liver functioning tests.
- Impact on the physical activity and nutrient intake

Phase II (c) Washout effect of vitamin-D supplementation on serum 25(OH)D status of T2DM subjects

- In terms of vitamin-D status
- On the anthropometric and blood pressure measurements
- Lipid profile and HbA1c levels of the subjects

PHASE II (A): SCREENING AND COLLECTION OF BASELINE DATA

Background Information of the subjects

In all 209 T2DM subjects were enrolled from a diabetic clinic. The background information of subjects enrolled is displayed in Table 4.2.1. Of the subjects enrolled 77% were local residents while 23% were from out-of-station. About 88.5% subjects were Hindus, 8.6% belonged to the Muslim community and 2.4% were Jain. Information on educational background of the subjects revealed that about 6% of the women were illiterate. Among the rest, majority (32.5%) were graduates. Marital status showed that majority of the subjects were married (91.1%) and about 92% lived in nuclear families. Majority of the women (81.5%) were housewives. Rest of the population was either engaged in service (25.4%) or had their own business (21.5%). Majority of the subjects (42.9%) had per capita income greater than Rs 10,000/- thus suggesting a high income group.

Table 4.2.1 BACKGROUND INFORMATION OF THE SUBJECTS (n, %)

Background Information	Females (n=108)	Males (n=101)	Total (n=209)
City of residence			
Vadodara	79 (73.1)	81 (80.2)	160 (76.6)
Out-of-station	29 (26.9)	20 (19.8)	49 (23.4)
Religion			
Hindu	93 (86.1)	92 (91.1)	185 (88.5)
Muslim	11 (10.2)	7 (6.9)	18 (8.6)
Christian	1 (0.9)	0	1 (0.5)
Jain	3 (2.8)	2 (2.0)	5 (2.4)
Education			
Illiterate	6 (5.6)	0	6 (2.9)
Primary	16 (14.8)	3 (3.0)	19 (9.1)
SSC	23 (21.3)	24 (23.8)	47 (22.5)
HSC	30 (27.8)	20 (19.8)	50 (23.9)
Graduate	28 (25.9)	40 (39.6)	68 (32.5)
Postgraduate	5 (4.6)	12 (11.9)	17 (8.1)
Others	0	2 (2.0)	2 (1.0)
Marital Status			
Unmarried	1 (0.9)	3 (3.0)	4 (1.9)
Married	95 (88.0)	97 (96.0)	192 (91.9)
Divorcee	1 (0.9)	0	1 (0.5)
Widow/widower	11 (10.2)	1 (1.0)	12 (5.7)
Occupation			
Housewife	88 (81.5)	0	88 (42.1)
Service	13 (12.0)	40 (39.6)	53 (25.4)
Business	6 (5.6)	39 (38.6)	45 (21.5)
Retired	1 (0.9)	22 (21.8)	23 (11.0)
Type of family			
Nuclear	71 (65.7)	77 (76.2)	148 (70.8)
Joint	33 (30.6)	20 (19.8)	53 (25.4)
Extended	4 (3.7)	4 (4.0)	8 (3.8)
Per Capita Income (Rs) Range		600 - 60,000	
<5000	32 (30.8)	27 (26.7)	59 (28.8)
5000-10,000	33 (31.7)	25 (24.8)	58 (28.3)
>10,000	39 (37.5)	49 (48.5)	88 (42.9)

Values in parenthesis indicate percent

Age-wise Distribution of the subjects

The subjects enrolled were between the ages 30 to 65 years. As seen from Table 4.2.2 the mean age of subjects was 52.7 years. Majority of the subjects (47.4%) belonged to the age group 51-60 years, followed by 26.3% in the 41-50 years group.

Family History of the subjects

The family history of diseases among the subjects is depicted in Table 4.2.3. The information revealed that the family history was highest for diabetes mellitus (65.1%) followed by hypertension (55%) and then coronary heart disease (19.6%). About 11.5% population had a family history of Asthma and 11% had for Cancer.

Medical History of the subjects

The information on self reported medical history of the subjects is given in Table 4.2.4. It revealed that prevalence of hypertension was maximum (57.9%) followed by dyslipidemia (22.5%) which is quiet expected as they are the most common co-morbidities of diabetes mellitus. The prevalence for both the conditions was high among males as compared to females. Thyroidism was present in 15.3% of the subjects followed by osteoporosis (7.7%) which was much higher among the females as compared to males (13% vs 2% respectively).

Health Check-up Pattern of the subjects

As the subjects were diabetics the health check up pattern was also studied among them. The information revealed that about 25% of the subjects were not going for regular health checkups and they did not even keep a track of their blood sugar levels (Table 4.2.5). Maximum numbers of subjects (28.6%) were going for quarterly check-ups as the HbA1c levels could also be examined at that time. About 15.8% went to get their blood estimations done every month and 14.8% went for check up every six months.

Duration of Diabetes among the subjects

The information on number of years when diabetes was detected among the subjects was also collected which is shown in Table 4.2.6. It was observed that the mean time with disease was 6.1 years among the subjects with duration ranging from 1 to 28 years. Females suffered from diabetes for a longer period as compared to males (6.9 years vs 5.4 years respectively). About 45.9% of the population had diabetes from less than five years, while nearly 19% were living with diabetes for more than ten years.

Table 4.2.2 AGE WISE DISTRIBUTION OF THE SUBJECTS (n, %)

Age (Years)	**Females (n=108)**	**Males (n=101)**	**Total (n=209)**
30-40	12 (11.1)	8 (7.9)	20 (9.6)
41-50	31 (28.7)	24 (23.8)	55 (26.3)
51-60	51 (47.2)	48 (47.5)	99 (47.4)
61-65	14 (13.0)	21 (20.8)	35 (16.7)
Mean age (Mean ± SD)	53.2 ± 8.2	52.2 ± 7.6	52.7 ± 7.8

Values in parenthesis indicate percent

TABLE 4.2.3 FAMILY HISTORY OF DISEASES AMONG SUBJECTS (n, %)

	Females (n=108)	Males (n=101)	Total (n=209)
Diabetes Mellitus			
History Present	73 (67.6)	63 (62.4)	136 (65.1)
Parents	72 (66.7)	63 (62.4)	135 (64.6)
Siblings	42 (38.9)	27 (26.7)	69 (33.0)
Both	23 (21.3)	19 (18.8)	42 (20.1)
Grandparents + family	3 (2.8)	1 (0.99)	4 (1.9)
Hypertension			
History present	63 (58.3)	52 (51.5)	115 (55.0)
Parents	63 (58.3)	52 (51.5)	115 (55.0)
Siblings	27 (25.0)	20 (19.8)	47 (22.5)
Both	15 (13.9)	8 (7.9)	23 (11.0)
Hyperlipidemia			
History Present	9 (8.3)	3 (2.9)	12 (5.7)
Parents	9 (8.3)	3 (2.9)	12 (5.7)
Siblings	2 (1.8)	1 (0.99)	3 (1.4)
CHD			
History Present	25 (23.1)	16 (15.8)	41 (19.6)
Parents	25 (23.1)	16 (15.8)	41 (19.6)
Siblings	9 (8.3)	5 (4.9)	14 (6.7)
Both	2 (1.8)	0	2 (0.95)
Thyroid			
History Present	11 (10.2)	6 (5.9)	17 (8.1)
Parents	11 (10.2)	6 (5.9)	17 (8.1)
Siblings	6 (5.6)	4 (3.9)	10 (4.8)
Both	0	1 (0.99)	1 (0.5)
Asthma			
History Present	12 (11.1)	12 (11.9)	24 (11.5)
Parents	12 (11.1)	12 (11.9)	24 (11.5)
Siblings	4 (3.7)	2 (1.9)	6 (2.9)
Both	1 (0.9)	0	1 (0.5)
Grandparents + family	0	1 (0.99)	1 (0.5)
Cancer			
History Present	9 (8.3)	14 (13.9)	23 (11.0)
Parents	9 (8.3)	14 (13.9)	23 (11.0)
Siblings	3 (2.8)	3 (2.9)	6 (2.9)
Both	0	1 (0.99)	1 (0.5)
Grandparents + family	1 (0.9)	0	1 (0.5)
Stroke			
History Present	2 (1.8)	2 (1.9)	4 (1.9)
Parents	2 (1.8)	2 (1.9)	4 (1.9)
Siblings	2 (1.8)	1 (0.99)	3 (1.4)
Both	1 (0.9)	0	1 (0.5)

Values in parenthesis indicate percent

TABLE 4.2.4 MEDICAL HISTORY OF THE SUBJECTS (SELF REPORTED) (n, %)

Condition	Females (n=108)	Males (n=101)	Total (n=209)
Hypertension	52 (48.1)	69 (68.3)	121 (57.9)
CHD	3 (2.8)	6 (5.9)	9 (4.3)
Dyslipidemia	22 (20.4)	25 (24.8)	47 (22.5)
Stroke	0	1 (1.0)	1 (0.5)
Thyroidism	29 (26.9)	3 (3.0)	32 (15.3)
Cancer	2 (1.9)	1 (1.0)	3 (1.4)
Asthama	4 (3.7)	1 (1.0)	5 (2.4)
Rheumatoid Arthritis	3 (2.8)	1 (1.0)	4 (1.9)
Osteoporosis	14 (13.0)	2 (2.0)	16 (7.7)
Others	7 (6.5)	11 (10.9)	18 (8.6)

Values in parenthesis indicate percent

TABLE 4.2.5 REGULAR HEALTH CHECK-UP PATTERN OF THE SUBJECTS (n, %)

Duration of Health check-up	Females (n=108)	Males (n=101)	Total (n=209)
No regular check-up	27 (25.7)	24 (24.5)	51 (25.1)
Every month	12 (11.4)	20 (20.4)	32 (15.8)
Every two months	12 (11.4)	10 (10.2)	22 (10.8)
Quarterly	33 (31.4)	25 (25.5)	58 (28.6)
Half yearly	14 (13.3)	16 (16.3)	30 (14.8)
Yearly	7 (6.7)	3 (3.1)	10 (4.9)

Values in parenthesis indicate percent

TABLE 4.2.6 DURATION OF DIABETES AMONG THE SUBJECTS (n, %)

Duration of Diabetes (years)	Females (n=108)	Males (n=101)	Total (n=209)
Mean ± SD	6.9 ± 5.5	5.4 ± 4.8	6.1 ± 5.2
Range		1-28 years	
(n, %)			
< 5 years	57 (52.8)	39 (38.6)	96 (45.9)
5-10 years	35 (32.4)	39 (38.6)	74 (35.4)
> 10 years	16 (14.8)	23 (22.8)	39 (18.7)

Values in parenthesis indicate percent

Precipitating factors for T2DM among the subjects

The various reasons which would have been responsible for precipitating diabetes among the subjects were also studied which is shown in Table 4.2.7. Majority of the subjects (75%) did not know if any such factor affected them. Among the ones that were reported, stress or emotional disturbance was the reason in 15.8%, followed by infections in 5.7% of the subjects.

Symptoms of T2DM among the subjects

Regarding the information on the symptoms of diabetes mellitus experienced by the subjects, it was seen that 56% of the subjects did not experience any symptoms and came to know about presence of diabetes on doing a blood test (Table 4.2.8). However in rest the most commonly experienced symptoms were fatigue (23.4%), unexplained weight loss (20.6%), frequent urination (16.3%) and increased thirst (14.3%).

Treatment regimen followed by the subjects

The type of treatment that the subjects were following for controlling their blood sugar levels is discussed in Table 4.2.9. Most of the subjects (85.6%) were taking oral drugs as therapeutic measure to control their blood sugar levels. About 13% were on a combination therapy of drugs and insulin, while only a small fraction of the subjects (1.4%) were taking only insulin.

BLOOD GLUCOSE LEVELS OF THE SUBJECTS

AS THE ENROLMENT OF THE SUBJECTS WAS DONE FROM A DIABETIC CLINIC, THE SUBJECTS USUALLY CAME WITH THEIR FASTING AND POST LUNCH SUGAR EXAMINATIONS DONE THE PREVIOUS DAY. THESE VALUES WERE RECORDED AS A SECONDARY DATA. FROM 209 ENROLLED SUBJECTS, 113 (54.1%) HAD BOTH THE PARAMETERS DONE. THE MEAN VALUES ARE DEPICTED IN TABLE 4.2.10 AND REVEALED THAT BOTH THE PARAMETERS WERE HIGH AMONG FEMALES AS COMPARED TO MALES HOWEVER WERE NOT STATISTICALLY SIGNIFICANT.

History of surgeries among the subjects

The history of surgeries the enrolled subjects had undergone is shown in Table 4.2.11. About 38% of the subjects had undergone surgeries when the data was collected. Maximum subjects (24%) had got their cataract removed, which is again quiet expected as in diabetes the eyes are affected early. Removal of fibroid or cyst (17.7%) was the next highest surgery reported among the subjects, followed by removal of appendix (13.9%) and fissure or piles (11.4%).

TABLE 4.2.7 PRECIPITATING FACTORS OF T2DM AMONG THE SUBJECTS (SELF REPORTED) (n, %)

Factor	Females (n=108)	Males (n=101)	Total (n=209)
Emotions/Stress	18 (16.7)	15 (14.9)	33 (15.8)
Surgery	4 (3.7)	1 (1.0)	5 (2.4)
Infections	4 (3.7)	8 (7.9)	12 (5.7)
Pregnancy	2 (1.9)	0	2 (1.0)
Don't know	80 (74.1)	77 (76.2)	157 (75.1)

Values in parenthesis indicate percent

TABLE 4.2.8 SYMPTOMS OF T2DM AMONG THE SUBJECTS (SELF REPORTED) (n, %)

Symptom	Females (n=108)	Males (n=101)	Total (n=209)
No symptoms	64 (59.3)	53 (52.5)	117 (56.0)
Polyuria (Frequent urination)	21 (19.4)	13 (12.9)	34 (16.3)
Polydipsia (Increased thirst)	17 (15.7)	13 (12.9)	30 (14.3)
Polyphagia (Increased hunger)	7 (6.5)	3 (2.9)	10 (4.8)
Neuropathy (Loss of sensation)	1 (0.9)	0	1 (0.5)
Fatigue	26 (24.1)	23 (22.8)	49 (23.4)
Unexplained weight loss	19 (17.6)	24 (23.8)	43 (20.6)
Retinopathy (Blurred vision)	5 (4.6)	7 (6.9)	12 (5.7)
Slow healing of wounds	3 (2.8)	2 (2.0)	5 (2.4)
Odema	2 (1.9)	2 (2.0)	4 (1.9)
Confusion / Irritability	2 (1.9)	2 (2.0)	4 (1.9)

Values in parenthesis indicate percent

TABLE 4.2.9 TREATMENT REGIMEN FOLLOWED BY THE SUBJECTS (n, %)

Treatment regimen for Diabetes	Females (n=108)	Males (n=101)	Total (n=209)
ORAL DRUGS	93 (86.1)	86 (85.1)	179 (85.6)
INSULIN	0	3 (3.0)	3 (1.4)
ORAL DRUGS + INSULIN	15 (13.9)	12 (11.9)	27 (12.9)

Values in parenthesis indicate percent

TABLE 4.2.10 BLOOD SUGAR VALUES OF THE SUBJECTS (SECONDARY DATA) (MEAN ± SD)

Parameter	Females (n=55)	Males (n=58)	Total (n=113)	t-test *p* value
FASTING BLOOD SUGAR (FBS)	155.6 ± 58.2	143.4 ± 39.8	149.3 ± 49.8	0.198^{EVNA}
POST PRANDIAL SUGAR (PP_2)	221.4 ± 93.9	207.2 ± 73.3	214.1 ± 83.9	0.373^{EVNA}

EVNA=EQUAL VARIANCE NOT ASSUMED

TABLE 4.2.11 HISTORY OF SURGERIES AMONG THE SUBJECTS (n, %)

History of Surgery	Females (n=108)	Males (n=101)	Total (n=209)
No surgeries	72 (66.9)	58 (57.4)	130 (62.2)
Had surgeries	36 (33.3)	43 (42.6)	79 (37.8)
Type of surgeries			
Piles/ Fissure	3 (8.3)	6 (13.9)	9 (11.4)
Cataract	7 (19.4)	12 (27.9)	19 (24.0)
Appendix	7 (19.4)	4 (9.3)	11 (13.9)
Cyst/ Fibroid	8 (22.2)	6 (13.9)	14 (17.7)
Kidney related	3 (8.3)	3 (7.0)	6 (7.6)
Knee replacement	3 (8.3)	0	3 (3.8)
Hernia/ Prostrate	0	5 (11.6)	5 (6.3)
Spin surgery	2 (5.6)	0	2 (2.5)
Heart surgeries	1 (2.8)	6 (13.9)	7 (8.9)
ENT (Tonsils, moles in nose/ear)	1 (2.8)	3 (7.0)	4 (5.1)
Others	3 (8.3)	6 (13.9)	9 (11.4)

Values in parenthesis indicate percent

About 9% of the subjects also reported heart surgeries with more number of males undergoing it than females.

History of fractures among the subjects

Among the subjects enrolled about 34% had a history of fractures as displayed in Table 4.2.12. The history of fractures were reported highest for legs (41.7%) among the subjects, then for hand fractures (30.5%) followed by fractures in ankle (11.1%) and shoulder (9.7%).

Nutritional supplements consumed by the subjects

About 35.4% of the population were taking nutritional supplements of some kind (Table 4.2.13). Multivitamin supplements (51.3%) were the most commonly consumed by the subjects as they were readily prescribed by the doctor for general well-being. Calcium, vitamin-B12 supplements for its deficiency rectification and ayurvedic medicines for control of diabetes were taken by about 19% of the subjects. Around 16% of the subjects were taking vitamin-D supplements also.

Dependence Syndrome among the subjects

The Dependence Syndrome pattern of the subjects is displayed in Table 4.2.14. Mostly the dependence was seen among male subjects only as still for women to get addicted to some bad habit is considered a taboo. Only 2.8% women subjects reported padiki dependence. While among males about 19% had tobacco addiction, 6% were cigarette smokers and 5% consumed gutka.

Dietary practices followed by the subjects

The dietary practises followed by the subjects are given in Table 4.2.15. Majority of the subjects (64.6%) were consuming vegetarian diets followed by non-vegetarians (27.8%). About 89.5% of the subjects preferred tea as a beverage on regular basis and most of the subjects (96.7%) consumed iodized salt. Regarding oil consumption practises only 5.8% were practising rotation of oil and most of them (75%) did it at every three months. About 10.5% of the subjects were discarding the left over oil after deep frying and about 18% were using it again for deep frying. Majority of the subjects (80.4%) were using the left over oil in sautéing vegetables or kneading dough for making bhakris, rotis or parathas. An attempt was made to study the amount of oil, salt and sugar consumed by the subjects per day. The data revealed that per day oil (49.9 ± 23.3 g/day) and salt (9.4 ± 4.8 g/day) consumption was high among

TABLE 4.2.12 HISTORY OF FRACTURES AMONG THE SUBJECTS (n, %)

Site of Fracture	Females (n=108)	Males (n=101)	Total (n=209)
Had fractures	35 (32.4)	37 (36.6)	72 (34.4)
• Shoulder	3 (8.6)	4 (10.8)	7 (9.7)
• Hand	10 (28.6)	12 (32.4)	22 (30.5)
• Legs	18 (51.4)	12 (32.4)	30 (41.7)
• Ankle	4 (11.4)	4 (10.8)	8 (11.1)
• Wrist	1 (2.8)	4 (10.8)	5 (6.9)
• Knee	2 (5.7)	1 (2.7)	3 (4.2)
• Elbow	3 (8.6)	2 (5.4)	5 (6.9)
• Others	2 (5.7)	2 (5.4)	4 (5.5)

Values in parenthesis indicate percent

TABLE 4.2.13 NUTRITIONAL SUPPLEMENTS CONSUMED BY THE SUBJECTS (n, %)

	Females (n=108)	Males (n=101)	Total (n=209)
Supplements Taken	46 (42.6)	28 (27.7)	74 (35.4)
Types of supplements			
Vitamin D	11 (23.9)	1 (3.6)	12 (16.2)
Calcium	11 (23.9)	3 (10.7)	14 (18.9)
Vitamin B12	10 (21.7)	4 (14.3)	14 (18.9)
Multivitamin	16 (34.8)	22 (78.6)	38 (51.3)
Iron	1 (2.2)	0	1 (1.3)
Ayurvedic supplement	8 (17.4)	6 (21.4)	14 (18.9)

Values in parenthesis indicate percent

TABLE 4.2.14 DEPENDENCY SYNDROME AMONG THE SUBJECTS (n, %)

Habits	Females (n=108)		Males (n=101)		Total (n=209)	
	Past	Present	Past	Present	Past	Present
Tobacco	0	0	1 (1.0)	19 (18.8)	1 (0.5)	19 (9.1)
Pan	0	0	0	1 (1.0)	0	1 (0.5)
Padiki	0	3 (2.8)	0	3 (3.0)	0	6 (2.9)
Gutka	0	0	2 (2.0)	5 (5.0)	2 (1.0)	5 (2.4)
Cigarette	0	0	6 (6.0)	6 (6.0)	6 (2.9)	6 (2.9)
Alcohol	0	0	2 (2.0)	4 (4.0)	2 (1.0)	4 (1.9)

Values in parenthesis indicate percent

TABLE 4.2.15 DIETARY PRACTICES OF THE SUBJECTS (n, %)

Practices	Females (n=108)	Males (n=101)	Total (n=209)
Type of Diet			
Vegetarian	70 (64.8)	65 (64.4)	135 (64.6)
Non- vegetarian	29 (26.9)	29 (28.7)	58 (27.8)
Ovo- vegetarian	9 (8.3)	7 (6.9)	16 (7.7)
Hot beverage Consumption			
Tea	96 (88.9)	91 (90.1)	187 (89.5)
Coffee	6 95.6)	6 (5.9)	12 (5.8)
Type of Salt consumed			
Iodized	103 (95.4)	99 (98.0)	202 (96.7)
Non-iodized	5 (4.6)	2 (2.0)	7 (3.3)
Same type of oil used whole year			
Yes	100 (92.6)	96 (96.0)	196 (94.2)
No	8 (7.4)	4 (4.0)	12 (5.8)
Duration of Oil rotation			
2 months	1 (0.9)	0	1 (0.5)
3 months	6 (5.6)	3 (3.0)	9 (4.3)
6 months	1 (0.9)	1 (1.0)	2 (1.0)
Usage of left over deep fried oil			
Deep Frying	20 (18.5)	17 (16.8)	37 (17.7)
Other Usage	87 (80.6)	81 (80.2)	168 (80.4)
Discard The Oil	15 (13.9)	7 (6.9)	22 (10.2)
Oil consumption per person per day (g/day)			
Mean ± SD	50.9 ± 24.6	49.1 ± 22.2	49.9 ± 23.3
T test p-value	0.581		
Salt consumption per person per day (g/day)			
Mean ± SD	9.7 ± 5.2	9.2 ± 4.6	9.4 ± 4.8
T test p-value	0.464		
Sugar consumption per person per day (g/day)			
Mean ± SD	25.9 ± 17.9	25.9 ± 18.7	25.9 ± 18.3
T test p-value	0.991		

Values in parenthesis indicate percent

all the subjects. The mean per day sugar consumption was 25.9 ± 18.3 g/day, which was also in higher proportion specially keeping in mind that the subjects were diabetic patients. The difference in consumption amounts was not statistically significant between the genders for any of the ingredients.

Type of oil consumed by the subjects

Regarding the type of oil consumed by the family eight of the respondents (two females and six males) could not reply as they did not have the information on this. As displayed in the Table 4.2.16, almost 39% of the subjects reported the usage of cotton seed oil for cooking as compared to other oils. The other predominant oils used were ground nut oil (27.7%) followed by sunflower (18.2%) or corn oil (16.3). A good variety and combinations of oil was reported by the subjects which they used for daily cooking. However blending of oil or use of oils in rotation was not so commonly practised by the subjects as seen in the previous table.

Type of milk consumed by the subjects

The milk consumption pattern of the subjects is given in Table 4.2.17. A variety of milk was consumed by the subjects with majority (37.7%) consuming the *Shakti* brand of Baroda Dairy. A large number of the subjects were consuming full fat milk in the form of *Gold* brand of Baroda Dairy (22.2%) and loosely sold buffalo milk (26.6%). 14.5% subjects were consuming loose cow's milk purchased from milk sellers and only 4% subjects consumed skim or toned milk which is actually an healthy option.

Non-invasive parameters for vitamin-D status

When the non-invasive determinants of vitamin-D were studied among the subjects, it was found that almost 10% of the subjects had dark coloured skin, which is regarded as a risk factor for vitamin-D deficiency, while most of the subjects belonged to the wheatish skin colour category (53.6%). A good proportion of the subjects (61.2%) were going out in the sun during day time between 10 am to 2 pm. About 65.6% of those going out in sun had sun exposure more than 20 minutes per day. The recommended level for sunlight exposure for appropriate vitamin-D synthesis is 20-25 minutes a day. However it was heartening to observe that the mean time of sun exposure was 30.7 minutes per day with highest frequency for 2-4 times a week (49.2%). Only one of the total subjects reported the use of sunscreen and only three females covered the body with sun-cost or dupatta while going out (Table 4.2.18).

TABLE 4.2.16 TYPE OF OIL CONSUMED BY THE SUBJECTS (n, %)

Type of oil	Females (n=106)	Males (n=95)	Total (n=201)
Groundnut oil	24 (22.2)	34 (33.7)	58 (27.7)
Cotton Seed oil	49 (45.4)	32 (31.7)	81 (38.7)
Corn oil	16 (14.8)	18 (17.8)	34 (16.3)
Sunflower oil	19 (17.6)	19 (18.8)	38 (18.2)
Mustard oil	1 (0.9)	3 (3.2)	4 (2.0)
Soyabean oil	2 (1.9)	1 (1.1)	3 (1.5)
Saffola Gold	8 (7.4)	6 (5.9)	14 (6.7)
Ricebran oil	1 (0.9)	0	1 (0.5)
Coconut oil	2 (1.9)	0	2 (1.0)

Values in parenthesis indicate percent

TABLE 4.2.17 TYPE OF MILK CONSUMED BY THE SUBJECTS (n, %)

Type of Milk	Females (n=108)	Males (n=99)	Total (n=207)
Cow	17 (15.7)	13 (13.1)	30 (14.5)
Buffalo	26 (24.1)	29 (29.3)	55 (26.6)
Skim milk	6 (5.5)	3 (3.0)	9 (4.3)
Shakti (toned)	39 (36.1)	39 (39.4)	78 (37.7)
Gold (full fat)	26 (24.1)	20 (20.2)	46 (22.2)

Values in parenthesis indicate percent

Table 4.2.18 NON-INVASIVE DETERMINANTS OF VITAMIN D STATUS AMONG THE SUBJECTS (n, %)

Determinants	Females (n=108)	Males (n=101)	Total (n=209)
Skin type			
• Fair	46 (42.6)	30 (29.7)	76 (36.4)
• Wheatish	59 (54.6)	53 (52.5)	112 (53.6)
• Dark	3 (2.8)	18 (17.8)	21 (10.0)
Use of sunscreen (sometimes)	0	1 (1.0)	1 (0.5)
Going out in sun	52 (48.1)	76 (75.2)	128 (61.2)
Frequency of sun exposure			
• Daily	18 (34.6)	32 (42.1)	50 (39.1)
• 2-4 times a week	27 (51.9)	36 (47.4)	63 (49.2)
• Once a week	7 (13.5)	8 (10.5)	15 (11.7)
Minutes of sunlight exposure per day (Mean ± SD)	33.3 ± 20.6	28.9 ± 18.3	30.7 ± 19.3
Sunlight exposure ≥20 min/day	35 (67.3)	49 (64.5)	84 (65.6)
Type of clothing			
• Sari	71 (65.7)	0	71 (34.1)
• Salwar-Kameez	58 (53.7)	0	58 (27.7)
• Pant-Shirt	0	101 (100.0)	101 (48.3)
• Hand gloves+ Face covered	3 (2.8)	0	3 (1.4)

Values in parenthesis indicate percent

Biophysical measurements of the subjects

The gender-wise anthropometric profile and blood pressure measurements of the subjects is showed in Table 4.2.19. The mean BMI and hip-circumference was found to be 28.3 Kg/m^2 and 102.2 cm respectively. Similarly the mean for parameters of abdominal obesity was also found to be on the higher side with mean WC of 94.8 cm, mean WHR of 0.93 and mean WSR of 0.59. The mean systolic blood pressure was 134.5 mmHg whereas mean diastolic blood pressure was 84.3 mmHg. Comparisons between male and female subjects revealed that height and WHR values were significantly higher in males while BMI, hip-circumference, WSR and percent body fat was significantly high among females. The mean blood pressure values were slightly above the physiologically normal range.

Prevalence of overweight/obesity among the subjects

The prevalence of overweight and obesity as classified by BMI among the subjects is depicted in Table 4.2.20. Majority of the subjects were falling in the category of obesity with a high percent prevalence of 71.3% and only 11% of the subjects studied had normal BMI. The abdominal obesity as assessed by WC, WSR and WHR revealed that a large proportion of the subjects had greater measurements, increasing the risk for Cardio Vascular Disease. The prevalence based on WC (73.7%), WSR (93.3%) was significantly higher among female as compared to male subjects, while for WHR (83.3%) the prevalence was significantly high among males.

Prevalence of Hypertension among the subjects

The prevalence of hypertension was studied using the latest JNC-VIII guidelines. The subjects were classified into three categories of normal, pre-hypertensive and hypertensive (Table 4.2.21). It was observed that for SBP, about 39.7% were hypertensive and nearly 39% were having the risk of getting hypertension. Similarly for DBP, about 32% of the subjects were in the hypertensive category and 48% were in the pre-hypertensive stage. The prevalence for pre-hypertension was more among females for both- SBP & DBP as compared to males.

When the blood pressure values were seen across the normotensive and hypertensive diabetic subjects, it was observed that both systolic and diastolic blood pressure was significantly higher among the hypertensive subjects (Table 4.2.22).

TABLE 4.2.19 ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS OF THE SUBJECTS (MEAN ± SD)

Variables	Females (n=108)	Males (n=101)	Total (n=209)	t-Test *p* value
Weight (Kg)	70.2 ± 12.8	72.6 ± 14.0	71.3 ± 13.4	0.196
Height (cm)	152.7 ± 5.3	165.6 ± 6.1	158.9 ± 8.6	**0.000*** **
BMI (Kg/m^2)	29.9 ± 5.0	26.5 ± 4.6	28.3 ± 5.1	**0.000*** **
WC (cm)	94.7 ± 10.7	94.9 ± 12.2	94.8 ± 11.4	0.866
HC (cm)	107.5 ± 12.5	96.7 ± 12.0	102.2 ± 13.4	**0.000*** **
WHR	0.88 ± 0.07	0.98 ± 0.07	0.93 ± 0.08	**0.000*** **
WSR	0.62 ± 0.07	0.57 ± 0.07	0.59 ± 0.07	**0.000*** **
% Body fat	41.3 ± 4.6	31.7 ± 5.7	36.7 ± 7.0	**0.000*** **
SBP (mmHg)	135.2 ± 20.1	133.6 ± 22.4	134.5 ± 21.2	0.599
DBP (mmHg)	83.9 ± 9.5	84.6 ± 10.5	84.3 ± 9.9	0.638

p < 0.001***

TABLE 4.2.20 PREVALENCE OF OVERWEIGHT & OBESITY AMONG THE SUBJECTS (n, %)

Category based on BMI	Females (n=108)	Males (n=101)	Total (n=209)	χ^2 *p* value
Underweight (BMI<18.5)	0	1 (1.0)	1 (0.5)	**0.003****
Normal (BMI:18.5 – 22.9)	5 (4.6)	18 (17.9)	23 (11.0)	
Overweight (BMI:23 – 24.9)	15 (13.9)	21 (20.8)	36 (17.2)	
Obese (BMI: ≥25)	88 (81.5)	61 (60.4)	149 (71.3)	
Based on % Body fat				
Body fat (F>30, M>20%)	106 (98.1)	97 (96.0)	203 (97.1)	0.362
Based on Anthropometric Indices				
WC (F≥80, M≥90 cm)	101 (93.5)	53 (52.5)	154 (73.7)	**0.000*** **
WSR (≥ 0.5)	106 (98.1)	89 (88.1)	195 (93.3)	**0.004****
WHR (F≥0.85, M≥0.9)	77 (71.3)	97 (96.0)	174 (83.3)	**0.000*** **

p< 0.001***, <0.01** Values in parenthesis indicate percent

TABLE 4.2.21 PREVALENCE OF HYPERTENSION AMONG THE SUBJECTS (n, %)

Criteria (JNC-8, 2014)		Females (n=108)	Males (n=101)	Total (n=209)
Systolic Blood Pressure (mmHg)	Normal (<120)	20 (18.5)	25 (24.8)	45 (21.5)
	Pre-hypertensive (120-139)	45 (41.7)	36 (35.6)	81 (38.8)
	Hypertensive (≥ 140)	43 (39.8)	40 (39.6)	83 (39.7)
Diastolic Blood Pressure (mmHg)	Normal (< 80)	22 (20.4)	20 (19.8)	42 (20.1)
	Pre-hypertensive (80-89)	53 (49.1)	48 (47.5)	101 (48.3)
	Hypertensive (≥90)	33 (30.6)	33 (32.7)	66 (31.6)

Values in parenthesis indicate percent

TABLE 4.2.22 BLOOD PRESSURE VALUES (mmHg) ACROSS NORMOTENSIVE & HYPERTENSIVE SUBJECTS (MEAN ± SD)

	Normotensive (n=38)	Hypertensive (n=171)	t-Test *p* value
Systolic Blood Pressure	107.4 ± 5.2	140.5 ± 18.5	**0.000*****EVNA**
Diastolic Blood Pressure	75.7 ± 5.8	86.2 ± 9.7	**0.000*****EVNA**

p< 0.001*** EVNA=EQUAL VARIANCE NOT ASSUMED

DETAILED RISK FACTOR ANALYSIS

THE DETAILED RISK FACTOR ANALYSIS IN THE FORM OF INFORMATION ON PHYSICAL ACTIVITY PATTERN, NUTRIENT INTAKE AND DIETARY PATTERN AND BIOCHEMICAL PARAMETERS WAS DONE ON 114 SUBJECTS ONLY DUE TO BUDGETARY CONSTRAINTS AND ALSO BECAUSE NOT ALL THE ENROLLED SUBJECTS WERE READY FOR BLOOD TESTS. HENCE HERE ON, THE RESULTS ARE PRESENTED FOR THE 114 SUBJECTS ON WHOM THE DETAIL RISK FACTOR ANALYSIS WAS CARRIED OUT.

Physical activity pattern of the subjects

An overview of the self reported physical activity pattern of the subjects as assessed by International Physical Activity (Short) Questionnaire is given in Table 4.2.23. The subjects were categorised according to their physical activity under 3 heads – low, moderate and high physical activity level. About 31.6% of the subjects were physically less active, in which most of the subjects were females (38.5%). As the population was diabetic, doing some form of physical activity is a must for them to maintain their blood sugar levels, and same was advised to them by doctors also. Hence it was found that a good number of subjects i.e. about 68% were moderately active; with more men (74.2%) falling in this category. The mean hours of sitting was found to be 5.9 ± 1.5 with females spending longer time in sitting activities as compare to their male counter-parts (6.1 vs 5.7 hours per day).

Nutrient intake of the subjects

The nutrient intake of the subjects is given in Table 4.2.24 (A). Information on nutrient intake was collected by 24 hour dietary recall method. The mean value for each of the nutrient was calculated as per National Institute of Nutrition's 2010 guidelines. Intake of calcium, vitamin-C, β carotene and dietary fibre was higher among female subjects while for rest of the nutrients males had a higher intake. However none of the nutrient intake was found to be statistically significant among both the genders.

Females were able to meet about 63% of the RDA for energy and 59% for proteins while males could meet around 56% of RDA energy and 57% for proteins (Table 4.2.24 B). Fat intake was very high among all the subjects and the percent RDA was more than double for both males and females. Vitamin-C intake of the subjects was more than adequate. The β carotene intake was <50% of RDA for both female and male subjects. Even the iron intake was less than 50% for females and about 62% for males.

TABLE 4.2.23 LEVELS OF PHYSICAL ACTIVITY AMONG SUBJECTS (n, %)

Physical Activity Level	Females (n=52)	Males (n=62)	Total (n=114)
Low	20 (38.5)	16 (25.8)	36 (31.6)
Moderate	31 (59.6)	46 (74.2)	77 (67.5)
High	1 (1.9)	0	1 (0.9)
Hours spent in sitting (Mean ± SD)	6.1 ± 1.6	5.7 ± 1.5	5.9 ± 1.5

Values in parenthesis indicate percent

TABLE 4.2.24(A) NUTRIENT INTAKE OF THE SUBJECTS (MEAN ± SD)

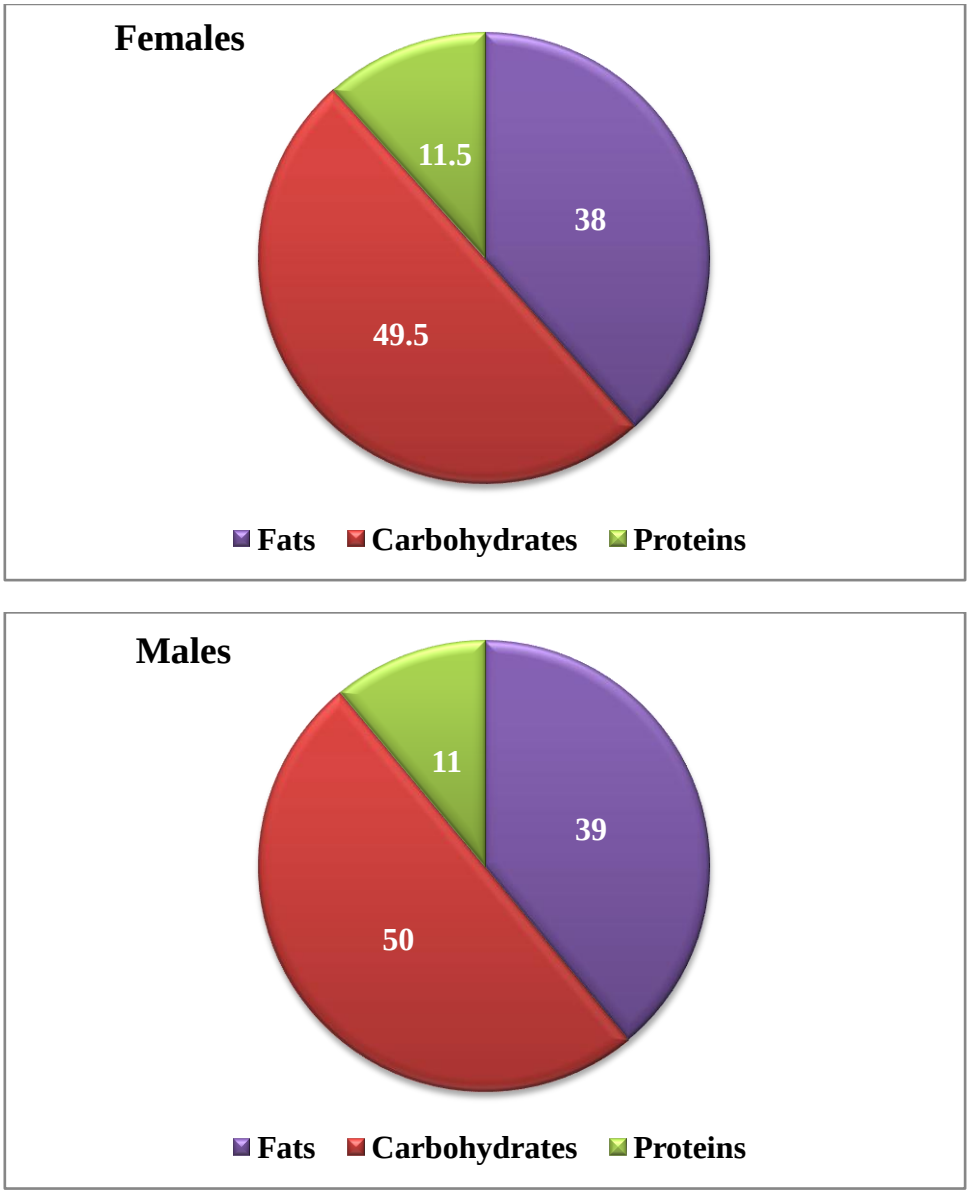
Nutrients	Females (n=52)	Males (n=62)	Total (n=114)	t-Test *p* value
Energy (Kcal)	1195 ± 339	1292 ± 285	1248 ± 313	0.100
Protein (gm)	32.6 ± 10.9	34.4 ± 8.3	33.6 ± 9.5	0.307
Fat (gm)	50.0 ± 18.0	54.3 ± 16.4	52.4 ± 17.2	0.186
Carbohydrates (gm)	146.7 ± 534	159.5 ± 41.0	153.6 ± 47.3	0.153
Iron (mg)	9.7 ± 4.6	10.5 ± 3.8	10.1 ± 4.2	0.287
Calcium	483 ± 269	457 ± 199	469 ± 233	0.565
β carotene (µg)	1488 ± 1571	1013 ± 1042	1250 ± 346	0.111
Vitamin C (mg)	64.9 ± 60.5	61.1 ± 68.1	62.9 ± 64.5	0.750
Crude Fibre (gm)	4.8 ± 2.1	5.1 ± 2.1	4.9 ± 2.1	0.403
Total Dietary Fibre	10.6 ± 5.9[#]	9.9 ± 4.3[#]	10.2 ± 5.1[#]	0.453
Insoluble Dietary Fibre	8.1 ± 4.7[#]	7.5 ± 3.5[#]	7.8 ± 4.1[#]	0.444
Soluble Dietary Fibre	2.5 ± 1.4[#]	2.4 ± 0.9[#]	2.5 ± 1.2[#]	0.499

[#]As reported by NIN for listed foods

TABLE 4.2.24(B) PERCENT RDA OF THE SUBJECTS

Nutrients	% RDA	
	Females (n=52)	Males (n=62)
Energy (Kcal)	62.9	55.7
Protein (gms)	59.3	57.3
Fat (gms)	250.0	217.2
Iron (mg)	46.2	61.8
Calcium (mg)	80.5	76.2
β carotene (µg)	31.0	21.1
Vitamin C (mg)	162.2	152.7

FIGURE 4.2.1 PERCENT ENERGY (KCAL) FROM MACRONUTRIENTS



The percent energy from macronutrients revealed that fats contributed about 38% & 39% of the total calories in females and males respectively. This resulted in lower percent of calories for females & males from carbohydrates, which was around 50% and even lower from proteins (about 11%) (Figure 4.2.1). THUS IT WAS SEEN THAT THE SUBJECTS WERE NOT HAVING A BALANCED DIETARY PATTERN AND THE DISTRIBUTION OF CALORIES COMING FROM MAJOR MACRO-NUTRIENTS WAS ALSO NOT APPROPRIATE.

CONSUMPTION FREQUENCY OF FOOD SOURCES FOR VITAMIN-D

The food frequency for some commonly eaten vitamin-D rich foods for the subjects was collected using a food frequency questionnaire. The information regarding it is depicted in Table 4.2.25. Major food sources of vitamin D are milk and some milk products, eggs and certain varieties of fish. As majority of the subjects were vegetarian, the consumption of eggs, fish and other non-vegetarian foods was very less. Among those who consumed them the highest frequency for egg was weekly for both males and females, for fish and chicken it was weekly for females and monthly for males, while for mutton consumption it was monthly for both the genders.

The frequency pattern of consumption of vitamin D rich food sources indicated that only few foods were eaten on a regular basis by the subjects. Highest consumption on daily basis was of ghee (63.2%) followed by milk (45.6%). Frequency of consuming curd (50%) and buttermilk (47.4%) by most of the subjects was found to be once a week. Maximum consumption of paneer (64.9%), butter (42.1%) and cheese (35.1%) was on monthly basis.

There were about 42.1% subjects who did not consume milk at all, which is an easily available source for vitamin-D. Thus the vitamin D levels were calculated across those who consumed milk and the non-consumers. It was observed that milk consumers had slightly higher levels of vitamin D than the non-consumers (14.5±10.2 vs 13.7±5.9 ng/mL), though the levels were statistically non-significant (Figure 4.2.2).

BIOCHEMICAL ESTIMATIONS OF THE SUBJECTS

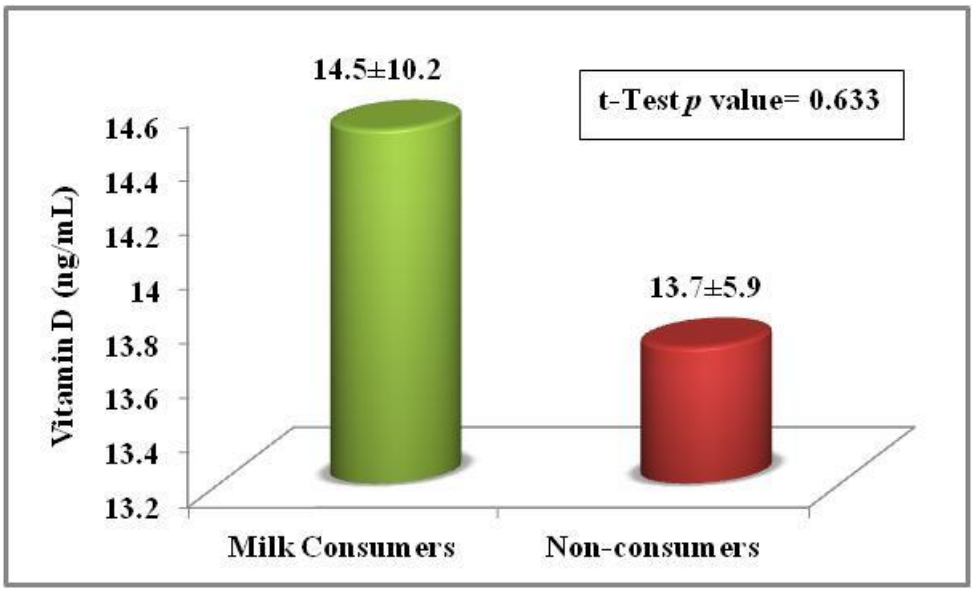
The following tables give the information regarding the various biochemical parameters estimated among the subjects. The estimations include vitamin-D status of the subject, iron status, HbA1c values, lipid profile, thyroid hormones, liver function test and kidney profile.

TABLE 4.2.25 FREQUENCY OF CONSUMPTION OF VITAMIN D RICH FOODS (n, %)

FOOD ITEMS	Females (n=52)					Males (n=62)				
	Daily	Weekly	Monthly	Occasionally	Never	Daily	Weekly	Monthly	Occasionally	Never
MILK	24 (46.2)	6 (11.5)	2 (3.8)	1 (1.9)	19 (36.5)	28 (45.2)	10 (16.1)	2 (3.2)	0	22 (35.5)
CURD	12 (23.1)	34 (65.4)	4 (7.7)	0	2 (3.8)	19 (30.6)	23 (37.1)	10 (16.1)	0	10 (16.1)
CHEESE	0	3 (5.8)	19 (36.5)	0	30 (57.7)	0	3 (4.8)	21 (33.9)	2 (3.2)	36 (58.1)
PANEER	0	5 (9.6)	30 (57.7)	1 (1.9)	16 (30.8)	0	10 (16.1)	34 (54.8)	2 (3.2)	16 (25.8)
BUTTERMILK	18 (34.6)	28 (53.8)	4 (7.7)	0	2 (3.8)	18 (29.0)	26 (41.9)	9 (14.5)	0	9 (14.5)
BUTTER	0	3 (5.8)	21 (40.4)	2 (3.8)	26 (50.0)	1 (1.6)	2 (3.2)	27 (43.5)	2 (3.2)	30 (48.4)
GHEE	30 (57.7)	13 (25.0)	2 (3.8)	0	7 (13.5)	42 (67.7)	6 (9.7)	3 (4.8)	3 (4.8)	11 (17.7)
EGG	1 (1.9)	6 (11.5)	5 (9.6)	0	40 (76.9)	1 (1.6)	8 (12.9)	7 (11.3)	3 (4.8)	43 (69.4)
FISH	0	4 (7.7)	3 (5.8)	0	45 (86.5)	1 (1.6)	3 (4.8)	6 (9.7)	1 (1.6)	49 (79.0)
MUTTON	0	1 (1.9)	5 (9.6)	0	46 (88.5)	0	2 (3.2)	5 (8.1)	2 (3.2)	54 (87.1)
CHICKEN	0	5 (9.6)	3 (5.8)	0	44 (84.6)	0	4 (6.5)	6 (9.7)	0	50 (80.6)

Values in parenthesis indicate percent

FIGURE 4.2.2 SERUM VITAMIN D LEVELS OF THE SUBJECTS ACROSS THEIR MILK CONSUMPTION PATTERN (MEAN ± SD)



VITAMIN-D STATUS OF THE SUBJECTS

Serum 25(OH)D was taken as the indicator of vitamin-D status among the population under study. The mean value for the subjects was found to be 14.2 ng/ml, which was much lower than the recommended optimum level of >30 ng/ml required for maintaining good health indicating a high level of sub-optimal vitamin-D status among the subjects. Female subjects had lower level of vitamin-D as compared to males though not statistically significant, but suggesting a higher prevalence of deficiency among them (Table 4.2.26).

The vitamin-D status of the subjects as categorised into deficiency, insufficiency and sufficiency groups based on their serum 25(OH)D levels is shown in Table 4.2.27. It was seen that almost 87% of the subjects were vitamin-D deficient having levels less than 20 ng/ml and only about 5% were in the sufficiency range with levels more than 30 ng/ml. As expected from the mean serum 25(OH)D levels from the previous table the prevalence of deficiency was higher among females as compared to male subjects (92.3% vs 85.5% respectively) though it was not statistically significant. The subjects identified as vitamin-D deficient were further classified into sub-category of mild, moderate and severe deficiency. As seen from Figure 4.2.3, about 60.5% subjects were falling under mild category and 28.1% in moderate category with higher number of females as compared to males in both categories. It was a relief to see that none of the subjects had severe deficiency i.e. levels <5 ng/mL.

Prevalence of anaemia among the subjects

The iron status of the subjects is given in Table 4.2.28 which also depicts the normal range for the parameters studied. The mean values for total iron, total iron binding capacity (TIBC) and percent transferrin saturation were in normal range for all the subjects. The mean haemoglobin of the subjects was 13.4 ± 1.6 g/dl. The prevalence of anaemia based on the haemoglobin values classified as per WHO 2001 guidelines was also mapped among the subjects. Around 25% of the subjects were found to be anaemic. Of these, 17.5% had mild anaemia and 7.9% had moderate anaemia. However it was good to see that none of the subjects was severely anaemic. The overall prevalence of anaemia was significantly higher among females as compared to male subjects (Table 4.2.29).

Glycemic profile of the subjects

The glycemic profile of the subjects is revealed in Table 4.2.30. As suggested by the medical ethics committee for human research, to study the glycemic profile only the glycoslated Hb (HbA1c) levels were examined. The mean value was 8.7% which was above the desired 7%,

TABLE 4.2.26 VITAMIN D LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameter	Females (n=52)	Males (n=62)	Total (n=114)	t-Test *p* value
25Hydroxy Vitamin D	13.0 ± 6.9	15.2 ± 10.1	14.2 ± 8.8	0.20

TABLE 4.2.27 VITAMIN D STATUS OF THE SUBJECTS (n, %)

Serum 25(OH)D ng/ml	Females (n=52)	Males (n=62)	Total (n=114)	χ^2 value
Deficiency (<20)	48 (92.3)	53 (85.5)	101 (88.6)	2.197
Insufficiency (20-≤ 30)	3 (5.8)	4 (6.5)	7 (6.1)	
Sufficiency (>30)	1 (1.9)	5 (8.1)	6 (5.3)	

Values in parenthesis indicate percent

FIGURE 4.2.3 GENDERWISE SUB-CLASSIFICATION OF VITAMIN-D DEFICIENCY (%)

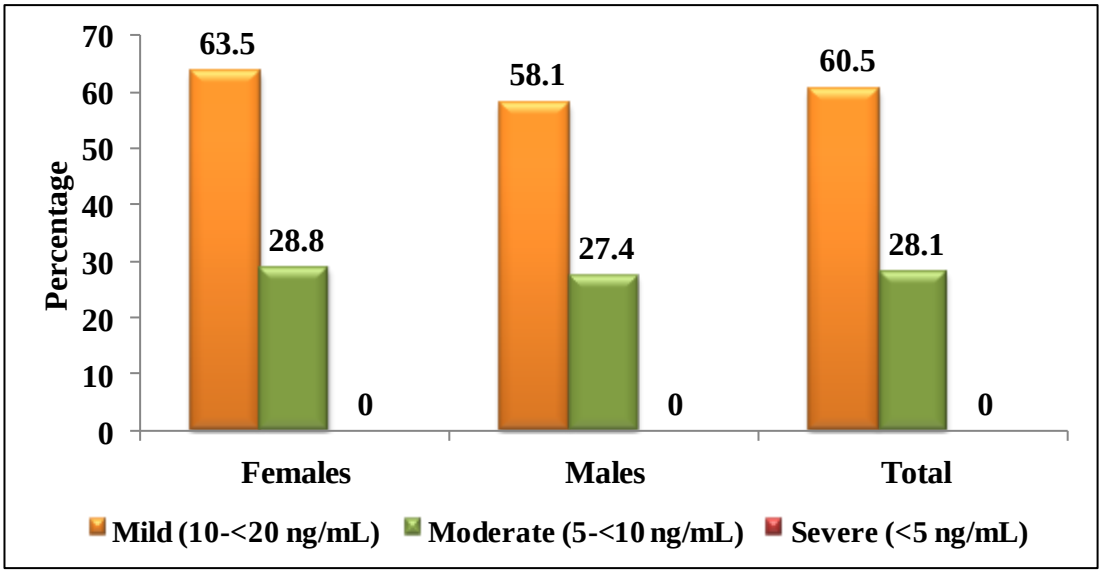


TABLE 4.2.28 IRON STATUS OF THE SUBJECTS (Mean ± SD)

Parameters	Normal Range	Females (n=52)	Males (n=62)	Total (n=114)
Hb (gm/dl)	Males >13 Females >12	12.3 ± 1.2	14.2 ± 1.4	13.4 ± 1.6
Iron (mcg/dl)	Male: 70-180 Female: 60-180	66.8 ± 18.8	84.6 ± 26.4	76.6 ± 24.8
TIBC (mcg/dl)	Male: 225-535 Female: 215-535	368.3 ± 52.0	362.5 ± 37.1	365.2 ± 44.3
% Transferrin Saturation	13-45	18.4 ± 5.1	23.5 ± 7.3	21.2 ± 6.9

TABLE 4.2.29 PREVALENCE OF IRON DEFICIENCY ANAEMIA AMONG SUBJECTS (n, %)

Classification based on Hb values	Females (n=52)	Males (n=62)	Total (n=114)	χ^2 value
Normal >12 (Females) >13 (Males)	37 (71.2)	48 (77.4)	85 (74.6)	
Mild (Females 11–11.9) (Males 11-12.9)	7 (13.5)	13 (21.0)	20 (17.5)	
Moderate (8 – 10.9)	8 (15.4)	1 (1.6)	9 (7.9)	
Severe (< 8)	0	0	0	
Total Anemic subjects	15 (28.8)	14 (22.6)	29 (25.4)	**7.851***

$p<0.05$* Values in parenthesis indicate percent

TABLE 4.2.30 GLYCEMIC PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameters	Females (n=52)	Males (n=62)	Total (n=114)	*p* value
Glycoslated Hb (HbA1c) (%)	8.7 ± 1.7	8.7 ± 1.5	8.7 ± 1.6	0.895
Average Blood Glucose (ABG)	203.7 ± 55.5	202.6 ± 49.9	203.1 ± 52.3	0.910
Aberration in Glycemic profile (n, %)				
HbA1c > 7 %	45 (86.5)	51 (82.3)	96 (84.2)	0.532
ABG ≥ 150 mg%	44 (84.6)	50 (80.6)	94 (82.5)	0.579

Values in parenthesis indicate percent

indicating unsatisfactory control according to ATP III guidelines. The mean value for average blood glucose (ABG) was 203 mg/dl which also indicated that necessary action should be taken to control blood sugar levels and maintain it in physiological normal range. Glucose control as seen by HbA1c, revealed that around 84% subjects had levels greater than 7% and similar percent of the subjects (82.5%) had ABG values above 150 mg/dl.

Prevalence of Dyslipidemia among the subjects

The lipid profile of the subjects as given in Table 4.2.31 showed that, values for total cholesterol, serum triglyceride and atherogenic fractions TC/H, TG/H and LDL/HDL ratio were all in normal range in both the genders. However the female subjects had significantly higher levels of total cholesterol as compared to their male counter parts. The mean values of LDL-C were higher among the subjects of both genders. The HDL-C levels were significantly lower among males as compared to females. However females had lower levels in regard to the physiological desirable values for them. Another indicator used was AIP. An improvisation of the indicator of small dense LDL, TAG/H, is its log transformed values, known as the Atherogenic Index of Plasma (AIP), is an efficient quantitative indicator of atherogenicity. The mean values of atherogenic index of plasma (AIP) and HsCRP which signals inflammation were also on the higher end among the subjects. Females again had significantly higher levels of Hs-CRP as compared to males.

When the data was segregated based on the standard cut off values, it was observed that around 35% had hypercholesterolemia with females having significant higher prevalence and almost same percent of the subjects had triglyceridemia with no significant difference among the genders. An alarming observation was that about 65% of the subjects had elevated LDL cholesterol levels and protective HDL lipoprotein was low in 50% of the subjects. TG/H ratio, which represents small dense lipoprotein was found to be high in 50.4% of the subjects (Table 4.2.32). AIP is an efficient quantitative indicator of atherogenicity and it was found that around 88% of the subjects were falling into high risk category. The inflammatory marker, HsCRP was high in nearly 79% of the subjects. These results indicate a high percent of the population under study had undesirable lipid, atherogenic and inflammatory parameters. All these aberrations indicate great risk of CVDs among them and calls for measures to address and reverse them to avoid later complications in diabetes mellitus.

TABLE 4.2.31 LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameters	Females (n=52)	Males (n=62)	Total (n=114)	t Test *p* value
Total Cholesterol	193.2 ± 40.0	177.0 ± 36.0	184.4 ± 38.6	**0.027*** EVNA**
Triglycerides	148.5 ± 63.1	137.3 ± 53.5	142.4 ± 58.0	0.308
LDL Cholesterol	113.2 ± 29.2	105.5 ± 26.3	109.0 ± 27.8	0.146
HDL Cholesterol	48.8 ± 9.8	42.0 ± 9.3	45.1 ± 10.1	**0.000*** EVNA**
VLDL-C	32.1 ± 21.9	27.0 ± 11.0	29.4 ± 17.0	0.111
TC/HDL Ratio	4.1 ± 1.3	4.3 ± 1.0	4.2 ± 1.2	0.272
LDL/HDL Ratio	2.4 ± 0.7	2.6 ± 0.8	2.5 ± 0.7	0.120
TAG/HDL Ratio	3.2 ± 1.9	3.5 ± 1.7	3.4 ± 1.8	0.409
AIP (log10 TG/H)	0.46 ± 0.2	0.49 ± 0.2	0.48 ± 0.2	0.540
HsCRP	0.51 ± 0.3	0.29 ± 0.3	0.38 ± 0.3	**0.000*** EVNA**

p< 0.001***, <0.05* EVNA=Equal variance not assumed

TABLE 4.2.32 PREVALENCE OF DYSLIPIDEMIA & INFLAMMATION AMONG THE SUBJECTS (n, %)

Parameter	Females (n=52)	Males (n=62)	Total (n=114)	χ^2 *p* value
TC ≥ 200 mg/dl	26 (50.0)	14 (22.6)	40 (35.1)	**0.002****
TG ≥ 150 mg/dl	19 (37.3)	21 (33.9)	40 (35.4)	0.708
LDL-C ≥ 100 mg/dl	36 (70.6)	36 (60.0)	72 (64.9)	0.244
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	30 (57.7)	27 (43.5)	57 (50.0)	0.132
TC/HDL ≥ 5	7 (13.5)	15 (24.2)	22 (19.3)	0.148
TAG/HDL ≥ 3	23 (45.1)	34 (54.8)	57 (50.4)	0.303
LDL/HDL ≥ 3.5	5 (9.8)	6 (10.0)	11 (9.9)	0.973
Atherogenic Index of Plasma (AIP)				
<0.11 (low risk)	1 (2.0)	2 (3.2)	3 (2.7)	0.583
0.11-0.21 (moderate risk)	3 (6.1)	7 (11.3)	10 (9.0)	
>0.21 (high risk)	45 (91.8)	53 (85.5)	98 (88.3)	
Inflammatory marker HsCRP				
≥ 0.1 mg/dl	49 (94.2)	41 (66.1)	90 (78.9)	**0.000****

p < 0.001***, <0.01** Values in parenthesis indicate percent

Thyroid hormone levels among the subjects

To study the thyroid gland functioning among the subjects, Thyroid Stimulating Hormone (TSH), Total Triiodothyronine (T_3) and Total Thyroxine (T_4) levels were examined which are depicted in table 4.2.33. The mean value for TSH was found to be within the normal physiological range. Similarly the values for TT_3 and TT_4 were also within the normal range for subjects of both the genders.

Kidney profile of the subjects

The kidney profile of the subjects as shown in Table 4.2.34, revealed that all the parameters i.e serum calcium, blood urea nitrogen (BUN), creatinine, uric acid and BUN-creatinine ratio were in normal range as per the physiological needs, thus suggesting no kidney functioning disorders among the subjects.

Liver Function test among the subjects

Liver Function Tests are one of the most commonly used screening blood tests, whether for the investigation of suspected liver disease or simply as 'routine' blood analysis. These tests were also performed for the study population to examine the levels of various liver enzymes among them. Similar to the kidney profile, all the liver parameters were also found to be in physiological normal range suggesting healthy liver profile of the subjects and thus no apparent presence of liver disorders (Table 4.2.35).

Aberrations in the biochemical parameters

On the basis of the normal values as mentioned in the above tables for the iron status, liver and kidney profile, the prevalence of aberrations was calculated among the subjects which is displayed in Table 4.2.36. It was observed that among the subjects for liver function tests, aberration in Alkaline Phosphatase levels were significantly higher among female subjects. Similar significant high prevalence of abnormal SGPT and GGT levels was also observed among female subjects as compared to males. Among the kidney functioning parameters, significantly high prevalence of aberrated creatinine levels was observed for the male subjects as compared to females, while prevalence of high uric acid and BUN/creatinine ratio was observed among female subjects as compared to males.

TABLE 4.2.33 THYROID HORMONES LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Females (n=52)	Males (n=62)	Total (n=114)
Thyroid Stimulating Hormone (TSH) µIU/ml	0.30-5.5	3.7 ± 4.1	3.5 ± 3.5	3.6 ± 3.8
Total Triiodothyronine (T_3) ng/dl	60-200	109.7 ± 20.3	108.6 ± 17.5	109.1 ± 18.7
Total Thyroxine (T_4) µg/dl	4.5-12.0	9.8 ± 1.8	9.0 ± 1.9	9.4 ± 1.9

Table 4.2.34 KIDNEY PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Females (n=52)	Males (n=62)	Total (n=114)
Calcium (mg/dl)	8.8-10.6	9.6 ± 0.3	9.6 ± 0.4	9.6 ± 0.3
BUN (mg/dl)	7.9-20	10.1 ± 2.8	9.6 ± 2.8	9.8 ± 2.8
Creatinine (mg%)	Male: 0.6-1.1 Female: 0.5-0.8	0.6 ± 0.1	0.7 ± 0.1	0.67 ± 0.1
Uric Acid (mg/dl)	Male: 3.5-7.2 Female: 2.6-6.0	5.3 ± 1.5	4.8 ± 1.1	5.1 ± 1.4
BUN/ Sr. Creatinine	9:1- 23:1	16.9 ± 5.0	13.3 ± 4.0	14.9 ± 4.8

TABLE 4.2.35 LIVER PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Females (n=52)	Males (n=62)	Total (n=114)
Alkaline Phosphatase (U/L)	Male: 53-128 Female: 42-98	98.2 ± 21.3	82.3 ± 22.6	89.6 ± 23.3
Total Bilirubin (mg/dl)	0.3-1.20	0.57 ± 0.2	0.66 ± 0.2	0.62 ± 0.2
Direct Bilirubin (mg/dl)	0-0.2	0.18 ± 0.08	0.19 ± 0.05	0.18 ± 0.06
Indirect Bilirubin (mg/dl)	0-0.9	0.39 ± 0.1	0.47 ± 0.2	0.43 ± 0.2
SGOT (U/L)	Male: 0-37 Female: 0-31	21.9 ± 11.8	23.0 ± 10.3	22.5 ± 10.9
SGPT (U/L)	Male: 13-40 Female: 10-28	24.5 ± 12.9	27.7 ± 15.1	26.3 ± 14.2
GGT (U/L)	Male: 0-55 Female: 0-38	28.4 ± 16.7	31.1 ± 18.5	29.9 ± 17.7
Total Protein (gm/dl)	6.6-8.3	7.6 ± 0.3	7.5 ± 0.5	7.5 ± 0.4
Serum Albumin (gm/dl)	3.5-5.2	4.1 ± 0.5	4.3 ± 0.3	4.2 ± 0.4
Serum Albumin/Globulin	0.9-2.0	1.2 ± 0.2	1.4 ± 0.2	1.3 ± 0.2

TABLE 4.2.36 ABERRATIONS IN BIOCHEMICAL PARAMETERS AMONG THE SUBJECTS (n, %)

Parameters	Females (n=52)	Males (n=62)	Total (n=114)	χ^2 *p* value
Iron (mcg/dl)	22 (43.1)	20 (32.3)	42 (37.2)	0.234
TIBC (mcg/dl)	0	0	0	--
% Transferrin Saturation	8 (15.7)	4 (6.5)	12 (10.6)	0.113
TSH (µIU/ml)	9 (17.3)	9 (14.8)	18 (15.8)	0.301
T3 (µg/dl)	0	0	0	--
T4 (µg/dl)	7 (13.5)	3 (4.8)	10 (8.8)	0.105
Alkaline Phosphatase (U/L)	24 (46.1)	6 (9.7)	30 (26.3)	**0.000*** **
Total Bilirubin (mg/dl)	0	2 (3.2)	2 (1.8)	0.191
Direct Bilirubin (mg/dl)	15 (28.8)	24 (38.7)	39 (34.2)	0.269
Indirect Bilirubin (mg/dl)	0	2 (3.2)	2 (1.8)	0.191
SGOT (U/L)	6 (11.5)	2 (3.2)	8 (7.0)	0.084
SGPT (U/L)	15 (28.8)	9 (14.8)	24 (21.1)	**0.017***
GGT (U/L)	11 (21.2)	5 (8.1)	16 (14.0)	**0.045***
Total Protein (gm/dl)	1 (1.9)	5 (8.1)	6 (5.3)	0.321
Serum Albumin (gm/dl)	2 (3.9)	0	2 (1.8)	0.116
Serum Albumin/Globulin	1 (1.9)	1 (1.6)	2 (1.8)	0.9
Calcium (mg/dl)	0	0	0	--
BUN (mg/dl)	12 (23.1)	14 (22.6)	26 (22.8)	0.950
Creatinine (mg%)	4 (7.7)	12 (19.3)	16 (14.0)	**0.015***
Uric Acid (mg/dl)	12 (23.1)	6 (9.7)	18 (18.8)	**0.001****
BUN/ Sr. Creatinine	11 (21.2)	6 (9.7)	17 (14.9)	**0.037***

p <0.001***, <0.01**, <0.05* Values in parenthesis indicate percent

ANALYSIS BY SEGREGATING THE SUBJECTS BASED ON VITAMIN-D LEVELS

To get a clarity regarding the determinants responsible for low serum vitamin-D levels among subjects further analysis was done by segregating the subjects based on their serum 25(OH)D levels into two groups- less than 20 and more than or equal to 20 ng/ml. About 92.3% of the females and 85.5% of males had vitamin-D levels less than 20 ng/ml (Table 4.2.37). Table 4.2.38 shows the anthropometric and blood pressure measurements across the vitamin-D levels among the groups. It was observed that all the measurements were higher for the low vitamin-D group. Significant higher values were observed for waist stature ratio and percent body fat in the low vitamin-D group when compared with the high vitamin-D group.

NON-INVASIVE PARAMETERS ACROSS VITAMIN-D LEVELS

An attempt was made to study the non-invasive parameters which could act as risk factors for poor vitamin-D status among individuals. The univariate analysis of the variables acting as risk factors for low vitamin-D levels is depicted in Table 4.2.39. It was seen that having high BMI, waist circumference, WHR and WSR had high odds for poor vitamin-D status, though none of the factors were found to be statistically significant among the groups. High diastolic BP also had very high odds for poor vitamin-D status but fell short of statistical significance. But these results do signify that high BMI, presence of abdominal obesity and elevated blood pressure levels may act as prominent risk factors for poor vitamin-D status.

Factors like having hypertension or history of fractures, consuming vegetarian diet or no tea, low physical activity, belonging to fair skin type and having exposure to sun for less than 20 minutes per day were also considered. it was observed that none of the factors had statistical significant relevance with the vitamin-d status of the subjects; however factors like having sun exposure less than 20 minutes per day and having a fair skin complexion had higher odds for low vitamin-d levels among the subjects, thus suggesting that if the lifestyle is made active more so in sunlight then the vitamin-d levels can be improved.

LIPID PROFILE OF THE SUBJECTS ACROSS VITAMIN-D LEVELS

The lipid profile, athrogenic indices and inflammatory marker was also studied across the low and high vitamin-D groups (Table 4.2.40). The univariate analysis in the form of odds ratio for the prevalence of dyslipidemia across the vitamin-D groups revealed that hypercholesterolemia was significantly high among the low vitamin-D group with very high

TABLE 4.2.37 DISTRIBUTION OF THE SUBJECTS BASED ON THEIR VITAMIN-D LEVELS (n, %)

Vitamin-D Level	Females (n=52)	Males (n=62)	Total (n=114)
< 20 ng/ml	48 (92.3)	53 (85.5)	101 (88.6)
≥20 ng/ml	4 (7.7)	9 (14.5)	13 (11.4)

Values in parenthesis indicate percent

TABLE 4.2.38 ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS ACROSS VITAMIN-D STATUS OF THE SUBJECTS (MEAN ± SD)

Parameters	Vitamin D <20 ng/ml (n=101)	Vitamin D ≥20 ng/ml (n=13)	t-Test *p* value
Weight (kg)	72.5 ± 14.2	68.5 ± 10.9	0.331
Body Mass Index	28.5 ± 5.3	25.8 ± 4.5	0.088
Waist Circumference	96.0 ± 11.8	90.9 ± 10.8	0.142
Hip Circumference	102.0 ± 14.6	99.2 ± 11.2	0.501
WHR	0.95 ± 0.08	0.92 ± 0.09	0.268
WSR	0.6 ± 0.08	0.56 ± 0.07	**0.040***
% Body Fat	36.9 ± 7.1	32.6 ± 6.6	**0.043***
SBP	139.9 ± 21.7	138.1 ± 25.9	0.774
DBP	84.9 ± 9.9	83.2 ± 10.8	0.569

p <0.05*

TABLE 4.2.39 NON-INVASIVE RISK FACTORS FOR LOW VITAMIN-D LEVELS AMONG THE SUBJECTS (n, %)

Parameters	Vitamin D <20 ng/ml (n=101)	Vitamin D ≥20 ng/ml (n=13)	OR	χ^2 *p* value	95% CI
Gender (Female)	48 (47.5)	4 (30.8)	0.49	0.253	0.14-1.69
Age (≥ 40 years)	76 (75.2)	10 (76.9)	0.91	0.894	0.23-3.57
High BMI (≥ 23)	89 (88.1)	10 (76.9)	2.23	0.260	0.53-9.24
High WC (F≥80, M≥90 cm)	75 (74.2)	7 (53.8)	2.47	0.123	0.76-8.03
High WSR (≥ 0.5)	93 (92.1)	11 (84.6)	2.11	0.370	0.39-11.23
High WHR (F≥0.85, M≥0.9)	88 (87.1)	9 (69.2)	3.0	0.088	0.8-11.19
High % BF (F>30, M>20)	99 (98.0)	13 (100.0)	--	--	--
High SBP (≥ 120 mmHg)	87 (86.1)	10 (76.9)	1.86	0.379	0.45-7.62
High DBP (≥ 80 mmHg)	77 (76.2)	1 (7.7)	6.4	0.09	0.55-73.9
HTN present	50 (49.5)	5 (38.5)	1.56	0.453	0.48-5.12
Having Fractures	40 (39.6)	6 (46.1)	0.76	0.650	0.24-2.44
Vegetarian diet	71 (70.3)	11 (84.6)	0.43	0.279	0.08-2.06
Low Physical activity	33 (32.7)	3 (23.1)	1.62	0.480	0.42-6.27
Sun exposure < 20 mins/day	27 (26.7)	2 (15.4)	2.38	0.299	0.44-12.75
Fair skin type	44 (43.5)	3 (23.1)	2.57	0.157	0.66-9.91

Values in parenthesis indicate percent

TABLE 4.2.40 PREVALENCE OF DYSLIPIDEMIA ACROSS VITAMIN-D LEVELS OF THE SUBJECTS (n, %)

Parameter	Vitamin D <20 ng/ml (n=101)	Vitamin D ≥20 ng/ml (n=13)	OR	χ^2 *p* value	95% CI
TC ≥ 200 mg/dl	39 (38.6)	1 (7.7)	7.5	**0.027***	0.9-60.3
TG ≥ 150 mg/dl	35 (35.0)	5 (38.5)	0.86	0.806	0.3-2.8
LDL-C ≥ 100 mg/dl	66 (67.3)	6 (46.2)	2.4	0.132	0.7-7.7
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	51 (50.5)	6 (46.2)	1.19	0.768	0.4-3.8
TC/HDL ≥ 5	20 (19.8)	2 (15.4)	1.35	0.704	0.3-6.6
TAG/HDL ≥ 3	51 (51.0)	6 (46.2)	1.21	0.742	0.4-3.8
LDL/HDL ≥ 3.5	10 (10.2)	1 (7.7)	1.36	0.776	0.2-11.6
AIP ≥ 0.21	96 (95.0)	12 (100.0)	--	--	--
HsCRP ≥ 0.1 mg/dl	83 (82.2)	7 (53.8)	3.95	**0.018***	1.2-13.2

p <0.05* Values in parenthesis indicate percent

odds for being a risk factor. The prevalence of inflammation was also found to be significantly higher in the low vitamin-D group with OR 3.95 (95% CI 1.2-13.2) as represented in Figures 4.2.4 and 4.2.5. Prevalence of conditions like hypertriglyceridemia, aberrated LDL-C, atherogenic indices and low HDL-C levels also showed high odds of having low vitamin-D levels thus identifying them as probable risk factors for poor vitamin-D status, though none of the parameters were statistically significant.

Aberrations in biochemical parameters across vitamin-D levels

The prevalence of aberrations across the vitamin-D groups in biochemical parameters like the iron status, glycemic profile, thyroid hormones, liver and kidney profile is presented in table 4.2.41. The prevalence for the iron parameters- Hb and % transferine saturation, and thyroid hormone-TSH was more in the low vitamin-D group. The liver enzymes Alkaline Phosphatase, SGPT and GGT the levels were aberrated more in the low vitamin-D group, while SGOT was more aberrated in high vitamin-D group. The creatinine and uric acid levels which are good indicators of kidney function were more aberrated among the high vitamin-D group and low vitamin-D group respectively. However, none of the risk factor was found to be statistically significant among the groups.

ANALYSIS ACROSS VITAMIN-D QUARTILES

To study the effect of vitamin-D levels on mean values of the parameters associated with anthropometry, lipid profile, iron status, glucose control, thyroid hormones and liver & kidney profile the data of all the above parameters was inspected across quartiles of the serum vitamin-D levels of the subjects as depicted in the Tables 4.2.42 to 4.2.46. One way analysis of variance was applied for the difference among the quartiles and then a post hoc test was carried out to see how the groups are different from each other statistically.

Genderwise distribution of subjects across vitamin-D quartiles

The gender-wise distribution of the subjects as per the vitamin-D quartiles showed that first and third quartile had more of females- 28.8% and 30.8% respectively as compared to males. These quartiles had twenty-nine subjects. While the second and the fourth quartile had more of males- 27.4% and 29% respectively as compared to females, which consisted of twenty-eight subjects each (Table 4.2.42).

FIGURE 4.2.4 PREVALENCE OF HYPERCHOLESTEROLEMIA ACROSS VITAMIN-D LEVELS OF THE SUBJECTS (n, %)

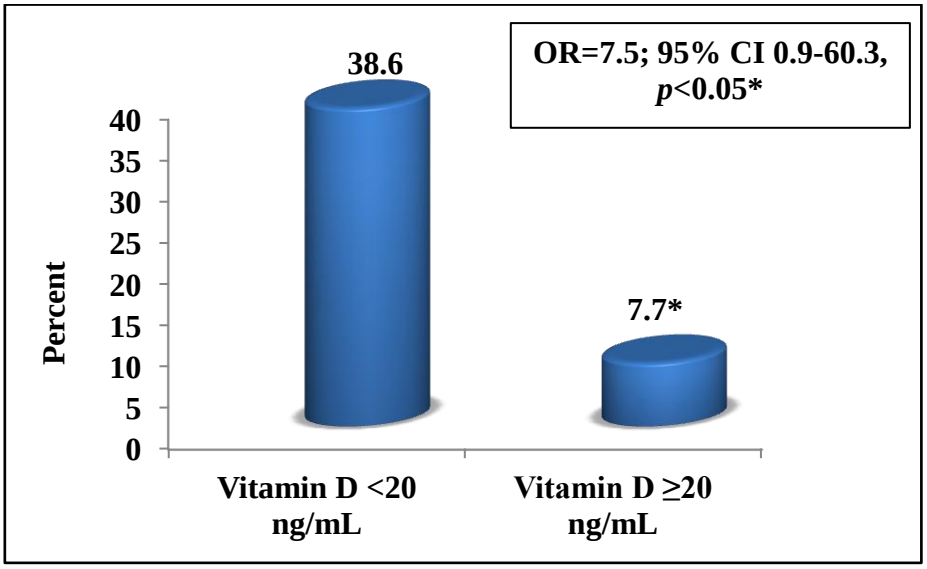


FIGURE 4.2.5 PREVALENCE OF INFLAMMATION ACROSS VITAMIN-D LEVELS OF THE SUBJECTS (n, %)

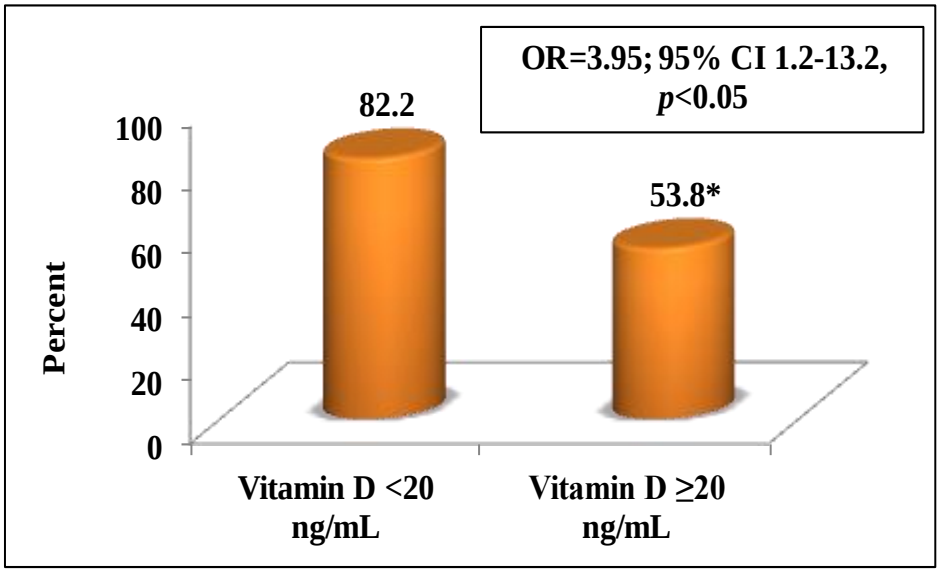


TABLE 4.2.41 ABERRATIONS IN BIOCHEMICAL PARAMETERS OF THE SUBJECTS ACROSS THEIR VITAMIN-D LEVELS (n, %)

Parameters	Vitamin D <20 ng/ml (n=101)	Vitamin D ≥20 ng/ml (n=13)	χ^2 *p* value
Hb (gm/dl)	26 (25.7)	3 (23.1)	0.975
Iron (mcg/dl)	35 (35.0)	7 (53.8)	0.186
% Transferrin Saturation	12 (12.0)	0	--
ABG (mg/dl)	83 (82.2)	11 (84.6)	0.828
HbA1c (%)	85 (84.2)	11 (84.6)	0.966
TSH (µIU/ml)	18 (17.8)	0	--
T4 (µg/dl)	7 (6.9)	3 (23.1)	0.053
Alkaline Phosphatase (U/L)	28 (27.7)	2 (15.4)	0.375
Total Bilirubin (mg/dl)	2 (2.0)	0	--
Direct Bilirubin (mg/dl)	36 (35.6)	3 (23.1)	0.369
Indirect Bilirubin (mg/dl)	2 (2.0)	0	--
SGOT (U/L)	7 (6.9)	1 (7.7)	0.919
SGPT (U/L)	22 (21.8)	2 (15.4)	0.567
GGT (U/L)	15 (14.9)	1 (7.7)	0.484
Total Protein (gm/dl)	5 (4.9)	1 (7.7)	0.777
Serum Albumin (gm/dl)	2 (2.0)	0	--
Sr. Albumin/Globulin ratio	2 (2.0)	0	--
BUN (mg/dl)	21 (20.8)	5 (38.5)	0.153
Creatinine (mg %)	13 (12.9)	3 (23.1)	0.538
BUN/Sr Creatinine ratio	15 (14.8)	2 (15.4)	0.529
Uric Acid (mg/dl)	18 (17.8)	0	--

Values in parenthesis indicate percent

TABLE 4.2.42 DISTRIBUTION OF THE SUBJECTS BASED ON VITAMIN-D QUARTILES (n, %)

Vitamin-D Quartiles	Females (n=52)	Males (n=62)	Total (n=114)
1st (5.26-9.73 ng/ml)	15 (28.8)	14 (22.6)	29 (25.4)
2nd (9.79-12.26 ng/ml)	11 (21.2)	17 (27.4)	28 (24.6)
3rd (12.3-15.2 ng/ml)	16 (30.8)	13 (21.0)	29 (25.4)
4th (15.38-61.6 ng/ml)	10 (19.2)	18 (29.0)	28 (24.6)

Values in parenthesis indicate percent

Biophysical measurements across vitamin-D quartiles

The anthropometric and blood pressure measurements across the quartiles are shown in Table 4.2.43 (A). It was observed that of all the parameters studied, waist-circumference, WSR and the percent body fat showed a significant decrease from 1^{st} to 4^{th} quartile. The post hoc analysis revealed that weight and WSR decreased significantly from 1^{st} to 4^{th} quartile. The post HOC analysis revealed that weight significantly reduced from 1^{st} to 3^{rd} quartile while percent body fat reduced significantly from 1^{st} to 4^{th} quartile (Table 4.2.43 (B)). Waist-circumference, hip-circumference and BMI were significantly different at 1^{st} to 3^{rd} and 1^{st} to 4^{th} quartile. It was only WSR which was significantly different at three points- 1^{st} to 2^{nd}, 1^{st} to 3^{rd} and 1^{st} to 4^{th} quartile (Table 4.2.43 (B)). Thus this data displayed a very positive trend of with the increase in vitamin-D levels the anthropometric measurements decreased substantially.

Lipid profile of the subject across vitamin-D quartiles

The lipid profile values across the various quartiles of vitamin-D are shown in Table 4.2.44 (A). The bad cholesterol -LDL showed a significant declining trend from 2^{nd} to 4^{th} quartile. Among rest of the parameters none showed a significant trend, though nearly all of them decreased with the increase in vitamin-D levels. The post hoc analysis (Table 4.2.44 (B)) revealed that HDL-C was significantly increased for 1^{st} vs 3^{rd}, while total cholesterol and LDL-C decreased significantly from 2^{nd} to 4^{th} and 3^{rd} to 4^{th} quartile which is a very positive trend. TC/H and LDL-C/HDL-C ratios were significantly decreased across 1^{st} to 4^{th} and 2^{nd} to 4^{th} quartile respectively.

ANALYSIS BY SEGREGATING SUBJECTS ON DURATION OF DIABETES

It is believed that with the increase in duration of a disease the clinical and biochemical profile also changes. Keeping this notion in view the subjects were segregated according to their years of diabetes into two groups- those having diabetes from five or less years and more than five years. The next set of tables deals with anthropometric and blood pressure measurements, biochemical parameters and nutrient intake across these groups.

The duration of diabetes ranged between one to twenty-eight years among the subjects. The gender-wise distribution of the subjects showed that more number of females (65.4%) had diabetes from less than five years while more males (54.8%) suffered from diabetes from a

TABLE 4.2.43 (A) ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS OF THE SUBJECTS ACROSS VITAMIN-D QUARTILES (MEAN ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (5.26-9.73) (n=29)	2nd (9.79-12.26) (n=28)	3rd (12.3-15.2) (n=29)	4th (15.38-61.6) (n=28)	
Weight (Kg)	76.1 ± 15.6	73.5 ± 12.2	68.5 ± 13.7	70.0 ± 13.3	0.155
Height (cm)	158.8 ± 6.8	161.3 ± 7.6	158.1 ± 10.2	160.9 ± 8.4	0.394
WC (cm)	100.9 ± 10.6	95.5 ± 12.3	92.7 ± 11.1	92.4 ± 11.8	**0.022***
HC (cm)	107.4 ± 12.9	99.9 ± 13.8	99.3 ± 14.2	99.9 ± 15.2	0.095
WHR	0.94 ± 0.06	0.96 ± 0.09	0.94 ± 0.09	0.93 ± 0.09	0.621
WSR	0.64 ± 0.07	0.59 ± 0.08	0.59 ± 0.08	0.58 ± 0.08	**0.023***
BMI	30.2 ± 6.3	28.3 ± 4.6	27.3 ± 4.9	26.6 ± 4.7	0.054
Body fat (%)	38.9 ± 6.9	36.4 ± 6.8	36.7 ± 6.0	33.4 ± 8.0	**0.032***
Systolic BP (mmHg)	137.3 ± 18.4	138.5 ± 19.3	142.8 ± 26.3	140.4 ± 24.4	0.802
Diastolic BP (mmHg)	84.3 ± 9.6	85.7 ± 10.6	85.5 ± 11.7	83.3 ± 8.0	0.795

$p<0.05$*

TABLE 4.2.43 (B) POST-HOC TEST FOR ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS ACROSS VITAMIN-D QUARTILES

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
Weight	0.466	**0.038***	0.098	0.178	0.354	0.678
WC	0.082	**0.008****	**0.006****	0.357	0.313	0.923
HC	0.046	**0.030***	**0.047***	0.876	0.992	0.869
WSR	**0.037***	**0.022***	**0.004****	0.850	0.405	0.514
BMI	0.174	**0.038***	**0.009****	0.475	0.207	0.574
% Body Fat	0.178	0.238	**0.003****	0.858	0.106	0.071

p <0.01**, <0.05*

Table 4.2.44 (A) Lipid Profile & Hs-CRP Levels of the Subjects Across Vitamin-D Quartiles (Mean ± SD)

Parameters	Vitamin-D Quartiles (ng/ml)				ANOVA *p* value
	1st (5.26-9.73) (n=29)	2nd (9.79-12.26) (n=28)	3rd (12.3-15.2) (n=29)	4th (15.38-61.6) (n=28)	
Total Cholesterol	184.0 ± 37.4	190.6 ± 49.3	193.9 ± 33.1	168.8 ± 28.8	0.068
Triglycerides	142.9 ± 42.2	149.7 ± 63.3	148.4 ± 58.4	128.2 ± 65.7	0.494
LDL Cholesterol	108.5 ± 28.1	118.2 ± 30.1	112.7 ± 25.4	97.5 ± 24.9	**0.043***
HDL Cholesterol	42.9 ± 10.6	44.0 ± 10.1	48.5 ± 10.7	44.9 ± 8.2	0.162
VLDL Cholesterol	33.1 ± 25.8	29.1 ± 13.5	29.7 ± 11.7	25.6 ± 13.1	0.437
TC/HDL Ratio	4.5 ± 1.4	4.4 ± 1.1	4.1 ± 1.1	3.9 ± 1.0	0.193
LDL/HDL Ratio	2.6 ± 0.8	2.7 ± 0.7	2.4 ± 0.7	2.2 ± 0.8	0.108
TAG/HDL Ratio	3.5 ± 1.4	3.6 ± 1.8	3.3 ± 1.9	3.1 ± 2.1	0.760
AIP (log10 TG/H)	0.51 ± 0.2	0.52 ± 0.2	0.46 ± 0.2	0.43 ± 0.2	0.373
HsCRP	0.46 ± 0.4	0.36 ± 0.3	0.38 ± 0.3	0.35 ± 0.3	0.572

p <0.05*

Table 4.2.44 (B) Post-Hoc Test For Lipid Profile & Hs-CRP Levels Across Vitamin-D Quartiles

Parameters	1st vs 2nd	1st vs 3rd	1st vs 4th	2nd vs 3rd	2nd vs 4th	3rd vs 4th
Total Cholesterol	0.512	0.322	0.133	0.744	**0.034***	**0.014***
HDL-C	0.667	**0.033***	0.443	0.090	0.738	0.173
LDL-C	0.192	0.556	0.131	0.458	**0.007****	**0.037***
TC/HDL-C	0.751	0.239	**0.049***	0.394	0.100	0.415
LDL/HDL-C	0.703	0.262	0.061	0.145	**0.030***	0.441

p <0.01**, <0.05*

longer duration. There were more subjects in the lower duration group as compared to the higher duration group (54.4% and 45.6% respectively) (Table 4.2.45).

Biophysical measurements across duration of Diabetes

The anthropometric and blood pressure measurements of the subjects across the duration of diabetes are depicted in Table 4.2.46. It was observed that nearly all the measurements except WHR and systolic blood pressure were significantly lower in the group who had diabetes from a shorter period as compared to those suffering from more than five years of disease. This may be due to effect of regular physical activity as advised by doctor to control weight and thus blood sugar levels.

When the prevalence of obesity and abdominal obesity was observed across the groups for duration of diabetes, it was seen that the prevalence of both the conditions was high in the shorter duration group. This is obvious as the measurements were also higher among them as compared to the other group. But only the prevalence of high waist-circumference was significantly more among the subjects having diabetes from a shorter time period (Table 4.2.47).

VITAMIN-D STATUS OF THE SUBJECTS ACROSS DURATION OF DIABETES

Table 4.2.48 (A) shows the serum vitamin-D and glycemic profile of the subjects across their duration of diabetes. It was seen that mean serum 25(OH)D level was significantly higher in the subjects with duration of diabetes more than five years as compared to the other group. This may be due to some form of vitamin-D taken by the subjects under doctor's guidance during the course of disease. When the status of vitamin-D was studied across the duration of diabetes it was seen that the prevalence of deficiency (levels less than 20ng/ml) was significantly more in the shorter duration group as compared to the other group. But the prevalence of sufficiency (levels more than 30ng/ml) was more in the longer duration group (Table 4.2.48 (B)). This trend is in line with the previous table depicting the serum 25(OH)D levels.

GLYCEMIC PROFILE OF THE SUBJECTS ACROSS DURATION OF DIABETES

The glycemic profile of the subjects across their duration of diabetes is given in Table 4.2.49. The mean values for HbA1c and average blood sugar were higher in the subjects with duration of diabetes more than five years as compared to the other group. This may be due to the fact that as the disease duration increases the control of blood sugar becomes difficult.

TABLE 4.2.45 DISTRIBUTION OF THE SUBJECTS BASED ON DURATION OF DIABETES (n, %)

Vitamin-D Level	Females (n=52)	Males (n=62)	Total (n=114)
≤ 5 years	34 (65.4)	28 (45.2)	62 (54.4)
> 5 years	18 (34.6)	34 (54.8)	52 (45.6)
Range	1-28 years		

Values in parenthesis indicate percent

TABLE 4.2.46 ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS ACROSS DURATION OF DIABETES OF THE SUBJECTS (MEAN ± SD)

Parameters	≤ 5 years (n=62)	> 5 years (n=52)	t-Test *p* value
Weight (kg)	74.5 ± 14.6	69.2 ± 12.5	**0.040*** EVNA
Body Mass Index	29.5 ± 5.4	26.5 ± 4.7	**0.002**** EVNA
Waist Circumference	98.4 ± 12.0	91.9 ± 10.6	**0.003**** EVNA
Hip Circumference	104.2 ± 14.9	98.7 ± 12.9	**0.036*** EVNA
WHR	0.95 ± 0.07	0.94 ± 0.09	0.478
WSR	0.62 ± 0.08	0.57 ± 0.07	**0.000***** EVNA
% Body Fat	38.3 ± 6.9	34.1 ± 6.8	**0.002**** EVNA
SBP	142.4 ± 21.5	136.5 ± 22.6	0.155
DBP	86.4 ± 9.8	82.7 ± 10.0	**0.049*** EVNA

p <0.001***, 0.01**, <0.05* Values in parenthesis indicate percent
EVNA= Equal variance not assumed

Table 4.2.47 PREVALENCE OF OVERWEIGHT AND OBESITY AMONG THE SUBJECTS ACROSS THEIR DURATION OF DIABETES (n, %)

Category based on BMI	≤ 5 years (n=62)	> 5 years (n=52)	χ^2 *p* value
Normal (BMI:18.5 – 22.9)	6 (9.7)	9 (17.3)	0.078
Overweight (BMI:23 – 24.9)	8 (12.9)	13 (25.0)	
Obese (BMI: ≥25)	48 (77.4)	30 (57.7)	
Based on % Body fat			
Body fat (F>30, M>20%)	61 (98.4)	51 (98.1)	0.90
Based on Anthropometric Indices			
WC (F≥80, M≥90 cm)	50 (80.6)	32 (61.5)	**0.024***
WSR (≥ 0.5)	58 (93.5)	46 (88.5)	0.339
WHR (F≥0.85, M≥0.9)	54 (87.1)	43 (82.7)	0.511

p <0.05* Values in parenthesis indicate percent

TABLE 4.2.48 (A) VITAMIN D LEVELS OF THE SUBJECTS ACROSS THE DURATION OF DIABETES (MEAN ± SD)

Parameter	≤ 5 years (n=62)	> 5 years (n=52)	t-Test *p* value
25Hydroxy Vitamin D	12.2 ± 4.1	16.6 ± 1.9	**0.013*^{EVNA}**

p <0.05* EVNA= Equal variance not assumed

TABLE 4.2.48 (B) VITAMIN D STATUS OF THE SUBJECTS ACROSS THE DURATION OF DIABETES (n, %)

Serum 25(OH)D ng/ml	≤ 5 years (n=62)	> 5 years (n=52)	Total (n=114)	χ^2 value
Deficiency (<20)	59 (95.2)	42 (80.8)	101 (88.6)	**8.19***
Insufficiency (20-≤ 30)	3 (4.8)	4 (7.7)	7 (6.1)	
Sufficiency (>30)	0	6 (11.5)	6 (5.3)	

p <0.05* Values in parenthesis indicate percent

TABLE 4.2.49 HbA1c & AVERAGE BLOOD GLUCOSE (ABG) LEVELS OF THE SUBJECTS ACROSS THE DURATION OF DIABETES (MEAN ± SD)

Parameter	≤ 5 years (n=62)	> 5 years (n=52)	t-Test *p* value
HbA1c (%)	8.6 ± 1.4	8.8 ± 1.8	0.404
ABG (mg/dl)	199.5 ± 45.7	207.5 ± 59.4	0.416

LIPID PROFILE OF THE SUBJECTS ACROSS DURATION OF DIABETES

The mean lipid profile and Hs-CRP values are displayed in Table 4.2.50. It was observed that mean levels of all the parameters studied including the atherogenic index of plasma were higher for the group suffering from diabetes for a shorter period as compared to the group with longer duration of diabetes. However, only the level of total cholesterol and the inflammatory marker, Hs-CRP was significantly higher in the shorter duration group.

Similar to the trend observed in the previous table, the prevalence of dyslipidemia was higher among the group having diabetes from a shorter period as compared to the longer duration group. However none of the prevalence rate was statistically significant. But the prevalence of presence of inflammation was found to be significantly higher in the shorter duration group (Table 4.2.51).

Aberrations in biochemical parameters across duration of Diabetes

When the aberrations in other biochemical parameters like the iron status, thyroid hormones, kidney profile and liver function tests was studied it was observed that prevalence of none of condition was statistically significant across the groups based on duration of diabetes. The prevalence of aberrations for Hb, TT4, TSH, serum albumin and creatinine levels was less among the shorter duration group. Rest of the parameters were more aberrated in the longer duration group (Table 4.2.52).

PREVALENCE OF METABOLIC SYNDROME ACROSS VARIOUS GROUPS

Metabolic syndrome (MS) consists of multiple, interrelated risk factors of metabolic origin that appear to directly promote the development of atherosclerotic cardiovascular disease. This constellation of metabolic risk factors is strongly associated with type 2 diabetes mellitus or the risk for this condition. Hence an attempt was made to study the prevalence of MS in the study population segregated into various groups such as gender-wise, as per their vitamin-D status, vitamin-D quartiles and duration of diabetes. MS was defined on two criteria- the International Diabetes federation (IDF) classification given in 2005 and the guidelines given by Adult Treatment Panel-III in 2001. The following sub-set of tables deals with the prevalence of MS across these groups.

As observed from the Table 4.2.53(A), the gender-wise distribution showed a significant high prevalence of MS by both the criterias among the female subjects as compared to their male counter parts. Similar significant prevalence was observed for both the criterias in the

TABLE 4.2.50 LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS ACROSS THEIR DURATION OF DIABETES (MEAN ± SD)

Parameters	≤ 5 years (n=62)	> 5 years (n=52)	t-Test *p* value
Total Cholesterol	191.1 ± 37.6	176.5 ± 38.6	**0.044*** EVNA**
Triglycerides	148.3 ± 60.8	135.5 ± 54.3	0.243
HDL Cholesterol	45.2 ± 9.5	45.0 ± 10.8	0.912
LDL Cholesterol	112.5 ± 27.5	104.8 ± 27.8	0.146
VLDL-C	31.3 ± 20.7	27.0 ± 10.8	0.176
TC/HDL Ratio	4.3 ± 1.1	4.1 ± 1.2	0.196
LDL/HDL Ratio	2.5 ± 0.6	2.4 ± 0.9	0.457
TAG/HDL Ratio	3.5 ± 1.8	3.3 ± 1.8	0.555
AIP (log10 TG/H)	0.49 ± 0.2	0.46 ± 0.2	0.446
HsCRP	0.48 ± 0.3	0.28 ± 0.3	**0.000*** EVNA**

p < 0.001***, <0.05* EVNA=Equal variance not assumed

TABLE 4.2.51 PREVALENCE OF DYSLIPIDEMIA & INFLAMMATION ACROSS DURATION OF DIABETES OF THE SUBJECTS (n, %)

Parameter	≤ 5 years (n=62)	> 5 years (n=52)	χ^2 *p* value
TC ≥ 200 mg/dl	26 (41.9)	14 (26.9)	0.094
TG ≥ 150 mg/dl	23 (37.7)	14 (32.7)	0.579
LDL-C ≥ 100 mg/dl	43 (71.7)	29 (56.9)	0.103
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	33 (53.2)	24 (46.2)	0.452
TC/HDL ≥ 5	12 (19.4)	10 (19.2)	0.987
TAG/HDL ≥ 3	34 (55.7)	23 (44.2)	0.223
LDL/HDL ≥ 3.5	5 (8.3)	6 (11.8)	0.547
AIP ≥ 0.21	60 (100.0)	47 (92.1)	0.152
HsCRP ≥ 0.1 mg/dl	56 (90.3)	34 (65.4)	**0.001****

p < 0.01**

TABLE 4.2.52 ABERRATIONS IN BIOCHEMICAL PARAMETERS OF THE SUBJECTS ACROSS THEIR DURATION OF DIABETES (n, %)

Parameters	≤ 5 years (n=62)	> 5 years (n=52)	χ^2 *p* value
Hb (gm/dl)	16 (25.8)	15 (28.8)	0.995
Iron (mcg/dl)	26 (42.6)	16 (30.8)	0.194
% Transferrin Saturation	7 (11.5)	5 (9.6)	0.749
HbA1c (%)	54 (87.1)	42 (80.8)	0.356
ABG (mg/dl)	53 (85.5)	41 (78.8)	0.353
T4 (µg/dl)	4 (6.5)	6 (11.5)	0.339
TSH (µIU/ml)	8 (12.9)	10 (19.2)	0.617
Alkaline Phosphatase (U/L)	19 (30.6)	11 (21.1)	0.512
Total Bilirubin (mg/dl)	0	2 (3.8)	0.119
Direct Bilirubin (mg/dl)	22 (35.5)	17 (32.7)	0.754
Indirect Bilirubin (mg/dl)	0	2 (3.8)	0.119
SGOT (U/L)	5 (8.1)	3 (5.8)	0.633
SGPT (U/L)	16 (25.8)	8 (15.4)	0.161
GGT (U/L)	12 (19.4)	4 (7.7)	0.074
Total Protein (gm/dl)	4 (6.4)	2 (3.8)	0.630
Serum Albumin (gm/dl)	1 (1.6)	1 (2.0)	0.889
Sr. Albumin/Globulin ratio	0	2 (3.8)	0.119
BUN (mg/dl)	16 (25.8)	10 (19.2)	0.405
Creatinine (mg %)	7 (11.5)	9 (17.3)	0.653
BUN/Sr Creatinine ratio	9 (14.5)	8 (15.4)	0.697
Uric Acid (mg/dl)	11 (17.7)	7 (13.5)	0.724

Values in parenthesis indicate percent

TABLE 4.2.53 (A) PREVALENCE OF METABOLIC SYNDROME AMONG THE SUBJECTS (n, %)

Criteria	Females (n=52)	Males (n=62)	Total (n=114)	χ^2 *p* value
IDF, 2005	43 (82.7)	31 (50.0)	74 (64.9)	**0.000*** **
ATP III, 2001	48 (92.3)	41 (66.1)	89 (78.1)	**0.001** **

p<0.01**, <0.001*** Values in parenthesis indicate percent

TABLE 4.2.53 (B) PREVALENCE OF METABOLIC SYNDROME ACROSS VITAMIN-D STATUS OF THE SUBJECTS (n, %)

Criteria	Vitamin D <20 ng/ml (n=101)	Vitamin D ≥20 ng/ml (n=13)	OR	χ^2 *p* value	95% CI
IDF, 2005	67 (66.3)	7 (53.8)	0.59	0.374	0.18-1.89
ATP III, 2001	80 (79.2)	9 (69.2)	0.59	0.413	0.16-2.1

Values in parenthesis indicate percent

TABLE 4.2.53 (C) PREVALENCE OF METABOLIC SYNDROME ACROSS VITAMIN-D QUARTILES OF THE SUBJECTS (n, %)

Parameters	Vitamin-D Quartiles (ng/ml)				χ^2 p value
	1st (5.26-9.73) (n=29)	2nd (9.79-12.26) (n=28)	3rd (12.3-15.2) (n=29)	4th (15.38-61.6) (n=28)	
IDF, 2005	22 (75.9)	18 (64.3)	17 (58.6)	17 (60.7)	0.522
ATP III, 2001	24 (82.8)	22 (78.6)	23 (79.3)	20 (71.4)	0.771

TABLE 4.2.53 (D) PREVALENCE OF METABOLIC SYNDROME AMONG THE SUBJECTS BASED ON THEIR DURATION OF DIABETES (n, %)

Criteria	≤ 5 years (n=62)	> 5 years (n=52)	OR	χ^2 p value	95% CI
IDF, 2005	47 (75.8)	27 (51.9)	0.34	**0.008** **	0.15-0.76
ATP III, 2001	53 (85.5)	36 (69.2)	0.38	**0.037***	0.15-0.95

p<0.01**, 0.05* Values in parenthesis indicate percent

population with shorter duration of diabetes as compared to those having diabetes from more than five years (Table 4.2.53(D)). When the population was segregated based on their vitamin-D status, the prevalence was high in the group with lower vitamin-D values as compared to the high level group for both the IDF and ATP-III criteria (Table 4.2.53(B)). Similarly based on vitamin-D quartiles, the prevalence decreased up to 3rd quartile but increased in the 4th quartile for the IDF classification. While as per the ATP-III criteria the prevalence decreased in the 2nd quartile, increased in the 3rd and again declined in the 4th quartile. However the highest prevalence was observed in the 1st quartile with the lowest serum vitamin-D levels (Table 4.2.53(C)). The prevalence based on vitamin-D levels and vitamin-D quartiles though was not statistically significant for both the criterias of MS.

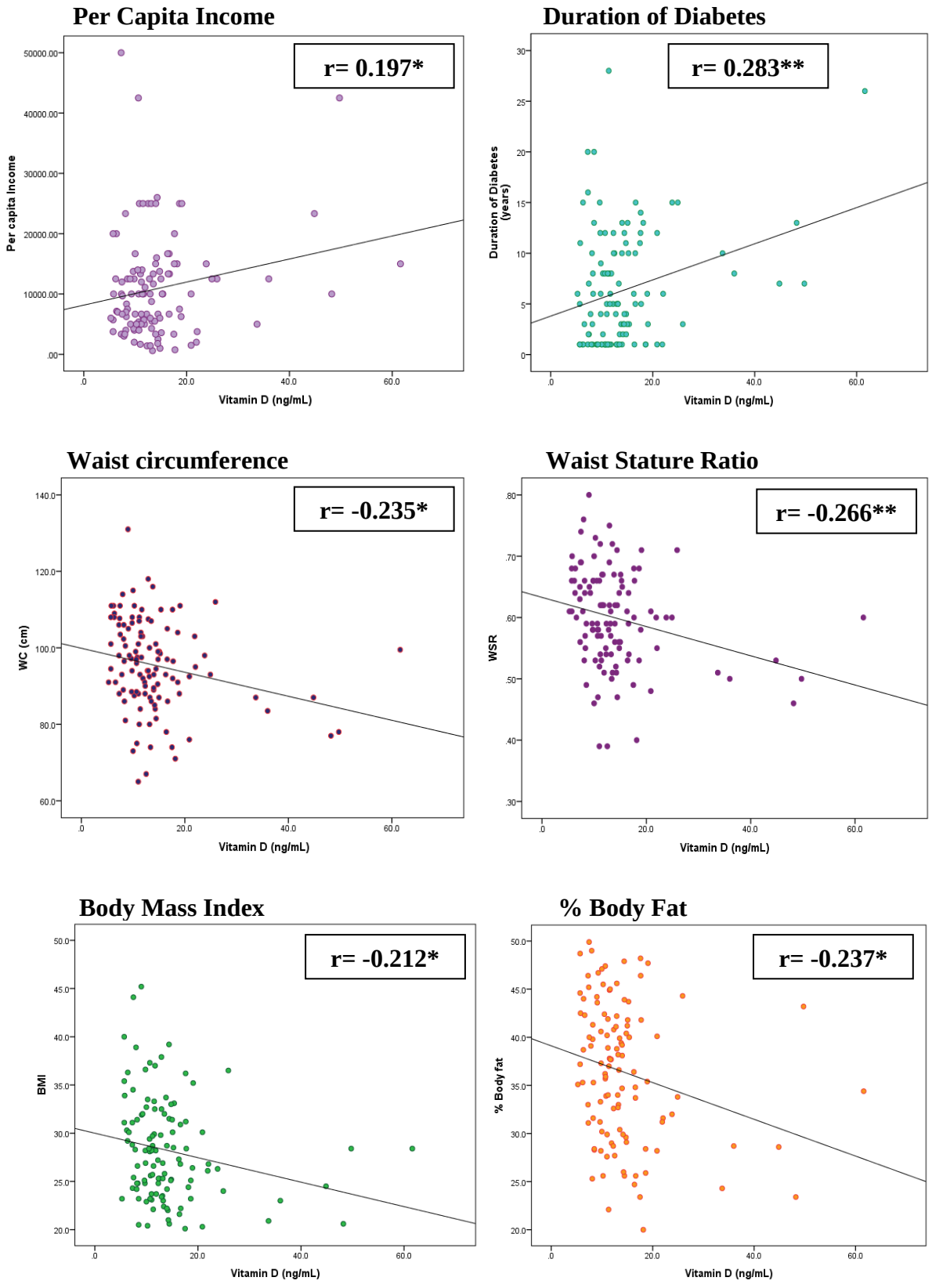
CORRELATIONS BETWEEN VITAMIN-D STATUS AND VARIOUS VARIABLES

From the previous analyses it was observed that the aberrations in many of the anthropometric and biochemical parameters significantly predict the outcome of vitamin-D status and clinical conditions. However to test the direction and extent of association between Vitamin D levels and these parameters the correlations between each of these were computed which are given in figures 4.2.6 and 4.2.7.

The correlations between vitamin-D levels and the non-invasive parameters are given in Figure 4.2.5. It was observed that duration of diabetes and per-capita income of the individual was positively significantly correlated with their vitamin-D levels i.e. as these increased the vitamin-D levels also increased. In the course of treatment for diabetes, the doctors usually prescribe multivitamins or pure vitamin-D supplementation to the subjects, which could be the probable reason for the positive correlation between the duration of disease with their vitamin-D levels. However a significant negative correlation was observed between the vitamin-D levels and anthropometric measurements-WC, WSR, BMI & % body fat, suggesting that reducing them would lead to rise in vitamin-D levels or vice versa.

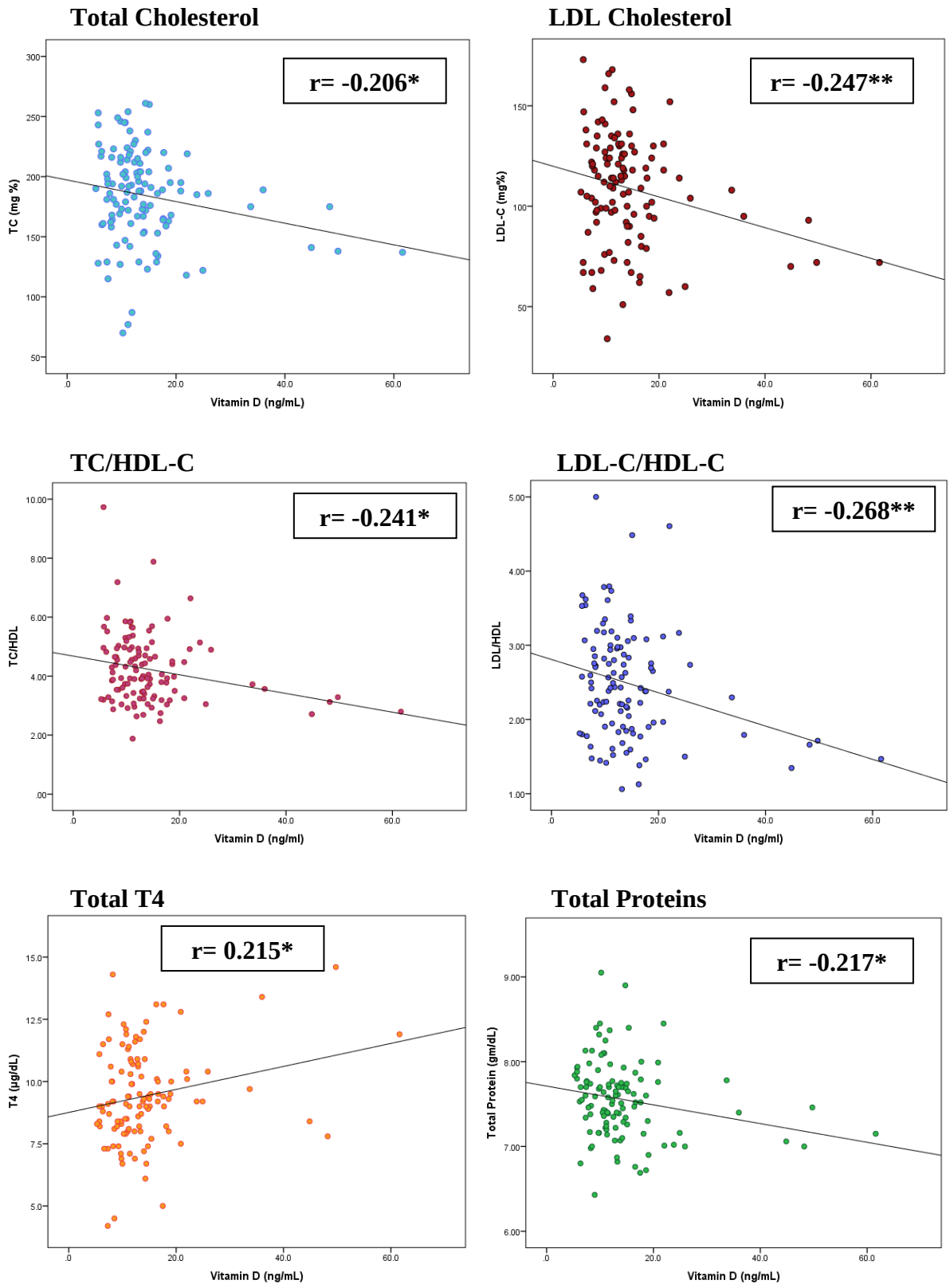
The correlations between vitamin-D levels and various biochemical parameters are shown in Figure 4.2.6. It is observed that except T4 levels, all the other mentioned parameters showed a significantly negative correlation with vitamin-D levels, suggesting that if vitamin-D is low in body more aberrations are expected in their levels leading to clinical conditions.

FIGURE 4.2.6 CORRELATIONS (PEARSON) BETWEEN VITAMIN-D LEVELS AND NON-INVASIVE PARAMETERS



p<0.01**, 0.05*

FIGURE 4.2.7 CORRELATIONS (PEARSON) BETWEEN VITAMIN-D LEVELS AND BIOCHEMICAL PARAMETERS



p<0.01**, 0.05*

MULTIVARIATE PREDICTORS OF VITAMIN-D STATUS

To investigate how multiple variables in this study synergistically predicted variations in the vitamin-D status of the subjects, a multivariate analysis was carried out on serum 25(OH)D levels as the dependent variable (Table 4.2.54). To study the determinants of low vitamin-D levels, continuous variables like age, duration of diabetes, WC, BMI, WHR, WSR, % body fat, TSH, T4, T_3, TC, HDL-C, LDL-C, TAG, AIP levels, Hs-CRP, creatinine, blood urea, uric acid, SGOT, SGPT, GGT, alkaline phosphatase, total proteins, and serum albumin were regressed. It was observed that the stepwise linear regression model that explained maximum amount of variation (23.3%) consisted of five variables-WSR, total T4, duration of diabetes, total proteins and LDL-C. Duration of diabetes and high levels of total T4 had positive coefficients with T4 having the strongest correlation with vitamin-D status. Total proteins and LDL-C were found to have a negative coefficient indicating they had a suppressive effect compared to the other two variables in the model.

TABLE 4.2.54 MULTIVARIATE PREDICTORS OF VITAMIN-D STATUS OF THE SUBJECTS (STEPWISE FORWARD LINEAR REGRESSION)

Predictor Variables	Adjusted r^2	Standardized β coefficients	*p* value
WSR	0.233	-0.171	0.057^{NS}
Total T4		0.273	**0.002****
Duration of Diabetes (years)		0.239	**0.009****
Total proteins		-0.204	**0.018***
LDL-C		-0.190	**0.031***

p<0.05*, <0.01**, NS – Non Significant

PHASE II (B): RANDOMISED CONTROL TRIAL TO STUDY THE IMPACT OF VITAMIN-D3 GRANULES ON SERUM 25(OH)D STATUS AND CARDIO-METABOLIC PROFILE OF SUBJECTS WITH T2DM

This phase is a randomised control trial (RCT) which was framed to evaluate the impact of vitamin-D3 granules supplementation on the serum 25(OH)D levels, anthropometric measurements, and other biochemical parameters in T2DM subjects. As described in the methods chapter in detail, out of 114 subjects on whom biochemical estimations were done seventy subjects with serum 25(OH)D <20ng/ml and who gave consent for participation were enrolled for this supplementation study. They were randomly divided into two groups- supplementation group (SC; n=40) and control group (CG; n=30). The supplementation group received 60,000 IU weekly vitamin-D3 (cholecalciferol) granules for eight weeks while the control arm didn't receive any intervention. Post data in the form of anthropometric measurements, 24-hour dietary recall, physical activity profile and biochemical estimations was collected at the end of eight weeks. The results of the study are as follows:

BACKGROUND INFORMATION OF THE SUBJECTS

The background characteristics of the seventy subjects of which forty were in supplementation group (22 males & 18 females) and thirty in control group (18 males & 12 females) is given in Table 4.2.55. The mean age of the subjects was 54.0±7.7 years and average duration of diabetes was 5.5±5.3 years. Majority of the subjects (80%) were on oral drugs therapy to control their blood sugar levels. Among them the prevalence of hypertension and dyslipidemia was high in the SG as compared to the controls with significant values for hypertension (65% in SG vs 36.7% in CG).

Vitamin-D status of the subjects post supplementation

At baseline, all study subjects were vitamin D-deficient with serum 25(OH)D levels less than 20 ng/ml. Change in serum 25(OH)D values in individual subjects performed at baseline and after Vitamin-D3 supplementation at eight weeks are given in Table 4.2.56. Serum 25(OH)D significantly increased in SG from 12.1±3.3 ng/ml to 43.6±16.2 ng/ml. The levels also increased in the CG significantly; however they still were in deficiency range (10.9±3.3 to 15.5±4.3 ng/ml).

After the supplementation eighty percent (32/40) of the subjects in the SG achieved the sufficiency status for serum 25(OH)D, while for the CG nearly eighty percent (23/30)

TABLE 4.2.55 BACKGROUND CHARACTERISTICS OF THE POPULATION FOR THE SUPPLEMENTATION STUDY (n, %)

Characteristic		Supplementation group (n=40)	Control Group (n=30)	χ^2 *p* value
Gender	Males	22 (55.0)	18 (60.0)	0.406
	Females	18 (45.0)	12 (40.0)	
Age (Mean ± SD)		55.0±7.6	52.7±7.8	0.988
Duration of Diabetes (Mean±SD)		6.3±5.8	4.3±4.5	0.151
Treatment regimen	Oral Drugs	30 (75.0)	24 (80.0)	0.738
	Insulin	1 (2.5)	1 (3.3)	
	Combine therapy	3 (7.5)	3 (10.0)	
Hypertension		26 (65.0)	11 (36.7)	**0.019***
Dyslipidemia		7 (17.5)	1 (3.3)	0.065
Consumption of drugs for Dyslipidemia		13 (32.5)	4 (13.3)	0.064
Smoking	Current	3 (7.5)	1 (3.3)	0.328
	Past	1 (2.5)	3 (10.0)	

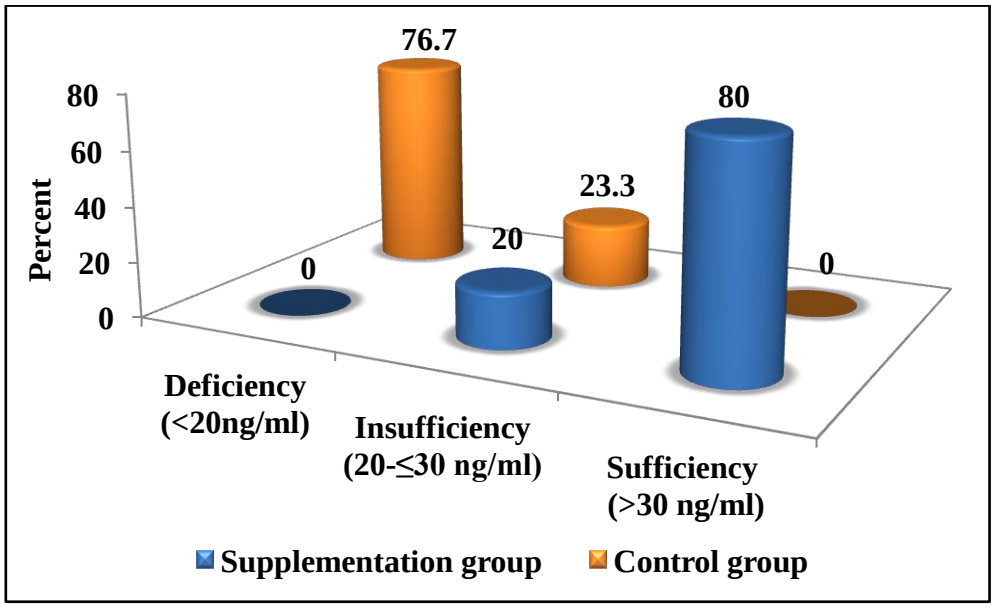
p <0.05* Values in parenthesis indicate percent

TABLE 4.2.56 VITAMIN-D LEVELS OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

	Supplementation group (n=40)	Control group (n=30)	Independent t (*p* value)
Pre	12.1±3.3	10.9±3.3	**0.000*** **
Post	43.6±16.2	15.5±4.3	
Paired t (*p* value)	**0.000*** **	**0.000*** **	

p<0.001***

FIGURE 4.2.8 VITAMIN-D STATUS OF THE SUBJECTS POST SUPPLEMENTATION (n=70) (n, %)



remained in the deficient range (Figure 4.2.8). Among the SG about twenty percent subjects shifted to the insufficiency range and none remained in the deficient category.

The gender-wise vitamin-D status of the subjects is given in table 4.2.57. It revealed that post supplementation more of females in SG attained the sufficiency status as compared to males in the group (83.3% & 77.3% respectively). While in the control group, more of males remained in the deficiency category as compared to females (77.8% & 75% respectively).

Biophysical measurements pre & post supplementation

A favourable trend was seen for the anthropometric measurements of the subjects taken at baseline and post supplementation which is depicted in table 4.2.58. It was observed that within the SG; weight (p=0.001**)**, waist circumference (p=0.025), Waist-Stature ratio (p=0.031), systolic blood pressure (p=0.035) and diastolic blood pressure (p=0.010) significantly decreased. A significant decline in systolic blood pressure (p=0.036) was also observed within the CG. After the supplementation, between the SG and CG almost all the anthropometric measurements were lower in the SG as compared to CG with weight (p=0.027) and waist circumference (p=0.012) having decreased significantly.

Lipid profile of the subjects' pre & post supplementation

Lipid parameters and Hs-CRP levels of the subjects were studied to see the impact of supplementation on their lipemic profile and inflammation (Table 4.2.59). It was observed that LDL-C decreased significantly (p<0.001) for both the groups after eight weeks of supplementation. The CG showed a significant decrease in total cholesterol (p=0.03) values as well, while for the SG lipid ratios TC/HDL-C (p=0.02) and LDL/HDL (p<0.001) decreased significantly. However between the SG and CG post supplementation, the SG had significantly lower levels for total cholesterol (p=0.037), LDL-C (p=0.035) and the ratios TC/HDL-C (p=0.022) and LDL/HDL (p=0.021). The rest of the parameters also showed a favourable decreasing trend among the SG at eight weeks, though the difference was non-significant when compared to CG. The inflammatory markers also decreased in the SG and slightly in CG, however the difference was not statistically significant.

The percent change in the lipid profile, atherogenic indices and HsCRP levels of the SG post eight weeks of supplementation is shown in Figure 4.2.9. The change in the percent prevalence of dyslipidemia and inflammation among the SG is given in Figure 4.2.10. It was

TABLE 4.2.57 GENDER-WISE VITAMIN-D STATUS OF THE SUBJECTS POST SUPPLEMENTATION (n, %)

Serum 25(OH)D ng/ml / Group		Deficiency (<20)	Insufficiency (20-≤ 30)	Sufficiency (>30)	χ^2 *p* value
Supplementation group	Males (n=22)	0	5 (22.7)	17 (77.3)	0.227
	Females (n=18)	0	3 (16.7)	15 (83.3)	
Control group	Males (n=18)	14 (77.8)	4 (22.2)	0	0.860
	Females (n=12)	9 (75.0)	3 (25.0)	0	

Values in parenthesis indicate percent

TABLE 4.2.58 ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

	Supplementation group (n=40)			Control group (n=30)			Inde.t *p* value
	Pre	Post	*p* value	Pre	Post	*p* value	
Weight	70.5±13.3	67.2±13.2	**0.001****	77.6±16.1	77.2±16.4	0.329	**0.027***
BMI	27.9±5.0	27.8±5.1	0.765	31.0±6.1	29.9±6.2	0.227	0.119
WC	95.4±11.2	92.9±10.7	**0.025***	99.2±13.5	100.2±12.9	0.248	**0.012***
HC	101.6±13	100.0±10.4	0.101	103±17.7	105.3±12.7	0.221	0.057
WHR	0.94±0.07	0.93±0.07	0.273	0.97±0.09	0.98±0.2	0.929	0.109
WSR	0.6±0.07	0.59±0.07	**0.031***	0.62±0.09	0.62±0.09	0.271	0.053
SBP	141.6±18.6	134.8±18.8	**0.035***	139±20.6	133.1±18.8	**0.036***	0.702
DBP	85.0±9.1	81.5±7.2	**0.010***	85.3±10.8	82.4±10.4	0.091	0.688

p <0.01**, 0.05*

TABLE 4.2.59 LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

	Supplementation group (n=40)			Control group (n=30)			Inde. t *p* value
	Pre	Post	*p* value	Pre	Post	*p* value	
TC	174.9±38.6	167.1±41.5	0.098	200.3±31.5	186.7±33.5	**0.03***	**0.037***
TG	137.9±62.9	128.1±54.9	0.268	146.5±50.9	166.1±98.9	0.243	0.065
HDL-C	44.1±10.1	44.8±11.2	0.287	45.9±9.2	43.9±8.9	0.062	0.713
LDL-C	106.1±24.8	83.9±29.0	**0.000*****	124.9±35.5	98.0±24.2	**0.000*****	**0.035***
VLDL-C	27.6±12.6	25.6±10.9	0.27	28.5±11	33.2±19.8	0.196	0.065
TC/H	4.1±1.1	3.8±0.9	**0.02***	4.3±1.1	4.4±1.2	0.606	**0.022***
TG/H	3.4±1.9	3.1±1.7	0.25	3.4±1.6	4.2±3.3	0.142	0.09
L/H	2.4±0.7	1.9±0.6	**0.000*****	4.3±9.3	2.3±0.7	0.236	**0.021***
AIP level	0.46 ± 0.2	0.43 ± 0.2	0.195	0.48 ± 0.2	0.52 ± 0.3	0.407	0.148
Hs-CRP	0.38 ± 0.3	0.33 ± 0.3	0.303	0.33 ± 0.3	0.32 ± 0.3	0.836	0.902[EVNA]

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

FIGURE 4.2.9 PERCENT CHANGE IN LIPID PROFILE OF THE SUPPLEMENTATION GROUP POST SUPPLEMENTATION

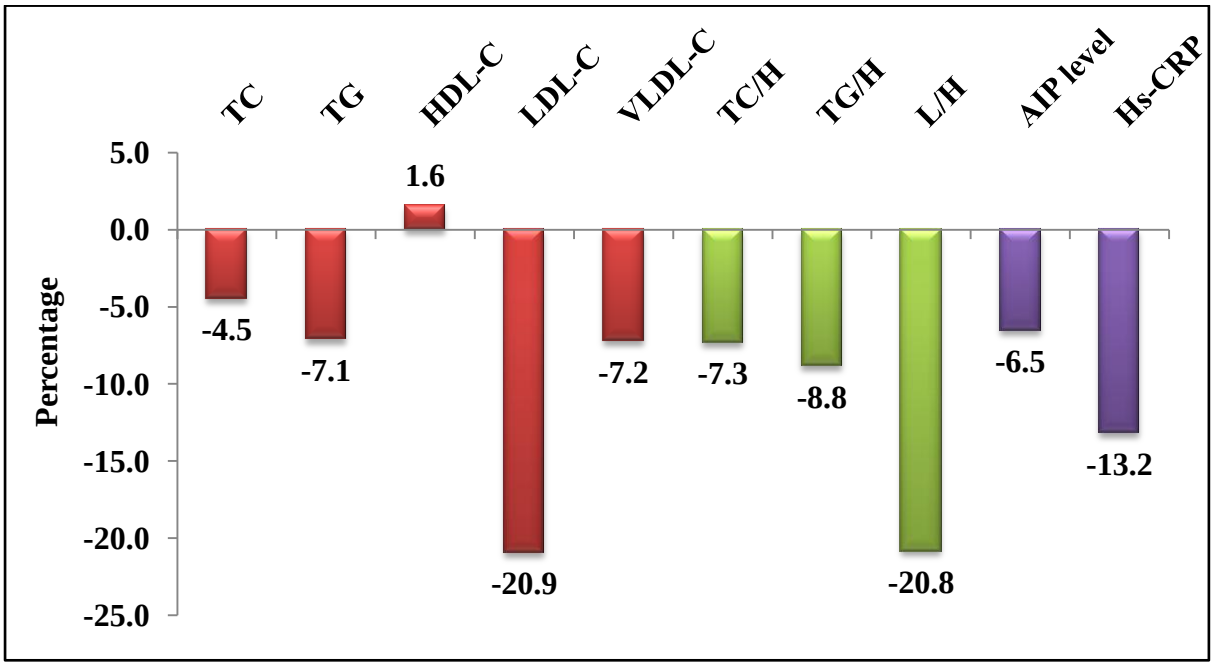
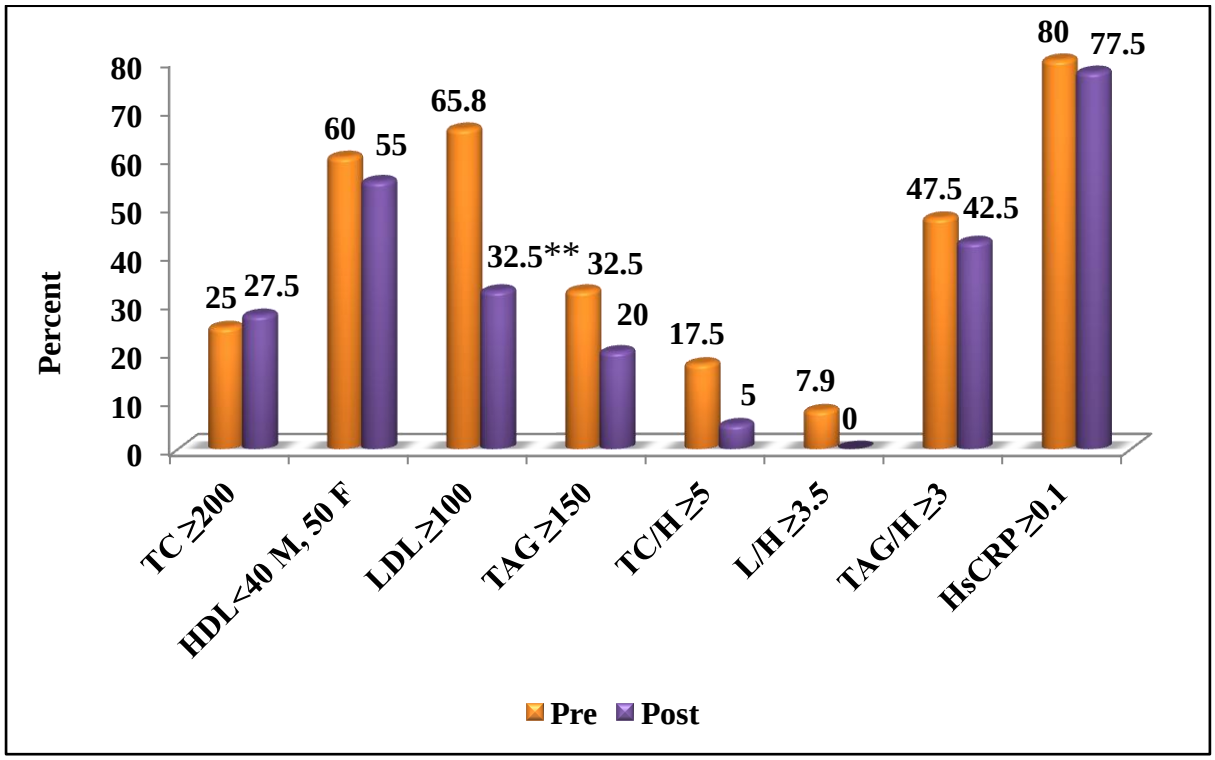


FIGURE 4.2.10 IMPACT OF VITAMIN-D SUPPLEMENTATION ON THE PREVALENCE OF DYSLIPIDEMIA & INFLAMMATION IN THE SUPPLEMENTATION GROUP



p<0.01**

observed that almost every parameter decreased (with significant reduction in LDL-C), except for prevalence of hypercholesterolemia which marginally increased by 2.5%.

Biochemical parameters among the subjects pre & post supplementation

As can be seen from Table 4.2.60 the iron status of the subjects revealed that haemoglobin and total iron binding capacity (TIBC) reduced significantly in both the groups post supplementation. A non-significant reduction was observed for HbA1c and ABG levels also in both the groups; however the reduction was more in the SG as compared to CG. The thyroid hormones also showed a non-significant decrease post supplementation in both the groups. No statistical significance was observed between the groups post supplementation for iron or glycemic status, but TSH values were significantly lower in the CG at the end or eight weeks. The percent change in the thyroid hormones and iron status for the SG is shown in Figure 4.2.11, while the change in glycemic parameters is given in Figure 4.2.12.

Liver and Kidney profile of the subjects' pre & post supplementation

The liver function tests and kidney profile values are depicted in Table 4.2.61. As it can be seen that in the liver function tests for the SG the alkaline phosphatase and total protein values decreased significantly. While for the CG the decrease was significant for SGPT, total protein and serum albumin values. No between the groups significance was observed for these tests post supplementation. The kidney profile of the subjects revealed that the values for serum calcium, creatinine and blood urea nitrogen (BUN) decreased post supplementation in both the groups, while uric acid and BUN/creatinine ratio values increased in both the groups. Between the groups analysis showed that the SG had significantly higher levels of BUN when compared to CG at the end of supplementation period. Figures 4.2.13 and 4.2.14 depicts the percent change in the liver function tests and kidney profile of the SG post eight weeks respectively.

Nutrient Intake of the subjects' pre & post supplementation

The pre and post supplementation nutrient intakes of the subjects are given in Table 4.2.62. There was no significant difference observed among the groups with respect to their intakes at the end of the supplementation period. However among the SG the intake of β carotene reduced significantly at eight weeks. For the CG the intake was found to significantly lower for macronutrients- energy, proteins and carbohydrates and as well for micronutrients- iron and vitamin-C.

TABLE 4.2.60 BIOCHEMICAL PARAMETERS OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

Parameters	Supplementation group (n=40)			Control group (n=30)			Independent t (*p* value)
	Pre	Post	*p* value	Pre	Post	*p* value	
Iron Status							
Hb (gm/dl)	13.5 ± 1.6	12.9 ± 1.5	**0.000*** **	13.9 ± 1.6	13.2 ±1.7	**0.000*** **	0.429
Iron (mcg/dl)	75.8 ±23.1	71.3 ±26.2	0.149	80.4 ±23.5	78.5 ±36.5	0.726	0.342
TIBC (mcg/dl)	359.2±43.5	346.8±41.8	**0.007** **	372.2±45.9	356.2±51.6	**0.009** **	0.416^{EVNA}
% Transferrin Saturation	21.3 ± 6.2	20.7 ± 7.3	0.466	21.9 ± 6.7	22.2 ± 10.4	0.842	0.471
Glycemic Status							
HbA1c (%)	8.7 ± 1.7	8.3 ± 1.7	0.062	8.5 ± 1.4	8.4 ± 1.7	0.607	0.791
ABG (mg/dl)	196.2±59.4	190.4±48.6	0.114	197.9±47.2	193.5±49.8	0.614	0.791
Thyroid Profile							
T3 (ng/dl)	109.1±18.5	106.2±17.2	0.194	107.5±12.9	106.2±14.4	0.486	0.995
T4 (µg/dl)	9.2 ± 1.7	9.2 ± 2.2	0.899	8.9 ± 1.6	9.1 ± 1.8	0.416	0.765
TSH (µIU/ml)	4.9 ± 5.7	3.9 ± 3.0	0.062	2.8 ± 1.4	2.4 ± 1.3	0.055	**0.009** **^{EVNA}

p<0.001***, <0.01** EVNA=Equal Variance Not Assumed

FIGURE 4.2.11 PERCENT CHANGE IN IRON STATUS & THYROID HORMONES OF THE SUPPLEMENTATION GROUP POST SUPPLEMENTATION

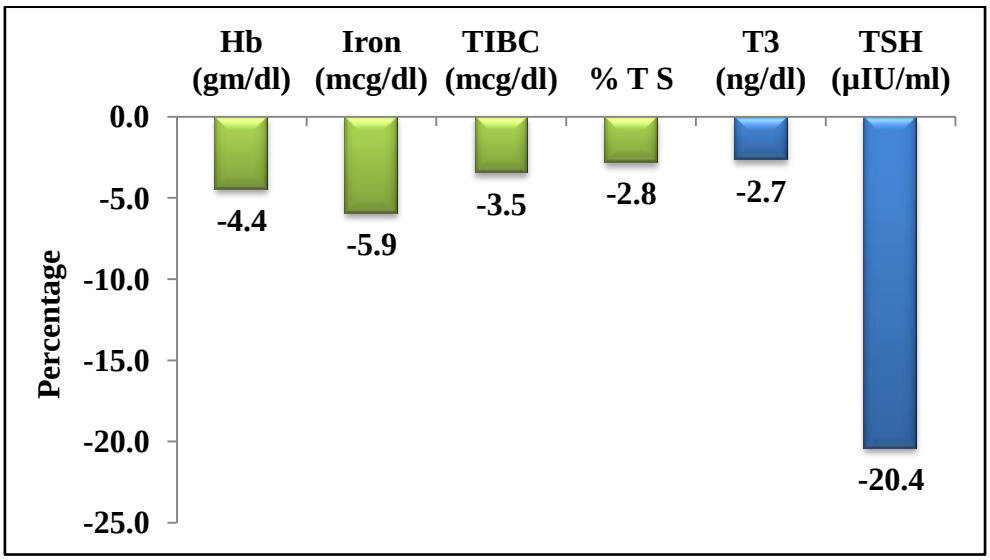


FIGURE 4.2.12 PERCENT CHANGE IN GLYCEMIC PROFILE OF THE SUPPLEMENTATION GROUP POST SUPPLEMENTATION

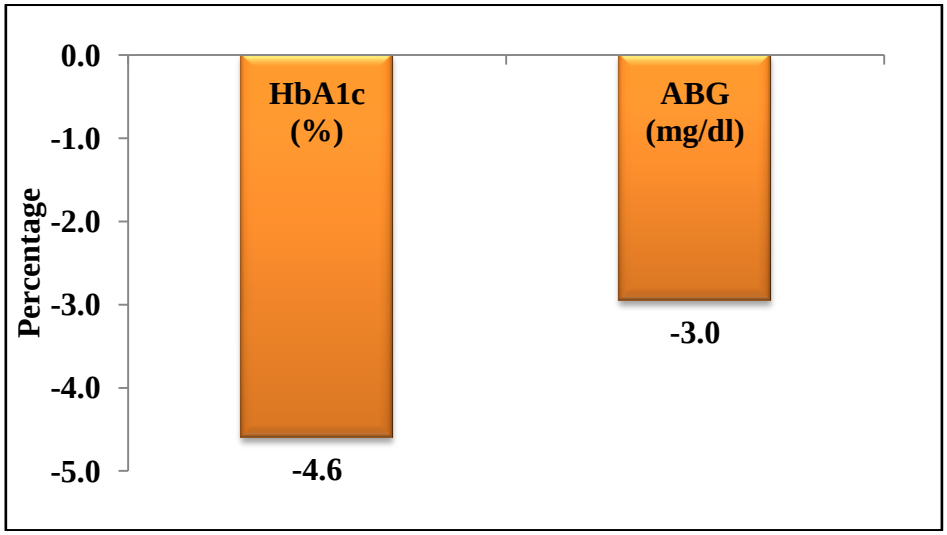


TABLE 4.2.61 LIVER & KIDNEY PROFILE OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

Parameters	Supplementation group (n=40)			Control group (n=30)			Independent t (*p* value)
	Pre	Post	*p* value	Pre	Post	*p* value	
Liver function Tests							
Alkaline Phosphatase (U/L)	88.4 ± 22.3	78.9 ± 19.8	**0.000*** **	89.9 ± 22.8	85.5 ± 22.9	0.117	0.20
Total Bilirubin (mg/dl)	0.63 ± 0.2	0.63 ± 0.2	0.905	0.67 ± 0.3	0.67 ± 0.3	0.908	0.497
Direct Bilirubin (mg/dl)	0.19 ± 0.08	0.19 ± 0.06	0.854	0.19 ± 0.05	0.19 ± 0.06	0.608	0.976
Indirect Bilirubin (mg/dl)	0.44 ± 0.19	0.44 ± 0.16	0.818	0.48 ± 0.2	0.49 ± 0.3	0.736	0.398
SGOT (U/L)	20.7 ± 7.9	20.7 ± 8.2	0.960	23.6 ± 10.8	21.6 ± 11.5	0.07	0.721
SGPT (U/L)	25.7 ± 13.3	26.3 ± 17.3	0.703	27.8 ± 11.4	24.2 ± 11.6	**0.011***	0.572
GGT (U/L)	28.8 ± 16.5	29.4 ± 18.6	0.677	30.4 ± 16.9	29.1 ± 15.3	0.622	0.958
Total Protein (gm/dl)	7.6 ± 0.4	7.4 ± 0.4	**0.000*** **	7.5 ± 0.4	7.3 ± 0.3	**0.001****	0.334
Serum Albumin (gm/dl)	4.2 ± 0.5	4.2 ± 0.2	0.578	4.24 ± 0.2	4.16 ± 0.2	**0.043***	0.466
Albumin/Globulin ratio	1.3 ± 0.2	1.3 ± 0.2	0.745	1.3 ± 0.2	1.3 ± 0.2	0.217	0.922
Kidney Profile							
Calcium (mg/dl)	9.4 ± 1.4	9.6 ± 0.3	0.445	9.6 ± 0.4	9.4 ± 0.3	0.091	0.061
BUN (mg/dl)	10.3 ± 2.4	10.8 ± 2.5	0.256	9.3 ± 2.6	9.3 ± 2.6	0.901	**0.024*^EVNA**
Creatinine (mg%)	0.69 ± 0.2	0.67 ± 0.2	0.188	0.67 ± 0.1	0.63 ± 0.1	**0.029***	0.280
BUN/Creatinine ratio	15.3 ± 4.6	16.5 ± 4.5	0.063	14.2 ± 4.3	15.1 ±4.8	0.082	0.226
Uric Acid (mg/dl)	5.4 ± 1.6	5.6 ± 1.3	0.262	4.8 ± 1.3	5.2 ± 1.6	**0.008****	0.249

p<0.001***, <0.01**, <0.05* EVNA=Equal Variance Not Assumed

FIGURE 4.2.13 PERCENT CHANGE IN LIVER FUNCTION TESTS OF THE SUPPLEMENTATION GROUP POST SUPPLEMENTATION

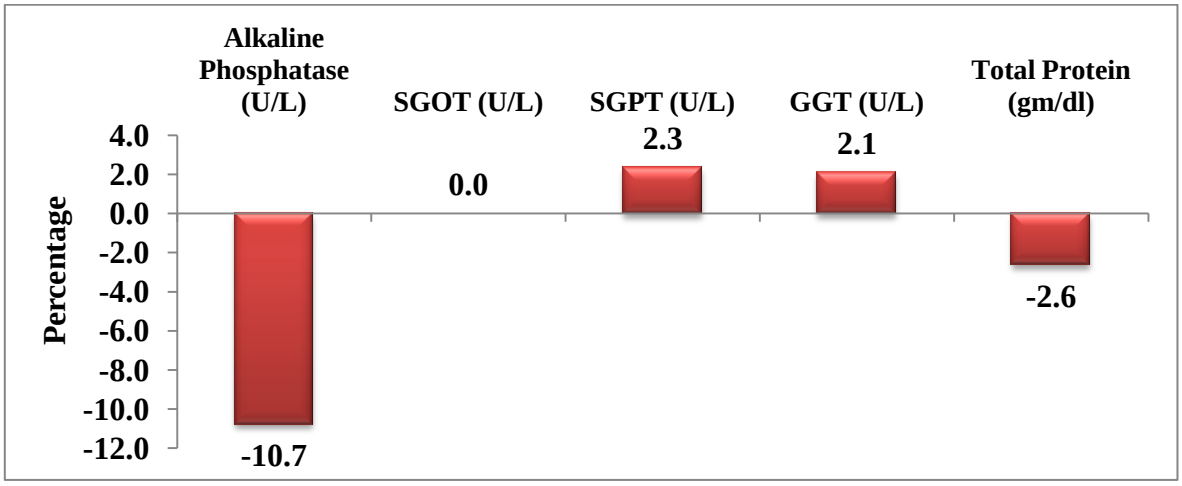


FIGURE 4.2.14 PERCENT CHANGE IN KIDNEY PROFILE OF THE SUPPLEMENTATION GROUP POST SUPPLEMENTATION

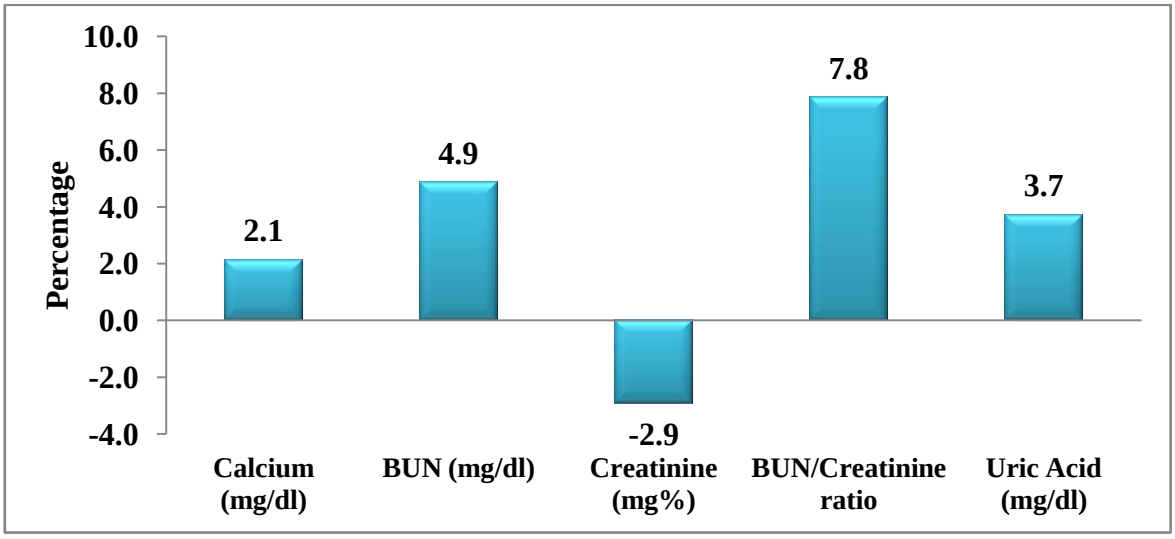


TABLE 4.2.62 NUTRIENT INTAKE OF THE SUBJECTS PRE & POST SUPPLEMENTATION [MEAN ± SD]

Nutrients	Supplementation group (n=40)			Control group (n=30)			Inde.t *p* value
	Pre	Post	*p* value	Pre	Post	*p* value	
Energy (Kcal)	1175 ± 284	1174 ±224	0.966	1268 ±334	1097 ±311	**0.012***	0.236
Protein (g)	30.6 ±8.0	32.4 ± 7.9	0.182	35.0 ± 9.9	30.2 ± 9.2	**0.038***	0.296
Fat (g)	50.7 ±14.6	49.2 ±13.5	0.536	47.9 ±18.1	44.7 ±19.4	0.337	0.254
CHO (g)	143 ± 44.0	144 ± 31.0	0.786	168 ± 45	138 ± 33	**0.002****	0.474
Calcium (mg)	415 ± 216	403 ± 196	0.439	454 ± 201	384 ± 173	0.059	0.677
Iron (mg)	9.48 ± 3.7	9.87 ± 4.1	0.595	10.78 ±4.0	8.67 ± 2.9	**0.01****	0.175
β carotene (µg)	1324 ±1215	452 ± 506	**0.011***	912 ±1144	802 ±1330	0.684	0.199
Vitamin C (mg)	60.6 ± 55.8	42 ± 37.0	0.084	71.9 ±58.4	46.6 ±44.4	**0.033***	0.643
Crude Fibre (g)	4.8 ± 2.1	4.3 ± 1.2	0.185	5.1 ± 1.9	4.5 ± 1.9	0.068	0.754
Total Fibre (g)	9.3 ± 4.6	9.4 ± 3.9	0.933	10.9 ± 4.3	10.5 ± 5.8	0.738	0.353
Insoluble Fibre	6.9 ± 3.5	7.3 ± 3.2	0.666	8.3 ± 3.6	8.1 ± 4.8	0.850	0.402
Soluble Fibre	2.3 ± 1.1	2.1 ± 0.9	0.247	2.6 ± 0.9	2.4 ± 1.1	0.30	0.215

p<0.01**, <0.05*

IMPACT OF SUPPLEMENTATION BASED ON INITIAL TOTAL CHOLESTEROL LEVELS OF THE SUBJECTS

An attempt was made to analyze the pre and post intervention lipid values, atherogenic indices and inflammatory marker depending upon the initial TC levels, i.e. subjects who had TC levels <200mg/dl and subjects who had TC levels ≥200mg/dl at the beginning of the supplementation. The results are displayed in tables 4.3.63 and 4.3.64.

Impact of supplementation on lipid profile of the subjects

The separate analysis revealed that among the supplementation group, subjects with both lower and higher TC levels showed significant reductions in LDL-C and L/H ratio. The control subjects with low initial TC showed significant decline only in LDL-C levels as compared to subjects who had high initial TC level, who saw only a reduction in TC, LDL-C values and L/H ratio. No significant trend was observed in both the groups with higher and lower initial TC levels for the inflammatory marker Hs-CRP (Table 4.2.63).

Impact of supplementation on biochemical parameters of the subjects

The impact of supplementation on various biochemical parameters is given in Table 4.2.64. As seen from the table, the serum 25(OH)D levels increased in both supplementation and control groups significantly irrespective of their initial TC levels. Similarly the haemoglobin levels were found to have decreased in both the groups significantly irrespective of their initial TC levels. For the subjects with lower TC levels, TIBC values also decreased significantly among both the SG & CG. While for the subjects in CG with higher TC levels the TSH reduced significantly.

TABLE 4.2.63 IMPACT OF VITAMIN-D SUPPLEMENTATION ON LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS BASED ON INITIAL TC LEVELS [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
TC <200 mg/dl	**n=30**			**n=14**		
TC	159.7 ± 31.2	154.2 ± 35.3	0.250	173.2 ± 18.5	178.1 ± 26.4	0.481
TG	122.7 ± 46.6	121.6 ± 50.8	0.888	144.9 ± 58.8	172.5 ± 98.5	0.243
HDL-C	41.5 ± 8.1	41.8 ± 8.9	0.630	42.2 ± 8.4	40.1 ± 9.3	0.170
LDL-C	97.8 ± 22.8	80.2 ± 22.3	**0.000*** **	100.3 ± 10.4	90.4 ± 17.1	**0.045***
VLDL-C	24.5 ± 9.3	24.3 ± 10.2	0.891	28.9 ± 11.7	34.5 ± 19.7	0.240
TC/H	3.8 ± 0.9	3.9 ± 1.1	0.081	4.7 ± 1.4	4.0 ± 1.3	0.072
TG/H	3.2 ± 1.5	3.1 ± 1.8	0.882	3.7 ± 1.9	4.9 ± 3.7	0.172
L/H	2.4 ± 0.8	1.9 ± 0.6	**0.000*** **	2.4 ± 0.6	2.4 ± 0.7	0.983
AIP level	0.45 ± 0.2	0.44 ± 0.2	0.771	0.51 ± 0.2	0.58 ± 0.3	0.288
Hs-CRP	0.32 ± 0.3	0.31 ± 0.3	0.935	0.26 ± 0.3	0.29 ± 0.3	0.585
TC ≥200 mg/dl	**n=10**			**n=16**		
TC	220.7 ± 14.8	205.6 ± 35.5	0.257	224.0 ± 18.3	194.2 ± 37.9	**0.001****
TG	183.8 ± 83.9	147.5 ± 65.1	0.194	147.8 ± 44.8	160.5 ± 102.2	0.609
HDL-C	51.8 ± 11.9	53.9 ± 12.8	0.309	49.2 ± 8.8	47.3 ± 7.1	0.222
LDL-C	129.5 ± 12.2	106.1 ± 28.0	**0.042***	146.5 ± 35.9	104.6 ± 27.9	**0.000*****
VLDL-C	36.7 ± 16.8	29.5 ± 12.9	0.195	28.2 ± 10.8	32.1 ± 20.4	0.484
TC/H	3.9 ± 0.9	4.4 ± 0.9	0.144	4.2 ± 1.0	4.5 ± 0.9	0.275
TG/H	4.0 ± 2.7	2.9 ± 1.4	0.159	3.1 ± 1.3	3.6 ± 2.9	0.528
L/H	2.6 ± 0.5	2.0 ± 0.6	**0.031***	3.1 ± 1.1	2.2 ± 0.7	**0.002****
AIP level	0.52 ± 0.3	0.41 ± 0.2	0.108	0.46 ± 0.2	0.47 ± 0.2	0.888
Hs-CRP	0.36 ± 0.3	0.41 ± 0.3	0.654	0.3 ± 0.2	0.36 ± 0.3	0.695

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

TABLE 4.2.64 IMPACT OF VITAMIN-D SUPPLEMENTATION ON BIOCHEMICAL PARAMETERS OF THE SUBJECTS BASED ON INITIAL TC LEVELS [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
TC <200 mg/dl	**n=30**			**n=14**		
25(OH)D (ng/ml)	11.9 ± 3.2	44.8 ± 16.5	**0.000*** **	10.3 ± 3.6	16.9 ± 4.8	**0.000*** **
Hb (gm/dl)	13.6 ± 1.7	12.9 ± 1.4	**0.000*** **	14.3 ± 1.1	13.7 ± 1.3	**0.002** **
Iron (mg/dl)	77.3 ± 24.4	72.5 ± 25.8	0.137	84.6 ±22.2	83.3±27.1	0.786
TIBC (mg/dl)	359.1±45.9	344.1±40.3	**0.003** **	362.4±43.4	340.4±33.2	**0.010** *
% TS	21.8 ± 6.7	21.2 ± 7.3	0.470	23.6 ± 6.5	24.7 ± 8.1	0.438
HbA1c (%)	8.7 ± 1.6	8.4 ± 1.7	0.163	8.4 ± 1.8	8.7 ± 2.3	0.566
ABG (mg/dl)	203.7±52.8	193.6±49.3	0.165	192.9±59.3	201.9±65.8	0.556
T3 (ng/dl)	109.4±17.0	106.6±17.9	0.290	108.2±10.4	108.9±13.9	0.846
T4 (µg/dl)	9.4 ± 2.2	9.2 ± 2.3	0.487	8.6 ± 1.2	9.1 ± 1.4	0.096
TSH (µIU/ml)	3.7 ± 3.1	4.5 ± 4.8	0.138	2.8 ± 1.2	2.7 ± 1.4	0.741
TC ≥200 mg/dl	**n=10**			**n=16**		
25(OH)D (ng/ml)	12.2 ± 3.7	40.1 ± 15.5	**0.000*** **	11.4 ± 3.1	14.1 ± 3.5	**0.013** *
Hb (gm/dl)	13.2 ± 1.7	12.6 ± 1.7	**0.003** **	13.5 ± 1.9	12.7 ± 1.9	**0.000*** **
Iron (mg/dl)	71.4 ± 18.7	67.9 ± 28.4	0.675	76.7 ± 24.7	74.2 ± 43.6	0.798
TIBC (mg/dl)	359.4±37.4	354.8±47.3	0.668	380.8±47.7	370.1±61.4	0.232
% TS	19.8 ± 4.1	19.2 ± 7.7	0.800	20.4 ± 6.8	20.0 ± 11.9	0.897
HbA1c (%)	8.3 ± 1.4	7.9 ± 1.6	0.401	8.6 ± 1.1	8.1 ± 1.0	0.080
ABG (mg/dl)	189.9±47.0	180.6±47.5	0.485	202.2±34.9	186.1±30.3	0.087
T3 (ng/dl)	108.4±23.4	105.2±15.6	0.470	107.0±15.1	103.9±14.9	0.190
T4 (µg/dl)	8.7 ± 1.2	9.1 ± 1.9	0.528	9.2 ± 1.9	9.1 ± 2.1	0.639
TSH (µIU/ml)	6.1 ± 8.1	4.1 ± 2.8	0.319	2.7 ± 1.6	2.2 ± 1.0	**0.010** *

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

IMPACT OF SUPPLEMENTATION BASED ON INITIAL TRIGLYCERIDE LEVELS OF THE SUBJECTS

The results of supplementation on the lipid values, atherogenic indices and inflammatory marker levels was also analyzed separately based on elevated and normal initial TAG levels, i.e. TAG<150mg/dl and TAG≥150mg/dl. The results are discussed in tables 4.2.65 and 4.2.66.

Impact of supplementation on lipid profile of the subjects

Post supplementation for the SG, among subjects with higher TAG levels, there was a significant reduction in TC & LDL-C, TC/H, L/H and atherogenic index of plasma (AIP) values, while for the CG significant reduction was seen only for LDL-C values. The decline in TAG levels among the higher level SG just missed the significance (p=0.05). LDL-C in both the groups and L/H ratio for the SG decreased significantly among subjects with lower initial TAG levels (Table 4.2.65).

Impact of supplementation on biochemical parameters of the subjects

The impact of supplementation on various biochemical parameters as stratified on their initial TAG levels is given in Table 4.2.66. The serum 25(OH)D levels increased in both supplementation and control groups significantly irrespective of their initial TAG levels. Similarly the haemoglobin levels were found to have decreased in both the groups significantly irrespective of their initial TAG levels. Subjects with lower initial TAG levels in the SG showed a significant decline for TIBC and HbA1c values, while in this category among the CG values for only TIBC reduced significantly.

TABLE 4.2.65 IMPACT OF VITAMIN-D SUPPLEMENTATION ON LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS BASED ON INITIAL TAG LEVELS [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
TAG <150mg/dl	**n=27**			**n=19**		
TC	170.0±34.7	170.1±42.3	0.978	196.8±35.8	190.4±33.9	0.274
TG	105.4±30.3	112.4±41.1	0.308	116.1±27.5	137.0±80.8	0.225
HDL-C	45.3 ± 9.6	46.2 ± 11.6	0.334	48.3 ± 9.9	45.7 ± 9.2	0.085
LDL-C	103.9±22.8	89.8±25.9	**0.000*** **	126.6±41.9	101.5±23.5	**0.003** **
VLDL-C	21.1 ± 3.0	22.5 ± 8.2	0.305	22.0 ± 6.6	27.4 ± 16.1	0.184
TC/H	3.8 ± 0.9	3.8 ± 0.8	0.461	4.3 ± 1.1	3.9 ± 1.1	0.148
TG/H	2.4 ± 0.9	2.5 ± 1.0	0.477	2.5 ± 0.8	3.3 ± 2.9	0.190
L/H	2.4 ± 0.6	1.9 ± 0.5	**0.000*** **	2.6 ± 1.1	2.3 ± 0.6	0.086
AIP level	0.36 ± 0.2	0.37 ± 0.2	0.609	0.38 ± 0.1	0.43 ± 0.3	0.290
Hs-CRP	0.32 ± 0.3	0.35 ± 0.3	0.586	0.32 ± 0.3	0.38 ± 0.3	0.424
TAG ≥150mg/dl	**n=13**			**n=11**		
TC	185.2±45.5	160.6±40.8	**0.037***	206.2±22.5	180.4±33.4	0.064
TG	205.5±59.6	160.6±66.9	0.050	198.9±36.9	216.4±110.7	0.637
HDL-C	41.4 ± 11.0	42.0 ± 10.1	0.656	41.9 ± 6.1	40.8 ± 7.6	0.485
LDL-C	110.9±29.3	81.1±27.1	**0.008** **	122.0±21.9	92.0±25.3	**0.008** **
VLDL-C	41.1±11.9	32.1±13.4	0.050	39.8±7.4	43.2±22.1	0.638
TC/H	3.9 ± 1.0	4.6 ± 1.3	**0.020***	4.6 ± 1.4	5.0 ± 0.7	0.384
TG/H	5.3 ± 1.9	4.1 ± 2.3	0.117	4.9 ± 1.6	5.7 ± 3.5	0.477
L/H	2.8 ± 0.9	1.9 ± 0.7	**0.002** **	2.9 ± 0.5	2.3 ± 0.8	0.058
AIP level	0.69 ± 0.2	0.56 ± 0.2	**0.020***	0.67 ± 0.1	0.68 ± 0.3	0.941
Hs-CRP	0.35 ± 0.3	0.31 ± 0.3	0.650	0.26 ± 0.2	0.24 ± 0.2	0.490

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

TABLE 4.2.66 IMPACT OF VITAMIN-D SUPPLEMENTATION ON BIOCHEMICAL PARAMETERS OF THE SUBJECTS BASED ON INITIAL TAG LEVELS [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
TAG <150 mg/dl	**n=27**			**n=19**		
25(OH)D (ng/ml)	12.1±3.2	45.6±15.9	**0.000*** **	10.9±3.4	16.1±4.8	**0.000*** **
Hb (gm/dl)	13.5±1.7	12.9±1.4	**0.000*** **	12.9±1.8	13.6±1.7	**0.001*** **
Iron (mg/dl)	76.0±25.9	72.8±27.0	0.409	78.1±22.4	81.2±36.6	0.663
TIBC (mg/dl)	358.2±47.2	345.6±40.3	**0.023***	376.5±48.8	354.4±48.1	**0.003****
% TS	21.4±6.7	21.1±7.3	0.778	21.1±6.6	23.3±10.9	0.308
HbA1c (%)	8.8±1.6	8.3±1.7	**0.049***	8.2±1.3	7.9±1.1	0.399
ABG (mg/dl)	206.1±54.0	190.7±50.0	0.052	186.9±42.6	181.5±31.5	0.540
T3 (ng/dl)	109.1±19.2	106.3±19.2	0.356	108.4±11.0	108.9±11.3	0.832
T4 (µg/dl)	9.1±2.2	9.1±2.4	0.754	9.2±1.1	9.2±1.3	0.983
TSH (µIU/ml)	4.3±5.1	3.4±2.9	0.076	9.2±1.1	9.2±1.3	0.983
TAG ≥150 mg/dl	**n=13**			**n=11**		
25(OH)D (ng/ml)	11.8 ± 3.6	39.5 ± 16.6	**0.000*** **	10.8 ± 3.4	14.4 ± 3.4	**0.006****
Hb (gm/dl)	13.4 ± 1.6	12.8 ± 1.6	**0.004****	14.4 ± 1.5	13.5 ± 1.6	**0.000*** **
Iron (mg/dl)	75.4±16.3	68.2±25.1	0.190	84.3±25.9	73.9±37.6	0.260
TIBC (mg/dl)	361.2±36.3	349.3±46.3	0.169	364.8±41.7	359.5±59.7	0.624
% TS	21.0±1.5	19.8±7.5	0.414	23.3±7.1	20.4±9.6	0.141
HbA1c (%)	8.2 ± 1.3	8.2 ± 1.6	0.982	9.1 ± 1.5	9.1 ± 2.4	0.989
ABG (mg/dl)	188.0±44.2	189.7±47.5	0.865	216.7±50.7	214.2±68.3	0.896
T3 (ng/dl)	109.0±17.7	106.0±12.8	0.307	106.1±16.2	101.5±18.4	0.235
T4 (µg/dl)	9.4 ± 1.5	9.4 ± 1.9	0.891	8.4 ± 2.2	8.8 ± 2.4	0.190
TSH (µIU/ml)	5.9 ± 7.0	4.7 ± 2.9	0.414	2.3 ± 0.9	2.4 ± 1.3	0.861

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

IMPACT OF SUPPLEMENTATION BASED ON DURATION OF DIABETES

As the duration of a disease also influences the biochemical parameters in an human body, an attempt was made to see the impact of the supplementation by stratifying the subjects based on their duration of diabetes- lower duration of ≤ 5 years and higher duration of >5 years. The results are depicted in tables 4.2.67 and 4.2.68.

Impact of supplementation on lipid profile of the subjects

The impact of supplementation on lipid parameters and Hs-CRP values revealed that a significant decline in the LDL-C and L/H ratio was noticed in the SG irrespective of their duration of diabetes. Similar trend was observed in the CG among the subjects with lower duration, while among the subject with higher duration only the LDL-C levels reduced significantly (Table 4.2.67).

Impact of supplementation on biochemical parameters of the subjects

As observed in the table for impact of supplementation on the biochemical parameters of the subjects stratified as per their initial TC and TG levels, for the duration of diabetes also the serum 25(OH)D levels increased significantly and haemoglobin levels decreased significantly in both supplementation and control groups irrespective of their duration of disease. A decline in TIBC values was seen in both the groups among the subjects who suffered from diabetes for a shorter period of time (Table 4.2.68).

TABLE 4.2.67 IMPACT OF VITAMIN-D SUPPLEMENTATION ON LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS STRATIFIED BY DURATION OF DIABETES [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
≤ 5 years	**n=22**			**n=23**		
TC	182.3 ± 40.7	176.7 ± 43.2	0.481	159.7±31.2	154.2±35.3	0.250
TG	147.6 ± 69.3	135.5 ± 58.8	0.194	135.5 ± 58.8	147.6 ± 69.3	0.399
HDL-C	43.6 ± 9.0	46.2 ± 9.4	0.059	47.3 ± 8.0	46.8 ± 8.7	0.568
LDL-C	109.3 ± 26.7	91.6 ± 28.2	**0.001****	127.9 ± 38.7	100.2 ± 25.8	**0.004****
VLDL-C	29.5 ± 13.8	27.1 ± 11.7	0.402	28.9 ± 11.7	35.6 ± 21.4	0.158
TC/H	3.8 ± 0.9	3.9 ± 0.9	0.156	4.4 ± 0.9	4.5 ± 1.3	0.660
TG/H	3.3 ± 1.9	2.9 ± 1.3	0.296	3.5 ± 1.7	4.6 ± 3.6	0.119
L/H	2.4 ± 0.6	1.9 ± 0.6	**0.001****	2.8 ± 0.9	2.4 ± 0.7	**0.027***
AIP level	0.46 ± 0.2	0.42 ± 0.2	0.305	0.49 ± 0.2	0.55 ± 0.3	0.305
Hs-CRP	0.44 ± 0.28	0.45 ± 0.3	0.920	0.35 ± 0.28	0.36 ± 0.3	0.791
>5 years	**n=18**			**n=7**		
TC	166.1 ± 34.9	155.3 ± 37.3	0.133	188 ± 28.3	174.5 ± 26.4	0.089
TG	126.2 ± 53.7	118.9 ± 50.1	0.475	135.6 ± 45.5	127.0 ± 54.9	0.399
HDL-C	40.7 ± 10.9	41.9 ± 13.8	0.378	45.1 ± 8.9	45.0 ± 8.9	0.903
LDL-C	102.3 ± 22.4	81.3 ± 23.2	**0.001****	115.3 ± 21.7	90.7 ± 17.4	**0.010***
VLDL-C	25.5 ± 10.7	23.8 ± 10.0	0.476	27.1 ± 9.1	25.4 ± 11.0	0.416
TC/H	3.9 ± 0.9	4.2 ± 1.2	0.074	4.0 ± 0.9	3.9 ± 1.7	0.809
TG/H	3.4 ± 1.9	3.2 ± 2.2	0.598	3.2 ± 1.4	2.9 ± 1.6	0.526
L/H	2.6 ± 0.9	2.0 ± 0.6	**0.001****	2.5 ± 0.8	2.1 ± 0.6	0.190
AIP level	0.47 ± 0.2	0.44 ± 0.2	0.452	0.4 ± 0.2	0.4 ± 0.2	0.415
Hs-CRP	0.18 ± 0.2	0.19 ± 0.2	0.869	0.7 ± 0.5	0.0 ± 0.2	0.338

p <0.01**, <0.05*

TABLE 4.2.68 IMPACT OF VITAMIN-D SUPPLEMENTATION ON BIOCHEMICAL PARAMETERS OF THE SUBJECTS STRATIFIED BY DURATION OF DIABETES [MEAN ± SD]

	Supplementation Group			Control Group		
	Pre	**Post**	**Paired t *p* value**	**Pre**	**Post**	**Paired t *p* value**
≤ 5 years	**n=22**			**n=23**		
25(OH)D (ng/ml)	12.1 ± 3.3	44.0 ± 15.6	**0.000*** **	11.5 ± 2.8	15.9 ± 4.5	**0.000*** **
Hb (gm/dl)	13.2 ± 1.7	12.8 ± 1.6	**0.000*** **	13.7 ± 1.6	12.9 ± 1.7	**0.000*** **
Iron (mg/dl)	72.9 ± 23.2	70.3 ± 27.4	0.543	79.3 ± 23.7	79.2 ± 39.8	0.983
TIBC (mg/dl)	356.3±36.6	339.7±31.9	**0.004** **	379.3±45.9	360.4±54.4	**0.014** *
% TS	20.4 ± 5.7	20.5 ± 7.1	0.942	21.3 ± 7.1	22.2 ± 11.4	0.619
HbA1c (%)	8.4 ± 1.4	8.1 ± 1.8	0.300	8.5 ± 1.3	8.4 ± 1.8	0.848
ABG (mg/dl)	194.4±46.1	186.9±51.0	0.354	197.6±44.9	195.5±52.9	0.834
T3 (ng/dl)	113.4±19.2	110.1±14.5	0.216	109.3±12.1	108.5±13.8	0.717
T4 (µg/dl)	9.7 ± 1.7	9.8 ± 1.9	0.901	9.2 ± 1.4	9.4 ± 1.5	0.192
TSH (µIU/ml)	5.4 ± 6.7	3.6 ± 2.5	0.804	2.6 ± 1.3	2.4 ± 1.3	0.282
>5 years	**n=18**			**n=7**		
25(OH)D (ng/ml)	11.9 ± 3.4	43.2 ± 17.4	**0.000*** **	8.9 ± 4.3	13.9 ± 3.6	**0.003** **
Hb (gm/dl)	13.7 ± 1.5	12.9 ± 1.3	**0.000*** **	14.5 ± 1.6	13.9 ± 1.7	**0.036** *
Iron (mg/dl)	79.4 ± 23.0	72.5 ± 25.4	0.150	84.0 ± 24.2	76.3 ± 25.2	0.476
TIBC (mg/dl)	362 ± 51.6	355.4±51.1	0.334	349.0±40.4	342.5±41.9	0.386
% TS	22.3 ± 6.8	20.9 ± 7.8	0.272	23.8 ± 5.6	22.2 ± 6.9	0.550
HbA1c (%)	8.8 ± 1.7	8.4 ± 1.6	0.201	8.5 ± 1.7	8.1 ± 1.4	0.463
ABG (mg/dl)	207.3±57.2	194.5±46.5	0.207	198.7±57.9	187.0±40.5	0.520
T3 (ng/dl)	103.9±16.7	101.4±19.4	0.525	101.7±14.8	98.7±14.7	0.372
T4 (µg/dl)	8.6 ± 2.1	8.5 ± 2.3	0.717	8.1 ± 2.0	7.8 ± 2.0	0.490
TSH (µIU/ml)	4.2 ± 4.3	4.1 ± 3.5	0.771	3.3 ± 1.4	2.5 ± 1.1	0.061

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

PHASE II (C) WASHOUT EFFECT OF VITAMIN-D SUPPLEMENTATION ON SERUM 25(OH)D STATUS OF T2DM SUBJECTS

This phase is in continuation of the supplementation study, carried out to assess the sustainability of vitamin-D levels after sixteen weeks of supplementation in the T2DM subjects. The washout effect of supplementation was studied on the serum 25(OH)D status, lipid profile, HbA1c values and anthropometric measurements on the available forty-eight subjects (SC; n=35 and CG; n=13). The results are discussed below-

Gender-wise distribution of subjects

The gender wise distribution of the subsets for the washout effect among the supplementation and control groups is given in Table 4.2.69. In this phase, forty-eight subjects- 25 females (52.1%) and 23 males (47.9%) were available at the end of 16 weeks of the study. In the SG there were 54.3% and 45.7% females and males respectively while in CG the distribution was 46.1% and 53.8% respectively.

Vitamin-D status of the subjects at washout period

Change in serum 25(OH)D values in individual subjects performed at baseline, at 8 weeks and at 16 weeks of washout period after cholecalciferol supplementation are shown in Table 4.2.70. The serum vitamin-D levels increased significantly in SG after eight weeks of supplementation, but dropped down significantly at 16 weeks (8 weeks of washout period), though the levels were significantly higher as compared to baseline. Similar trend was observed for CG, however throughout the study period this group had deficient vitamin-D levels. About 83% (29/35) of the subjects attained sufficiency levels of serum vitamin-D after 8 weeks of supplementation, but only 14% (5/35) would sustain it after 16 weeks in the SG. While in CG after 16 weeks hundred percent of the subjects (13/13) reverted back to deficiency status (Figure 4.2.15).

Biophysical measurements of the subjects at washout period

The anthropometric parameters of the subjects revealed that there was a significant decrease in waist circumference (WC) among SG at end of 16 weeks, while other measurements decreased non-significantly. In the CG also small non-significant decrease was observed for all anthropometric measurements. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) increased in both the groups from 8 to 16 weeks of the study, but the SG had significantly lower DBP at 16 weeks as compared to CG (p=0.041) (Table 4.2.71 (A)).

TABLE 4.2.69 GENDER-WISE DISTRIBUTION OF THE SUBJECTS IN SUPPLEMENTATION & CONTROL GROUPS (n, %)

	Supplementation group	Control group	Total
Females	19 (54.3)	6 (46.1)	25 (52.1)
Males	16 (45.7)	7 (53.8)	23 (47.9)
Total	35 (72.9)	13 (27.1)	48 (100.0)

Values in parenthesis indicate percent

TABLE 4.2.70 MEAN SERUM VITAMIN-D LEVELS IN SUBJECTS AT BASELINE, POST SUPPLEMENTATION & WASHOUT PERIOD (n=48) [MEAN ± SD]

Parameter		Supplementation Group (n=35)	Control Group (n=13)	t test *p* value
Vitamin-D	Baseline	11.8±3.3	11.2±3.1	0.587
	Post data	43.4±16.3	17.7±4.2	**0.000*** **
	Washout	22.2±6.5	13.6±2.3	**0.000*** **
F-value		**0.000*** **	**0.000*** **	

p<0.001***

FIGURE 4.2.15 VITAMIN-D STATUS OF THE SUBJECTS AT POST & WASHOUT PERIOD (n=48)

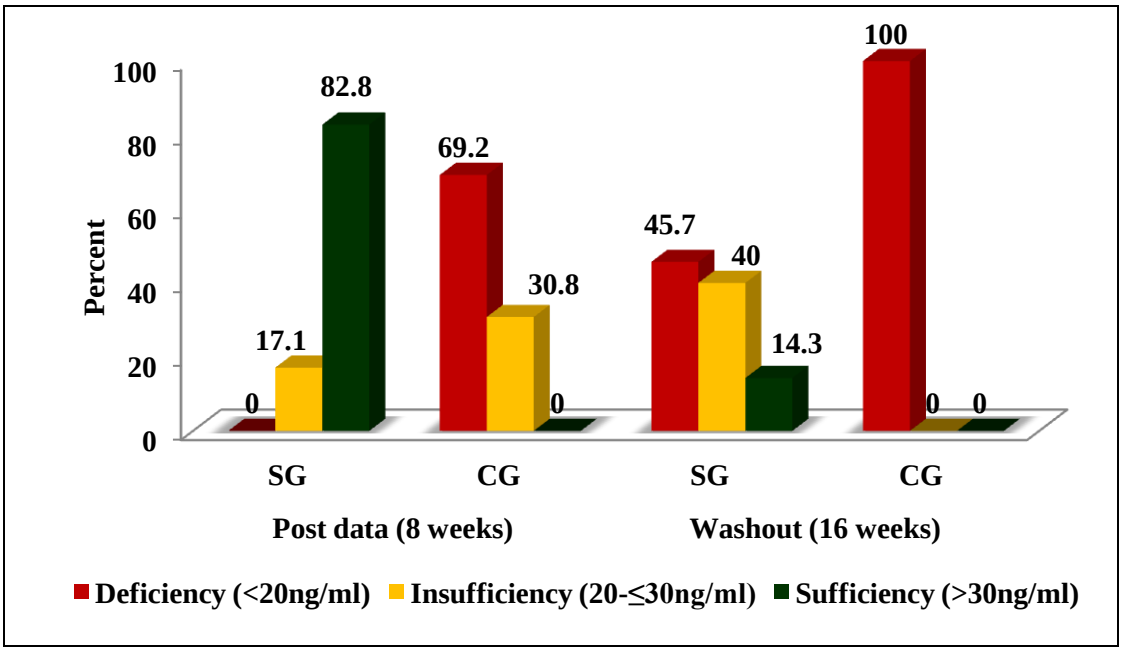


TABLE 4.2.71 (A) BIOPHYSICAL MEASUREMENTS OF THE SUBJECTS AT BASELINE, POST SUPPLEMENTATION & WASHOUT PERIOD (n=48) [MEAN ± SD]

Parameter		Supplementation Group (n=35)	Control Group (n=13)	t test *p* value
Weight (Kg)	Baseline	71.3 ± 12.1	74.5 ± 14.9	
	Post data	69.6 ± 11.5	73.4 ± 13.9	0.354
	Washout	69.6 ± 11.5	73.6 ± 14.5	0.326
F-value		0.794	0.976	
WC (cm)	Baseline	96.5±10.3	97.9±13.1	
	Post data	93.5±10.3	99.7±13.8	0.099
	Washout	89.6±9.0	94.5±12.3	0.134
F-value		**0.015***	0.590	
HC (cm)	Baseline	103.4 ± 11.3	103.0 ± 17.3	
	Post data	100.8 ± 9.9	104.5 ± 12.5	0.291
	Washout	100.1 ± 9.6	103.8 ± 12.2	0.262
F-value		0.376	0.964	
WHR	Baseline	0.94±0.06	0.96±0.09	
	Post data	0.93±0.08	0.95±0.08	0.335
	Washout	0.89±0.07	0.91±0.09	0.566
F-value		0.058	0.314	
WSR	Baseline	0.61 ± 0.07	0.062 ± 0.09	
	Post data	0.59 ± 0.07	0.63 ± 0.09	0.117
	Washout	0.56 ± 0.05	0.59 ± 0.07	0.10
F-value		0.367	0.615	
BMI	Baseline	28.2 ± 4.8	29.9 ± 5.9	
	Post data	28.0 ± 4.7	29.4 ± 5.8	0.403
	Washout	27.7 ± 4.4	29.5 ± 5.9	0.268
F-value		0.903	0.971	
% Body Fat	Baseline	36.9 ± 6.6	37.9 ± 7.8	
	Post data	35.9 ± 6.8	37.4 ± 6.8	0.528
	Washout	36.4 ± 6.8	38.1 ± 6.6	0.437
F-value		0.843	0.965	
SBP (mmHg)	Baseline	142.1±17.5	143.5±18.9	
	Post data	135.1±18.1	134.2±19.4	0.880
	Washout	138.7±20.2	145.7±16.2	0.270
F-value		0.299	0.250	
DBP (mmHg)	Baseline	85.9±9.1	86.4±10.8	
	Post data	82.0±7.5	83.8±9.2	0.501
	Washout	83.9±7.6	89.3±8.6	**0.041***
F-value		0.141	0.350	

p<0.05*

TABLE 4.2.71 (B) LSD POST-HOC TEST FOR ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS OF SUPPLEMENTATION GROUP

Parameter	Pre to Post	Pre to Washout	Post to Washout
WC	0.208	**0.004****	0.096
WHR	0.721	**0.027***	0.062
DBP	**0.048***	0.313	0.327

p<0.01**, <0.05*

In order to see that how the groups were different from each other at the points of data collection i.e. baseline, post supplementation (8 weeks) and washout effect (16 weeks), LSD post hoc test was performed. The test showed that for SG, WC (p=0.004) and WHR (p=0.027) decreased significantly from baseline to washout period, while DBP (p=0.048) showed significant decrease from baseline to post supplementation (Table 4.2.71(B)).

Lipid profile of the subjects at washout period

The lipid profile of the subjects at 8 and 16 weeks of the study is displayed in Table 4.2.72 (A). All the parameters reduced after 8 weeks of supplementation in SG with significant drop in LDL-C and LDL/HDL ratio; however the levels of both these parameters increased significantly after the washout period at 16 weeks. The CG did not show favourable changes in the lipid parameters at the end of both 8 and 16 weeks except for LDL-C and LDL/HDL ratio which showed similar trend to supplementation group. The post hoc test as shown in table 4.2.72 (B) revealed that LDL-C levels significantly decreased at 8 weeks in both the groups (SG p=0.004, CG p=0.012) and increased significantly at the end of 16 weeks (SG p=0.000, CG p=0.002). A significant drop in LDL/HDL ratio was observed in SG post supplementation (p=0.009) and after washout (p=0.000) while for CG significance it was seen at end of washout period (p=0.003).

HbA1c levels of the subjects at washout period

The mean HbA1c and average blood glucose levels of the subjects are shown in Table 4.2.73. A non-significant decrease in HbA1c & ABG values was observed in SG at both 8 and 16 weeks of the study period. While for CG a slight non-significant decrease was seen at 8 weeks which again increased at the end of 16 weeks. In the post hoc analysis also no statistical significance was observed at any point of the study

TABLE 4.2.72 (A) MEAN LIPID LEVELS IN SUBJECTS AT BASELINE, POST SUPPLEMENTATION & WASHOUT PERIOD (n=48) [MEAN ± SD]

Parameter		Supplementation Group (n=35)	Control Group (n=13)	t test *p* value
TC (mg%)	Baseline	176.2±40.1	204.4±30.1	0.152 0.092
	Post data	169.6±41.6	188.6±35.4	
	Washout	181.5±40.5	202.5±27.6	
F-value		0.479	0.383	
HDL-C (mg%)	Baseline	44.3±10.6	48.5±10.9	0.845 EVNA 0.937
	Post data	45.2±11.7	45.9±11.1	
	Washout	43.6±11.5	43.8±7.7	
F-value		0.834	0.507	
LDL-C (mg%)	Baseline	106.8±25.4	124.1±23.6	0.181 0.086
	Post data	85.1±29.5	97.6±25	
	Washout	112.9±34.4	131.5±27.5	
F-value		**0.000*** **	**0.004** **	
VLDL-C (mg%)	Baseline	28.9 ± 12.7	28.8 ± 10.3	0.474 EVNA 0.683
	Post data	26.8 ± 11.2	30.6 ± 17.4	
	Washout	28.9 ± 16.8	31.0 ± 11.8	
F-value		0.371	0.914	
TAG (mg%)	Baseline	145±63.5	144.6±51.7	0.475 EVNA 0.683
	Post data	133.9±56.2	153±87.2	
	Washout	144.6±84.3	155.1±59	
F-value		0.751	0.917	
TC/H	Baseline	4.1 ± 1.1	4.1 ± 1.4	0.191 0.328
	Post data	3.9 ± 0.9	4.3 ± 1.3	
	Washout	4.4 ± 1.2	4.8 ± 0.9	
F-value		0.443	0.443	
L/H	Baseline	2.5±0.8	2.6±0.8	0.240 0.273
	Post data	1.9±0.7	2.2±0.6	
	Washout	2.7±1.0	3.1±1.7	
F-value		**0.013***	**0.013***	
TG/H	Baseline	3.5 ± 1.9	3.2 ± 1.8	0.509 EVNA 0.858
	Post data	3.2 ± 1.8	3.9 ± 3.5	
	Washout	3.6 ± 2.5	3.8 ± 2.0	
F-value		0.792	0.792	

p<0.05*, <0.01**, <0.001*** EVNA=Equal Variance Not Assumed

TABLE 4.2.72 (B) LSD POST-HOC TEST FOR LIPID PARAMETERS OF THE SUBJECTS

Parameter		Pre to Post	Pre to Washout	Post to Washout
Supplementation Group	LDL-C	**0.004****	0.410	**0.000*****
	L/H	**0.009****	0.239	**0.000*****
Control Group	LDL-C	**0.012***	0.460	**0.002****
	L/H	0.206	0.073	**0.003****

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

TABLE 4.2.73 SERUM HbA1c & AVERAGE BLOOD GLUCOSE LEVELS IN SUBJECTS AT BASELINE, POST SUPPLEMENTATION & WASHOUT PERIOD (N =48) [MEAN ± SD]

Parameter		Supplementation Group (n=35)	Control Group (n=13)	t test *p* value
HbA1c (%)	Baseline	8.4±1.5	8.1±1.3	0.621 0.933
	Post data	8.2±1.7	7.9±1.1	
	Washout	8.1±1.6	8.0±1.2	
F-value		0.890	0.890	
ABG	Baseline	195.4 ± 50.4	184.4 ± 42.9	0.619 0.929
	Post data	187.7 ± 49.7	180.2 ± 32.5	
	Washout	186.5 ± 45.4	185.3 ± 34.7	
F-value		0.708	0.933	

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DISCUSSION

Vitamin D from cutaneous synthesis, dietary or supplemental intake, is transported to the fat where it can be stored or to the liver for the first step of activation, the hydroxylation to 25-hydroxyvitamin D [25(OH)D], which is the major circulating form of vitamin D and measured to assess a patient's vitamin D status. Vitamin D modulates insulin receptor gene expression and insulin secretion; it is an interesting environmental candidate for type 2 diabetes mellitus pathogenesis and development (Ortlepp et al., 2003). However, less is known on the association between vitamin D and type 2 diabetes mellitus. Also it has been observed that in developing countries, onset of diabetes generally occurs in individuals at younger age i.e. around 46-65 years. Hence in the present study the vitamin D deficiency among the T2DM subjects of age 30-65 years was mapped and an effort was made to identify the determinants of vitamin-D status. Also a randomised control trial with supplementation of cholecalciferol granules for eight weeks was conducted to study its impact on serum vitamin D levels of the deficient subjects and their cardio-metabolic profile. The sustainability of the improved vitamin D levels was checked after a washout period of eight weeks post supplementation. The results obtained are discussed below-

In the developing countries like India, a continuous and rapid increase in the prevalence of T2DM has been occurring which mainly could be attributed to rapid transition in lifestyle, accompanied by a rising epidemic of obesity (Ramachandran et al., 2010). Therefore, the first phase of the study planned as a formative research in which T2DM subjects were enrolled, their background information was collected and prevalence of risk factors was analysed.

Hypertension is a common co-morbidity in diabetes and vice versa. Diabetes and hypertension coexist in approximately 40-60% of the patients with T2DM (Sowers et al., 2001). Studies from India have also shown that about 50% of the diabetic individuals in India have hypertension (Singh et al., 1996). Similarly Diabetes Mellitus is considered as an independent risk factor for cardiovascular disease. This is because T2DM is a component of the metabolic cluster, which is associated with other risk factors like insulin resistance, dyslipidemia, hypertension, abdominal obesity and prothrombotic state (Reaven, 1993). However there is inadequate data on stroke and CVD in India.

A review of CVD in T2DM from India also commented on the paucity of literature on the subject (Sridhar, 2002). In one of the presentations to an endocrine centre in South it was

revealed that 1.12% of the T2DM subjects (189 out of 16,570 subjects) had a diagnosis of CVD (Feinglass et al., 1999). In the recent study also hypertension was the most prevalent medical condition among the subjects and the self reported prevalence was around 57.9% while for CVD the prevalence was 4.3%. Based on the estimated levels of lipid parameters, hypercholesterolemia and hypertriglyceridemia was present in around 35% of the subjects, while high LDL-cholesterol levels seen in 65% of the subjects, which is quite alarming. About half of the population studied had suboptimum levels of the protective HDL-cholesterol which worsens the risk of cardiovascular events among the diabetic subjects.

Supporting these results, data from Chennai and also results published by Mohan et al (1996), showed that the prevalence of complications of T2DM was as follows: retinopathy 23.7%, nephropathy 5.5%, peripheral neuropathy 25.5%, CHD 11.4% and stroke 0.9%. Prevalence of hypertension was also very high (38.2%) (Ramachandra et al.,1999). In the present study retinopathy was reported by 5.7% and neuropathy and stroke by 0.5% of the subjects.

Obesity and specifically abdominal obesity is another major risk factor for diabetes mellitus. It has been observed that Asian Indians have a higher percentage of body fat, for a given BMI, when compared with the white population (Banerji et al., 1999; Gallagher et al., 1996) and small increments in weight trigger glucose intolerance in the susceptible subjects (Shelgikar et al., 1991). The current study also observed a very high prevalence of both obesity and abdominal obesity among the diabetic subjects. Based on BMI (<23 kg/m^2), around 71% of the subjects were obese and an alarmingly high percent (97%) had high percent body fat, while abdominal obesity was present in 74% of the subjects.

The results obtained in our study are in line with the results reported by Akhter et al., (2015) in a recent study among 175 Pakistani T2DM subjects. The mean age of the subjects was 54.1±12 years and duration of diabetes was 8.1 years. Mean HbA1c was 8.1% which similar to our study (8.7%). Hypertension was present in 65.7% of the subjects and prevalence of dyslipidemia was 60%. In the study total and central body fat correlated significantly with BMI (r=0.68, p <0.001) and waist circumference (r=0.66, p<0.001). The authors concluded that high body fat percentage, waist circumference were seen especially in woman and central body fat percentage in both sexes among patients with type 2 diabetes mellitus in Pakistan.

The major objective of the study was to map the prevalence of vitamin D deficiency among the T2DM subjects. The mean serum 25(OH)D levels of the diabetic subjects were much below

the optimum requirement of >30 ng/mL, signalling a high prevalence of deficiency. About 88.6% of the subjects were found to be vitamin D deficient, with females showing a non-significant higher prevalence as compared to males. Similar high prevalence has been reported by many authors worldwide. Taheri et al, (2012) reported a prevalence of 83.3% among T2DM subjects as compared to 75.6% among the healthy subjects aged 20-80 years in Iran. Subramanian et al (2011) among Asian Indians reported a prevalence of 57.6% among the diabetic subjects with suboptimal serum vitamin D levels among them (11.0±7.5 ng/mL). Tiwari et al (2012), reported that 57.3% of T2DM subjects (mean age 51 years) had vitamin D deficiency (<50 nmol/l) and about 17.6% of them had severe vitamin D deficiency (<25 nmol/l). In the present study to the relief of the researchers no subject fell in the severe deficiency category.

Human and animal studies have shown that vitamin D and calcium are involved in glycemic metabolism, and that altered vitamin D and calcium concentrations play a role in the development of diabetes. The relationship between vitamin D, calcium and insulin was first elucidated in 1967, when Milner and Hales showed that in animal, calcium and magnesium, which are tightly regulated by the vitamin D system; were essential for insulin secretion (Milner & Hales, 1967). Thus one of the promising strategies towards improving glucose levels would be vitamin D supplementation. Vitamin D2 and vitamin D3 are available as oral over-the-counter supplements.

The prime objective of the second phase of the study was to investigate the impact of vitamin D supplementation in the form of cholecalciferol (vitamin-D3) granules on the serum 25(OH)D levels and the glycemic and lipemic parameters among T2DM subjects. Currently, it is a common practice by physicians in India to prescribe a vitamin-D3 (cholecalciferol) sachet of 1500 µg (60,000 IU) to be taken each week for 6–8 weeks for overt or occult vitamin-D deficiency among the general as well as diseased population (Goswami, Gupta, Ray, Singh, & Tomar, 2008). Thus keeping this and the Endocrine Society's Practice Guidelines recommendation for treatment strategies in mind, a dose of 60,000 IU oral vitamin-D3 was given weekly for eight weeks to T2DM subjects in the current study. The Endeocrine society's guidelines are for patients with vitamin D deficiency depending on age and underlying medical conditions. In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, at least 6000–10,000 IU/day of vitamin D to treat

vitamin D deficiency are recommended, followed by maintenance therapy of at least 3000–6000 IU/day (Holick et al., 2011).

The results of the present study showed that after eight weeks of supplementation, mean serum 25(OH)D levels increased to the range of >30 ng/mL, which is currently considered adequate or sufficient for bone health & extra skeletal functions in about eighty percent of the supplemented subjects. This indicated that cholecalciferol supplementation to achieve higher levels of serum vitamin D remains a promising adjuvant therapy for T2DM patients. Similar significant increase in serum 25(OH)D levels after supplementation have been reported among T2DM subjects by various authors with different baseline serum 25(OH)D levels, cholecalciferol doses used, duration of study, and response to supplementation (Talaei, Mohamadi, & Adgi, 2013; Heshmat et al., 2012; Sugden et al., 2008).

There are several reports that have shown an inverse relationship between vitamin D levels and obesity, even in different definition of obesity according to weight, BMI and waist circumference (Need et al, 2005; Konradsen et al., 2008) and this correlation was not dependent on dose of vitamin D supplements provided to the subjects (Garcia-Bailo et al., 2011). However the results are conflicting. Similarly low levels of 25- hydroxyvitamin D are also associated with many markers of cardiovascular disease; for example, hypertension (Lind et al, 1995) and increased vascular resistance (Holick et al, 2005). In many small supplementation studies, interventions to increase 25-hydroxyvitamin D have shown to reduce blood pressure in populations at risk of cardiovascular disease (Pfeifer et al., 2001; Krause et al., 1998).

In a prospective interventional study conducted to investigate the effects of vitamin D3 supplementation (2000 IU vitamin D3) on the metabolic profiles of Saudi T2DM subjects pre- and post vitamin D supplementation over an 18-month period, it was observed that there was a significant improvement in the circulating levels of 25-hydroxyvitamin D from baseline to 6 months (32.2 ± 1.5 vs. 57.7 ± 1.4 nmol/l, $p<0.001$), and these levels remained unchanged over the course of the supplementation period. However levels remained below normal at end of 18 months after the onset of treatment and no change was reported in the blood pressure, BMI and glucose levels of the subjects (Al-Daghri et al., 2012).

In another prospective interventional study among 499 Saudi T2DM subjects divided in various groups based on their medication profile; who received 2000 IU vitamin D3 daily for one year and the control group with no intervention, it was observed that circulating 25(OH)D

concentrations improved in all patient groups. No significant changes were observed in the BMI and glucose in any of the intervention groups across the follow-ups. The observation for BMI values was similar even after stratification for gender. There was a significant decrease in the mean systolic pressure and increase in mean diastolic blood pressure from baseline to twelve months among males. While among females blood pressure remained stable along the follow-ups at both six months and twelve months (Alkharfy et al., 2013).

A randomized double-blind clinical trial conducted among 42 diabetics was designed to investigate the effect of injection of vitamin D on insulin resistance and anthropometric parameters in T2DM. The intervention group received a single intramuscular injection of 300,000 IU of vitamin D3 and placebo group received no treatment. The age range of patients was 37–79 years with a mean of 56 years and diabetes duration of 5 ± 7 years (mean ± SD). Three months after vitamin D injection, HbA1c, anthropometric factors and HOMA index in intervention group stayed constant, however, serum 25(OH)D was significantly increased (mean difference post supplementation 22.4 ± 39.9 ng/mL, p =0.007) (Heshmat et al., 2012).

One of the studies conducted among 34 T2DM subjects in Scotland, reported that a single large dose of oral vitamin D (100 000 IU vitamin D2 or Placebo) improved the endothelial function in patients with Type 2 diabetes and vitamin D insufficiency. Vitamin D supplementation increased 25-hydroxyvitamin D levels by 15.3 nmol/l relative to placebo and significantly improved the flow mediated vasodilatation (FMD) of the brachial artery by 2.3%. The improvement in FMD remained significant after adjusting for changes in blood pressure. Vitamin D supplementation significantly decreased systolic blood pressure by 14 mmHg compared with placebo among the subjects (Sugden et al., 2008).

The results of the present study however differed with many of the above mentioned studies which reported no significant changes in either anthropometric measurements of blood pressure after vitamin D supplementation. A significant decrease in weight and waist circumference post supplementation was observed in the current study, hence indicating that vitamin-D3 supplementation can alter the risk of abdominal obesity which is one of the proven risk factors for T2DM. Also significant reductions in both systolic and diastolic blood pressure; which is considered as a surrogate marker for cardiovascular risk, were observed in the present study among the vitamin-D supplemented subjects. Ethnicity of the subjects and duration of diabetes may be one of the possible reasons for these differences among the present study and the quoted ones.

Vitamin D is also essential for normal insulin secretion in response to glucose and also for maintenance of glucose tolerance. Vitamin D supplementation is considered as one of the novel strategies toward prevention and control of T2DM. However in the present study vitamin-D supplementation did not significantly lower the HbA1c levels among the subjects. This might be due to a short study period which was unable to trace profound changes in the parameter.

Witham et al (2010) also reported that vitamin D intake (at different dosage) had no effects on insulin resistance or on HbA1c values. A systematic review of fifteen trials also reported no significant improvement in fasting glucose, HbA1c or insulin resistance in those treated with vitamin D compared to placebo (George, Pearson, & Witham, 2012). Thus these results call for research of longer duration to elicit the impact of vitamin-D on the glycemic parameters.

However a before-after study conducted by Talaei et al (2013) among 100 Irani T2DM subjects, 30-70 years of age; who received 50,000 IU vitamin D3 orally per week for eight weeks showed significant improvements in serum fasting plasma glucose (FPG), insulin and in HOMA-IR after treatment with vitamin D, suggesting that vitamin D supplementation could reduce insulin resistance in T2DM. The mean levels at baseline and at the end, for serum 25(OH)D concentration were 43.03±19.28 and 60.12±17.2 (p=0.02), FPG were 138.48±36.74 and 131.02±39 mg/dl (p=0.05), for insulin, 10.76±9.46 and 8.6±8.25 μIu/ml (p=0.028) and for HOMA-IR, 3.57±3.18 and 2.89±3.28 (p=0.008) respectively.

Regarding effect of vitamin-D on lipids the literature is sparse and existing trials did not show consistent and significant vitamin D effects on blood lipids warranting further studies for clarification (Zittermann, Gummert, & Börgermann, 2011). However, there is evidence to support that vitamin D supplementation can independently improve cardiovascular risk. One mechanism may involve direct promotion of large HDL particle formation, via elevations in serum apolipoprotein A1 (ApoA1) concentrations, a process that increases reverse cholesterol transport (Kazlauskaite et al., 2010).

Al-Daghri et al (2012) in their study conducted among T2DM subjects, who received 2000 IU D3 tablet per day for eighteen months, reported a significant improvement in the lipid profile of subjects. A significant decrease in LDL cholesterol (baseline = 4.4 ± 0.8 mmol/L vs 18 months = 3.6 ± 0.8 mmol/L, p<0.001) and total cholesterol (baseline = 5.4 ± 0.2 mmol/L vs 18 months = 4.9 ± 0.3 mmol/L, p<0.001) was noted, as well as with a significant improvement in HOMA-β function (p=0.002). Worthy to note was the non-significant increase in HDL

cholesterol across time points. Other metabolic parameters that changed significantly were an increase of serum calcium (p=0.003); insulin (p<0.001) and HOMA-IR (p<0.001). Of note was the significant decrease in serum albumin levels (p<0.001). the present study also reported a significant decrease in total cholesterol and LDL-C and lipid ratios, while a non-significant increase in HDL-C in the supplemented group.

The current study for the first time evaluated serum 25(OH)D, HbA1c and lipid profile at eight weeks and again at sixteen weeks to assess the vitamin-D status after withdrawal of vitamin-D supplementation among T2DM subjects. There has been no study till date to the best of our knowledge, for comparison of serum 25(OH)D at sixteen weeks after a supplementation with eight week oral cholecalciferol therapy. The results showed that after eight weeks of supplementation, the mean serum 25(OH)D levels had increased to the sufficiency range in about 80% of the supplemented subjects, but a significant drop in serum 25(OH)D levels after washout period in supplementation group (43.4±16.3 to 22.2±6.5 ng/ml) was observed. Thus, despite efficacy of such a quick supplementation schedule, at eight weeks normal 25(OH)D could not be maintained for a washout period of further eight weeks in sufficiency range (>30 ng/ml).

Two studies looking at the washout effect of vitamin-D supplementation undertaken among Indian population though not among diabetics but in other conditions have reported similar findings. One of the studies was conducted among subjects with chronic kidney disorder (CKD) on maintenance hemodialysis receiving cholecalciferol granules (60,000 IU once a week) for 6 weeks. After supplementation serum 25(OH)D increased significantly (p<0.001), but dropped significantly (p=0.04) at 12 weeks of supplementation (Bansal et al., 2014).

In another study among healthy Indians with chronic hypovitaminosis D, significant rise in serum 25(OH)D were reported at 8 weeks of supplementation with 60,000 IU cholecalciferol, however after one year of follow up these levels could not be maintained in sufficient range (Goswami et al., 2008).

In both these studies the mean 25(OH)D level was significantly higher as compared to the baseline at the washout period, which is true for the present study also. These studies though not conducted among T2DM subjects do point out to the fact that vitamin-D levels are not maintained at >30 ng/ml range after few weeks of supplementation and hence maintenance

therapy on continuous basis is needed. Recommendations and consensuses for the appropriate dose and duration for each vitamin D status should also be framed.

The present phase of the study also made an effort to see the washout effect of vitamin-D supplementation on anthropometric, lipid parameters and HbA1c among T2DM subjects. A favourable change was seen in most of the parameters in supplementation group after eight weeks. An improvement in lipid profile was also observed among the supplemented group; however the levels could not be sustained after the washout period. As discussed earlier studies have reported a change in anthropometric measurements and glycemic and lipemic profile in diabetic subjects after vitamin-D supplementation, however no study has looked into the washout effect of the supplementation dose on these parameters. But these findings can be used for comparisons if trials looking into the washout effect of vitamin-D supplementation on cardio-metabolic profile are conducted in future.

Salient observations

Thus from the results and discussion of this phase the following salient findings can be enlisted-

Formative research

- The mean age of the subjects was 52.7 years and they were suffering from T2DM from an average of 6.1 years.
- About 28.6% of the subjects were going for check-ups every three months and most of the subjects (85.6%) were on oral drugs as medications.
- Majority of the subjects were consuming vegetarian diets (64.6%) and predominantly they failed to meet their daily RDA with respect to both macro and micronutrients. However the fat intake was very high among the subjects.
- A high prevalence of overweight and obesity was observed among the subjects.
- Vitamin D deficiency was present in 87% of the subjects with no significant gender differences.
- Among the clinical conditions, females were significantly more anemic than men (28.8% vs 22.6%, $p<0.05$). The mean HbA1c levels were also high among the subjects (8.7±1.6%).
- Hypercholesterolemia was present in 35% of the population with females having significantly higher prevalence compared to males (50% vs 22.6%, $p<0.01$).

- Metabolic syndrome (MS) as per IDF classification was present in 65% of the subjects, while as per ATP-III criteria 78% of the subjects showed the presence of MS. Females had significantly higher prevalence as compared to males irrespective of the classification used.
- WSR, percent body fat and hypercholesterolemia was significantly high among the subjects with vitamin-D levels <20 ng/mL.
- Across the vitamin D quartiles, waist-circumference, WSR, percent body fat and LDL cholesterol showed a significant declining trend from lower to higher quartile.
- Prevalence of obesity and vitamin D deficiency was more among the subjects with duration of diabetes less than five years.
- Total T4, duration of diabetes, total proteins and LDL-C emerged as predictor variables of vitamin D levels.

Supplementation study and washout effect

- A significant rise of 31.5 ng/mL in serum 25(OH)D levels was observed among the supplementation group as compared to controls. However, after the washout period of eight weeks, the serum 25(OH)D levels decreased significantly by 21.4 ng/mL in the supplementation group.
- Weight and waist circumference decreased significantly in the supplementation group as compared to the controls after eight weeks of supplementation. However after sixteen weeks only waist circumference showed a significant decrease
- Post supplementation, the experimental group had significantly lower levels for total cholesterol and LDL-C as compared to the control group. None of the lipid parameter which reduced after eight weeks of supplementation could sustain the favorable decline after the washout period.
- No impact on glycemic parameters was observed in the groups after the supplementation period.

Positives and negatives of the study

Few limitations of the research need to be acknowledged. Firstly, the study included subjects with T2DM with no other major secondary complication of T2DM, and therefore findings are limited to this disease condition only. Since variations in metabolic changes differ not only by age or gender but also by the presence of the disease itself, the results cannot be generalised for

the population on a whole. Secondly the differences in various parameters were not seen based on various medication regimes (though it was not changed during the study period), which may interfere with the vitamin-D metabolism. And thirdly the duration of eight weeks and the sample size may have proved to be small to observe prominent impact on many of the parameters, as with large sample size and longer duration, changes in various parameters might have been more profound.

However the findings have added to the existing knowledge of role of vitamin-D and its supplementation in improving biophysical measurements and biochemical profile in T2DM and will support further research carried out to study the impact of vitamin-D3 supplementation among the diabetic adult population of India.

Brief conclusion

The results of the current study indicate that weekly 60,000 IU of cholecalciferol supplementation for eight weeks in Indians adults with T2DM would correct their serum 25(OH)D levels to the vitamin D-sufficient range and showed a positive impact on many of the anthropometric measurements and lipid parameters among them. However, such quick supplementation would not maintain the 25(OH)D levels in the sufficient range for the following eight weeks among the subjects.

PHASE III

COMPARISON BETWEEN DIABETIC AND NON-DIABETIC POPULATIONS

As the study involved two populations- one which was apparently healthy and specifically did not suffer from diabetes mellitus and the other which was enrolled from a diabetic clinic i.e the population having type-II diabetes mellitus, a comparison between the two populations for various parameters was studied. The parameters compared were biophysical measurements, physical activity pattern and the nutrient intake of the subjects and the biochemical estimations. The results of the same are depicted from Tables 4.3.1 to 4.3.14.

Biophysical measurements of the populations

The anthropometric measurements revealed that almost all the parameters were higher in the diabetic population as compared to the non-diabetic subjects (Table 4.3.1). Weight, waist-circumference, WHR, BMI, percent body fat and blood pressure were significantly higher among the diabetic subjects than non-diabetic subjects.

Prevalence of overweight/obesity among the subjects

When the prevalence of obesity was studied among the populations, it was seen that based on the BMI criteria the diabetic population had significantly higher proportion of obese subjects as compared to the non-diabetic population (68.4% vs 48.8%) (Table 4.3.2). The diabetic subjects also had significantly higher prevalence of high percent body fat levels among them as compared to their non-diabetic counter parts. The prevalence of abdominal obesity based on WC, WHR and WSR showed that for all the three indices the prevalence was higher in non-diabetic population; however it was not statistically significant.

Nutrient Intake of the populations

The nutrient intake of the subjects is given in Table 4.3.3. The data depicted that the intake of all the macro as well as micronutrients was lower among the diabetic subjects. However significant difference was seen only for energy, proteins, carbohydrates, iron, dietary calcium and fibre intake.

Physical Activity Pattern of the subjects

The physical activity levels of both the populations when compared showed that the diabetic population had higher proportion of subjects doing moderate activity, while significantly more number of non-diabetic subjects were engaged in high level physical activity (Table

TABLE 4.3.1 ANTHROPOMETRIC & BLOOD PRESSURE MEASUREMENTS OF THE SUBJECTS (MEAN ± SD)

Measurements	Diabetics (n=114)	Non-Diabetics (n=129)	t-Test *p* value
Weight (Kg)	72.0 ± 13.9	65.0 ± 14.8	**0.000*****EVNA**
Height (cm)	159.7 ± 8.4	158.1 ± 9.4	0.154
WC (cm)	95.4 ± 11.8	92.5 ± 11.1	**0.047***EVNA
HC (cm)	101.7 ± 14.3	99.9 ± 10.1	0.280
WHR	0.94 ± 0.08	0.92 ± 0.07	**0.047***EVNA
WSR	0.59 ± 0.08	0.6 ± 0.07	0.201
BMI	28.1 ± 5.3	25.9 ± 4.7	**0.001****EVNA
Body fat (%)	36.4 ± 7.1	33.7 ± 6.5	**0.003****EVNA
Systolic BP (mmHg)	139.7 ± 22.1	128.7 ± 16.7	**0.000*****EVNA
Diastolic BP (mmHg)	84.7 ± 10.0	80.8 ± 10.3	**0.003****EVNA

p < 0.001***,<0.01**, <0.05* EVNA= equal variance not assumed

Table 4.3.2 PREVALENCE OF OVERWEIGHT AND OBESITY AMONG THE SUBJECTS (n, %)

Category based on BMI	Diabetics (n=114)	Non-Diabetics (n=129)	χ^2 *p* value
Normal (BMI:18.5 – 22.9)	15 (13.2)	39 (29.5)	**0.002****
Overweight (BMI:23 – 24.9)	21 (18.4)	27 (20.9)	
Obese (BMI: ≥25)	78 (68.4)	63 (48.8)	
Based on % Body fat			
Body fat (F>30, M>20%)	112 (98.2)	116 (89.9)	**0.007****
Based on Anthropometric Indices			
WC (F≥80, M≥90 cm)	82 (71.9)	105 (81.4)	0.08
WSR (≥ 0.5)	104 (91.2)	116 (89.9)	0.729
WHR (F≥0.85, M≥0.9)	97 (85.1)	111 (86)	0.832

p<0.01** Values in parenthesis indicate percent

TABLE 4.3.3 NUTRIENT INTAKE OF THE SUBJECTS (MEAN ± SD)

Nutrients	Diabetics (n=114)	Non-Diabetics (n=129)	t-Test *p* value
Energy (Kcal)	1248 ± 313	1492 ± 424	**0.000*****EVNA**
Protein (gm)	33.6 ± 9.5	40.6 ± 12.8	**0.000*****EVNA**
Fat (gm)	52.4 ± 17.2	55.7 ± 21.3	0.184
Carbohydrates (gm)	153.6 ± 47.3	200.9 ± 57.5	**0.000*****EVNA**
Iron (mg)	10.1 ± 4.2	12.0 ± 5.8	**0.004****EVNA**
Calcium	469 ± 233	581.8 ± 374.7	**0.005****EVNA**
β carotene (μg)	1250 ± 346	1701 ± 3014	0.207
Vitamin C (mg)	62.9 ± 64.5	78.9 ± 76.2	0.079
Crude Fibre (gm)	4.9 ± 2.1	5.8 ± 2.6	**0.005****EVNA**
Total Dietary Fibre	10.2 ± 5.1#	12.8 ± 6.5#	**0.001****EVNA**
Insoluble Dietary Fibre	7.8 ± 4.1#	9.5 ± 5.0#	**0.003****EVNA**
Soluble Dietary Fibre	2.5 ± 1.2#	3.2 ± 1.6#	**0.000*****EVNA**

#as reported by NIN for listed foods

p < 0.001***, <0.01** EVNA= equal variance not assumed

TABLE 4.3.4 LEVELS OF PHYSICAL ACTIVITY AMONG SUBJECTS (n, %)

Physical Activity Level	Diabetics (n=114)	Non-Diabetics (n=129)	χ^2 value
Low	36 (31.6)	32 (24.8)	**0.000***
Moderate	77 (67.5)	76 (58.9)	
High	1 (0.9)	21 (16.3)	
Hours spent in sitting (Mean ± SD)	5.9 ± 1.5	4.5 ± 1.6	**0.000*****EVNA**

p<0.001*** Values in parenthesis indicate percent

4.3.4). The mean hours spent in sitting activities were significantly higher among the diabetic population.

VITAMIN-D STATUS OF THE POPULATIONS

The serum vitamin-D levels of the subjects are displayed in Table 4.3.5. The mean level in both the populations was less than 20 ng/ml, indicating a high prevalence of vitamin-D deficiency among the subjects.

The subjects were divided into various categories of vitamin-D deficiency based on their serum 25(OH)D levels as given in Figure 4.3.1. It was observed that in both the populations about 88% of the subjects were vitamin-D deficient. About 4% of the non-diabetics and 5% of the diabetics were found to be vitamin-D sufficient with serum levels more than 30 ng/ml. Thus irrespective of the presence or absence of diabetic condition a high prevalence of vitamin-D deficiency was observed among the subjects. However it was a relief to observe that when VDD sub-classified into mild, moderate and severe categories majority of the subjects in both the groups had mild VDD and only 1.6% of the subjects among the non-diabetics showed the presence of severe deficiency (Figure 4.3.2).

Prevalence of Anaemia among the subjects

The iron status of the subjects is given in Table 4.3.6. The information revealed that mean haemoglobin levels were significantly lower for the non-diabetic population as compared to the diabetics. Keeping this trend in mind the prevalence of anaemia was studied among the subjects, which is shown in Table 4.3.7. It was seen that the non-diabetic population had significantly higher number of subjects with anaemia as compared to the diabetic population (41.1% vs 25.4%). None of the subjects in both the populations were severely anaemic.

Prevalence of Hyperlipidemia among the subjects

The mean levels of lipid profile and inflammatory marker, HsCRP for the subjects is given in Table 4.3.8. The data showed that levels of serum triglycerides, the atherogenic indices, AIP values and HsCRP were significantly higher among the diabetic subjects, and on the other hand the good cholesterol HDL levels were significantly lower among these subjects. This trend pointed towards a higher prevalence of hyperlipidemia among the diabetics as compared to their non-diabetic counter parts.

The previously feared trend was clearly observed in terms of prevalence of hyperlipidemia and inflammation among the diabetic subjects as seen from Table 4.3.9. The prevalence of

TABLE 4.3.5 VITAMIN D LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameter	Diabetics (n=114)	Non-Diabetics (n=129)	t-Test *p* value
25Hydroxy Vitamin D	14.2 ± 8.8	13.7 ± 7.2	0.589

FIGURE 4.3.1 VITAMIN D STATUS OF THE SUBJECTS (%)

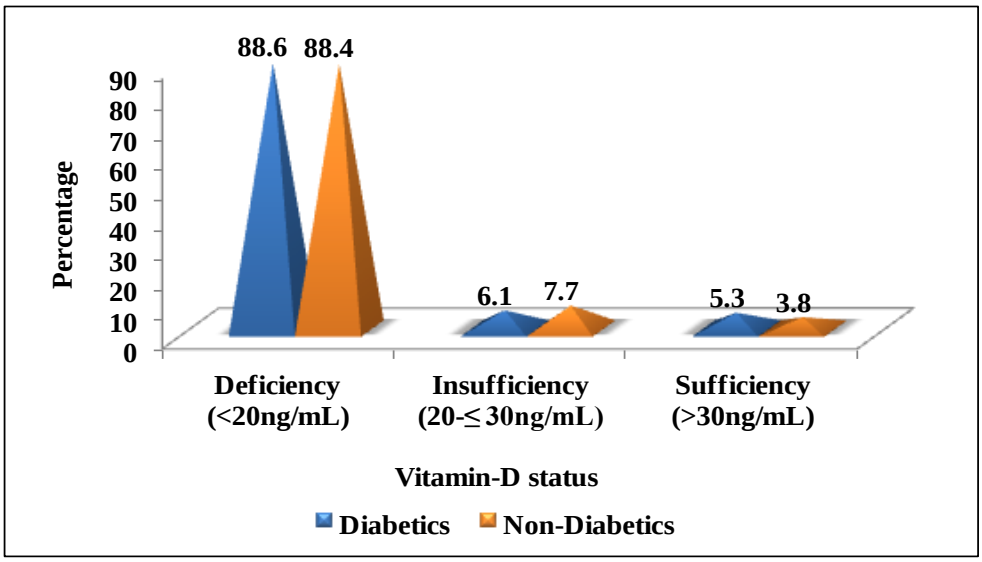


FIGURE 4.3.2 SUB-CLASSIFICATION OF VITAMIN D DEFICIENCY AMONG THE SUBJECTS (%)

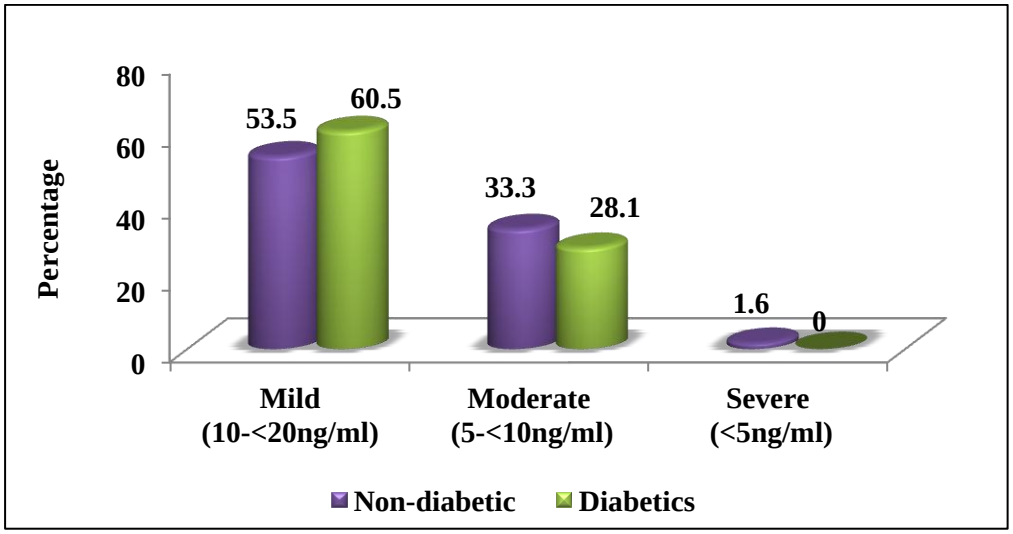


TABLE 4.3.6 IRON STATUS OF THE SUBJECTS (Mean ± SD)

Parameters	Normal Range	Diabetics (n=114)	Non-Diabetics (n=129)	t-test *p* value
Hb (gm/dl)	Males >13 Females >12	13.4 ± 1.6	12.5 ± 1.4	**0.000*****[EVNA]
Iron (mcg/dl)	Male: 70-180 Female: 60-180	76.6 ± 24.8	76.5 ± 27.9	0.965
TIBC (mcg/dl)	Male: 225-535 Female: 215-535	365.2 ± 44.3	371.2 ± 56.8	0.364
% Transferrin Saturation	13-45	21.2 ± 6.9	21.2 ± 8.2	0.998

$p < 0.001$*** EVNA= equal variance not assumed

TABLE 4.3.7 PREVALENCE OF IRON DEFICIENCY ANAEMIA AMONG SUBJECTS (n, %)

Classification based on Hb values	Diabetics (n=114)	Non-Diabetics (n=129)	χ^2 value
Normal >12 (Females) >13 (Males)	85 (74.6)	76 (58.9)	
Mild (Females 11–11.9) (Males 11-12.9)	20 (17.5)	41 (31.8)	
Moderate (8 – 10.9)	9 (7.9)	12 (9.3)	
Severe (< 8)	0	0	
Total Anemic subjects	29 (25.4)	53 (41.1)	**7.26***

$p<0.05$* Values in parenthesis indicate percent

TABLE 4.3.8 LIPID PROFILE & HS-CRP LEVELS OF THE SUBJECTS (MEAN ± SD)

Parameters	Diabetics (n=114)	Non-Diabetics (n=129)	t test *p* value
Total Cholesterol	184.4 ± 38.6	191.9 ± 34.3	0.113
Triglycerides	142.4 ± 58.0	121.6 ± 67.7	**0.011*^{EVNA}**
LDL Cholesterol	109.0 ± 27.8	111.1 ± 28.8	0.567
HDL Cholesterol	45.1 ± 10.1	52.1 ± 12.4	**0.000***^{EVNA}**
VLDL-C	29.4 ± 17.0	24.3 ± 13.5	**0.012*^{EVNA}**
TC/HDL Ratio	4.2 ± 1.2	3.8 ± 0.9	**0.000***^{EVNA}**
LDL/HDL Ratio	2.5 ± 0.7	2.2 ± 0.6	**0.012*^{EVNA}**
TAG/HDL Ratio	3.4 ± 1.8	2.6 ± 2.2	**0.003**^{EVNA}**
AIP (log10 TG/H)	0.48 ± 0.2	0.3 ± 0.3	**0.000***^{EVNA}**
HsCRP	0.38 ± 0.3	0.2 ± 0.2	**0.000***^{EVNA}**

p< 0.001***, <0.01**, <0.05* EVNA=Equal variance not assumed

TABLE 4.3.9 PREVALENCE OF HYPERLIPIDEMIA & INFLAMMATION AMONG THE SUBJECTS (n, %)

Parameter	Diabetics (n=114)	Non-Diabetics (n=129)	χ^2 *p* value
TC ≥ 200 mg/dl	40 (35.1)	53 (41.1)	0.337
TAG ≥ 150 mg/dl	40 (35.4)	25 (19.4)	**0.005****
LDL-C ≥ 100 mg/dl	72 (64.9)	83 (64.3)	0.933
HDL-C <40 mg/dl (Male) <50 mg/dl (Female)	57 (50.0)	54 (41.9)	0.204
TC/HDL ≥ 5	22 (19.3)	12 (9.3)	**0.025***
TAG/HDL ≥ 3	57 (50.4)	30 (23.3)	**0.000*****
LDL/HDL ≥ 3.5	11 (9.9)	3 (2.3)	**0.012***
Atherogenic Index of Plasma (AIP)			
<0.11 (low risk)	3 (2.7)	24 (18.6)	**0.000*****
0.11-0.21 (moderate risk)	10 (9.0)	16 (12.4)	
>0.21 (high risk)	98 (88.3)	89 (69.0)	
Inflammatory marker HsCRP			
≥ 0.1 mg/dl	90 (78.9)	80 (60.0)	**0.000*****

p< 0.001***, <0.01**, <0.05* Values in parenthesis indicate percent

hypertriglyceridemia, inflammation, altered atherogenic indices and atherogenic index of plasma was significantly higher in the diabetic population as compared to non-diabetic subjects. The diabetic subjects thus posed a higher risk for CVDs.

Levels of Thyroid Hormones among the subjects

Thyroid Stimulating Hormone (TSH), Total Triiodothyronine (T_3) and Total Thyroxine (T_4) levels were examined for the populations and the levels were found to be in the physiologically normal range. The values across genders as depicted in Table 4.3.10 showed that mean values for T3 were significantly higher for non-diabetic subjects, while that of T4 were higher for diabetic subjects. Both the elevations were seen only among the female subjects of the respective populations suggesting thyroid dysfunction.

Kidney Profile of the subjects

The kidney profile of the subjects as shown in Table 4.3.11; revealed that all the parameters were in the physiologically normal range for subjects of both the populations.

Liver Profile of the subjects

Similar to the kidney profile, all the liver parameters were also found to be in physiological normal range suggesting healthy liver profile of the subjects and thus no apparent presence of liver disorders (Table 4.3.12). The alkaline phosphatase and GGT levels were significantly higher among the diabetic subjects as compared to their non-diabetic counterparts.

Prevalence of Metabolic Syndrome among the subjects

The prevalence of metabolic syndrome among the populations was studied using the guidelines given by the International Diabetes federation (2005) and the Adult Treatment Panel-III (2001). According to both the criterias the diabetic population had significantly higher prevalence as compared to the non-diabetic population (Figure 4.3.3). This is a matter of concern as it indicates the presence of multiple risk factors among the subjects but is obvious too, as diabetics are more prone to unfavourable alterations in their cardio-metabolic parameters due to physiological changes because of the presence of the disease.

Correlations between vitamin-D levels and various variables among the populations

As seen from the previous tables a high prevalence of overweight/obesity and hyperlipidemia was observed among the subjects, hence correlations between vitamin-D levels and these parameters was studied in both populations The summary of correlations is given in Table 4.3.13. The anthropometric measurements- WC, WSR, BMI, percent body and lipid

TABLE 4.3.10 GENDER WISE THYROID HORMONES LEVELS OF THE SUBJECTS (MEAN ± SD)

Gender	Parameter	Diabetics (n=114)	Non-Diabetics (n=129)	t test *p* value
Females	TSH µIU/ml	3.7 ± 4.1	3.9 ± 3.9	0.827
	T_3 ng/dl	109.7 ± 20.3	117.6 ± 20.2	**0.029*** EVNA**
	T_4 µg/dl	9.9 ± 1.9	8.6 ± 1.4	**0.000*** EVNA**
Males	TSH µIU/ml	3.6 ± 3.5	2.7 ± 1.4	0.095
	T_3 ng/dl	108.6 ± 17.5	111.2 ± 18.2	0.453
	T_4 µg/dl	8.9 ± 1.9	8.6 ± 1.6	0.279
Total	TSH µIU/ml	3.6 ± 3.8	3.5 ± 3.2	0.678
	T_3 ng/dl	109.1 ± 18.7	115.3 ± 19.7	**0.012*** EVNA**
	T_4 µg/dl	9.4 ± 1.9	8.6 ± 1.5	**0.001** EVNA**

p< 0.001***, <0.01**, <0.05* EVNA=Equal variance not assumed

TABLE 4.3.11 KIDNEY PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Diabetics (n=114)	Non-Diabetics (n=129)	t test *p* value
Calcium (mg/dl)	8.8-10.6	9.6 ± 0.3	9.4 ± 0.4	**0.000*****[EVNA]**
BUN (mg/dl)	7.9-20	10.1 ± 2.8	9.7 ± 3.5	0.789
Creatinine (mg%)	Male: 0.6-1.1 Female: 0.5-0.8	0.6 ± 0.1	0.7 ± 0.4	0.508
Uric Acid (mg/dl)	Male: 3.5-7.2 Female: 2.6-6.0	5.3 ± 1.5	5.2 ± 1.6	0.301
BUN/ Sr. Creatinine	9:1- 23:1	14.9 ± 4.8	15.3 ± 5.1	0.653

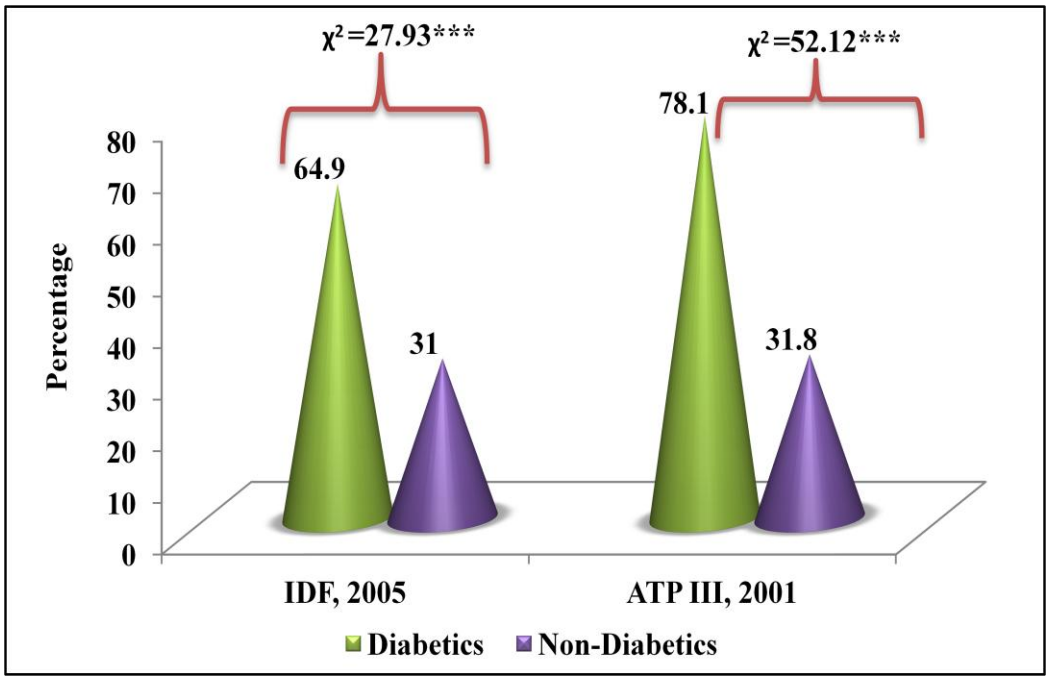
p< 0.001*** EVNA=Equal variance not assumed

TABLE 4.3.12 LIVER PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Normal Range	Diabetics (n=114)	Non-Diabetics (n=129)	t test *p* value
Alkaline Phosphatase (U/L)	Male: 53-128 Female: 42-98	89.6 ± 23.3	82.6 ± 20.9	**0.016*****[EVNA]**
Total Bilirubin (mg/dl)	0.3-1.20	0.62 ± 0.2	0.6 ± 0.2	0.258
Direct Bilirubin (mg/dl)	0-0.2	0.18 ± 0.06	0.17 ± 0.05	0.237
Indirect Bilirubin (mg/dl)	0-0.9	0.43 ± 0.2	0.4 ± 0.2	0.209
SGOT (U/L)	Male: 0-37 Female: 0-31	22.5 ± 10.9	24.2 ± 10.5	0.214
SGPT (U/L)	Male: 13-40 Female: 10-28	26.3 ± 14.2	25.8 ± 16.2	0.814
GGT (U/L)	Male: 0-55 Female: 0-38	29.9 ± 17.7	24.6 ± 14.5	**0.012*****[EVNA]**
Total Protein (gm/dl)	6.6-8.3	7.5 ± 0.4	7.6 ± 0.3	0.857
Serum Albumin (gm/dl)	3.5-5.2	4.2 ± 0.4	4.3 ± 0.3	0.555
Serum Albumin/Globulin	0.9-2.0	1.3 ± 0.2	1.3 ± 0.2	0.429

p<0.05* EVNA=Equal variance not assumed

FIGURE 4.3.3 PREVALENCE OF METABOLIC SYNDROME AMONG THE SUBJECTS (%)



p <0.001***

TABLE 4.3.13 CORRELATIONS BETWEEN VITAMIN-D LEVELS AND NON-INVASIVE PARAMETERS

Variables	Diabetics (n=114)	Non-Diabetics (n=129)
	Pearson 'r' value	
WC (cm)	-0.235*	
WSR	-0.266**	
BMI	-0.212*	
Body fat (%)	-0.237*	-0.246**
Total Cholesterol	-0.206*	
LDL Cholesterol	-0.247**	-0.184*

p <0.01**, <0.05*

parameters- total cholesterol and LDL-C showed a negative significant correlation with the vitamin-D levels among the diabetic subjects, whereas among the non-diabetic subjects only percent body fat and LDL-C were negatively significantly correlated. Thus from this analysis it was observed that only percent body fat and LDL-C were the variables showing significant correlation with the vitamin-D levels of the subjects in both the populations. Both the parameters showed a negative correlation, hence suggesting that abnormal elevated levels may compromise vitamin D levels or vice versa among the subjects.

Predictor Variables of vitamin-D status among the populations

A comparison for the various variables which emerged out as predicators of serum vitamin-D status in the stepwise multivariate analysis for both the populations was done which is presented in Table 4.3.14. It was observed that for the diabetic group; WSR, Total T4 and LDL-C were the significant predictors. While among the non-diabetics; percent body fat, thyroid hormones-TSH & T3 and LDL-C emerged as the predictors. It was noticed that all the variables in both the populations emerged as suppressors i.e. with their higher levels the vitamin-D was seen to be reduced. Though only LDL-C was the common predictor between the populations, it is to be seen that the other predictors were related to abdominal obesity and functioning of thyroid gland. Hence it is very important to monitor them closely and adopt measures to prevent obesity and thyroid dysfunction.

TABLE 4.3.14 SUMMARY OF PREDICTORS VARIABLES OF VITAMIN-D STATUS (STEPWISE FORWARD LINEAR REGRESSION)

Diabetics (n=110)		Non-Diabetics (n=129)	
Predictor variables	***β*-coefficient**	**Predictor variables**	***β*-coefficient**
WSR	-0.255**	% Body fat	-0.200*
T4	0.215*	T3	-0.209*
LDL-C	-0.210*	TSH	-0.239**
		LDL-C	-0.168*

p<0.05*, <0.01**

DISCUSSION

Diabetes Mellitus is a dreadful non-communicable disease. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. In the past decade, interest in diabetes mellitus and vitamin D metabolism has grown. More recent studies suggest a deleterious effect of low vitamin D (hypovitaminosis D) on general health. And specific studies propose an association between hypovitaminosis D and the aetiology and progression of type 2 diabetes mellitus (T2DM). As the present study involved both T2DM subjects and apparently normal ones, a comparison was made between them for their vitamin-D status and other parameters.

Due to the role of vitamin D in many of the pathways related to glucose metabolism, it is not wrong to assume that patients with T2DM are more prone to vitamin D deficiency. However in the present study a high prevalence was observed in both the diabetic as well as non-diabetic subjects. The results revealed that the mean vitamin-D levels were very low in both the populations and around 88% of the subjects in both the groups were vitamin-D deficient.

The results are little contrary with those of a cross-sectional study conducted in Iran among 100 type 2 diabetic patients and 100 healthy subjects aged between 20–80 years, which revealed that the prevalence of vitamin-D deficiency was 83.3% among diabetic patients and 75.6% among healthy subjects adjusted for age and gender. The authors further reported that the serum 25(OH)D levels (22.08 ± 15.20 vs 22.22 ± 10.03, p=0.75) and BMI (26.22 ± 9.30 vs 26.26 ± 4.55,p=0.98) were comparable between diabetic subjects and healthy controls. Serum calcium was found to be significantly higher among the normal subjects as compared to the diabetics (8.94 ± 0.59 vs 9.14 ± 0.53, p=0.02). The findings also indicated that serum concentration of 25(OH)D correlated inversely with body mass index (BMI) in diabetic patients and healthy controls (Taheri et al., 2012). However, in our study BMI (28.1±5.3 vs 25.9±4.7, p<0.001) showed significant negative correlation among the diabetic subjects only, and serum calcium levels (9.6 vs 9.4 mg/dL) were found to be similar between the diabetic and non-diabetic subjects.

In another study by Subramanian et al (2011), carried out among 92 T2DM patients and equal number of non-diabetic patients matched for age, gender, BMI, waist circumference and total body fat in northern India also reported vitamin D deficiency to be significantly more prevalent among T2DM patients than the non-diabetic patients (57.6% vs 33.3%, p = 0.001). The

average concentration of serum 25(OH)D (ng/ml) was significantly lower for diabetic males than diabetic females (9.07±6.7 vs 12.6±7.6, $p = 0.02$).

The prevalence of obesity when studied was found to be significantly higher among the diabetic population. The diabetic subjects also had significantly higher prevalence of percent body fat. In one study on 66 white Spanish women with BMI between 24–35 kg/m^2, overweight and obese women were found to be at higher risk of vitamin-D deficiency, largely due to excess adiposity rather than inadequate intake (Rodrguez et al., 2009), thus indicating body fat to be one of the important indices in relation to vitamin-D status. However another study on 250 over weight and obese adults of different ethnicities demonstrated contrary results stating that the serum level of vitamin-D was inversely related to weight, higher waist circumference, and higher HbA1c, but not with adipose mass (McGill et al., 2008). Though this relationship needs to be further investigated, in our study WSR and percent body fat; both indicators of abdominal obesity emerged as significant predictors for poor vitamin-D status among the subjects.

Diabetes is considered a coronary heart disease (CHD) risk equivalent and it is frequently associated with various other cardiovascular (CV) risk factors. Dyslipidemia is a major risk factor for macrovascular complications in patients with T2DM. In our study also the prevalence of hyperlipidemia was high among the diabetic subjects with significant levels for hypertriglyceridemia, altered atherogenic indices and inflammation. The results are in line with the fact that diabetics are more prone to CVDs and show unfavourable alterations in the lipid levels. The constellation of *metabolic risk factors* is also strongly associated with type 2 diabetes mellitus or the risk for this condition and the primary goal of clinical management of the metabolic syndrome is to reduce risk for clinical atherosclerotic disease (Grundy et al., 2005). In view of this statement the prevalence of metabolic syndrome was found to be significantly higher among the diabetic subjects by both the ATP-III and IDF guidelines as compared to the non-diabetic subjects.

Salient observations

Thus from this comparison between the diabetic and non-diabetic groups the following salient findings were noticed-

- Subjects in both the groups were not consuming a balanced diet as their nutrient intake did not match the RDA, except for fat intake which exceeded the recommended allowance of 30 gm/day.
- A high prevalence of deficiency was mapped among the subjects of both the populations due to suboptimum vitamin-D levels among them.
- The diabetic population showed higher prevalence of almost all the metabolic conditions like anemia, hypertriglyceridemia along with altered atherogenic indices and increased levels of inflammatory marker Hs-CRP and metabolic syndrome as compared to the non-diabetic group.
- Within both the groups, indices of central obesity, thyroid dysfunction and LDL-C emerged as common and significant suppressors of optimum vitamin-D status.

Conclusion

Hence to briefly conclude this phase of the research, both the populations had a high prevalence of vitamin-D deficiency, but the anthropometric indices indicating the prevalence of obesity and the lipid levels indicating metabolic alterations were found to be significantly higher among the diabetic group. However it is recommended that necessary efforts should be put in to manage central obesity, thyroid function and ideal lipid levels by subjects in both the populations as these were identified as significant predictors for vitamin-D levels.

PHASE IV

Development of Nutrition Health Education (NHE) Material

Education continues to be a key component in the prevention and treatment of any disorder especially so for diabetes. The primary role of education is to enhance the subject's understanding of the disease/disorder and provide detailed training on non-pharmacologic interventions used in the management. More importantly, patient education is essential if patients are to make beneficial changes in lifestyle and to remain these changes over time (Tough, 1985). Interpersonal counselling using NHE material is one of the most cost-effective and useful methods to raise the knowledge levels of a population. Emphasising the issues of sunlight exposure for cutaneous production of vitamin-D, avoiding the use of sunscreen, exposing face, neck and arms to sunlight for at least 15-20 minutes twice a day between 10 am to 2 pm can help raise the serum vitamin-D levels naturally. Increasing outdoor physical activity helps in both exposing body to sunlight and improving vitamin-D metabolism for effective absorption and utilization. Physical activity also helps to control weight, blood sugar and blood pressure. Hence this aspect of lifestyle change should be highly propagated. Bringing a change in dietary pattern in form of consuming vitamin-D sources like milk and milk products, cod liver oil, fish and lean meat can act as a long term strategy for restoring vitamin-D levels in blood. Such a change should be encouraged in high risk population. All the above points along with importance of vitamin-D in progression or pathogenesis of T2DM, if incorporated in an NHE material, can form a strong tool for the population most affected or at risk of vitamin-D deficiency to attain and maintain good quality life.

The literature discussed does speculate the role of vitamin-D in the progression of non-communicable diseases more so in T2DM. Thus development of relevant NHE material addressing the key components of lifestyle and behaviour change in relation to blood vitamin-D levels is the need of the hour. Information regarding natural and dietary sources of vitamin-D, consequences of its deficiency and importance of increased outdoor physical activity needs to be shared among the high risk groups. Interpersonal counselling is an effective tool to impart knowledge and should be explored and used extensively to improve the quality of life of subjects with T2DM and vitamin-D deficiency. In the present study an attempt was made to develop the education material related to various aspects of vitamin-D and its role in T2DM, however its efficacy was not examined which can and should be taken up as another full-time research topic.