CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the findings of the study in accordance with the study objectives. The results are presented under the following sections:

Section I: Cross sectional survey of the T2DM subjects on metformin

Section II: Screening of B12 status among T2DM subjects on metformin

Section III: Intervention study (RCT) - Vitamin B12 and Calcium versus B12 supplementation

SECTION I: CROSS SECTIONAL SURVEY OF THE T2DM SUBJECTS ON METFORMIN

Section I presents the results of Phase I which was a cross sectional survey conducted on 245 T2DM adults on metformin. The results of section - I have been formulated under the following heads-

- The demography and the socio economic status of T2DM subjects
- Age and Duration of T2DM subjects
- Life style habits and Dietary pattern of T2DM subjects
- Anthropometry and Biophysical measurement of T2DM subjects
- Nutritional Status of T2DM subjects using various anthropometric indices: Gender Perspective
- Hypertension Profile of T2DM subjects
- Drug profile of the T2DM subjects on metformin
- Association of present metformin dosage and its side effects in T2DM subjects
- Assessment of Diabetic Peripheral Neuropathy (DPN) in T2DM subjects
- Association of DPN with Age duration of diabetes and nutritional status
- Association of DPN with drug therapy and metformin dosage
- Association between DPN and hypertension
- Assessment of Quality of Life (QoL) of T2DM adults using WHOQoL Bref
- Association between QoL and socio demographic characteristics
- Association between QoL and DPN in T2DM subjects
- Association between QoL and Nutritional status in T2DM subjects
- Highlights of Phase I

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THE DEMOGRAPHY AND THE SOCIO ECONOMIC STATUS OF THE T2DM SUBJECTS

The demography & Socioeconomic status of the T2DM subjects is given in Table 4.1. The study was conducted on a total of 245 T2DM adults on metformin in an urban setting at Sir Ganga Ram hospital, Delhi wherein there were more female subjects (162 of 245, 66.12%) than male subjects (83 of 245, 33.87%). The study sample represent a heterogeneous socio economic group with a mean income of Rs. 21,700.82 (21,700.82±18541.32) with a minimum per capita income (PCI) of Rs. 4000 and a maximum of Rs. 1,00,000. As depicted in Fig 4.1 there were similar proportion of T2DM subjects who belonged to PCI of less than equal to Rs. 10,000 (35%) and PCI of less than equal to Rs. 20,000 (34.5%) while very few (9.8%) belonged to PCI of more than Rs 40,000 but less than equal to Rs.1,00,000. There was one female subject who did not reveal her family income.

As regards occupation (Fig 4.2), majority (54%) of the subjects were housewives whereas a little less than one-third (24%) belonged to service class while very few had their own business (12%).

AGE AND DURATION OF DIABETES OF T2DM SUBJECTS

The age of the T2DM adults varied from 26 years to 96 years with a mean age of 58.28 years (58.28 ±11.20). Table 4.1 depicts that approx. three-fourth (74%) of the subjects were above 50years of age while a quarter (26%) of the population belonged to less than up to 50 years of age group.

The duration of diabetes of the sample studied varied from 4 months to 360 months that is up to 30 years. The mean duration of the diabetes was 91 months (91±84.87) that is ~ 7.58 years. Majority (192 of 245) had long standing diabetes of less than or equal to 10years while remaining 53 of 245 had duration of diabetes for more than 10years. When further categories were made with an interval of 5 years (Table 4.1) it was found that 50% of the population had diabetes for ≤ 5years while 28 % had diabetes for more than 5years but less than 10years while 22% had diabetes for more than 10years.

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TABLE 4.1: SOCIO DEMOGRAPHIC CHARACTERISTICS OF T2DM SUBJECTS ON METFORMIN

Descriptive Characteristics	Total (N=245)		Males (N=83)		Females (N= 162)	
	n	%	n	%	n	%
Age group (Y)						
≤50 y	65	26.5	24	28.9	41	25.3
>50 y	180	73.5	59	71.1	121	74.7
Duration of Diabetes						
≤5y	123	50.2	32	38.6	91	56.2
>5≤10y	69	28.2	28	33.7	41	25.3
>10y	53	21.6	23	27.7	30	18.5
Life Style Habits						
Alcohol						
Yes	11	4.49	5	6	6	3.7
No	234	95.51	78	94	156	96.3
Cigarette						
Yes	18	7.35	8	9.6	10	6.2
No	227	92.6	75	90.4	152	93.8
Tobacco						
Yes	12	4.9	4	4.8	8	4.9
No	233	95.1	79	95.2	154	95.1
Dietary Habits						
Veg	105	42.85	35	42.17	70	43.21
Non Veg	115	46.94	43	51.81	72	44.44
Ovo Veg	25	10.21	5	6.02	20	12.35
Milk Consumption (ml/day)						
<200	164	66.94	51	61.45	113	69.75
200-400	63	25.71	24	28.91	39	24.08
>500	18	7.35	8	9.64	10	6.17

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FIG.. 4.1: PERCENT DISTRIBUTION OF PCI OF T2DM ADULTS ON METFORMIN

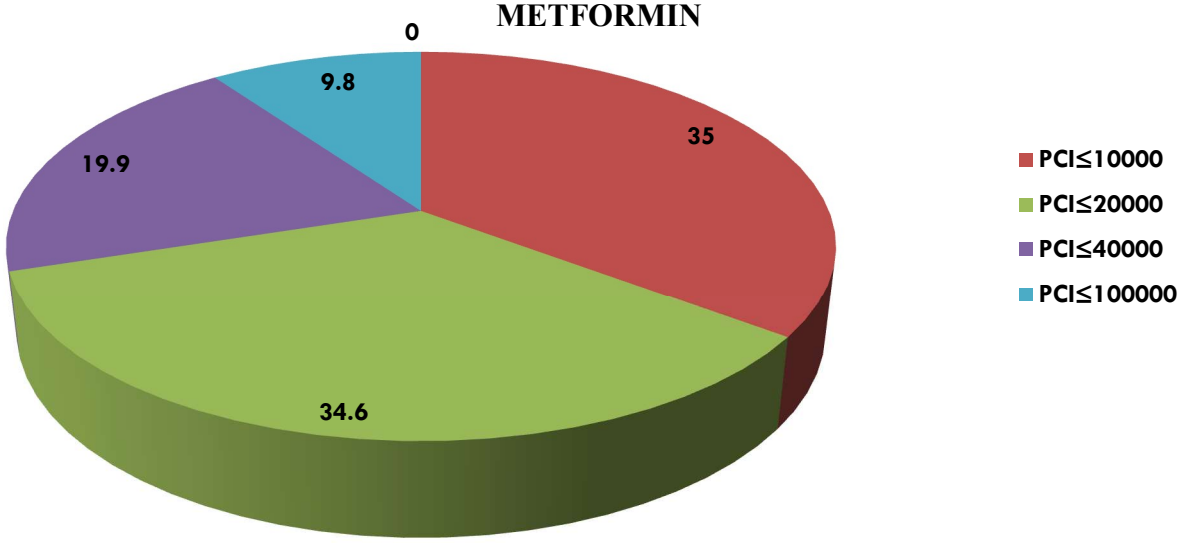
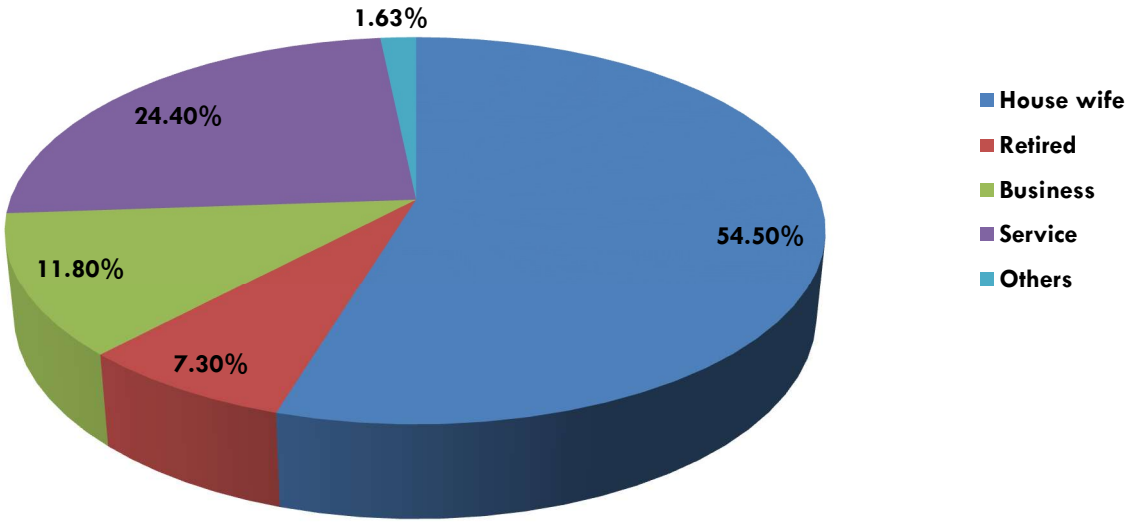


FIG. 4.2: PERCENTAGE DISTRIBUTION OF VARIOUS OCCUPATIONS AMONG T2DM ADULTS ON METFORMIN



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LIFE STYLE HABITS AND DIETARY PATTERN OF T2DM SUBJECTS

As regards the life style habits approx. 90% of the population reported that they did not drink alcohol nor did they consume cigarette or tobacco (Table 4.1). Around 10% of the population were ovo-vegetarians (who consumed egg but no other non- vegetarian food) while similar proportion of the population were vegetarians (~ 43%) and non-vegetarian (~ 47%).

Trends of milk consumption showed that only one quarter (26%) of the population had adequate consumption (200-400 ml milk) while majority (~ 67%) had low milk consumption (less than 200 ml) whereas mere 7% had good milk consumption (greater than 500 ml). Milk consumption trends were similar for males and females (Table 4.1) No striking gender differences were found in the dietary pattern except that the ovo vegetarianism was more prevalent among females (12%) than males (6%) (Table 4.1).

ANTHROPOMETRY AND BIOPHYSICAL MEASUREMENT IN T2DM SUBJECTS

A gender wise comparison of several anthropometry and biophysical variables of T2DM subjects on metformin is depicted in Table 4.2. The various anthropometric variables were similar between male and female subjects except for weight, waist circumference (WC) and hip circumference (HC). There was significant difference for weight measurement and HC ($p<0.05$) & WC ($p<0.01$) between male and female subjects ($p<0.05$) All the indices were higher for male T2DM subjects than female T2DM subjects.

Mean WC and HC of T2DM males and females in relation to BMI by Asia Pacific Classification WHO 2000 is depicted in Table 4.3 It was seen that amongst Males & Female subjects there were no significant differences in their WC & HC between the underweight, normal, overweight, obese and morbid obese T2DM adults when cross tabulated by BMI

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NUTRITIONAL STATUS OF T2DM SUBJECTS USING VARIOUS INDICES: GENDER PERSPECTIVE

Table 4.4 depicts the gender wise distribution of nutritional status of T2DM subjects on metformin using various indices like BMI, WHR and WC. By BMI, majority (40.4%) of T2DM subjects were obese whereas nearly one-quarter (20.40%) were morbidly obese. Amongst the overweight T2DM subjects approximately three-fourth of the female subjects were overweight (71.9%). Majority (~93%) of the T2DM adults had abnormal WHR (>0.9 for male and >0.85 for females). There was no significant difference in WHR between males and female subjects. Abdominal obesity by WC.>90 for males and WC>80 for females was present in ~76%. There was significant difference between males and female subjects with higher percentage of females (83%) than males (~64%) having abdominal obesity as indicated by WC ($p<0.01$).

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TABLE: 4.2: ANTHROPOMETRY, BP MEASUREMENT, AGE AND DIABETES DURATION OF T2DM ADULTS ON METFORMIN: GENDER PERSPECTIVE (MEAN± S.D)

Parameter	Total (N=245)	Males (N = 83)	Females (N = 162)	p Value
	Mean ± S.D.	Mean ± S.D.	Mean± S.D.	
Height (cm)	157.35±7.15	158.56 ±7.88	156.73±6.69	0.072
Weight (Kg)	66.20 ±12.92	68.48 ±9.59	65.02±14.22	0.025*
BMI (Kg/m2)	26.79 ±5.13	27.33 ±4.04	26.52±5.60	0.239
Waist Circumference (cm)	90.62 ±10.94	93.41 ±10.55	89.20±10.89	0.004**
Hip Circumference (cm)	95.30 ±14.74	98.22 ±20.13	93.81±10.78	0.026*
Waist Hip Ratio (WHR)	0.96 ±.09	0.96 ±0.10	0.96±0.08	0.622
Waist Stature ratio (WSR)	0.95±.09	0.97±10	0.95±0.08	0.361
Systolic BP (mmHg)	127.59±17.07	127.23±15.41	127.78±15.41	0.812
Diastolic BP (mmHg)	81.41±10.25	79.99±11.04	82.14±9.79	0.121
Age (Y)	58.28±11.19	59.14 ± 12.35	57.84± 10.57	0.389
Duration of Diabetes (m)	91.55±84.83	104.18± 90.76	85.07± 81.16	0.095

*p<0.05, **p<0.01

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TABLE: 4.3: MEAN WAIST CIRCUMFERENCE & HIP CIRCUMFERENCE IN RELATION TO THEIR BODY MASS INDEX (MEAN± S.D)

Nutritional Status (BMI)	Waist Circumference (cm)				Hip Circumference (cm)			
	Males (N = 83)		Females (N = 162)		Males (N = 83)		Females (N = 162)	
	n	Mean± S.D.	n	Mean± S.D.	n	Mean± S.D.	n	Mean ±S.D.
Total	83	93.41±10.55	162	89.20±10.89	83	98.22±20.13	162	93.81±10.78
Underweight[a]	2	92.50±0.71	4	85±8.91	2	98±0.00	4	83.25±6.99
Normal[a]	12	88.92±12.06	21	89.14±6.53	12	90.92±13.35	21	93.76±8.92
Overweight[a]	16	94.25±12.89	41	90.51±10.15	16	95.25±10.02	41	96.46±9.63
Obese[a]	35	94.54±9.30	64	89.81±11.50	35	103.46±28.1	64	93.73±10.77
Morbid[a] Obesity	18	93.56±10.25	32	86.84±12.95	18	95.89±7.06	32	91.91±12.86
F Value	.665		0.718		1.203		1.869	
p Value	.618		.581		.316		.119	

a=Classified by BMI as per Asia pacific classification: WHO 2000.

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TABLE: 4.4: PREVALENCE OF UNDERWEIGHT, NORMAL, OVERWEIGHT, OBESITY AND MORBID OBESITY AMONG T2DM ADULTS ON METFORMIN: GENDER PERSPECTIVE

Body Mass Index (BMI=Kg/m^2)	**Total (N=245)**		**Males (N=83)**		**Females (N=162)**	
	N	**%**	**n**	**%**	**n**	**%**
Underweight[a]	6	2.44	2	2.4	4	2.5
Normal[a]	33	13.46	12	14.5	21	13
Overweight[a]	57	23.26	16	19.3	41	25.3
Obese[a]	99	40.4	35	42.2	64	39.5
Morbid Obesity[a]	50	20.4	18	21.7	32	19.8
χ^2 , p value	1.147, p = 0.887					
Waist Hip Ratio (WHR)	**Total (N=245)**		**Males (N=83)**		**Females (N=162)**	
	N	**%**	**n**	**%**	**n**	**%**
Abnormal[b]	227	92.65	78	93.98	149	91.98
Normal[b]	18	7.34	5	6.02	13	8.02
χ^2 , p value	χ^2 =0.321 , p=0.570					
Waist Circumference (WC)	**Total (N=245)**		**Males (N = 83)**		**Females (N = 162)**	
	N	**%**	**n**	**%**	**n**	**%**
Abnormal[c]	187	76.32	53	63.9	134	82.71
Normal[c]	58	23.67	30	36.1	28	17.28
χ^2 , p value	χ^2 =10.76 , p= 0.001**					
Waist Stature Ratio (WSR)	**Total (N=245)**		**Males (N = 83)**		**Females (N = 162)**	
	N	**%**	**n**	**%**	**n**	**%**
Abnormal[d]	244	99.59	82	98.80	162	100
Normal	1	0.41	1	1.2	0	0
χ^2 , p value	N.A					

a= BMI as per Asia pacific classification: WHO 2000, *p < .05, ***p< .001, b=WHO cut offs for W.C.
c=WHO cut offs for W.H.R, d W.S.R ≥0.55 for males&≥0.53 for females.

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HYPERTENSION PROFILE OF T2DM ADULTS

Gender wise distribution on the basis of their stage of hypertension is depicted in Fig 4.3. Overall majority (62%) of the T2DM subjects were pre hypertensive followed by hypertension stage I and stage II. Proportion of males subjects (72.3%) were higher than female subjects (57.4%) in pre hypertension category whereas proportion of female subjects (33.3%) were higher in stage I hypertension than male subjects (18.1%). But these results were non-significant by Chi square analysis

The changing trends in prevalence of hypertension by BMI using Asia Pacific Classification is depicted in Table 4.5. Surprisingly it was seen that one- third of the underweight T2DM adults had stage I hypertension and 66.7% of them fell in pre hypertension category.

DRUG PROFILE OF T2DM ADULTS ON METFORMIN

As regards drug history of T2DM subjects on metformin (N=245) it was found that present metformin dosage was in multiples of 500mg varying from as low as 500mg to 2500 mg. The most common present metformin dosage was 1000mg as more than half of the population (57.6%) was on 1000mg whereas most common past metformin dosage was 500 mg (46.9%) as reported in the old prescriptions. The mean of present metformin dosage was 1057.14±449.04mg while the mean of past metformin dosage was 867.35±467.99 mg.

ASSOCIATION OF PRESENT METFORMIN DOSAGE AND SIDE EFFECTS OF METFORMIN

As depicted in Table 4.6 it was found that there was a highly significant association between gastrointestinal (GI) side effects of metformin with their present metformin dose($p<0.001$). An increasing trend of the subjects reported metallic taste as side effect with the increase in present dosage of metformin. Almost three-fourth (70%) of the population had reported metallic taste as the most common side effects with the metformin dosage upto 2500 mg/day. This could influence in a negative way the food intake of the subjects compromising the dietary needs

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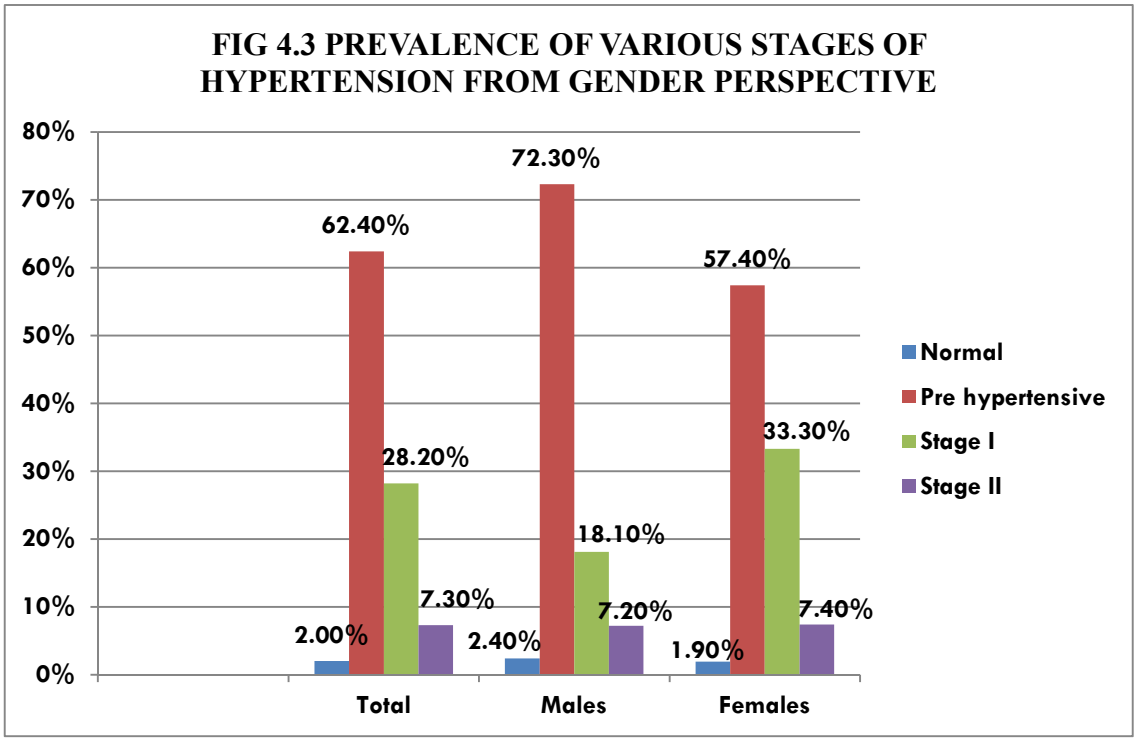

TABLE 4.5: THE CHANGING TREND IN PREVALENCE OF HYPERTENSION BY NUTRITIONAL STATUS

Nutritional Status	BP Measurement								
	Total	Normal BP[b]		Pre Hypertension[b]		Stage I Hypertension[b]		Stage II Hypertension[b]	
	N	n	%	n	%	n	%	n	%
Underweight[a]	6	0	0	4	66.7	2	33.3	0	0
Normal[a]	33	1	3	18	54.5	11	33.3	3	9.1
Overweight[a]	57	1	1.8	35	61.4	16	28.1	5	8.8
Obese I/ Obese[a]	99	2	2	61	61.6	30	30.3	6	6.1
Morbid Obesity[a]	50	1	2	35	70	10	20	4	8

a=Classified by BMI as per Asia pacific classification: WHO 2000., b=Classified by JNC VIII classification

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TABLE 4.6: DISTRIBUTION OF SIDE EFFECTS OF METFORMIN BY PRESENT METFORMIN DOSE

Present Metformin Dose (mg/day)	Total (N)	GI Side Effects of Metformin					
		No Side Effects		Anorexia		Metallic Taste	
		n	%	n	%	n	%
500	54	38	70.4	7	13	9	16.7
1000	141	97	68.8	16	11.3	28	19.9
≥ 1500 - 2000	50	11	22	4	8	35	70
p Value		0.000***					

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ASSESSMENT OF DIABETIC PERIPHERAL NEUROPATHY (DPN) IN T2DM ADULTS

Assessment of DPN was done by MNSI (Annexure V) consisting of two parts: history questionnaire and physical assessment questionnaire.

DPN BY MNSI HISTORY QUESTIONNAIRE

Two questions: 'Muscle cramps in legs/feet' and 'Feel weak most of the time' were not included in scoring of MNSI history score. Whereas a "yes " response to the other two questions: 'Distinguish hot water from cold' and 'Sense feet when you walk' was given a score of 0 for yes response. Rest all the questions were given a score of 1 for 'yes' response and MNSI history score was a sum total of 13 questions.

MNSI history questionnaire score varied from 0 to 10 for 245 T2DM adults screened by MNSI. The mean of MNSI history score among T2DM adults was 3.88 ± 2.79. There was no significant difference in MNSI history score between male and female subjects (p=0.765).

As regards MNSI history (Table 4.7 and Fig 4.4) the results showed that as high as 67.8% showed numbness in their feet/ legs & nearly half of them (53.5%) had burning pain in their legs/feet. While only one-third (33%) of them said that their feet was too sensitive to touch. As high as 64% got muscle cramps in their legs/ feet. Very few (10%) of them had an open sore on their foot. Approximately 42% of them felt weak all over most of the time and their symptoms were worse at night. A little more than one third (37%) reported to hurt their legs when they walked. However, amputation cases were very rare (0.4%). By chi square it was found that there were no significant differences between male and female subjects for any of the MNSI history questions. Fig 4.4 shows distribution of 'yes' response of MNSI history questions among the total T2DM adults on metformin. It showed that only 6.1% T2DM adults answered 'Yes' for the question if their doctor has ever told you that you have diabetic neuropathy which shows that DPN was under diagnosed.

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DPN BY MNSI PHYSICAL ASSESSMENT (DPN SCORE)

MNSI physical assessment score was called as DPN score which varied from 0 to 7.5. For the 245 T2DM adults screened by MNSI, the mean DPN scores was 2.14± 1.98. There was no significant difference in mean scores of DPN between males and females.

Almost three fourth (180 of 245 ,73.5%) of the population were suffering from DPN as assessed by MNSI physical assessment score (DPN score>0) which was alarmingly high (Fig 4.5). As depicted in Fig 4.6 it was found that there was no significant difference between male and female subjects with regard to the prevalence of DPN(p=0.545).

Fig 4.7 depicts various grades of DPN which shows that most common grade of DPN was low DPN (39.2%) *(MNSI physical assessment score/DPN score greater than 0 but less than equal to 2.5)* followed by high DPN (34.3%) *(MNSI physical assessment score/DPN score greater than 2.5).* Almost one quarter (26.4%) of the population was free of DPN *(MNSI physical assessment score/DPN score was 0).* Fig 4.8 depicts the percent distribution of gender among various grades of DPN. Fig 4.9 depicts the prevalence of grades of DPN across gender which shows that there was no significant difference between the two genders in various grades of DPN (p=0.741).

ASSOCIATION OF DPN WITH AGE, DURATION OF DIABETES AND NUTRITIONAL STATUS

As depicted in Table 4.8 it was seen that there was no significant difference between the age as well as the duration of diabetes among those who were DPN positive (DPN scores >0) in comparison to those who had no DPN(DPN=0). Distribution of T2DM subjects based on duration of diabetes by their DPN grades is shown in Table 4.9. It was seen that duration of diabetes showed no trends with the grades of DPN. Long standing diabetes alone had no effect on DPN. Other factors like glycemic control had crucial role in manifestation of DPN which is discussed in Phase II.

Nutritional status as assessed by BMI, WC, WHR showed no significant difference between those who were DPN positive (DPN scores >0) in comparison to those who had no DPN(DPN=0) (Table 4.8).

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TABLE 4.7: MNSI HISTORY QUESTIONS RELATED TO DPN AMONG T2DM ADULTS ON METFORMIN: GENDER PERSPECTIVE

MNSI History Questions	Males (N=83)		Females (N=162)		Total (N=245)	
	n	%	n	%	n	%
1.Are your legs and/or feet numb?						
Yes	51	61.4	115	71	166	67.8
No	32	38.6	47	29	79	32.2
2.Do you ever have any burning pain in your legs and /or feet?						
Yes	44	53	87	53.7	131	53.5
No	39	47	75	46.3	114	46.5
3.Are your feet too sensitive to touch?						
Yes	48	29.6	33	39.8	164	33.1
No	50	70.4	114	60.2	164	66.9
4.Do you get muscle cramps in your legs and/or feet?						
Yes	50	60.2	107	66	157	64.1
No	33	39.8	55	34	88	35.9
5.Do you have any prickling feelings in your legs or feet?						
Yes	37	44.6	71	43.8	108	44.1
No	46	55.4	91	56.2	137	55.9
6.Does it hurt when the bed covers touch your skin?						
Yes	24	28.9	55	34	79	32.2
No	59	71.1	107	66	166	67.8
7.When you get into tub or shower, are you able to tell the hot water from the cold water?						
Yes	64	77.1	121	74.7	185	75.5
No	19	22.9	41	25.3	60	24.5
8.Have you ever had an open sore on your foot?						

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Yes	12	14.6	13	8.1	25	10.3
No	70	85.4	147	91.9	217	89.7
9.Has your doctor ever told you that you have diabetic neuropathy						
Yes	4	4.8	11	6.8	15	6.1
No	79	95.2	151	93.2	230	93.9
10.Do you feel weak all over most of the time?						
Yes	39	47	63	38.9	102	41.6
No	44	53	99	61.1	143	58.4
11.Are your symptoms worse at night?						
Yes	29	34.9	71	43.8	100	40.8
No	54	65.1	91	56.2	145	59.2
12.Do your legs hurt when you walk?						
Yes	33	39.8	58	35.8	91	37.1
No	50	60.2	104	64.2	154	62.9
13.Are you able to sense your feet when you walk?						
Yes	71	85.5	149	92	220	89.8
No	12	14.5	13	8	25	10.2
14.Is the skin on your feet so dry that it cracks open?						
Yes	29	34.9	65	40.1	94	38.4
No	54	65.1	97	59.9	151	61.6
15.Have you ever had amputation?						
Yes	1	1.2	0	0	1	0.4
No	82	98.8	162	100	244	99.6

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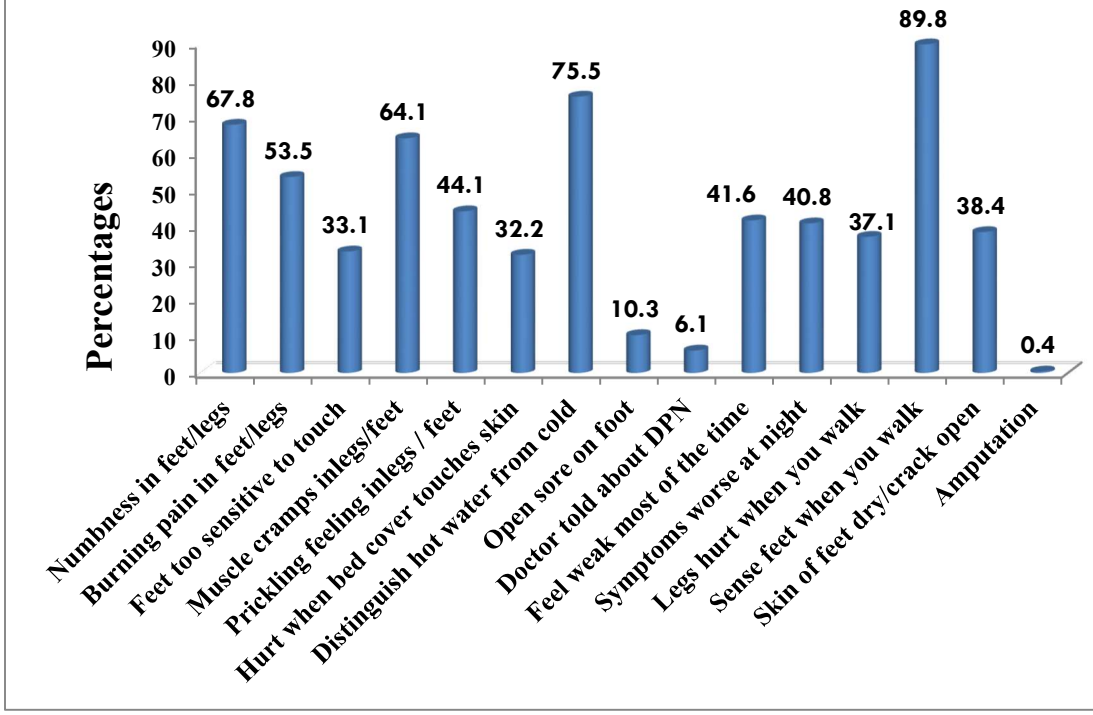
FIG 4.4 'YES'RESPONSE TO MNSI HISTORY QUESTIONS FOR T2DM ADULTS ON METFORMIN



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ASSOCIATION OF DPN WITH DRUG THERAPY AND METFORMIN DOSAGE

Further analysis was done for DPN and drug therapy (use of only metformin was mono therapy, use of metformin+ one drug was dual therapy and use of metformin with more than one drug was defined as multiple therapy) as shown in Table 4.10 which shows no trend. It was seen that there was no association between DPN and drug therapy.

As regards metformin dosage it was found that there was no significant difference between the present as well as past metformin dosage among those who were DPN positive (DPN scores >0) in comparison to those who had no DPN(DPN=0) (Table 4.8).

DPN grades were also related with present and past metformin dose but no trend was found indicating that there was no significant association between various grades of DPN and metformin dosage (Table 4.11).

FIG 4.5: PREVALENCE OF DPN AMONG T2DM ADULTS ON METFORMIN

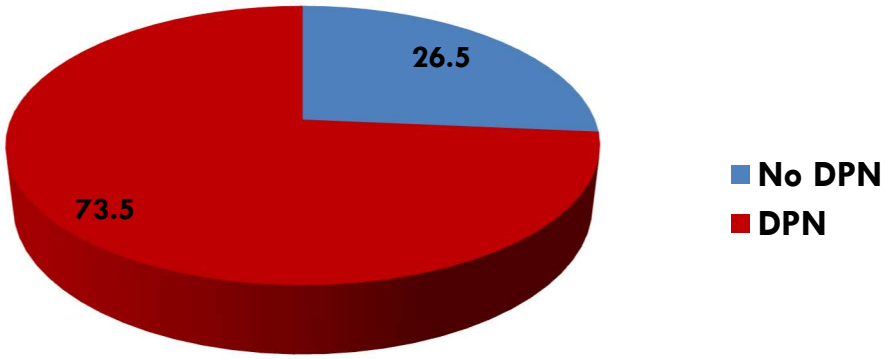

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FIG 4.6: PERCENT PREVALENCE OF DPN FROM GENDER PERSPECTIVE

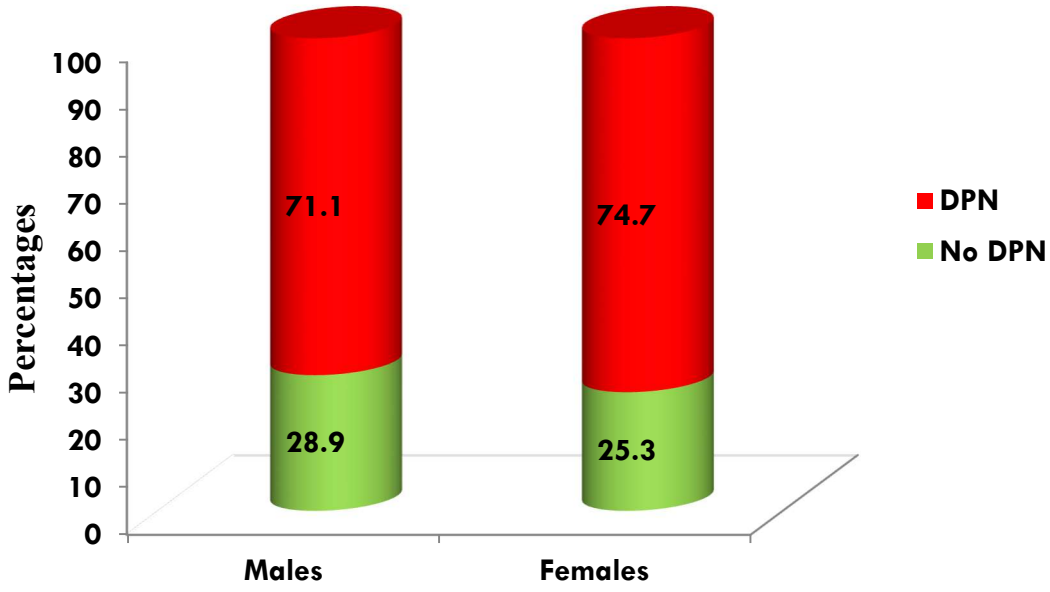

FIG 4.7 PERCENT PREVALENCE OF VARIOUS GRADES OF DPN AMONG T2DM ADULTS ON METFORMIN

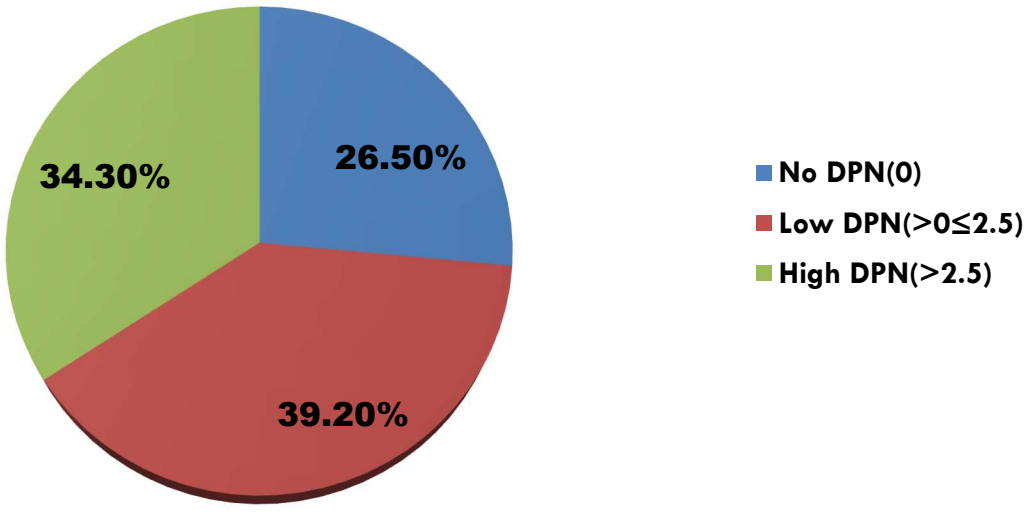

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FIG. 4.8: PERCENT DISTRIBUTION OF GENDER AMONG VARIOUS GRADES OF DPN

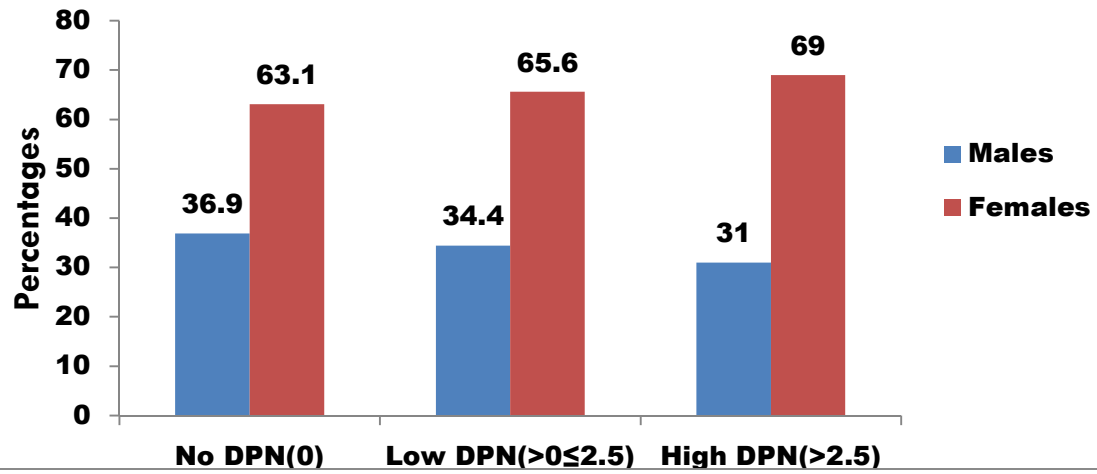
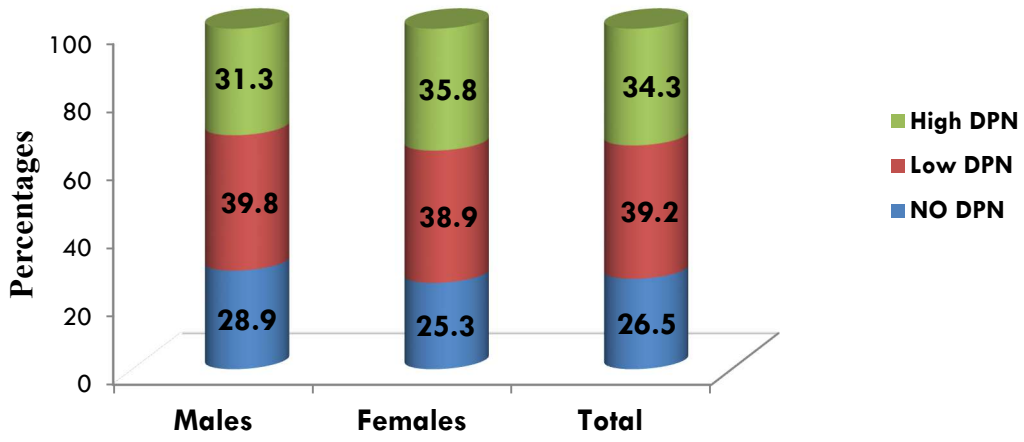


FIG 4.9: PREVALENCE OF VARIOUS GRADES OF DPN ACROSS GENDER



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ASSOCIATION OF DPN AND HYPERTENSION

There was no significant difference in the mean BP of those who were DPN positive (DPN scores >0) in comparison to those who had no DPN (DPN=0). Table 4.12 shows cross tabulation of various stages of hypertension and DPN grades. It was seen that among various categories of hypertension distribution of T2DM adults on metformin was more or less similar irrespective of the grade of DPN. Among those who had no DPN there were 3 subjects with normal BP and among those who had low grade DPN and high grade DPN there was 1 subject in each group. To do chi square test none of the cells should have count less than 5 so the normal BP category was combined with prehypertensive category as shown in Table 4.12. Further there was no significant association between hypertension and DPN grades.

ASSESSMENT OF QOL OF T2DM ADULTS USING WHOQOL BREF

The quality of life of T2DM adults was assessed by 26 questions out of which the first two questions, 'How would you rate your quality of life? ' and 'How satisfied are you with your health?' were individually measured as the Overall quality of Life and General Health facet respectively. However, the remaining 24 questions were grouped under the four domains of Quality of Life Index (QLI): Physical Health, Psychological Health, Social Relationship and Environment

The overall quality of life of T2DM adults is shown in Table 4.13 which depicts that more than half (54%) of T2DM adults reported that their quality of life was 'good'. But when asked specific questions from other facets of WHOQoL Bref as described in following text, it depicted poor state of quality of life. As regards overall quality of health it was found that by chi square there was no significant difference between male and female subjects.

The satisfaction of health in life of T2DM adults is shown in Table 4.14 which depicts that majority (57%) of them reported that they are neither satisfied nor dissatisfied with their health. As regards satisfaction of health it was found that by chi square there was no significant difference between the two genders

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TABLE 4.8: COMPARISON OF AGE, DURATION OF DIABETES AND METFORMIN DOSAGE AMONG THOSE WITH DPN AND THOSE WITHOUT DPN

Parameter	No DPN (DPN scores=0) (N=65)	DPN (DPN>0) (N=180)	p value
Duration of DM (months)	100±97.14	88.44±80.06	0.348
Age (Y)	56.74±13.18	58.84±10.37	0.248
Present metformin dosage(mg)	1092.31±499.16	1044.44±430.31	0.463
Past metformin dosage	938.46±511.64	841.67±449.94	0.153
BMI	26.79±3.79	26.94±5.10	0.826
WC.	90.00±9.97	90.85±11.28	0.592
HC.	93±10.86	96.13±15.86	0.142
WHR	0.97±0.09	0.95±.09	0.143
Systolic B.P	126.62±21.89	127.94±15.01	0.592
Diastolic B.P	79.78±11.12	81.99 ± 9.89	0.137

TABLE 4.9: DISTRIBUTION OF T2DM ADULTS BASED ON THE DURATION OF DIABETES BY THE DPN GRADES OF THE T2DM ADULTS ON METFORMIN

DPN Scores	Total	Duration of DM<10Y		Duration of DM>10Y	
		n	%	n	%
No DPN (0)	65	49	75	16	24.6
Low DPN (Score>0≤ 2.5)	96	81	84.4	15	15.6
High DPN (Score>2.5)	84	62	73.8	22	26.2
p value	0.181				

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TABLE 4.10: DISTRIBUTION OF T2DM ADULTS BASED ON THE PRESENT DRUG THERAPY BY THE DPN GRADES OF THE T2DM ADULTS ON METFORMIN

DPN Grades	Total	Mono therapy		Dual therapy		Multi therapy	
	N	n	%	n	%	n	%
No DPN (0)	65	20	30.8	38	58.5	7	10.8
Low DPN (Score >0≤2.5)	96	29	30.2	62	64.6	5	5.2
High DPN (Score>2.5)	84	26	31	54	64.3	4	4.8
p value	0.604						

TABLE 4.11: ASSOCIATION BETWEEN DPN GRADES AND METFORMIN DOSAGE

DPN grades	Total	Present Metformin Dosage				Past Metformin Dosage			
		≤1000mg		>1000mg		≤1000 mg		>1000mg	
	N	n	%	n	%	n	%	n	%
No DPN (DPN scores =0)	65	50	76.9	15	23.1	55	84.6	10	15.4
Low DPN (DPN scores >0≤2.5)	96	76	79.2	20	20.8	86	89.6	10	10.4
High DPN (DPN scores > 2.5)	84	69	82.1	15	17.9	72	85.7	12	14.3
P value		0.729				0.603			

TABLE 4.12: PREVALENCE OF VARIOUS STAGES OF HYPERTENSION BY THE DPN GRADES OF THE T2DM ADULTS ON METFORMIN

DPN Grades	Total	Normal and Pre Hypertensive		Hypertensive Stage I		Hypertensive Stage II	
	N	n	%	%	n	n	%
No DPN (0)	65	43	66.2	18	27.7	4	6.2
Low DPN Score(>0≤2.5)	96	60	62.5	26	27.1	10	10.4
High DPN Score(>2.5)	84	55	65.5	25	29.8	4	4.8
P value	0.673						

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TABLE 4.13: OVER ALL QUALITY OF LIFE AMONG T2DM ADULTS ON METFORMIN BY GENDER

Likert Scale	Overall quality of life	T2DM Females (N=162)	T2DM Males (N=83)	Total (N=245)
How would you rate your quality of life?				
1	**Very Poor**	2(1.2)	0 (0)	2(0.8)
2	**Poor**	20 (12.3)	7 (8.4)	27(11)
3	**Neither poor nor good**	44(27.2)	14(16.9)	58(23.7)
4	**Good**	79(48.8)	54(65.1)	133(54.3)
5	**Very good**	17(10.5)	8(9.6)	25(10.2)

() Value in parenthesis are percentages

TABLE 4.14: SATISFACTION WITH HEALTH AMONG T2DM ADULTS ON METFORMIN BY GENDER

Likert Scale	General Health	T2DM Females (N=162)	T2DM Males (N=83)	Total (N=245)
How satisfied are you with your health?				
1	**Very dissatisfied**	3(1.9)	0(0)	3(1.2)
2	**Dissatisfied**	22(13.6)	9(10.8)	31(12.7)
3	**Neither satisfied nor dissatisfied**	91(56.2)	48(57.8)	139(56.7)
4	**Satisfied**	45(27.8)	24(28.9)	69(28.2)
5	**Very satisfied**	1(0.6)	2(2.4)	3(1.2)

() Value in parenthesis are percentages

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Table 4.15 depicts the physical domain of quality of life index (QLI) of T2DM subjects on metformin by gender. It shows that majority (41%) of the T2DM adults felt that physical pain prevents them from doing what they need to do to a moderate extent. Across the gender higher female subjects (43%) than male subjects (37%) felt so. Majority of the T2DM adults (60%) felt that they need some medical treatment to function in their daily life to a moderate amount. Many T2DM adults felt that they had enough energy for everyday life to a extent of moderate (36%) and mostly (33%). More than half of the adults (62%) felt that they are able to get around to neither poor nor good amount. Around 39.6% T2DM adults felt that they were satisfied with their sleep. More than half (52 %) of the T2DM adults felt satisfied with their ability to perform daily living activities and 53.5% felt their capacity to do work was also satisfactory. By chi square no significant gender differences were found with regard to the various facets of physical domain.

Table 4.16 depicts psychological domain of QLI of T2DM adults on metformin by gender. It showed that majority (48.6%) of the T2DM adults on metformin enjoyed their life very much. Around 36% felt their life to be meaningful only to a moderate amount. Majority (44.5%) of the T2DM adults felt that they were able to concentrate very well, however as high as approximately 32% could concentrate only to a moderate amount. Majority (39.6%) of the adults felt that they were able to accept their bodily appearance. Nearly54.3% of the adults felt that they were satisfied with themselves however more than one fourth (26.1%) were neither satisfied nor dissatisfied with themselves. Majority (61%) of the adults seldom experienced blue mood, despair, anxiety, and depression. By chi square there were no significant differences between male and female subjects were seen for any of the facets of psychological domain.

Table 4.17 depicts social relationship domain of QLI of T2DM adults on metformin by gender. It shows that more than half (53.9%) of the adults were satisfied with their personal relationship. Majority (62%) of the adults felt that they were satisfied with their sex while only a few (2%) were very dissatisfied. More than half (54.3%) of the T2DM adults were satisfied with the fact that they got support from their friend. Chi square showed no significant differences between males and females for any of the facets of social relationship domain.

Table 4.18 shows environment domain of QLI of T2DM adults on metformin by gender. Majority (34.7%) felt that they feel safe in their daily life to a moderate amount.

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TABLE 4.15: PHYSICAL DOMAIN OF QOL OF T2DM ADULTS ON METFORMIN BY GENDER

Likert Scale	Physical Health	T2DM Females (N=162)	T2DM Males (N=83)	Total (N=245)
To what extent do you feel that physical pain prevents you from doing what you need to do?				
1	Not at all	1(0.6)	0(0)	1(0.4)
2	A Little	28(17.3)	11(13.3)	39(15.9)
3	A moderate amount	70(43.2)	31(37.3)	101(41.2)
4	Very much	47(29)	32(38.6)	79(32.2)
5	Extreme Amount	16(9.9)	9(10.8)	25(10.2)
How much do you need any medical treatment to function in your daily life?				
1	Not at all	4(2.5)	0(0)	4(1.6)
2	Little	26(16)	10(12)	36(14.7)
3	A moderate amount	96(59.3)	51(61.4)	147(60.0)
4	Very much	36(22.2)	21(25.3)	57(23.3)
5	An extreme amount	0(0)	1(1.2)	1(0.4)
Do you have enough energy for everyday life?				
1	Not at all	4(2.5)	1(1.2)	4(1.6)
2	A Little	23(14.2)	7(8.4)	29(11.8)
3	Moderately	59(36.4)	17(20.5)	62(25.3)
4	Mostly	48(29.6)	52(62.7)	136(55.5)
5	Completely	28(17.3)	6(7.2)	14(5.7)
How well are you able to get around?				
1	Very Poor	3 (1.9)	1(1.2)	4(1.6)
2	Poor	22(13.6)	7(8.4)	29(11.8)
3	Neither poor nor good	45(27.8)	17(20.5)	62(25.3)
4	Good	84(51.9)	52(62.7)	136(55.5)
5	Very good	8(4.9)	6(7.2)	14(5.7)
How satisfied are you with your sleep?				
1	Very dissatisfied	4(2.5)	1(1.2)	5(2)
2	Dissatisfied	19(11.7)	6(7.2)	25(10.2)
3	Neither Satisfied nor dissatisfied	57(35.2)	25(30.1)	82(33.5)
4	Satisfied	58(35.8)	39(47)	97(39.6)
5	Very Satisfied	24(14.8)	12(14.5)	36(14.7)
How satisfied are you with your ability to perform your daily living activities				
1	Very dissatisfied	2(1.2)	0(0)	2(0.8)
2	Dissatisfied	21(13)	8(9.6)	29(11.8)
3	Neither Satisfied nor dissatisfied	54(33.3)	21(25.3)	75(30.6)
4	Satisfied	78(48.1)	50(60.2)	128(52.2)
5	Very Satisfied	7(4.3)	4(4.8)	11(4.5)
How satisfied are you with your capacity to work?				
1	Very dissatisfied	5(3.1)	0(0)	5(2)
2	Dissatisfied	17(10.5)	7(8.4)	24(9.8)
3	Neither Satisfied nor dissatisfied	47(29)	20(24.1)	67(27.3)
4	Satisfied	83(51.2)	48(57.8)	131(53.5)
5	Very Satisfied	10(6.2)	8(9.6)	18(7.3)

() Value in parenthesis are percentages

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TABLE 4.16: PSYCHOLOGICAL DOMAIN OF QOL OF T2DM ADULTS ON METFORMIN CROSS TABULATED BY GENDER

Likert Scale	Psychological Health	T2DM Females (N=162)	T2DM Males (N=83)	Total (N=245)
How much do you enjoy life?				
1	Not at all	2(1.2)	0(0)	2(0.8)
2	Little	34(21)	14(16.9)	48(19.6)
3	A moderate amount	44(27.2)	20(24.1)	64(26.1)
4	Very much	75(46.3)	44(53)	119(48.6)
5	An extreme amount	7(4.3)	5(6)	12(4.9)
To what extent do you feel your life to be meaningful?				
1	Not at all	3(1.9)	0(0)	3(1.2)
2	Little	32(19.8)	14(16.9)	46(18.8)
3	A moderate amount	60(37)	29(34.9)	89(36.3)
4	Very much	38(23.5)	16(19.3)	54(22)
5	An extreme amount	29(17.9)	24(28.9)	53(21.6)
How well are you able to concentrate?				
1	Not at all	4(2.5)	0(0)	4(1.6)
2	Little	31(19.1)	9(10.8)	40(16.3)
3	A moderate amount	50(30.9)	28(33.7)	78(31.8)
4	Very much	70(43.2)	39(47)	109(44.5)
5	Extremely	7(4.3)	7(8.4)	14(5.7)
Are you able to accept your bodily appearance?				
1	Not at all	2(1.2)	0(0)	2(0.8)
2	A Little	22(13.6)	10(12)	32(13.1)
3	Moderately	58(35.8)	21(25.3)	79(32.2)
4	Mostly	58(35.8)	39(47)	97(39.6)
5	Completely	22(13.6)	13(15.7)	35(14.3)
How satisfied are you with yourself?				
1	Very dissatisfied	4(2.5)	0(0)	4(1.6)
2	Dissatisfied	18(11.1)	8(9.6)	26(10.6)
3	Neither satisfied nor dissatisfied	46(28.4)	18(21.7)	64(26.1)
4	Satisfied	84(51.9)	49(59)	133(54.3)
5	Very satisfied	10(6.2)	8(9.6)	18(7.3)
How often do you have negative feelings such as blue mood, despair, anxiety, depression?				
1	Never	3(1.9)	0(0)	3(1.2)
2	Seldom	22(13.6)	10(12)	32(13.1)
3	Quite often	25(15.4)	5 (6)	30(12.2)
4	Very often	92(56.8)	57(68.7)	149(60.8)
5	Always	20(12.3)	11(13.3)	31(12.7)

() Value in parenthesis are percentages

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TABLE 4.17: SOCIAL RELATIONSHIP DOMAIN OF QOL OF T2DM ADULTS ON METFORMIN BY GENDER

Likert Scale	Psychological Health	T2DM Females (N=162)	T2DM Males (N=83)	Total (N= 245)
How satisfied are you with your personal relationship?				
1	Very dissatisfied	5(3.1)	0(0)	5(2)
2	Dissatisfied	19(11.7)	8(9.6)	27(11)
3	Neither satisfied nor dissatisfied	45(27.8)	19(22.9)	64(26.1)
4	Satisfied	83(51.2)	49(59)	132(53.9)
5	Very satisfied	10(6.2)	7(8.4)	17(6.9)
How satisfied are you with your sex life?				
1	Very dissatisfied	4(2.5)	1(1.2)	5(2)
2	Dissatisfied	13(8)	5(6)	18(7.3)
3	Neither satisfied nor dissatisfied	43(26.5)	14(16.9)	57(23.3)
4	Satisfied	95(58.6)	57(68.7)	152(62)
5	Very satisfied	7(4.3)	6(7.2)	13(5.3)
How satisfied are you with the support you get from your friend?				
1	Very dissatisfied	3(1.9)	0(0)	3(1.2)
2	Dissatisfied	20(12.3)	9(10.8)	29(11.8)
3	Neither satisfied nor dissatisfied	46(28.4)	17(20.5)	63(25.7)
4	Satisfied	83(51.2)	50(60.2)	133(54.3)
5	Very satisfied	10(6.2)	7(8.4)	17(6.9)

() Value in parenthesis are percentages

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TABLE 4.18 ENVIRONMENT DOMAIN OF QOL OF T2DM ADULTS ON METFORMIN BY GENDER

Likert Scale	Physical Health	T2DM Females (N=162)	T2DM Males (N=83)	Total (N=245)
How safe do you feel in your daily life?				
1	Not at all	4(2.5)	0(0)	4(1.6)
2	Little	24(14.8)	12(14.5)	36(14.7)
3	A moderate amount	56(34.6)	29(34.9)	85(34.7)
4	Very much	50(30.9)	22(26.5)	72(29.4)
5	Extremely	28(17.3)	20(24.1)	48(19.6)
How healthy is your physical environment?				
1	Not at all	4(2.5)	0(0)	4(1.6)
2	Little	30(18.5)	12(14.5)	42(17.1)
3	A moderate amount	42(25.9)	18(21.7)	60(24.5)
4	Very much	69(42.6)	45(54.2)	114(46.5)
5	Extremely	17(10.5)	8(9.6)	25(10.2)
Have you enough money to meet your needs?				
1	Not at all	3(1.9)	0(0)	3(1.2)
2	A Little	24(14.8)	10(12)	34(13.9)
3	Moderately	61(37.7)	25(30.1)	86(35.1)
4	Mostly	53(32.7)	35(42.2)	88(35.9)
5	Completely	21(13)	13(15.7)	34(13.9)
How available to you is the information that you need in your day-to -day life?				
1	Not at all	7(4.3)	0(0)	7(2.9)
2	A Little	20(12.3)	11(13.3)	31(12.7)
3	Moderately	45(27.8)	20(24.1)	65(26.5)
4	Mostly	64(39.5)	35(42.2)	99(40.4)
5	Completely	26(16)	17(20.5)	43(17.6)
To what extent do you have the opportunity for lesiure activities?				
1	Not at all	5(3.1)	1(1.2)	6(2.4)
2	A Little	22(13.6)	7(8.4)	29(11.8)
3	Moderately	51(31.5)	24(28.9))	75(30.6)
4	Mostly	64(39.5)	40(48.2)	104(42.4)
5	Completely	20(12.3)	11(13.3)	31(12.7)
How satisfied are you with the conditions of your living place?				
1	Very dissatisfied	5(3.1)	1(1.2)	6(2.4)
2	Dissatisfied	17(10.5)	6(7.2)	23(9.4)
3	Neither satisfied nor dissatisfied	28(17.3)	10(12)	38(15.5)
4	Satisfied	102(63)	53(63.9)	155(63.3)
5	Very satisfied	10(6.2)	13(15.7)	23(9.4)

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How satisfied are you with your access to health services?				
1	Very dissatisfied	4(2.5)	1(1.2)	5(2)
2	Dissatisfied	23(14.2)	9(10.8)	32(13.1)
3	Neither satisfied nor dissatisfied	42(25.9)	15(18.1)	57(23.3)
4	Satisfied	69(42.6)	41(49.4)	110(44.9)
5	Very satisfied	24(14.8)	17(20.5)	41(16.7)
How satisfied are you with your transport?				
1	Very dissatisfied	3(1.9)	0(0)	3(1.2)
2	Dissatisfied	19(11.7)	9(10.8)	28(11.4)
3	Neither satisfied nor dissatisfied	38(23.5)	10(12)	48(19.6)
4	Satisfied	95(58.6)	58(69.9)	153(62.4)
5	Very satisfied	7(4.3)	6(7.2)	13(5.3)

() Value in parenthesis are percentages

TABLE 4.19: TOTAL MEAN SCORES AND PERCENT MEAN SCORES OF DOMAINS OF WHO QOL OF T2DM ADULTS

Domains of WHOQOL-Bref	**Minimum Score obtained**	**Maximum Score obtained**	**Maximum attainable score**	**Total (N=245) Mean±SD**	**Percent Mean score (Mean Score/Maximum attainable score)*100**
Physical health	13	30	35	23.15±2.93	66.14
Psychological health	11	25	30	19.56±3.39	65.2
Social Relation	3	15	15	10.68±2.35	71.2
Environment	9	39	40	28.41± 6.57	71.03

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Majority (46.5%) felt that their physical environment was very much healthy while approx one fourth (24.5 %) of the subjects said that their physical environment was healthy to a moderate amount. Very few (1.2%) said they did not have enough money to meet their needs. This attributes to the availability of the government health services as regards medication for T2DM adults at affordable prices. Approx. 36% felt that they have enough money to meet their needs mostly while similar proportion (35%) felt that they have enough money to meet their needs moderately. Majority (40.4%) felt that they have most of the information that they need in their day-to -day life. Majority (42.2%) felt that they mostly have opportunity to perform leisure activities. Nearly 63.3% felt that they were satisfied with the conditions of their living place. About 45% felt that they were satisfied to their access to health services. Majority (62.4%) felt that they were satisfied with their transport facilities to a little extent. By chi square there were no significant differences between the two genders in any facet of environment domain.

Table 4.19 depicts total mean scores for the four domains of QoL. The % mean scores of the four domains of QoL ranged in between 65% to 71%. Among the four domains of WHOQoL Bref the highest % mean scores were for Social relationship and Environment domain indicating that the study population had relatively more satisfaction of their personal relationship, sexual activity, social support and their environmental health. Moreover, lowest mean score was observed for psychological health indicating not very good bodily image, positive feelings, self-esteem, personal beliefs and concentration also having negative feelings.

ASSOCIATION OF QOL AND SOCIO DEMOGRAPHIC CHARACTERISTICS

As depicted in Table 4.20 the association between the four mean domain scores of QoL and socio demographic characters like **sex, age, PCI** were seen by student t test. Association between **gender and QoL** revealed that there was no significant difference in mean domain scores of QoL between males and females. Association between mean **duration of diabetes and QoL** showed that there were no significant differences in mean scores in any of the four domains between the age wise categories for 5y or 7y or 10y.

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As depicted in Table 4.21 it was found that the facet 'satisfaction with health' obtained significantly lower mean scores for the older (age>50yrs) than in the younger T2DM adults($p<0.05$). However overall quality of life obtained lower mean scores for T2DM subjects with lower per capita income (PCI≤20,000) in comparison to those who had PCI>20,000 ($p<0.05$) (Table 4.22).

There were significantly lower mean domain scores in three domains: physical health, psychological health and environment for older (>50 y) T2DM subjects ($p<0.05$). In Physical domain there were significantly lower mean scores among several facets like 'physical pain prevents from doing what you need', 'enough energy for every day life' , 'ability to get around' and 'satisfaction with sleep' for older (age>50y) than the younger T2DM adults on metformin ($p<0.05$) (Table 4.21). In Psychological domain there were significantly lower mean scores among several facets like 'enjoyment in life', 'feeling life meaningful' and 'ability to concentrate' for older (age>50y) than the younger T2DM adults on metformin ($p<0.05$) (Table 4.21). In Environment domain there were significantly lower mean scores among several facets like 'safety in daily life', 'enough money to meet daily needs', 'availability of information in day to day life' and 'opportunity for leisure activities' for older (age>50y) than the younger T2DM adults on metformin ($p<0.05$) (Table 4.21).

Association between **PCI and QoL** revealed that there were significantly lower mean domain scores in three domains: psychological health($p<0.05$), social relationship($p<0.05$) and environment($p<0.01$) for T2DM adults with lower per capita income (PCI≤20,000) in comparison to those who had PCI>20,000. In Psychological domain there were significantly lower mean scores among only two facets: 'ability to concentrate' and 'accept bodily appearance' for lower PCI group (≤ Rs. 20,000) than in the higher PCI (Rs>20,000) T2DM adults on metformin ($p<0.05$) (Table 4.22). In Social Relationship domain there were significantly lower mean scores among all three facets: 'satisfaction with sex life', 'satisfaction with personal life' and 'satisfaction with support from friend' for lower PCI group (≤ Rs. 20,000) than in the higher PCI (Rs>20,000) T2DM adults on metformin ($p<0.05$) (Table 4.22). In Environment domain there were significantly lower mean scores among several facets like 'safety in daily life', 'how

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healthy is physical environment', 'Enough money to meet daily needs', 'ability of information needed for daily life',' opportunity for leisure activity', 'satisfaction with living place', satisfaction with access to health service' for lower PCI group (≤ Rs. 20,000) than in the higher PCI (Rs>20,000) T2DM adults on metformin ($p<0.05$) (Table 4.22).

ASSOCIATION OF QOL AND DPN

The overall quality of life and satisfaction with health obtained significantly lower mean scores for those suffering with peripheral neuropathy (DPN scores >0) in comparison to those without neuropathy (DPN score=0) ($p=0.000$ and $p=0.007$ respectively). As depicted in Table 4.23 and Fig 4.10 it was found that those with DPN had lower domain scores for all the four domains of WHOQoL-Bref. However the difference between the two groups were very significant for Physical domain ($p<0.05$) and highly significant for psychological health, social health and environment domain of WHOQoL - Bref.($p<0.001$). The association of DPN with QoL was also seen by Pearson correlation and it was found that DPN scores had a highly significant weak negative correlation with all the four domains of WHO QoL Bref ($r= -0.442$, $p=0.000$ for DPN scores and Physical domain, $r= -0.435$,$p=0.000$ for DPN scores and Psychological domain,$r= -0.478$,$p=0.000$ for DPN scores and social relationship and $r= -0.484$, $p=0.000$ for DPN and Environment domain).It can be said that as the DPN scores increased the domain scores decreased among T2DM adults on metformin.

ASSOCIATION OF QOL AND NUTRITIONAL STATUS

As depicted in Table 4.24 by ANOVA it was found that there were no significant differences between underweight, normal, overweight, obese grade I and obese grade II (classified by BMI) as regards the four domains of WHOQol Bref. The association of QoL with nutritional status by BMI was also seen by Pearson correlation and it was found that BMI showed very weak non-significant correlation with all the four domains of WHOQol Bref ($r= -0.061$, $p=0.340$ for BMI and Physical domain, $r= -040$,$p=0.531$ for M=BMI and Psychological domain, $r= -0$,$p=0.200$ for BMI and social relationship and $r= -0.041$, $p=0.523$ for DPN and Environment domain)

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TABLE 4.20: COMPARISON OF THE WHO BREF MEAN SCORES IN FOUR DOMAINS ACCORDING TO SEX, AGE AND PER CAPITA INCOME

Domains of WHOQoL Bref	Socio Demographic Characteristics		p value
	Male (N= 83)	**Females (N= 162)**	
Physical health	23.53±2.54	22.95±3.10	0.143
Psychological health	20.13±3.20	19.26±2.45	0.056
Social Relationship	11.07±2.11	10.48±2.42	0.060
Environment	27.85±6.73	29.51±6.15	0.061
	Age≤50yrs (N=65)	Age >50yrs (N=180)	p value
Physical health	23.80±2.29	22.91±3.10	.016*
Psychological health	20.42±3.22	19.24±3.40	.017*
Social Relationship	11±1.89	10.56±2.49	.197
Environment	29.98±5.51	27.84±6.84	.024*
	PCI ≤Rs. 20,000	PCI > Rs. 20,000	p value
Physical health	22.95±3.11	23.64±2.43	0.62
Psychological health	19.28±3.51	20.25±3.01	.031*
Social Relationship	10.46±2.49	11.21±1.91	.012*
Environment	27.73±6.94	30.3±5.39	.006**

*p<0.05,**p<0.001

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TABLE 4.21: COMPARISON OF MEAN SCORES FOR OVERALL QUALITY OF HEALTH, SATISFACTION OF HEALTH AND VARIOUS FACETS OF PHYSICAL HEALTH DOMAIN, PSYCHOLOGICAL HEALTH DOMAIN AND ENVIRONMENT DOMAIN ACROSS AGE

Various facets of WHO QoL Bref	Age≤50yrs (N=65)	Age>50yrs (N=180)	P value
How would you rate your quality of life?	3.78±.78	3.56±0.86	.067
How satisfied are you with your health?	3.31±.56	3.10±0.73	.039*
Physical Domain questions/facets			
To what extent do you feel that physical pain prevents you from doing what you need to do?	3.55±0.848	3.29±0.89	.038*
How much do you need any medical treatment to function in your daily life?	3.17±.52	3.02±0.72	.134
Do you have enough energy for everyday life?	3.80±.89	3.37±0.96	.002*
How well are you able to get around?	3.69±.61	3.46±90	.020*
How satisfied are you with your sleep?	3.75±.77	3.47±.98	.021*
How satisfied are you with your ability to perform your daily living activities	3.62±.63	3.43±.84	.063
How satisfied are you with your capacity to work?	3.66±.64	3.50±.91	.125
Psychological Domain questions/facets			
How much do you enjoy life?	3.58±0.93	3.29±.85	.022*
To what extent do you feel your life to be meaningful?	3.71±1.03	3.34±1.06	.018*
How well are you able to concentrate?	3.58±0.86	3.28±.87	.018*
Are you able to accept your bodily appearance?	3.71±.79	3.47±.96	.053
How satisfied are you with yourself?	3.69±.63	3.50±.90	.065
How often do you have negative feelings such as blue mood, despair, anxiety, depression?	3.86±.81	3.65±.92	.102
Environment domain questions/facets			
How safe do you feel in your daily life?	3.77±.96	3.41±1.02	.015*
How healthy is your physical environment?	3.66±.89	3.39±0.96	.051
Have you enough money to meet your needs?	3.72±0.86	3.38±.95	.012*
How available to you is the information that you need in your day-to -day life?	3.83±.86	3.48±1.05	.008*
To what extent do you have the opportunity for leisure activities?	3.75±0.75	3.42±.99	.006*
How satisfied are you with the conditions of your living place?	3.80±.67	3.63±.92	.123
How satisfied are you with your access to health services?	3.75±.85	3.56±1.02	.140
How satisfied are you with your transport?	3.69±.68	3.56±.84	.198

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TABLE 4.22: COMPARISON OF MEAN SCORES FOR OVERALL QUALITY OF HEALTH, SATISFACTION OF HEALTH AND VARIOUS FACETS OF PSYCHOLOGICAL HEALTH DOMAIN, SOCIAL RELATIONSHIP AND ENVIRONMENT DOMAIN ACROSS PCI

Various facets of WHO QoL Bref	PCI≤Rs 20,000 (N=171)	PCI>20,000 (N=73)	P value
How would you rate your quality of life?	3.55±.89	3.78±.71	.033*
How satisfied are you with your health?	3.10±.69	3.29±.70	.053
Psychological Domain questions/facets			
How much do you enjoy life?	3.31±0.90	3.52±.82	.088
To what extent do you feel your life to be meaningful?	3.39±1.09	3.58±1.00	.205
How well are you able to concentrate?	3.30±0.93	3.53±0.73	.035*
Are you able to accept your bodily appearance?	3.44±0.96	3.75±0.80	.010*
How satisfied are you with yourself?	3.49±0.88	3.70±0.72	.057
How often do you have negative feelings such as blue mood, despair, anxiety, depression?	3.65±.96	3.84±.71	.094
Social relationship questions/facets			
How satisfied are you with your sex life?	3.44±.91	3.73±.69	.009**
How satisfied are you with your personal relationship?	3.55±0.83	3.75±.64	.040*
How satisfied are you with the support you get from your friend?	3.46±0.88	3.73±.69	.013*
Environment domain questions/facets			
How safe do you feel in your daily life?	3.42±1.05	3.71±.92	0.041*
How healthy is your physical environment?	3.37±0.97	3.7±.86	.009*
Have you enough money to meet your needs?	3.38±.97	3.70±0.85	.015*
How available to you is the information that you need in your day-to -day life?	3.45±1.05	3.86±.87	.002**
To what extent do you have the opportunity for lesiure activities?	3.43±0.99	3.71±.79	.018*
How satisfied are you with the conditions of your living place?	3.60±.91	3.85±.72	.025*
How satisfied are you with your access to health services?	3.54±1.02	3.79±.87	.046*
How satisfied are you with your transport	3.54±86	3.70±.68	.135

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TABLE 4.23: COMPARISON OF MEAN DOMAIN SCORES OF VARIOUS DOMAINS OF WHOQOL-BREF IN BETWEEN THOSE WITH DPN AND WITHOUT DPN

Domains of WHOQoL-Bref	DPN (DPN Scores>0) (N=180)	NO DPN (DPN Scores=0) (N=65)	P value
Physical Health	22.84±3.07	24±2.3	0.002**
Psychological Health	19.11±3.46	20.78±2.85	0.000***
Social Relation	10.37±2.52	11.52±1.50	0.000***
Environment	27.42±7.0	31.14±4.17	0.000***

p<0.01,*p<0.001

FIG 4.10:COMPARISON OF MEAN DOMAIN SCORES OF QOL AMONG T2DM ADULTS WITH AND WITHOUT DPN

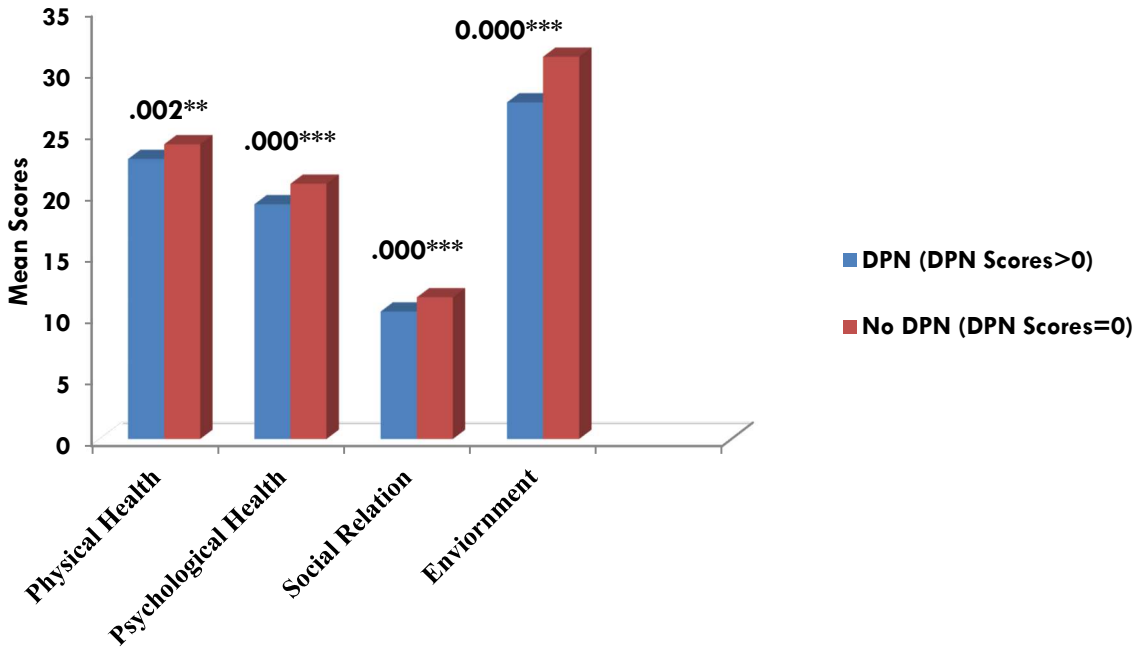

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TABLE 4.24: COMPARISON OF MEAN DOMAIN SCORES OF VARIOUS DOMAINS OF WHOQOL-BREF AMONG T2DM ADULTS WITH DIFFERENT NUTRITIONAL STATUS AS MEASURED BY BMI

WHO Qol Bref Domains	Underweight[a]	Normal[a]	Over weight[a]	Obese grade I[a]	Morbid[a] obese	F Value	P value
Physical Domain	23.67±3.07	22.79±2.61	22.91±2.52	23.76±2.83	22.38±3.53	2.241	0.065
Psychological Domain	20.83±4.62	19.64±3.22	19.05±3.24	20.04±3.17	18.96±3.39	1.434	0.223
Social Relationship	10.83±2.86	10.21±2.07	10.70±1.88	11.11±2.37	10.08±2.78	2.015	0.093
Environment	30.67±9.14	27.67±6.57	27.82±5.97	29.66±6.16	26.82±7.40	2.051	0.088

a=Classified by BMI as per Asia pacific classification :WHO 2000.

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HIGHLIGHTS OF PHASE I:

- The cross sectional survey of T2DM adults on metformin revealed that the study population had more females than males belonging to a wide age range between 26-96 y with mean age of 58.2y and three-fourth of the population were above 50y of age. The mean duration of diabetes was ~8 y and majority had long standing diabetes upto 10y which belonged to heterogneous socio economic group with a mean PCI of Rs. 21,700.82±18541.32.
- Majority did not consume alcohol or tobacco or cigarette. The study population had similar proportions of vegetarian and non-vegetarian while majority had low milk consumption (less than 200 ml) and there were no gender differences.
- Majority of T2DM adults suffered from over nutrition problem as majority were obese by BMI and had abnormal W.H.R and increased waist circumference and it was more so in females than males. Majority of the T2DM adults were pre hypertensive followed by hypertension stage I.
- There was a highly significant association between GI side effects of metformin with their present metformin dose ($p<0.001$). An increasing trend of the proportion of population reporting metallic taste as side effects was seen with the increase in present dosage of metformin. Almost three-fourth (70%) of the population had reported metallic taste as the most common side effects with the metformin dosage upto 2500 pg/ml; detrimental to their food ingestion required for dietary compliance; crucial for maintaining euglycemia in order to prevent micro vascular secondary complications and neuropathy and thereby maintaining their quality of life.
- As regards DPN, three fourth of the population was suffering from DPN but it was of low grade. Majority reported milder symptoms of neuropathy like numbness in their feet/legs, burning pain in their legs/feet and muscle cramps in their legs/feet and rare reported amputation. MNSI history showed that DPN was under diagnosed by physician.
- Amongst all the four domains of QoL it was the psychological health which was the poorest and QoL was associated with age, PCI and DPN but not with the nutritional status.

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- Older type 2 diabetics (age>50y) had poor 'satisfaction with health' than the younger adults(age<50y) significantly. There were significantly lower mean domain scores in three domains: physical health, psychological health and environment for older (age >50 y).
- T2DM adults with lower PCI (PCI≤20,000) had significantly poor overall quality of life in comparison to those who had PCI>20,000($p<0.05$). There were significantly lower mean domain scores in three domains: psychological health($p<0.05$), social relationship($p<0.05$) and environment($p<0.01$) for T2DM adults with lower per capita income (PCI≤20,000) in comparison to those who had PCI>20,000.
- The 'overall quality of life' and 'satisfaction with health' obtained significantly lower mean scores for those suffering with peripheral neuropathy in comparison to those without neuropathy. Those with DPN had lower domain scores for all the four domains of WHOQoL-Bref indicating poor quality of life. By Pearson correlation there was a highly significant weak negative correlation between DPN and all the four domains of WHO QoL Bref.

SECTION II: SCREENING OF B12 AMONG T2DM SUBJECTS ON METFORMIN

In phase II all those subjects who gave consent by signing the study consent form (Annexure I) for blood estimations were selected for the study. In all the blood was collected on 155 T2DM adults out of 245 patients included in the cross sectional survey.

The results of Phase II are described under the following heads:

- Biochemical Profile of T2DM subjects
  - Serum B12 levels
  - Haemoglobin and Macrocytic anemia
  - Glycated haemoglobin
- Prevalence of B12 deficiency, grades of B12 deficiency and gender
- Association between B12 deficiency and anemia
- Association between B12 deficiency and diet
- Association between B12 deficiency and metformin dosage

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- B12 deficiency and side effects of the drug metformin and past metformin dosage
- Association of nutritional status (by BMI) with serum B12 levels and glycated hemoglobin
- Association of serum B12 deficiency with hypertension
- Association of serum B12 deficiency with Nutritional status
- Association of B12 deficiency with duration of Type 2 diabetes
- Association of B12 deficiency with glycemic control
- Association of B12 deficiency of T2DM adults with their recent drug therapy
- Association of B12 deficiency with DPN
- Receiver Operating Characteristics (ROC) curve analysis for DPN related to B12 deficiency
- Association of DPN with glycemic control
- Association of B12 deficiency and QoL
- Association of QoL and Glycemic control
- Risk Factors for B12 deficiency in T2DM adults on metformin
- Risk factors for diabetes peripheral neuropathy in T2DM adults on metformin
- Highlights of Phase II

BIOCHEMICAL PROFILE OF T2DM ADULTS

SERUM B12 LEVELS AMONG T2DM SUBJECTS

Fig 4.11 depicts the Box plot of serum B12 of T2DM adults on metformin. It can be easily seen from the graph that the distribution of serum B12 had several outliers: 559,575, 600, 634, 674, 727,785,814,960,1002,1033,1200,1500,1746 pg/ml. Considering these outliers in the data points the range of serum B12 was from 94-512 pg/ml with a median of 190pg/ml and mean of 287.31 pg/ml (Fig 4.11). From the box plot it can be said that 50% of the population lies in between 150pg/ml and 311pg/ml.

Further from the Table 4.25 it can be said that mean of serum B12 was 287 pg/ml. An active effort was made to exclude patients who had been given vitamin B12-containing supplements for any indication (review of available medical records was done, and

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patients were asked about the use of Vitamin B12-containing supplements), but these preparations are available over the counter, and it cannot be definitely said that patients had never taken these medications earlier. Further it was found that mean serum B12 levels were significantly different for males and females ($p<0.05$). The males had higher mean serum B12 levels than females (363.92±353.88vs. 250.83±170.62) (Table 4.25).

PREVALENCE OF B12 DEFICIENCY, GRADES OF B12 DEFICIENCY AND GENDER

It was found that more than half (**81 o**f 155, **52%**) of the study subjects were B12 deficient (**vitamin B12 deficiency was defined as serum B12 less than equal to 200 pg/m**l) (Table 4.26)

[The concentrations suggested for defining **B12 deficiency by de Benoist, (2008) in WHO technical consultation on folate and vitamin B12 deficiency are: < 150 pmol/L (203 pg/mL)** for serum vitamin B12. Conversion factor used for vitamin B12 is 0.737 which means 1pg/ml is equal to 0.737 pmol/L. So it means 200pg/ml serum B12 is equal to 150pmol/L. In **our study the cut off used to define B12 deficiency was ≤200pg/ml which can be considered as aprox .≤150pmol/L**.]

The most common levels of B12 observed were 150pg/ml. As depicted in Table 4.26 more proportion of females (58%) than males (40%) were suffering from B12 deficiency ($p<.05$).

Grades of B12 deficiency across gender: Fig 4.12 depicts various grades of vitamin B12 deficiency across gender. Majority (47 out of 155, 30.3%) of them had mild B12 deficiency(150-200pg/ml). 20.6% (32 of 155) had moderate B12 deficiency(101-149pg/ml). Only 1.3% (2 of 155) had severe B12 deficiency (B12≤ 100pg/ml).

There was no statistically significant difference between various grades of B12 deficiency across gender ($p=0.125$)

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HEMOGLOBIN AND MACROCYTIC ANEMIA AMONG T2DM SUBJECTS

Hemoglobin of T2DM subjects on metformin varied from 7.6-14.6 g/dl with a median of 12.2g/dl, mode of 11g/dl and mean of 12.25±1.41g/dl (Table 4.25). It was found that there was no significant difference between the mean hemoglobin levels of male and female T2DM subjects. The overall prevalence of anemia was 40% with 64% & 28.6% in males and females respectively. The difference in prevalence in anemia between male and female subjects was significant ($p<0.001$). This could be attributed to the practice of prescribing iron supplements more commonly to female subjects than male subjects. With regard to severity of anemia it was found that majority (34%) had mild grade anemia and there were more males (58%) than females (22.9) (Fig 4.13). A total of 75 T2DM adults were on iron supplements of which 46 were females and 29 were males. It was observed that the mean Hb levels in subjects given iron supplements was 12.99 g/dl versus 11.55g/dl in non-supplemented group and this difference was highly significant($p<0.001$). Further, there was highly significant difference in the prevalence of anemia in subjects taking iron supplementation (21.3%) versus non-iron supplemented group (78.7%) ($p<0.001$) (Fig 4.14).

No case of macrocytic anemia was observed among the subjects.

GLYCATED HEMOGLOBIN (HbA1c) AND GENDER

HbA1c of T2DM adults on metformin varied from 6.4-12% with a median of 8.10%, mode of 7% and mean of 8.44 (Table 4.25). There was no significant difference between the mean glycated hemoglobin levels of males and females ($p=0.176$). Majority (71%) of the population had poor glycemic (HbA1c >7%) control. There was a trend that more females (30.6%) than males (26%) had good glycemic control but this was not significant ($p=0.566$).This may be due to the fact that females being more conscious of their body image visited the dietitian more commonly than males; contact with dietitian made females compliant to dietary advice, crucial for glycemic control beyond their compliance to oral hypoglycemic agents.

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FIG 4.11: BOX PLOT FOR SERUM B12 DISTRIBUTION OF T2DM ADULTS ON METFORMIN

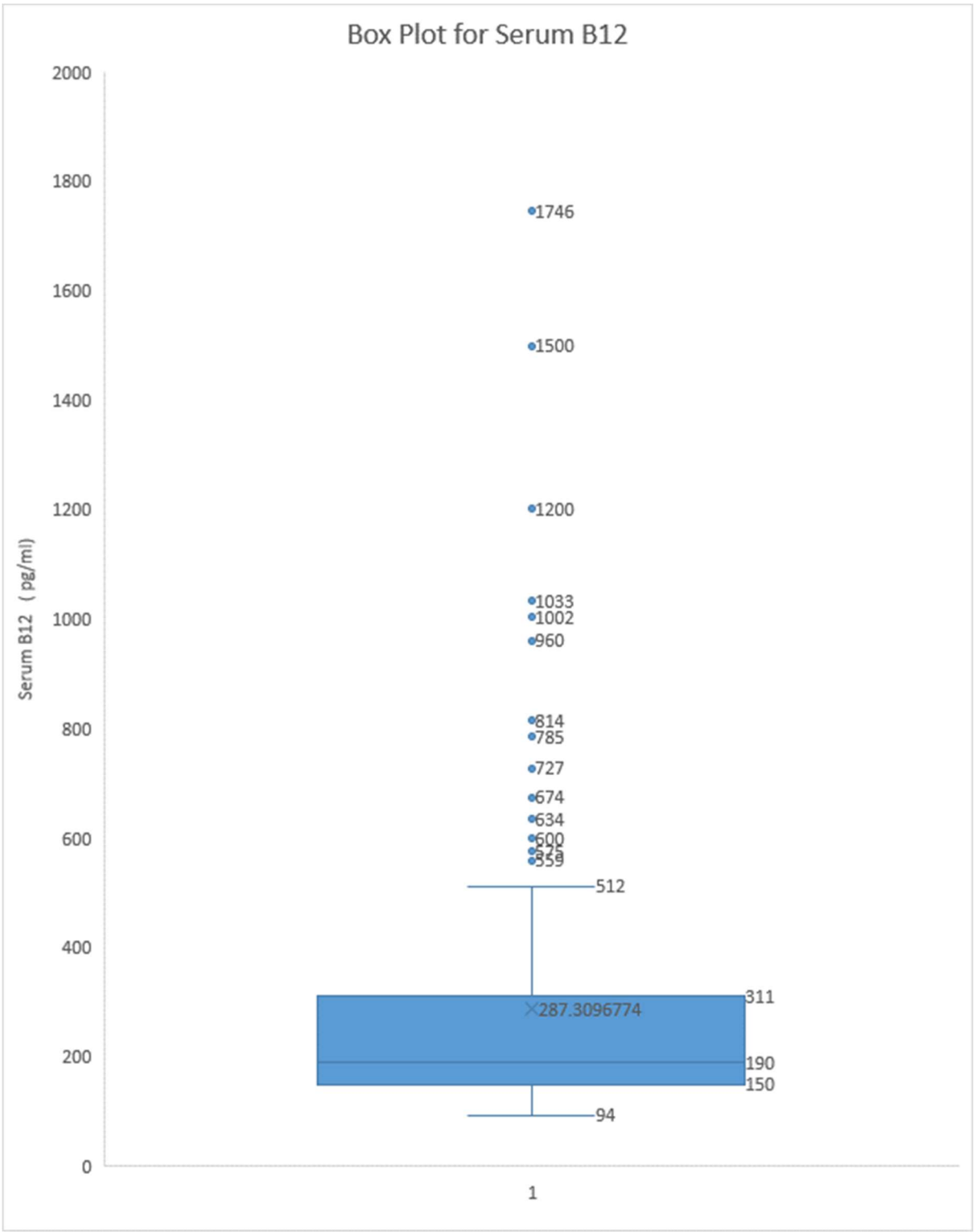

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TABLE 4.25: BIOCHEMICAL PROFILE OF T2DM ADULTS BY GENDER (MEAN±S.D)

Biochemical Profile	Range	Total (T=155)	Total (T=150)	Males (T=50)	Females (T=105)	P value
		Mean±S.D	Mean±S.E	Mean±S.D	Mean±S.D	
Serum B12(pg/ml)	94-1746	287.31±249.63	287.31±20.5	363.92± 353.88	250.83± 170.62	.036*
HbA1c(%)	6.4 -12	8.44±1.64	8.44±0.110	8.70±1.63	8.32±1.64	.176
Haemoglobin (g/dl)	7.6-14.6	12.25±1.26	12.25±0.10	12.37±1.22	12.19±1.28	.399

*p<0.05

TABLE 4.26: PREVALENCE OF B12 DEFICIENCY, GLYCEMIC CONTROL AND ANEMIA AMONG T2DM ADULTS ON METFORMIN BY GENDER

Biochemical Parameter	Total (N=155)		Males (N= 50)		Females (N=105)		p value
	N	%	N	%	N	%	
Serum B12(pg/ml)							
≤200(Deficient)	81	52.3	20	40	61	58.1	.035*
>200(Normal)	74	47.7	30	60	44	41.9	
HbA1c(%)							
≤7(Euglycemia)	45	29.03	13	26	32	30.5	0.566
>7(Poor glycemia)	110	70.97	37	74	73	69.5	
Haemoglobin							
Normal	93	60	18	36	75	71.4	0.000***
Anemic	62	40	32	64	30	28.6	

*p<0.05,***p<0.001

FIG 4.12: GRADES OF B12 DEFICIENCY AMONG T2DM ADULTS ACROSS GENDER

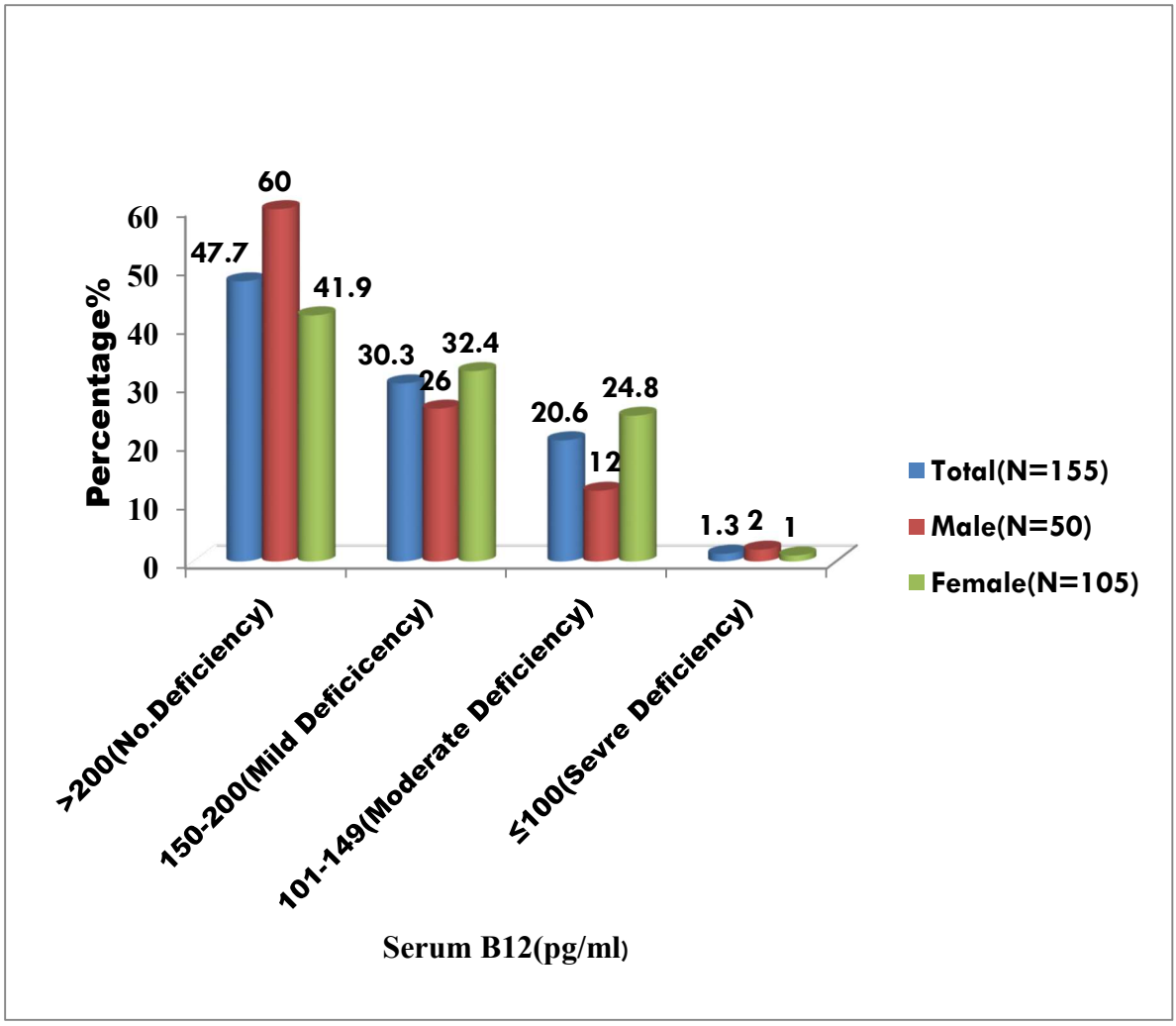

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FIG. 4. 13: GRADES OF ANEMIA AMONG T2DM ADULTS ON METFORMIN ACROSS GENDER

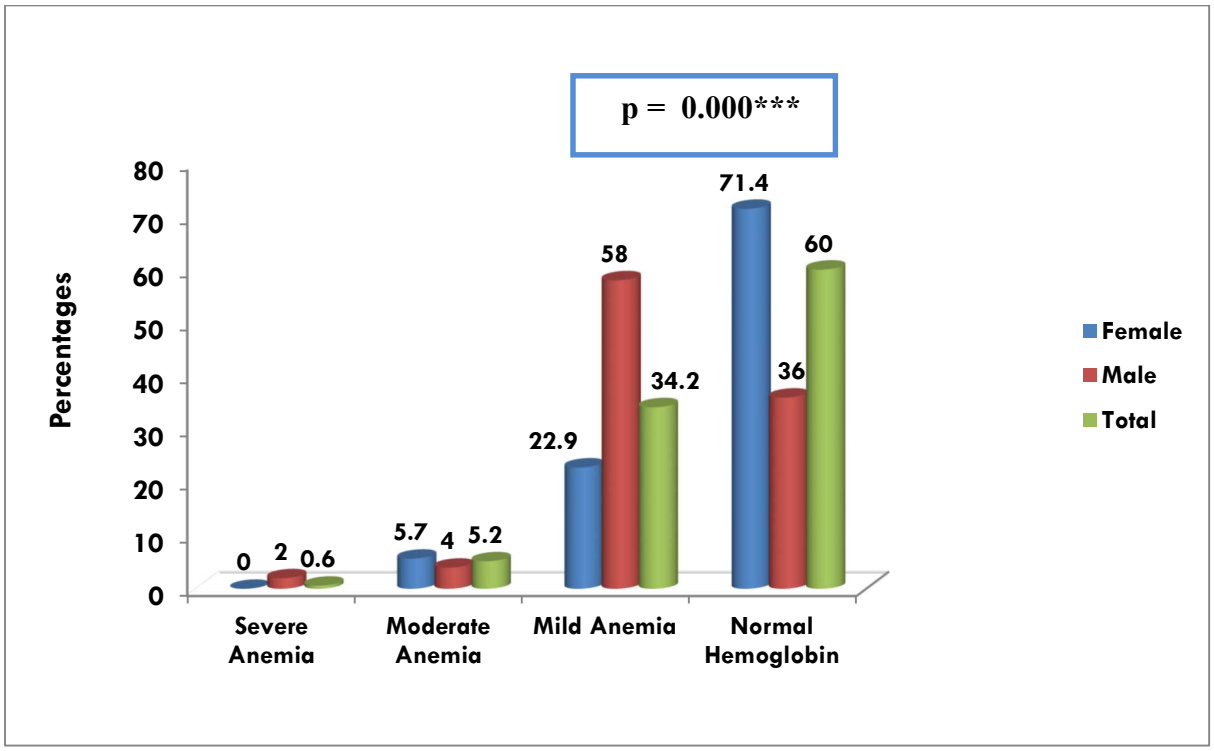
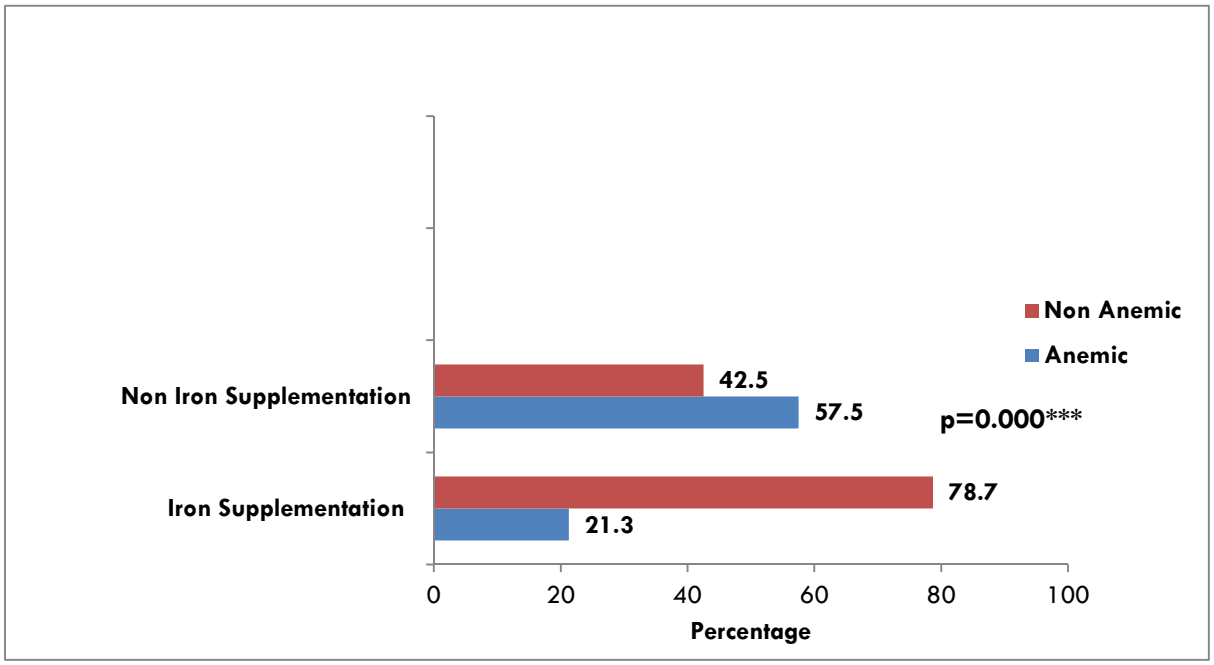


FIG 4.14: PREVALENCE OF ANEMIA AMONG THOSE ON IRON SUPPLEMENTATION AND NON IRON SUPPLEMENTATION



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ASSOCIATION BETWEEN B12 DEFICIENCY AND ANEMIA

Cell morphology and CBC showed no positive cases of macrocytic anemia. This showed the absence of any clinical vitamin B12 deficiency in the studied sample. This also indicates that though there was biochemical B12 deficiency at tissue levels there were no cases of macrocytic anemia for B12 levels for as low as 94pg/ml. This study showed low serum B12 levels associated with metformin use but we did not find metformin use to be associated with overt B12 deficiency or clinical B12 deficiency.

As depicted in Table 4.27 it was found that there was no significant difference in the prevalence of anemia among B12 deficient and those with normal B12 levels($p=0.264$). Serum vitamin B12 deficient patients did not have a higher prevalence of anemia.

When the means of hemoglobin of B12 deficient group were compared with those with normal B12 levels (Table 4.28) it was found that there was no significant difference between the two groups. This showed that vitamin B12 deficiency as defined by serum vitamin B12 levels had no impact on hematological parameter (hemoglobin) assessed in our study.

ASSOCIATION BETWEEN B12 DEFICIENCY AND DIET:

As depicted in Table 4.29 and Fig 4.15 it was found that there was significantly higher proportion of vegetarians (54.3%) among those who were B12 deficient (≤200pg/ml) in comparison to those who had normal B12 levels(>200pg/ml) ($p<0.05$). On the contrary there were significantly higher proportion of non-vegetarians (including ovovegetarians) (66.2%) among those who had normal B12 levels($p<0.05$).

Further the trends of milk consumption showed that there were no significant differences in milk consumption patterns in between the B12 deficient group and those with normal B12 levels (Table 4.29).

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TABLE 4.27 PREVALENCE OF ANEMIA AMONG B12 DEFICIENT AND THOSE WITH NORMAL B12

Hemoglobin status	Total	Normal B12 (>200pg/ml) (N=74)		B12 deficient (≤200pg/ml) (N=81)		P value
		N	%	N	%	
Normal	93	41	55.4	52	64.2	0.264
Anemic#	62	33	44.6	29	35.8	

#Hb<13 for males and Hb<12g/dl for females

TABLE 4.28 COMPARISON OF MEANS OF SEVERAL PARAMETERS IN BETWEEN B12 DEFICIENT AND NORMAL B12 LEVELS

Parameter	Normal B12 (B12>200pg/ml) (N=74)	B12 deficient (B12≤200pg/ml) (N=81)	P Value
Hemoglobin	12.05±1.46	12.42±1.34	0.103
Glycated hemoglobin (HbA1c%)	8.29±1.53	8.59±1.74	0.257
DPN scores	1.55±2.03	3.1±1.86	0.000***
MNSI History score	2.80±2.48	4.9±2.79	0.000***
Age of patients(Y)	58.07±12.32	59 ±8.75	0.591
Duration of diabetes(m)	90.26±91.65	95.69±74.28	0.685
Present metformin dosage	1092.59±447	1054.05±41	0.21
Past metformin dosage	790.12±417.31	871.62±475.86	0.258

***p<0.001

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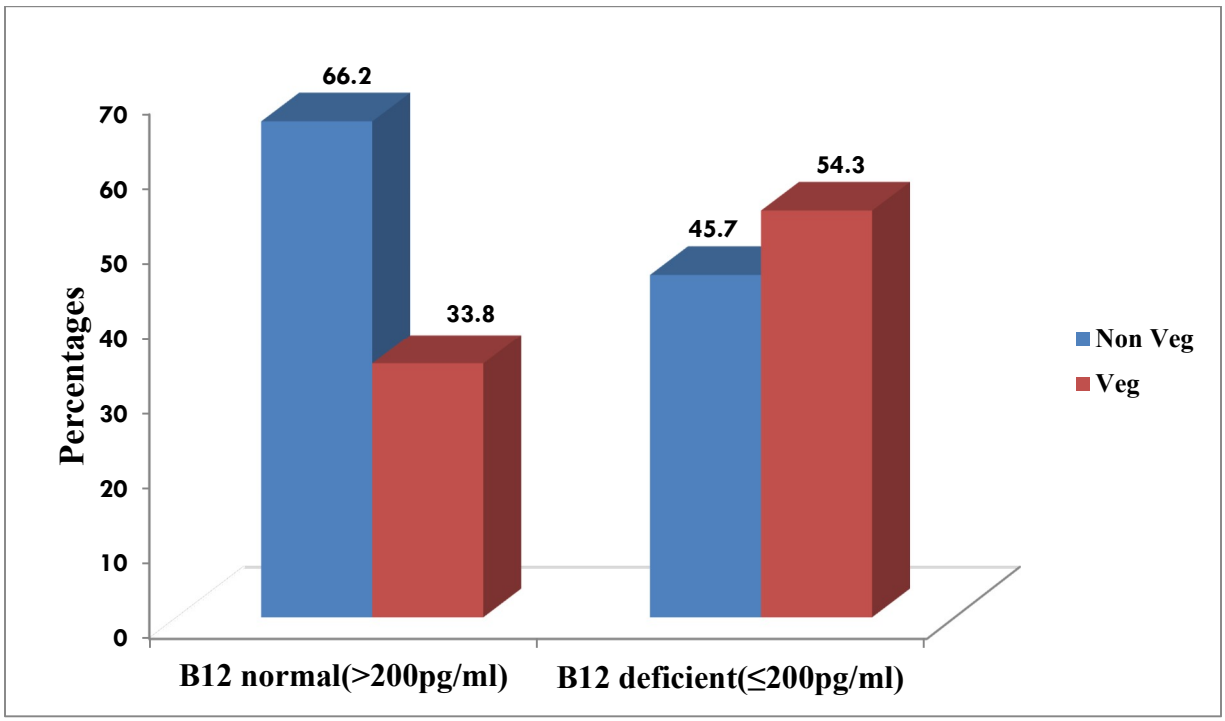
TABLE 4.29: ASSOCIATION BETWEEN B12 DEFICIENCY AND DIET

Dietary Pattern	B12 Normal (>200pg/ml) (N= 74)		B12 deficient (≤200pg/ml) (N= 81)		P value
	n	%	n	%	
Non Veg	49	66.2	37	45.7	0.010*
Veg	25	33.8	44	54.3	
Milk Consumption					
<200 ml	42	56.8	57	70.4	0.109
200-400ml	24	32.4	21	25.9	
>400ml	8	10.8	3	3.7	

*p<0.05

FIG 4.15: VEGETARIANISM VERSUS NON VEGETAIANISM ACROSS B12 DEFICIENCY



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ASSOCIATION BETWEEN B12 DEFICIENCY AND METFORMIN DOSAGE:

The mean present metformin dosage among those with B12 deficiency was 1092.59± 447 mg while that of those with normal B12 was 1054.05±410mg. This showed that dose of metformin was only slightly higher in those with B12 deficiency than those with normal B12 levels. However, this difference was not statistically significant (p= 0.21) as mentioned in Table 4.28 shown before.

Further a comparison of distribution of serum B12 across present metformin dosage was made by box plot as shown in Fig 4.16. It can be seen from the Fig. 4.16 that the mean of serum B12 falls but median remains almost similar as one moves across the increasing metformin dosage from 500mg to 1000mg to ≥1500mg. However, when the mean serum B12 of three groups with different metformin dosage was compared by ANOVA as shown in Table 4.30 there was no statistically significant difference in mean serum B12 levels between the three groups of 500mg, 1000mg and 1500mg dosage.

After removing the outliers (cases with serum B12>512 pg/ml) when the means of three groups with different metformin dosage was compared by ANOVA as shown in Table 4.31 there was no significant fall in mean serum B12 levels between the three groups of 500mg, 1000mg and 1500mg dosage.

As depicted in Table 4.32, by chi square there was no significant difference in the proportion of T2DM adults in various groups with different present metformin dosage in between B12 deficient individuals than those with normal B12 levels.

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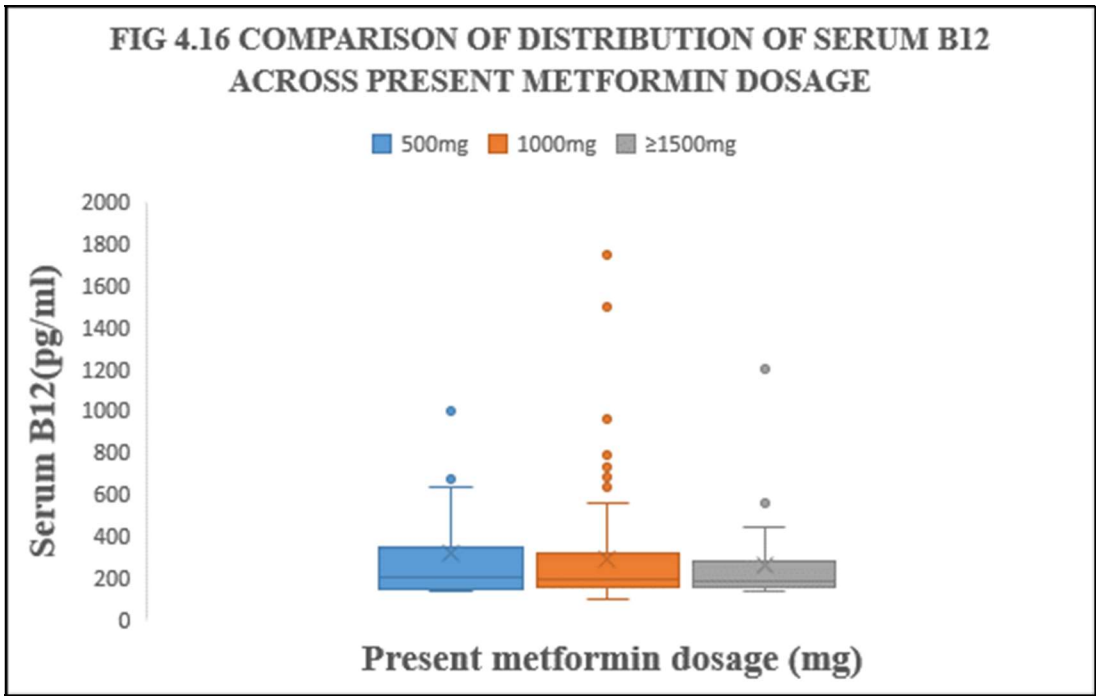

TABLE 4.30: COMPARISON OF SERUM B12 LEVELS AMONGST VARIOUS GROUPS WITH DIFFERENT METFORMIN DOSAGE

Present Metformin Dosage (mg)	N	Minimum	Maximum	Mean± S.D.	ANOVA
500	28	133	1033	312.86±256.90	
1000	95	94	1746	290.91±263.618	F=0.433
≥1500	32	136	1200	254.28±199.02	P=0.649
Total	155	94	1746	287.31±249.63	

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TABLE 4.31: COMPARISON OF SERUM B12 LEVELS AMONGST VARIOUS GROUPS WITH DIFFERENT METFORMIN DOSAGE AFTER REMOVING THE OUTLIERS

Present Metformin Dosage (mg)	N	Minimum	Maximum	Mean±S.D.	ANOVA
500	22	133	352	192.82±64.26	
1000	85	94	512	216.84±93.36	F=0.672
≥1500	30	136	437	212.57±80.47	P=0.513
Total	137*	94	512	212.04±86.46	

*After removing outliers from 155 total 137 T2DM adults were left

TABLE 4.32 DISTRIBUTION OF T2DM ADULTS IN VARIOUS DOSES OF PRESENT METFORMIN DOSE ACROSS B12 DEFICIENCY

B12 status	N	500mg		1000mg		1500mg		2000mg	
		n	%	n	%	n	%	n	%
>200pg/ml	74	14	18.9	45	60.8	8	10.8	7	9.5
≤200pg/ml	81	14	17.3	50	61.7	5	6.2	12	14.8
P value	0.581								

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B12 DEFICIENCY AND SIDE EFFECTS OF THE DRUG METFORMIN AND PAST METFORMIN DOSAGE

Of the reported gastro intestinal (GI) side effects of metformin like nausea, vomiting, diarrhea, anorexia and metallic taste the population under study reported metallic taste (29%) as their most common GI problems followed by anorexia (11%). However greater proportion of the population (59.6%) reported no GI side effects.

Further association between the serum B12 levels and GI side effects of metformin was seen as depicted in Table 4.33. T2DM adults with B12 deficiency had more GI side effects in comparison to those with normal B12 levels. There was a significant difference in proportion of various GI side effects between those who were B12 deficient versus those had normal B12 levels ($p<0.05$) (Table 4.33). However, no significant association was found between recent serum B12 status and past metformin dosage.

ASSOCIATION OF NUTRITIONAL STATUS (BY BMI) WITH SERUM B12 LEVELS AND GLYCATED HEMOGLOBIN

Table 4.34 depicts mean serum B12 of male and female subjects and HbA1c levels of males and female subjects in relation to their Nutritional Status by BMI. Amongst males there was no significant difference in the serum B12 levels between the various categories of underweight, normal, overweight, obese and morbid obese as classified by BMI. Similar results were obtained for females as well.

As regards HbA1c also there was no significant association between various categories of nutritional status by BMI and glycated hemoglobin levels neither for males nor for females (Table 4.34)

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TABLE 4.33: ASSOCIATION BETWEEN SERUM B12 STATUS AND METFORMIN SIDE EFFECTS AND PAST METFORMIN DOSAGE

Serum B12 Status (pg/ml)	Total (N)	GI Side Effects of Metformin					
		No Side Effects		Anorexia		Metallic Taste	
		n	%	n	%	n	%
Normal (>200pg/ml)	74	36	48.6	12	16.2	26	35.11
Deficient (≤200pg/ml)	81	48	59.3	3	3.7	30	37
p Value		0.029*					
Serum B12 Status (pg/ml)	**Total (N)**	**Past Metformin Dosage (N.R = 2)**					
		500mg/day		**1000 mg/day**		**>1500 mg/day**	
		n	**%**	**n**	**%**	**n**	**%**
Deficient (≤200pg/ml)	80	45	70.4	7	13	9	16.7
Normal (>200pg/ml)	73	34	68.8	16	11.3	28	19.9
p Value	0.472	22		4	8	35	70

*p<0.05

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TABLE 4.34 MEAN SERUM B12 OF MALES AND FEMALES AND HbA1c LEVELS OF MALES A
RELATION TO THEIR NUTRITIONAL STATUS BY BMI

Nutritional Status (BMI)	Serum B12 (pg/ml)				HbA1c (%)		
	Males (N = 50)		Females (N = 105)		Males (N = 50)		
	N	Mean± S.D.	n	Mean± S.D.	n	Mean±S.D.	n
Underweight[a]	2	271.00±57.98	2	162.50±14.85	2	7.95±0.50	2
Normal[a]	8	245.62±161.59	15	214.47±124.43	8	8.72±1.26	15
Overweight[a]	7	515.71±578.41	26	231.15±158.39	7	9.39±1.79	26
Obese[a]	19	368.89±279.16	38	260.71±190.00	19	8.42±1.75	38
Morbid Obesity[a]	14	362.14±415.92	24	286.58±182.68	14	8.84±1.72	24
Total	50	363.92±353.87	105	250.83±170.62	50	8.70±1.63	105
F Value	.560		.560		.558		
p Value	.693		.678		.695		

a= BMI by Asia Pacific Classification WHO - 2000

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ASSOCIATION OF SERUM B12 DEFICIENCY WITH HYPERTENSION

Table 4.35 shows the prevalence of various stages of hypertension by serum B12 status in T2DM adults on metformin. It was found that among the 74 T2DM adults with normal B12 status there were only 2 adults who had normal BP and none among 81 T2DM adults with B12 deficiency had normal BP. The number of normal BP adults were combined with those who had Pre hypertension and a combined category was created as depicted in Table 4.35. It was found that there was no significant difference in proportion of various stages of hypertension between those who had B12 deficiency and those who had normal B12 levels.

ASSOCIATION OF SERUM B12 DEFICIENCY WITH NUTRITIONAL STATUS

Table 4.36 shows the prevalence of various stages of nutritional status (by BMI) across serum B12 status in T2DM adults on metformin. It was found that there were 2 subjects in underweight category among the proportion of B12 deficient individuals and other 2 subjects who were underweight had normal B12 status. Since we cannot apply chi square if any cell number is less than 5 the underweight category is not depicted in Table 4.36. However for normal, overweight, obese and morbid obese category no significant difference was found in between B12 deficient subjects and those with normal B12 levels.

Table 4.37 depicts the prevalence of abdominal obesity (measured by WC) and obesity by WHR across serum B12 status in T2DM adults on metformin. There was no significant difference in prevalence of abdominal obesity by WC in between those who had B12 deficiency versus those who had normal B12 levels. Similarly, there was no significant difference in prevalence of obesity by WHR in between those who had B12 deficiency versus those who had normal B12 levels.

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TABLE 4.35: PREVALENCE OF VARIOUS STAGES OF HYPERTENSION BY SERUM B12 STATUS IN T2DM ADULTS ON METFORMIN

Serum B12 Status (pg/ml)	BP Measurement						
	Total	Normal BP and Pre Hypertension		Stage I Hypertension		Stage II Hypertension	
	N	n	%	n	%	n	%
>200	74	46	62.2	20	27	8	10.8
≤200	81	56	69.1	23	28.4	2	2.5
p Value	0.106						

TABLE 4.36: NUTRITIONAL STATUS (BY BMI) OF T2DM ADULTS ON METFORMIN BY THEIR B12 STATUS

Serum B12 Status (pg/ml)	Nutritional Status (BMI)								
	Total	Normal		Over Weight		Obese		Morbid Obesity	
	N	n	%	N	%	n	%	N	%
>200	74	8	10.8	12	16.2	31	41.9	21	28.4
≤200	81	15	18.5	21	25.9	26	32.1	17	21
p Value	0.162								

TABLE 4.37: PREVALENCE OF ABDOMINAL OBESITY BY W.C. AND OBESITY BY WHR ACROSS SERUM B12 STATUS

Serum B12 status (pg/ml)	Total	Abdominal obesity present (by w.c.)		Abdominal obesity absent (by w..c.)	
	N	n	%	n	%
B12>200	74	52	70.3	22	29.7
B12≤200	81	66	81.5	15	18.5
P value		0.102			
Serum B12 status(pg/ml)		**Obesity present by W.H.R**		**Obesity absent by W.H.R**	
	N	**n**	**%**	**n**	**%**
B12>200	74	69	93.2	5	6.8
B12≤200	81	73	90.1	8	9.9
P value		0.484			

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ASSOCIATION OF B12 DEFICIENCY WITH DURATION OF TYPE 2 DIABETES

Table 4.38 shows distribution of T2DM adults on metformin based on duration of diabetes by serum B12 status. It was found that among those who were B12 deficient there were more proportion (25.9% vs 17.6%) of T2DM adults with longer duration of diabetes (>10y). But these results were not statistically significant.

Moreover, the distribution of serum B12 in various groups with different duration of diabetes was studied by constructing box plot as shown in Fig. 4.17. It was clearly evident from the box plot that there was a decrease in median of serum B12 with the increasing duration of diabetes among T2DM adults on metformin. This indicates that there was a trend of lower serum B12 in those with longer duration of diabetes among metformin users. By median test (Table 4.39 and Fig 4.18) it was found that there was no significant difference in the frequencies of median serum B12 between the three groups with different duration of diabetes. Further the median test was performed after removing the outliers which is depicted in Table 4.40 and Fig.4.19 which depicted that there was no significant difference in the frequencies of median serum B12 between the three groups with different duration of diabetes.

ASSOCIATION OF B12 DEFICIENCY WITH GLYCEMIC CONTROL

Table 4.41 shows distribution of T2DM adults on metformin based on HbA1c by serum B12 status. It was found that there was non-significant difference in the glycemic control of those who were B12 deficient than those who had normal B12 levels.

ASSOCIATION OF B12 DEFICIENCY OF T2DM ADULTS WITH THEIR RECENT DRUG THERAPY

Table 4.42 shows distribution of T2DM adults on metformin based on the use of their present combination of drugs by serum B12 status. Mono therapy is defined as only use of metformin while dual therapy means use of metformin along with one oral hypoglycemic agent whereas triple therapy includes use of metformin along with two oral hypoglycemic agents. Of those who were B12 deficient majority fell under dual therapy followed by mono therapy and triple therapy. Similar trends were also observed for those with normal B12 status. It can be said that B12 status had no significant association with present combination of drug therapy

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TABLE 4.38: DISTRIBUTION OF T2DM ADULTS ON METFORMIN BASED ON DURATION OF DIABETES MELLITUS BY SERUM B12 STATUS

Serum B12 Status (pg/ml)	Total (N = 155)	DM Duration < 10 Yrs		DM Duration > 10 Yrs	
		N	%	n	%
>200	74	61	82.4	13	17.6
≤200	81	60	74.1	21	25.9
p Value		0.209			

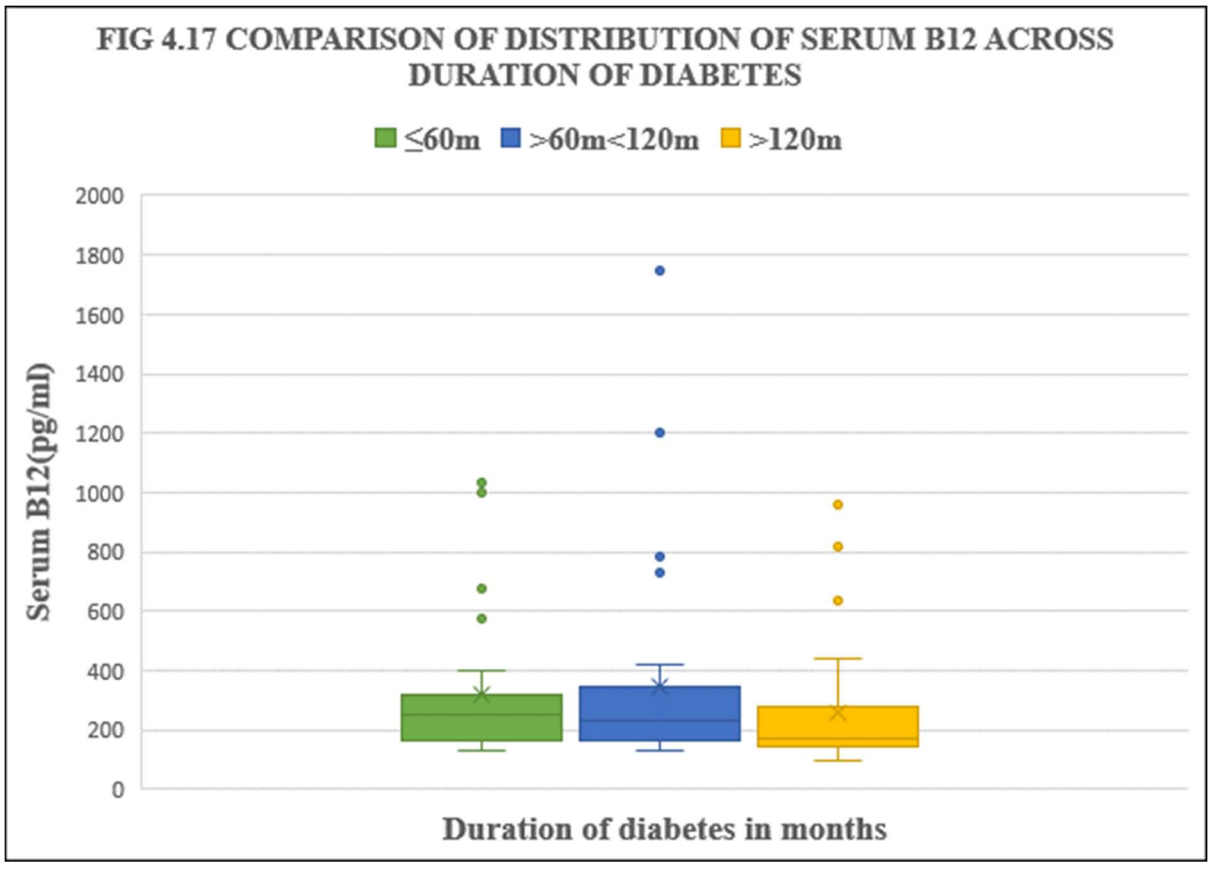

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TABLE 4.39: COMPARISON OF MEDIAN SERUM B12 IN THREE GROUPS WITH INCREASING DURATION OF DIABETES

Frequencies (n)			
	Diabetes Duration		
	<60 m	>60m≤120m	>120m
Serum B12(pg/ml) >Median	38	24	13
SerumB12(pg/ml) <=Median	37	22	21
Test statistics			
	Serum B12 (pg/ml)		
N	155		
Median	190		
Chi square	$\chi 2$= 1.823, d.f =2, p=0.402		

FIG 4.18: FREQUENCIES OF MEDIAN SERUM B12 IN THREE GROUPS WITH INCREASING DURATION OF DIABETES AMONG T2DM ADULTS ON METFORMIN (N=155)

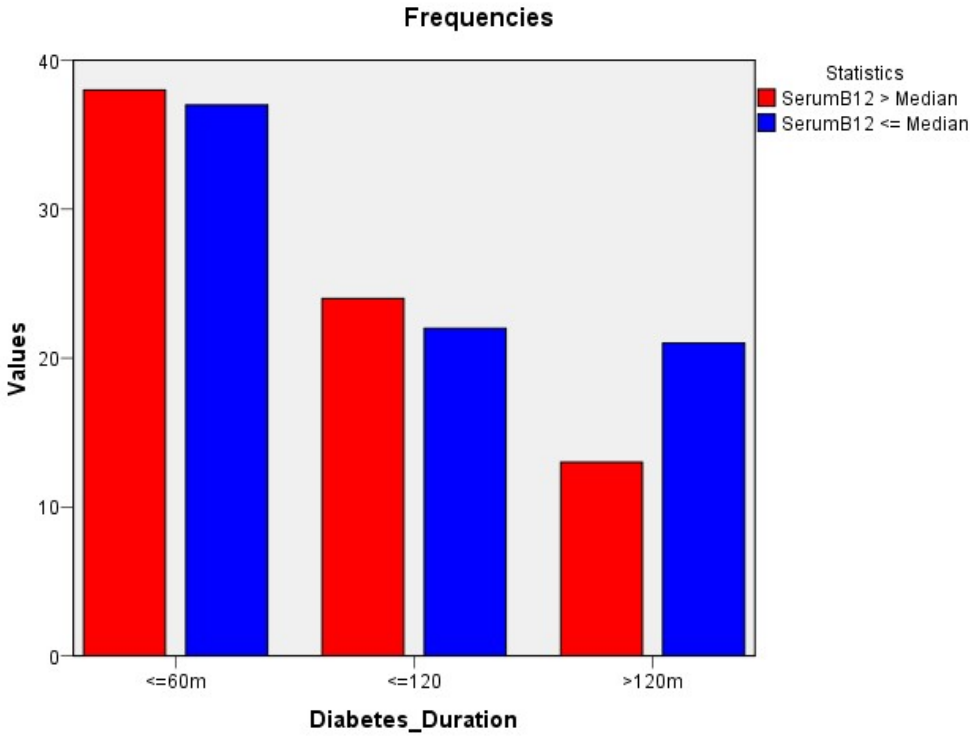

TABLE 4.40: COMPARISON OF MEDIAN SERUM B12 (WITHOUT OUTLIERS) WITH INCREASING DURATION OF DIABETES

Frequencies (n)			
	Diabetes Duration		
	<60 m	>60m≤120m	>120m
Serum B12(pg/ml) >Median	33	22	11
SerumB12(pg/ml) <=Median	32	19	20

Test statistics	
	Serum B12 (pg/ml)
N	137
Median	180
Chi square	$\chi 2$=2.669, d.f =2, p=0.263

FIG 4.19: FREQUENCIES OF MEDIAN SERUM B12(WITHOUT OUTLIERS) IN THREE GROUPS WITH INCREASING DURATION OF DIABETES AMONG T2DM ADULTS ON METFORMIN (N=137)

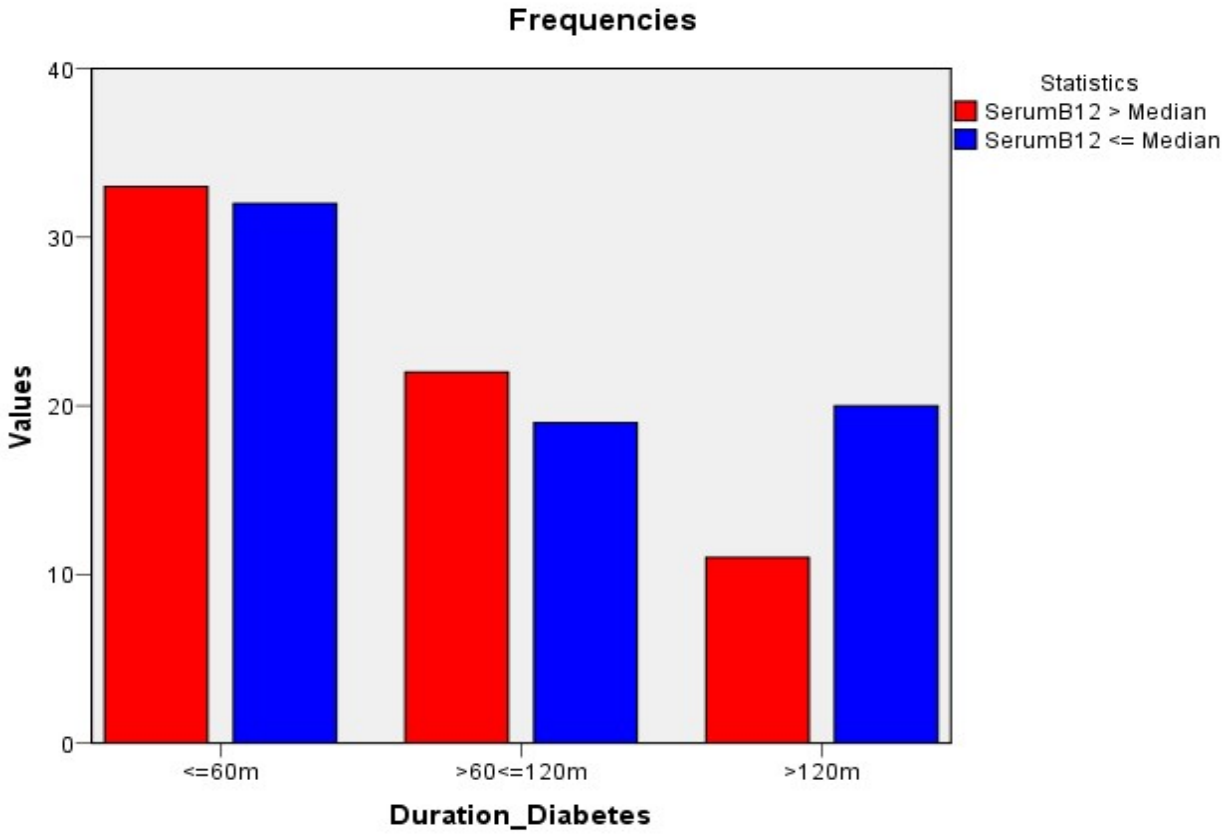

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TABLE 4.41: DISTRIBUTION OF T2DM ADULTS ON METFORMIN BASED ON HbA1c BY SERUM B12 STATUS

Serum B12 Status (pg/ml)	Total (N= 155)	HbA1c % (≤7)		HbA1c % (>7)	
		n	%	n	%
>200	74	24	53.5	50	45.5
≤200	81	21	46.7	60	54.5
p Value		0.373			

TABLE 4.42: DISTRIBUTION OF T2DM ADULTS ON METFORMIN BASED ON THE USE OF THEIR PRESENT COMBINATION OF DRUGS BY SERUM B12 STATUS

Serum B12 Status (pg/ml)	Present Combination of Therapy						
	Total (N = 155)	Mono Therapy		Dual Therapy		Triple Therapy	
		n	%	n	%	n	%
>200	74	24	32.4	44	59.5	6	8.1
<200	81	21	25.9	57	70.4	3	3.7
p Value		0.278					

`

ASSOCIATION OF B12 DEFICIENCY AND DPN

Prevalence of serum B12 status by DPN among the T2DM adults on metformin is shown in Table 4.43 and Fig. 4.20. It was seen that of those suffering from DPN whether low or high majority had B12 deficiency (58.2% and 70.5% respectively) in comparison to those with no DPN where B12 deficient population was only 15.4%. There was a significant association ($p = 0.000$) between DPN and B12 deficiency.

Further Pearson correlation between serum B12 and DPN scores came out to be non-significant ($r= - 0.127$, $p= 0.116$). Higher the DPN score lower the B12 status thus more was the occurrence of B12 deficiency. However, the strength of correlation was weak. Odds ratio between B12 deficiency and DPN was 10.0 (C.I. 3.89-26.) suggesting that those who are B12 deficient are ten times more likely to have DPN in comparison to those who are not B12 deficient.

RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE ANALYSIS FOR DPN RELATED TO B12 DEFICIENCY (FIG 4.21)

ROC curve analysis was used to evaluate the relationship between DPN and vitamin B12 deficiency. DPN scores as assessed by MNSI physical assessment were taken as 'test variable ' and B12 deficiency defined as less than equal to 200pg/ml was taken as 'state variable' or binary variable.

The objective to construct ROC curve was to determine the reflection point (cut off) of DPN score for B12 deficiency defined at ≤200pg/ml and B12 >200pg/ml as normal. The area under the curve (AUC) as depicted in Table 4.44 was calculated with 95% CI and the various coordinates of ROC are shown in Table 4.45

Among the several coordinates of the curve (Table 4.45) the DPN cut-off value of 1.75 would give a sensitivity of ~72% and specificity of ~67%. The DPN cut-off value of 2.25 gives a sensitivity of 60% and specificity of 74%. Ideally, one wants both sensitivity and specificity to be high, but typically, for a screening tool specificity is given more importance, so we select the 2.25 cut-off for DPN score to define Diabetic peripheral neuropathy as assessed by MNSI

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TABLE 4.43: PREVALENCE OF SERUM B12 STATUS BY DPN GRADES OF THE T2DM ADULTS ON METFORMIN

DPN Scores	Total	Serum B12 >200 pg/ml (N=74)		Serum B12 ≤200 pg/ml (N= 81)	
	N	n	%	n	%
No DPN (Scores =0)	39	33	84.6	6	15.4
Low DPN (Score≤2.5)	55	23	41.8	32	58.2
High DPN (Score>2.5)	61	18	29.5	43	70.5
p value	0.000***				

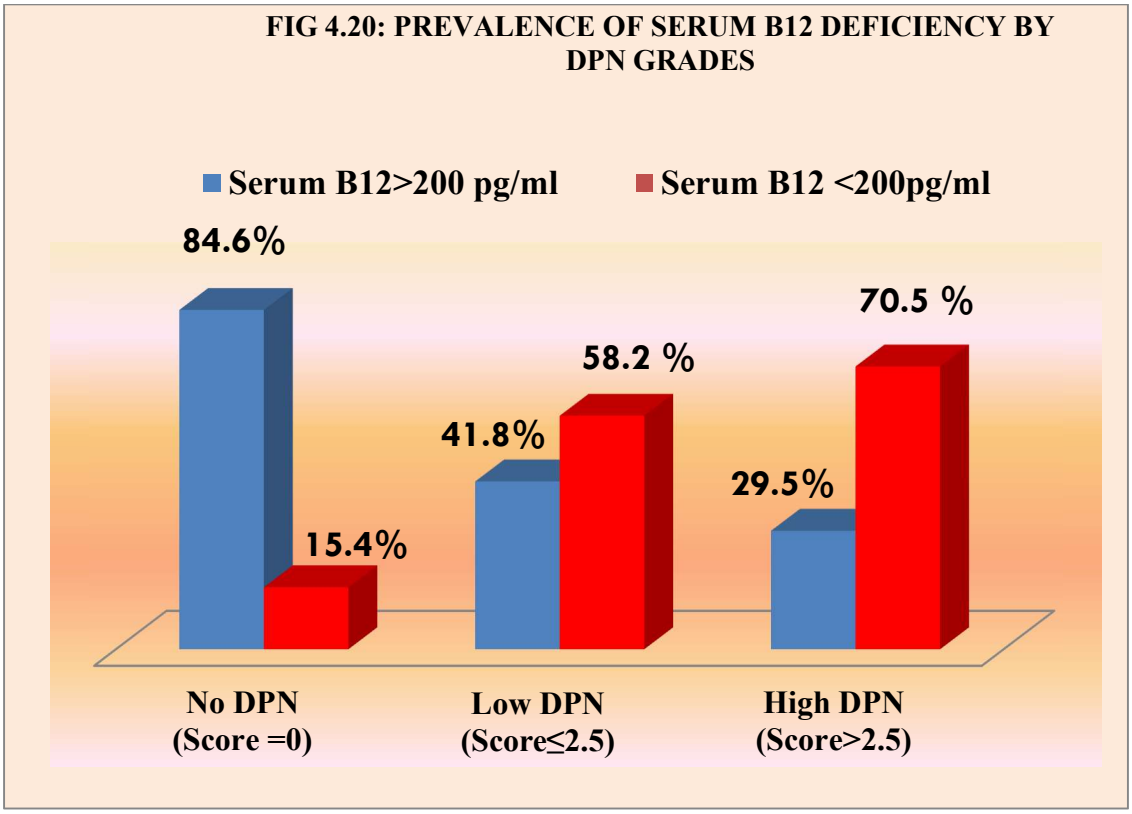

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TABLE 4.44: AREA UNDER THE CURVE FOR ROC CURVE ANALYSIS FOR DIABETIC PERIPHERAL NEUROPATHY RELATED TO B12 DEFICIENCY

AUC	S.E.	P value	C.I.
0.742	.041	.000***	0.661- 0.832

***P<0.001

TABLE 4.45: COORDINATES OF ROC CURVE ANALYSIS FOR DIABETIC PERIPHERAL NEUROPATHY RELATED TO B12 DEFICIENCY

Test variable: DPN score (Positive if greater than or equal to [a])	Sensitivity	1-Specificity
-1.000	1.000	1.000
.250	.926	.554
.750	.926	.541
1.250	.778	.378
1.750	.716	.324
2.250	.605	.257
2.750	.531	.243
3.250	.494	.162
3.750	.481	.149
4.500	.210	.108
5.250	.185	.108
5.750	.136	.081
6.250	0.000	.041
6.750	0.000	.027
7.250	0.000	.014
8.500	0.000	0.000

The test result variable(s): DPN Scores has at least one tie between the positive actual state group and the negative actual state group.

a = The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

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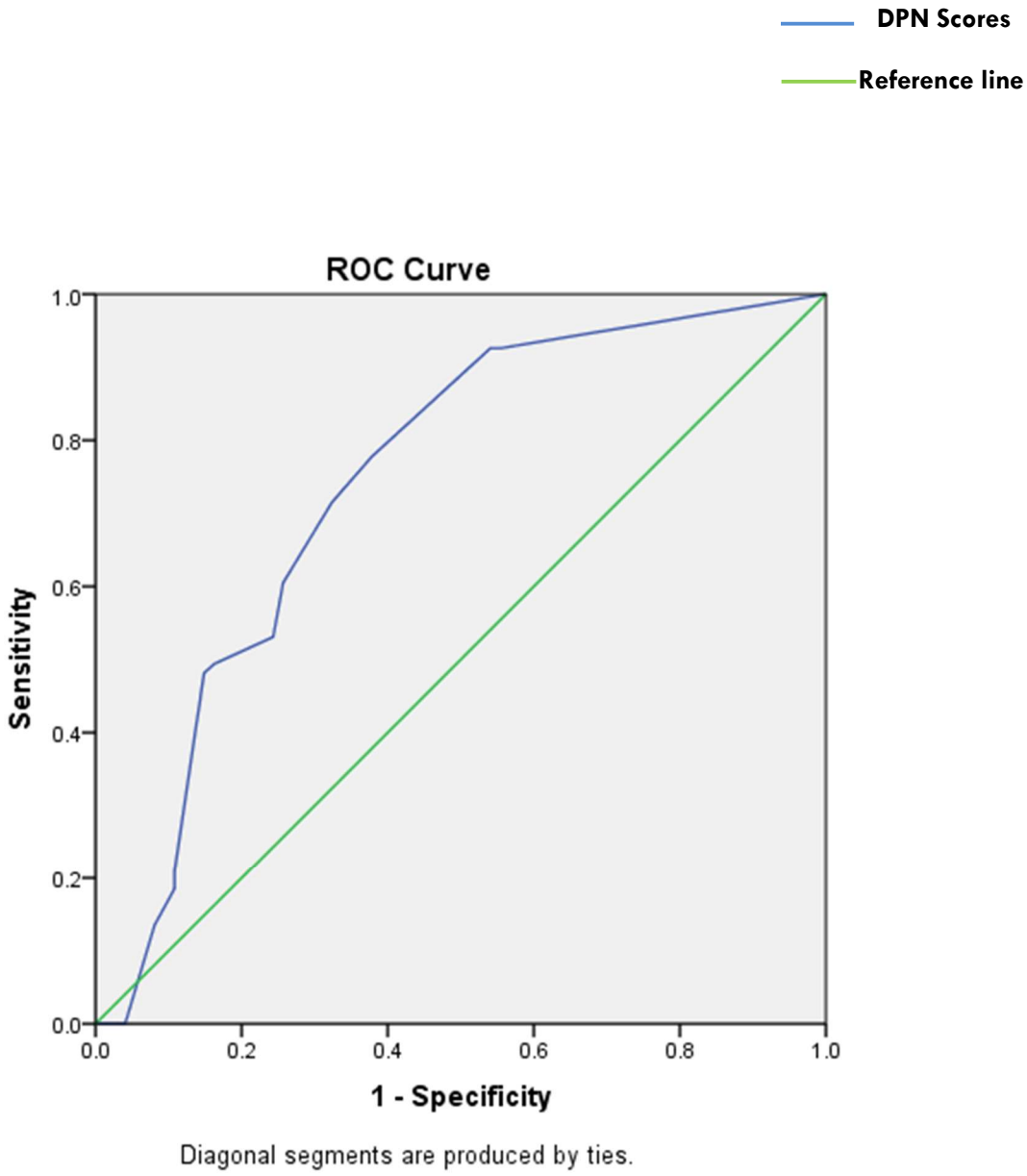
FIG 4.21 RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE FOR DIABETIC PERIPHERAL NEUROPATHY SCORE (DPN SCORE) RELATED TO VITAMIN B12 DEFICIENCY



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For the DPN score, the AUC value was 0.742 (95 C.I., 0.661- 0.832, p<0.001) and the **reflection point was 2.25** with a sensitivity of 60% and specificity of 74%.

From this ROC curve analysis, it can be said that when B12 screening is done among diabetics on metformin then those who have B12 deficiency (defined as serum B12≤ 200 pg/ml) are probable to have DPN scores of 2.25 (p< 0.001).

ASSOCIATION OF DPN WITH GLYCEMIC CONTROL

Further distribution of T2DM adults based on the glycated haemoglobin levels by their DPN grade was carried out (Table 4.46). It was found that there was significant association between grades of DPN and glycemic control (p<0.001).

Amongst the good glycemic control (HbA1c ≤7%) group the majority(53.8%) had no DPN and very few (8.2%) had high grade DPN (Table 4.46 and Fig 4.22). In contrast, the poor glycemic control (HbA1c>7%) group had a very high proportion of population with high grade DPN (~92%) indicating that poorer the glycemic control, higher the chances of getting DPN (P<0.001).

CORRELATION BETWEEN DPN AND GLYCEMIC CONTROL

Further correlation between glycemic control and DPN scores came out to be significant (r=0.381, P= 0.000). As the glycated haemoglobin increases the DPN score also increases. It can be said that poorer the glycemic control higher the DPN score.

ASSOCIATION OF B12 DEFICIENCY AND QOL

As depicted in Table 4.47 and Fig 4.23, it was found that the QoL domain scores of B12 deficient group was lower than that in the normal B12 group for all the four domains: Physical Health, Psychological health, Social relationship and Environment of WHOQOL Bref and this result was highly significant(p<0.001).

Further Pearson correlation between B12 and QoL domain scores showed that there was significant weak correlation between B12 and physical domain (r = 0.225, p=.005), B12 and psychological domain (r= 0.300, p=0.000), B12 and social relationship domain (r= 0.278, p=0.000) and B12 and environment domain(r= 0.313, p=0.000).

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TABLE 4.46: DISTRIBUTION OF T2DM ADULTS BASED ON THE GLYCATED HAEMOGLOBIN LEVELS BY THE DPN GRADES OF THE T2DM ADULTS ON METFORMIN

DPN Scores	Total	HbA1c >7% (Poor glycemic control)		HbA1c ≤7% (Good glycemic control)	
	N=155	n	%	n	%
No DPN (0)	39	18	46.2	21	53.8
Low DPN (Score>0≤2.5)	55	36	65.5	19	34.5
High DPN (Score>2.5)	61	56	91.8	5	8.2
p value	0.000**				

**P<0.001

FIG. 4.22: PREVALENCE OF GOOD GLYCEMIC CONTROL (HbA1c≤7) AND POOR GLYCEMIC CONTROL(HBA1c >7%) BY GRADES OF DPN

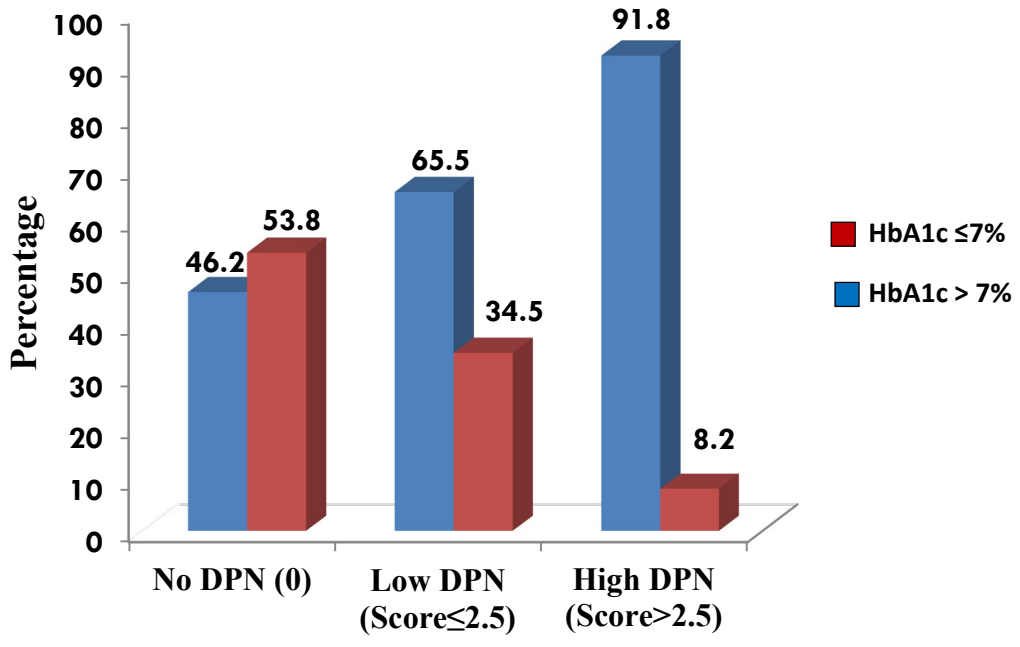

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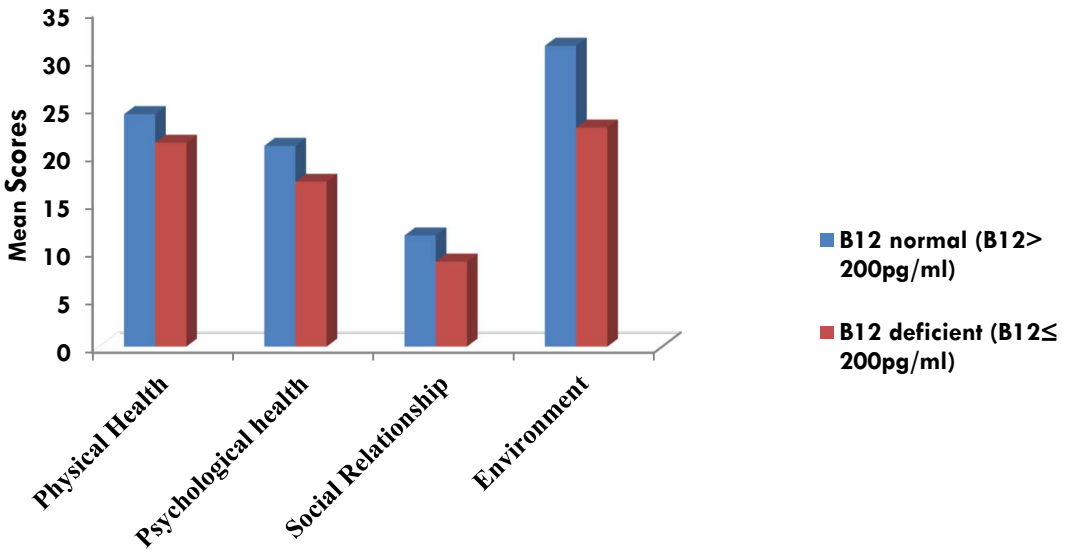
TABLE 4.47: COMPARISON OF MEAN SCORES OF VARIOUS DOMAINS OF WHOQOL-BREF IN BETWEEN B12 DEFICIENT AND NORMAL B12 GROUPS

Domains of WHOQOL-Bref	B12 Normal (B12>200 pg/ml) (N=74)	B12 deficient (B12≤200pg/ml) (N= 81)	P Value
Physical Health(d1)	24.26±2.52	21.28±3.15	0.000***
Psychological Health(d2)	20.96±2.70	17.21±3.11	0.000***
Social Relationship(d3)	11.62±1.69	8.88±2.59	0.000***
Environment(d4)	31.39±4.35	22.86±6.69	0.000***

***p<0.001

FIG.4.23 : QUALITY OF LIFE MEAN DOMAIN SCORES ACROSS B12 DEFICIENCY



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ASSOCIATION OF QOL AND GLYCEMIC CONTROL

As depicted in Table 4.48, it was found that the QoL domain scores of poor glycemic control was lower than that in the good glycemic control group for all the four domains: Physical Health, Psychological health, Social relationship and Environment of WHOQOL Bref and this result was significant for Physical health domain at $p<0.01$ while it was significant at $p<0.001$ for Psychological health, social relationship and environment domain of QoL.

Pearson correlation between HbA1c and QoL domain scores showed that there was significant negative weak correlation between HbA1c and physical domain ($r = -0.172$, $p=.032$*), HbA1c and psychological domain ($r=- 0.234$, $p=0.003$*), HbA1c and social relationship ($r= -0.192$, $p=0.017$*) and HbA1c and environment domain ($r= -0.227$, $p=0.005$**).

RISK FACTORS FOR B12 DEFICIENCY IN T2DM ADULTS ON METFORMIN

Among the several factors associated with B12 deficiency by odds (Table 4.49), the abdominal obesity (WC), BMI, WHR, glycated hemoglobin, duration of diabetes, present drug therapy, DPN presence, grade of DPN and diet were associated (OR>1). However, DPN presence or absent and diet emerged as the only significant risk factors for B12 deficiency on metformin treated T2DM adults ($p<0.001$ and $p<0.05$ respectively).

The odds of having B12 deficiency (≤ 200pg/ml) was 10.06 times higher (CI- 3.89-26.00) among T2DM adults on metformin if they were DPN positive (DPN scores>0) in comparison to those who had no DPN (DPN scores=0) ($p<0.001$).

The odds of having B12 deficiency (≤ 200pg/ml) was 2.33 times higher (CI-1.216-4.467) among T2DM adults on metformin if they were on a vegetarian diet than those on a non-vegetarian diet($p<0.05$).

RISK FACTORS FOR DIABETES PERIPHERAL NEUROPATHY IN T2DM ADULTS ON METFORMIN

Defining DPN deficiency as DPN scores ≥ 2.5 and DPN scores< 2.5 as no DPN and then several factors were associated by Odds Ratio as shown in Table 4.50. Among the several

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factors associated with DPN by odds (Table 4.50), hypertension, age, glycemic control (HbA1c), B12 deficiency and duration of T2DM were associated. (O.R> 1).However glycemic control (HbA1c ≤7) and B12 deficiency were the only significant risk factors for the occurrence of DPN among T2DM adults on metformin (p<0.001)

The odds of having DPN (DPN acores≥2.5) was 4.43 times higher (CI- 2.23-8.80) among T2DM adults on metformin if they were B12 deficient (B12≤200pg/ml) in comparison to those who had normal B12 (B12>200pg/ml) (p<0.001)

The odds of having DPN (DPN acores≥2.5) was 4.62 times higher (CI-2.03-10.51) among T2DM adults on metformin if they had poor glycemic control (HbA1c>7) in comparison to those who had good glycemic control (HbA1c ≤7) (p<0.001).

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TABLE 4.48: COMPARISON OF MEAN SCORES OF VARIOUS DOMAINS OF WHOQOL-BREF IN BETWEEN THOSE WITH GOOD GLYCEMIC CONTROL AND THOSE WITH POOR GLYCEMIC CONTROL

Domains of WHOQOL-Bref	Good Glycemic Control (HbA1c ≤7%) N= 45	Poor Glycemic Control (HbA1c >7%) N=110	p Value
Physical Health (d1)	23.60 ± 2.66	22.34 ± 3.37	0.015*
Psychological health(d2)	20.04 ± 2.65	18.57±3.67	.006**
Social relationship(d3)	11.09 ± 1.73	9.82±2.80	.001**
Environment(d4)	29.07 ± 4.92	26.06 ± 7.67	.004**

*P<0.005,**p<0.01

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TABLE 4.49: RISK FACTOR ASSOCIATION FOR B12 DEFICIENCY AMONG T2DM ADULTS ON METFORMIN (N=155)

Factors	Total N	B12 deficient (B12>200pg/ml) (N=74)	Normal B12 (B12≤200pg/ml) (N=81)	O.R	95% CI	P value
Hypertension Present(Includes Prehypertensives)	153	72	81	-	-	-
Hypertension Absent	2	2	0			
Abdominal obesity Present(by w.c.)	118	52	66	1.86	0.88-3.94	0.103
Abdominal obesity absent(by w.c.)	37	22	15			
BMI>23	133	65	68	1.38	0.55-3.45	0.489
BMI≤23	22	9	13			
WHR≥1	80	35	45	1.39	0.739-2.62	0.305
WHR<1	75	39	36			
Glycated Hb>7	110	50	60	1.37	0.684-2.74	0.374
Glycated Hb≤7	45	24	21			
Duration of DM>10yrs	121	61	60	1.64	0.75-3.57	0.21
Duration of DM≤10yrs	34	13	21			
Present Drug Therapy						
Mono Therapy	45	24	21	1.37	0.68-2.75	0.37
Multiple Drug Therapy	110	50	60			
High grade DPN (DPN Scores>2.5)	61	18	43	1.7	0.797-3.707	0.168
Low grade DPN (DPNscores>0≤2.5)	55	23	32			
No DPN (DPN scores=0)	39	33	6	10.06	3.89-26.00	0.000***
Yes DPN (DPN scores >0)	116	41	75			
Side Effect of Metformin						
Present	71	38	33	0.651	0.345-1.23	0.186
Absent	84	36	48			
Diet						
Vegetarian present	69	44	25	2.330	1.216-4.467	0.010*
Vegetarian absent (Ovovegetarian/Non vegetarian)	86	37	49			

*p<0.05, ***p<0.001

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TABLE 4.50: RISK FACTOR ASSOCIATION FOR DIABETES PERIPHERAL NEUROPATHY (DPN SCORES≥2.5) AMONG T2DM ADULTS ON METFORMIN

Factors	Total N	DPN Positive (DPN score≥ 2.5)	No DPN (DPN score<2.5)	O.R	95% CI	P value
Hypertension Present(Includes Prehypertensives)	240	92	148	2.48	0.27-22.59	0.403
Hypertension Absent	5	1	4			
Sex (F)	162	65	97	0.759	0.437-1.32	0.329
(M)	83	28	55			
Age≤50 (26-50)	65	20	45	1.53	0.83-2.81	0.163
Age>50 (>50-96)	180	73	107			
BMI≥23	206	71	135	0.406	0.203-0.814	0.009**
BMI<23	39	22	17			
Glycated Hb>7	110	59	51	4.62	2.03-10.51	0.000***
Glycated Hb≤7	45	9	36			
B12 deficiency (≤200pg/ml)	81	49	32	4.43	2.23-8.80	0.000***
Normal B12 (>200pg/ml)	74	19	55			
Metformin dosage>1000mg	50	15	35	0.64	0.32-1.25	0.193
Metformin dosage≤1000mg	195	78	117	0.64		
Duration of DM>5yrs	122	52	70	1.48	0.88-2.49	0.134
Duration of DM≤10yrs	123	41	82			

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

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HIGHLIGHTS OF PHASE II:

- By Box plot of serum B12 it was found that serum B12 had several outliers and the 50% of the population was in between 150pg/ml and 311pg/ml. Serum B12 outliers may be due to the fact that these patients would have taken B12 injections or B12 supplements in past however those on B12 injections/ supplements for past two months were excluded from the study by review of available medical records. But these preparations are available over the counter, and it cannot be surely said that patients had never taken these medications earlier.
- This study showed low serum B12 levels associated with metformin use but we did not find metformin use to be associated with overt B12 deficiency or clinical B12 deficiency as there were no positive cases of macrocytic anemia. Though there was biochemical B12 deficiency but B12 deficiency at tissue levels was not there for B12 levels as low as 94pg/ml.
- Vitamin B12 deficiency as defined by serum B12 levels had no impact on hematological parameter (Hb) assessed in our study as there was no significant difference in the prevalence of anemia among B12 deficient and those with normal B12 levels.
- Majority (60%) had normal hemoglobin and there were significantly greater proportion of males than females who were anemic ($p<0.001$). Those on iron supplements had higher Hb and prevalence of anemia was more among those who were not on iron supplements ($p<0.001$)Majority (71%) of the population had poor glycemic (HbA1c >7%) control with no significant gender difference.
- More than half (52%) of the population were B12 deficient (B12<200pg/ml) and B12 deficiency was more in females than males ($p<.05$).
- Among those who were B12 deficient there was significantly higher proportion of vegetarians in comparison to those who had normal B12 levels ($p<0.05$). The odds of having B12 deficiency (≤ 200pg/ml) was 2.33 times higher among T2DM adults on metformin if they were on a vegetarian diet than those on a non vegetarian diet($p<0.05$).

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- There was no significant association between the B12 deficiency and milk consumption.
- By box plot there was no significant association between serum B12 and metformin dosage.
- Of the reported GI side effects metallic taste was the most common and here was a significant difference in proportion of various GI side effects between those who were B12 deficient versus those had normal B12 levels ($p<0.05$). However, no significant association was found between recent serum B12 status and past metformin dosage.
- There was no significant association between B12 deficiency and nutritional status
- By box plot there was no significant association between B12 deficiency and duration of diabetes.
- There was significant difference in the glycemic control of those who were B12 deficient than those who had normal B12 levels ($p<0.05$).
- Of those suffering from DPN whether low or high majority had B12 deficiency in comparison to those with no DPN where B12 deficient population was only 15.4% ($p<0.001$)
- OR between B12 deficiency and DPN was 10.0 suggesting that those who are B12 deficient are ten times more likely to have DPN in comparison to those who are not B12 deficient.
- By ROC analysis it can be said that when B12 screening is done among diabetics on metformin then those who have B12 deficiency (defined as serum B12$\leq$ 200 pg/ml) are probable to have DPN scores of 2.25 ($p< 0.001$)
- The poor glycemic control (HbA1c>7%) group had a very high proportion of population with high grade DPN indicating that poorer the glycemic control, higher the chances of getting DPN ($p<0.001$).
- Pearson correlation between HbA1c and DPN scores was significant ($r=0.381$, $p=0.000$). As the glycated Hb increases the DPN score also increases. Poorer the glycemic control higher the DPN score

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- QoL domain scores of B12 deficient group was significantly lower than that in the normal B12 group for all the four domains: Physical Health, Psychological health, Social relationship and Environment of WHOQOL Bref ($p<0.001$).
- There was significant weak correlation between B12 and all four domains of QoL.
- QoL domain scores of poor glycemic control was lower than that in the good glycemic group for all the four domains of QoL($p<0.05$).
- Pearson correlation between HbA1c and QoL domain scores showed that there was significant negative correlation between HbA1c and all four domains.
- The odds of having DPN (DPN acores≥2.5) was 4.43 times higher among T2DM adults on metformin if they were B12 deficient (B12≤200pg/ml) in comparison to those who had normal B12 (B12>200pg/ml) ($p<0.001$)
- The odds of having DPN (DPN acores≥2.5) was 4.62 times higher among T2DM adults on metformin if they had poor glycemic control (HbA1c>7) in comparison to those who had good glycemic control (HbA1c ≤7) ($p<0.001$).

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SECTION III: INTERVENTION STUDY (RCT): VITAMIN B12 AND CALCIUM VERSUS B12 SUPPLEMENTATION

The section III present results of Phase III which was a randomized control trial which aimed to assess the effect of calcium (500 mg) with B12(1000µg) versus vitamin B12 (1000µg) supplementation for eight weeks on neuropathy, quality of life and vitamin B12 status in type 2 diabetes (T2DM) adults on metformin.

The results of section III have been formulated under the following heads:

- General profile and risk factor profile of the two supplementation group
- Impact of supplementation on glycated Hb, serum B12, MNSI physical assessment total scores/ DPN scores and MNSI history total scores
- Impact of supplementation on prevalence of glycemic control
- Impact of supplementation on prevalence of B12 deficiency
- Impact of supplementation on prevalence of DPN
- Impact of supplementation on MNSI history
- Impact of supplementation on MNSI history score
- Impact of supplementation on MNSI Physical Assessment:
- Impact of supplementation on MNSI Physical Assessment total score /DPN score:
- Impact of supplementation on QoL
- Impact of supplementation on Physical Health domain of QoL
- Impact of supplementation on Psychological Health domain of QoL
- Comparing the pre supplementation or baseline QoL characteristics of the two groups of supplementation for several responses to 26 questions of WHO QoL Bref
- Comparison of impact of supplementation in mean difference (Post-Pre) of serum B12, HbA1c, DPN score, MNSI history score and four domains of QoL in between the two supplementation groups
- Conclusion about supplementation in relation to nutrient-drug interaction (B12-metformin
- Highlights of Phase III

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GENERAL PROFILE AND RISK FACTOR PROFILE OF THE TWO SUPPLEMENTATION GROUP

The T2DM adults in intervention trial belonged to the age group of 42-77y with a mean age of 59 years. The two supplementation groups were matched for their age and duration of diabetes by applying student t test and it was found that they were similar for their age and duration of diabetes (p= 0.497 and p=0.530 respectively) (Table 4.51.a.).

The two supplementation groups were matched for sex, proportion of veg and non veg subjects (including ovo veg) and proportion of various levels of serum B12 by applying chi square test and it was found that the two supplementation groups were similar for the proportion of males and females, similar for the proportion of vegetarians and non-vegetarians (including ovo veg) and similar for the proportion of various levels of B12 [Table 4.51(a)].

The risk profile of the subjects in the two groups is depicted in Table 4.51(b). The two supplementation groups were matched for several risk parameters like WC and BP by student t test and it was found that they were similar for their BMI and WC (p= 0.168 and 0.359). Further the proportion of those with normal BMI and those with abnormal BMI (≥23) were similar as there was no significant difference between the two groups by chi square (p= 0.584). Similarly, the proportion of those with normal WC and those with abnormal WC (≥80 for females and ≥90 for males) were similar as there was no significant difference between the two groups by chi square (p=0.336). As regards BP it was seen that all the subjects in both the groups were hypertensive (BP >80/120). To conclude it can be said that the two supplementation groups were similar in risk profile.

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TABLE 4. 51(a): GENERAL PROFILE OF THE SUPPLEMENTATION GROUPS OF THE T2DM ADULTS (MEAN ± S.D, N, %)

General Profile	Ca+B12 group (N= 50)	Control Group (N=30)	P Value
Age (Y)	58.76 ± 9.04	60.10 ± 7.52	0.497
Duration of diabetes(months)	92.20±65.44	103.10±88.48	0.530
B12≤150	24(48)	20(66.7)	0.104
B12>150	26(52)	10(33.3)	
Veg	29(58)	15(50)	0.05
Non Veg and Ovo veg	21(42)	25(83)	
Females	39(78)	21(70)	0.424
Males	11(22)	9(30)	

() Value in parenthesis denote percentages

TABLE 4.51 (b): RISK FACTOR PROFILE OF THE SUPPLEMENTATION GROUPS OF THE T2DM ADULTS (MEAN ± S.D, N, %)

Parameter	Ca+ B12 Group (N= 50)	B 12 group (N=30)	P Value
Mean BMI	27.48±3.71	25.77±7.27	0.168
Normal BMI[a]	9 (18)	4(13.3)	0.584
Abnormal BMI(BMI>23)[a]	41(82)	26 (86.7)	
Mean W.C	90.46±10.20	92.63±10.18	0.359
Normal W.C[b]	11 (22)	4 (13.33)	0.336
Abnormal WC[b]	39 (78)	26 (86.66)	
Normal B.P.	0	0	N.A
Abnormal B.P.[C] (Pre hypertension+ Hypertension Stage I+ Hypertension Stage II)	50	30	

() Value in parenthesis denote percentages, a=BMI by Asia Pacific Classification 2000, b=WC.by WHO classification, c=B.P. by JNC VIII

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IMPACT OF SUPPLEMENTATION ON GLYCATED Hb, SERUM B12, MNSI PHYSICAL ASSESSMENT TOTAL SCORES/ DPN SCORES AND MNSI HISTORY TOTAL SCORES

In Table 4.52 the two groups of supplementation were matched for glycated Hb, Serum B12, DPN scores and MNSI history total score by applying student t test (Table 4.). It was found that before supplementation there was no difference in the mean glycated Hb ($p=0.80$), mean DPN scores($p=0.292$) and mean MNSI history total score($p=0.869$) of the T2DM adults between the two groups. However, as regards mean serum B12 levels before supplementation there was significant difference in between two groups ($p<0.05$).

After supplementation there was a highly significant ($p<0.001$) decrease in the mean glycated hemoglobin levels in both the groups however the decrease was more in B12 supplemented group than Ca+ B12 supplemented group (Table 4.52).

As regards serum B12, after supplementation there was a highly significant ($p<0.001$) increase in the mean serum B12 levels in both the groups however the increase in Ca+B12 group was more than that in B12 group. However, this increase in serum B12 levels cannot be attributed solely to Calcium supplementation as the mean serum pre supplementation values were higher in B12+ Ca group than the B12 group (Table 4.52).

As regards to DPN scores, after supplementation there was a highly significant ($p<0.001$) decrease in the mean DPN scores of B12 group than Ca+B12 group ($p<0.05$), indicating that B12 intake in T2DM improved DPN in T2DM adults (Table 4.52).

The data on MNSI History total scores, after supplementation showed that there was a highly significant ($p<0.001$) decrease in the mean MNSI History total scores.

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TABLE 4.52: IMPACT OF SUPPLEMENTATION ON GLYCATED HB, SERUM B12, DPN SCORES AND MNSI HISTORY TOTAL SCORES OF T2DM ADULTS

Parameter	Supplementation Stage	Ca+B12 group (N= 50)	B12 group (N= 30)	Student t test p value
Glycated Hb (HbA1c%)	Pre supplementation	8.55±1.72	8.66±1.82	0.80
	Post supplementation	6.54±3.94	3.51±4.74	0.005**
	Paired t test p value	0.000***(↓)	0.000***(↓)	
Serum B12 (pg/ml)	Pre supplementation	157.42±19.08	146.97±22.13	0.028*
	Post supplementation	295.70±60.08	180±37.46	0.000***
	Paired t test p value	0.000***(↑)	0.000***(↑)	
MNSI Physical Assessment Total score/ DPN Scores	Pre supplementation	3.38±1.82	2.93±1.84	0.292
	Post supplementation	3.09±1.97	1.98±1.81	0.014*
	Paired t test p value	.017* (↓)	0.000***(↓)	
MNSI History total score	Pre supplementation	4.96±2.43	5.07±3.33	0.869
	Post supplementation	4.18±2.1	4.30±3	0.834
	Paired t test p value	0.000(↓)	0.000(↓)	

*p<0.05, **p<0.005, ***p<0.001

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IMPACT OF SUPPLEMENTATION ON PREVALENCE OF GLYCEMIC CONTROL

As depicted in Table 4.53 at the beginning of supplementation none of the T2DM adults had normal glycemic control (HbA1c<6%). However, after supplementation over all 38.8% attained normal glycemic control. On the other hand, after supplementation the overall prevalence of poor glycemic control decreased from 73.8% to 48.8%. After supplementation subjects with normal glycemia were more in B12 group (63%) than that in Ca+B12 group. After supplementation, the prevalence of T2DM adults with poor glycemic control (HbA1c>7%) were more in Ca+B12 group (56%) than that in B12group (36.7%).

IMPACT OF SUPPLEMENTATION ON PREVALENCE OF B12 DEFICIENCY

Since we supplemented T2DM adults whose serum B12 were less than equal to 200pg/ml there was 100% B12 deficiency in pre supplementation stage. After supplementation among 80 T2DM adults, a total of 52 (65%) attained normal B12 levels (200pg/ml). There was a highly significant difference ($p<0.001$) in the % prevalence of B12 deficiency in between two supplementation groups (Table 4.54). It was seen that in Ca+B12 group, 46 out of 50 (92%) attained normal serum B12 levels while only 6 out of 30 (20%) attained normal B12 levels in B12 group and these results were highly significant (Fig. 4.24).

IMPACT OF SUPPLEMENTATION ON PREVALENCE OF DPN

As seen in Table 4.55, it can be said that before supplementation there was no significant difference in the % prevalence of DPN in between two groups ($p=0.410$) so the two groups were similar in their DPN prevalence before supplementation.

Over all after supplementation the prevalence of high DPN (DPN scores>2.5) decreased by 17.5% (61.25% to 43.75%) whereas prevalence of no DPN increased by 5% (6.25% to 11.25%).

However there was no significant difference in prevalence of low grade and high grade of DPN between the two groups after supplementation. (Table 4.55)

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TABLE 4.53: IMPACT OF SUPPLEMENTATION ON PREVALENCE OF GLYCEMIC CONTROL

Parameter	B12+Ca Group (N=50)		B12 Group (N=30)		Total (N=80)	
Glycemic control prevalence before supplementation						
HbA1c (Glycemic control)	**n**	**%**	**n**	**%**	**n**	**%**
HbA1c<6% (Normal)	0	0	0	0	0	0
HbA1c >6≤7% (Good)	9	18	12	40	21	26.2
HbA1c >7% (Poor)	41	82	18	60	59	73.8
P value #	0.030*					
Glycemic control prevalence after supplementation						
HbA1c (Glycemic control)	**n**	**%**	**n**	**%**	**n**	**%**
HbA1c<6% (Normal)	12	24	19	63.3	31	38.8
HbA1c -6-7% (Good)	10	20	0	0	10	12.5
HbA1c->8% (Poor)	28	56	11	36.7	39	48.8

*p<0.05, # Chi square is applied in between two categories of good and poor glycemic control

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TABLE 4.54 POST SUPPLEMENTATION PREVALENCE OF B12 DEFICIENCY

Serum B12	Ca+B12 Group (N=50)		B12 Group (N=30)		Total (N=80)	
	n	%	n	%	n	%
≤200pg/ml	4	8	24	80	28	35
>200pg/ml	46	92	6	20	52	65
P Value	P=0.000***					

P<0.001***

FIG. 4.24: POST SUPPLEMENTATION PREVALENCE OF B12 DEFICIENCY AND NORMAL B12 IN BETWEEN TWO GROUPS

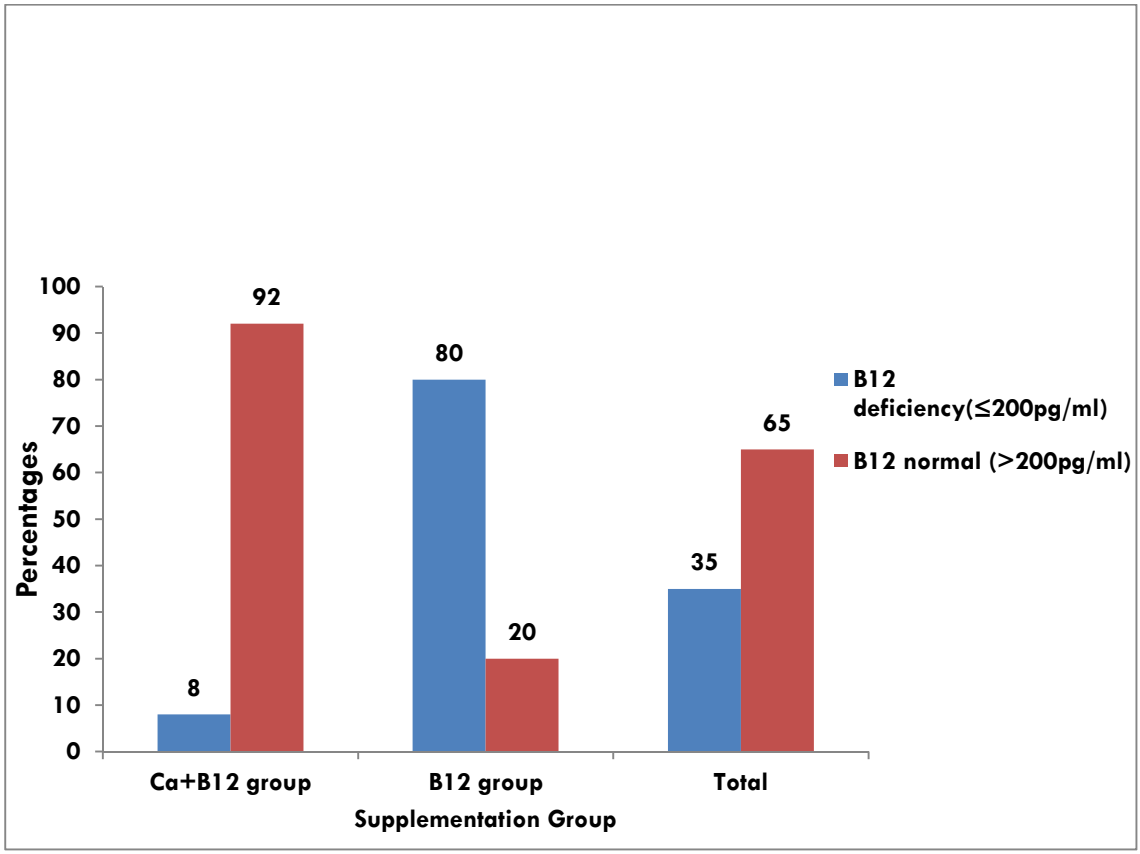

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TABLE 4.55: IMPACT OF SUPPLEMENTATION ON DPN PREVALENCE

Parameter	Ca+B12 group (N=50)		B12 Group (N=30)		Total (N=80)	
DPN prevalence before supplementation						
DPN	**n**	**%**	**n**	**%**	**n**	**%**
DPN scores=0 (NO DPN)	2	4	3	10	5	6.25
DPN scores ≤2.5>0 (Low DPN)	15	30	11	36.7	26	32.5
DPN scores >2.5 (High DPN)	33	66	16	53.3	49	61.25
P value	0.410#					
DPN prevalence after supplementation						
DPN	**n**	**%**	**n**	**%**	**n**	**%**
DPN score=0 (NO DPN)	2	4	7	23.3	9	11.25
DPN scores≤2.5>0 (Low DPN)	21	42	15	50	36	45
DPN scores>2.5 (High DPN)	27	54	8	26.7	35	43.75
P value	0.093#					

#Chi square applied in between two categories: DPN scores≤2.5>0 and DPN scores >2.5 as the category DPN score=0 has cell count less than 5

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IMPACT OF SUPPLEMENTATION ON MNSI HISTORY

Table 4.56 and Table 4.57 shows the comparison of 15 various questions asked regarding the symptoms of past DPN between the two supplementation group in pre and post supplementation stage respectively. By chi square there was no significant difference between the Ca+B12 group and B12 group for any of the 15 questions asked regarding the MNSI history.

IMPACT OF SUPPLEMENTATION ON MNSI HISTORY SCORE

IT was found that the MNSI history score after supplementation and before supplementation had same range from 0-10. However, the mean of MNSI history total score after supplementation decreased from 5.00±2.78 to 4.22±2.45 for the 80 T2DM adults.

Comparison of MNSI History total score between Ca+B12 group and B12 group in pre and post supplementation stage are shown in Table 4.58. Amongst the Ca+B12 group majority (32%) scored 7 while in B12 group majority (16.7%) scored 4 and in Ca+B12 group also there were 16% T2DM subjects who had score of 4. Chi square test was not applicable as several cells had count less than 5.

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TABLE 4.56: COMPARISON BETWEEN CALCIUM+B12 GROUP AND B12 GROUP FOR PRE SUPPLEMENTATION MNSI HISTORY

MNSI History Questions	Total (N=80)		Ca+B12 group (N= 50)		B12 group (N=30)		P value
	n	%	n	%	n	%	
1.Legs/feet numb							
Yes	44	55	30	60	14	46.7	0.246
No	36	45	20	40	16	53.3	
2.Burning pain in legs/feet							
Yes	27	33.7	18	36	9	30	0.583
No	53	66.3	32	64	21	70	
3.Feet Sensitive to touch							
Yes	30	37.5	16	32	14	46.7	0.190
No	50	62.5	34	68	16	53.3	
4.Muscle cramps in legs/feet							
Yes	39	48.8	23	46	16	53.3	0.343
No	41	51.2	27	54	14	46.7	
5.Prickly feelings in legs/feet							
Yes	26	32.5	17	34	9	30	0.712
No	54	67.5	33	66	21	70	
6.Hurt when bed cover touches skin							
Yes	39	48.7	27	54	12	40	0.225
No	41	51.3	23	46	18	60	
7.When into tub/shower, able to tell the hot water from cold water							
Yes	54	67.5	33	66	21	70	0.454
No	26	32.5	17	34	9	30	
8.Had an open sore on foot							
Yes	9	11.3	2	4	7	23.3	N.A

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No	71	88.7	48	96	23	76.7	
9.Doctor told you about diabetic neuropathy							
Yes	8	10	7	14	1	3.3	N.A
No	72	90	43	86	29	96.7	
10.Feel weak most of the time							
Yes	23	28.8	15	30	8	26.7	0.750
No	57	71.2	35	70	22	73.3	
11.Symptoms worse at night							
Yes	41	51.2	25	50	16	46.7	0.773
No	39	48.8	25	50	14	53.3	
12.Legs hurt when you walk							
Yes	40	50	26	52	14	46.7	0.644
No	40	50	24	48	16	53.3	
13.Sense your feet when you walk							
Yes	76	95	48	96	28	93.3	N.A
No	4	5	2	4	2	6.7	
14.Skin on feet so dry that it cracks open							
Yes	52	65	32	64	20	66.7	0.059
No	28	35	18	36	10	33.3	
15.Have you ever had an amputation							
Yes	0	0	0	0	0	0	N.A
No	80	100	50	100	30	100	

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TABLE 4.57: COMPARISON BETWEEN Ca+B12 AND B12 GROUP FOR POST SUPPLEMENTATION MNSI HISTORY

MNSI History Questions	Total (N=80)		Experimental (N= 50)		Control (N=30)		P value
	n	%	N	%	n	%	
1.Legs/feet numb							
Yes	36	45	20	40	16	53.3	0.246
No	44	55	30	60	14	46.7	
2.Burning pain in legs/feet							
Yes	27	33.8	18	36	9	30	0.583
No	53	66.2	32	64	21	70	
3.Feet Sensitive to touch							
Yes	30	37.5	16	32	14	46.7	0.190
No	50	62.5	34	68	16	53.3	
4.Muscle cramps in legs/feet							
Yes	39	48.8	23	46	16	53.3	0.525
No	41	51.2	27	54	14	46.7	
5.Prickly feelings in legs/feet							
Yes	26	32.5	17	34	9	30	0.712
No	54	67.5	33	66	21	70	
6.Hurt when bed cover touches skin							
Yes	39	48.8	27	54	12	40	0.225
No	41	51.2	23	46	18	60	
7.When into tub/shower, able to tell the hot water from cold water							
Yes	26	32.5	17	34	9	30	0.712
No	54	67.5	21	70	33	66	
8.Had an Open sore on foot							
Yes	9	11.2	7	23.3	2	4	N.A
No	71	88.8	23	76.7	48	96	
9.Doctor told you about diabetic neuropathy							
Yes	8	10	1	3.3	7	14	N.A
No	72	90	29	96.7	43	86	

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10.Feel weak most of the time							
Yes	23	28.7	15	30	8	26.7	0.750
No	57	71.2	35	70	22	73.3	
11.Symptoms worse at night							
Yes	41	51.2	25	50	16	53.3	0.773
No	39	48.8	25	50	14	46.7	
12.Legs hurt when you walk							
Yes	40	50	26	52	14	46.7	0.644
No	40	50	24	48	16	53.3	
13.Sense your feet when you walk							
Yes	4	5	2	4	2	6.7	0.596
No	76	95	48	96	28	93.3	
14.Skin on feet so dry that it cracks open							
Yes	52	65	32	64	20	66.7	.809
No	28	35	18	36	10	33.3	
15.Have you ever had an amputation							
Yes	0	0	0	0	0	0	N.A
No	80	100	50	100	30	100	

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TABLE 4.58: MNSI HISTORY TOTAL SCORE BETWEEN Ca+B12 AND B12 GROUP IN PRE AND POST SUPPLEMENTATION STAGE

Pre Supplementation Stage						
MNSI History Total score	**Ca+B12 group (N=50)**		**B12 group (N=30)**		**Total (N=80)**	
	n	**%**	**n**	**%**	**N**	**%**
0	1	2	2	6.7	3	3.8
1	2	4	4	13.3	6	7.5
2	8	16	1	3.3	9	11.2
3	6	12	4	13.3	10	12.5
4	8	16	5	16.7	13	16.2
5	0	0	1	3.3	1	1.2
6	2	4	3	10	5	6.2
7	16	32	0	0	16	20
8	7	14	3	10	10	12.5
9	0	0	3	10	3	3.8
10	0	0	4	13.3	4	5
Post Supplementation Stage						
MNSI History Total score	**Ca+B12 group (N=50)**		**B12 group (N=30)**		**Total (N=80)**	
	n	**%**	**n**	**%**	**N**	**%**
0	1	2	3	10	4	5
1	2	4	4	13.3	6	7.5
2	14	28	2	6.7	16	20
3	4	8	4	13.3	8	10
4	4	8	4	13.3	8	10
5	10	20	2	6.7	12	15
6	5	10	4	13.3	9	11.2
7	9	18	2	6.7	11	13.8
8	1	2	1	3.3	2	2.5
9	0	0	3	10	3	3.8
10	0	0	1	3.3	1	1.2

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IMPACT OF SUPPLEMENTATION ON MNSI PHYSICAL ASSESSMENT:

Various questions used to assess MNSI Physical Assessment were compared between the two groups in pre supplementation and post supplementation stage for right foot and left foot & is shown in Table 4.59. and Table 4.60 respectively. Chi square test was not applicable in majority of questions as there were several cells with count less than 5.

As regards MNSI physical assessment of right foot post supplementation it was found that there was significant difference in between Ca+B12 group and B12 group for vibration perception at great toe ($p<0.05$) (Table 4.59). This indicates that calcium supplementation along with B12 supplementation is better in improving vibration perception at great toe over and above B12 supplementation alone.

MNSI physical assessment score of right foot obtained in the study sample were 0, 0.5,1,1.5,2,2.5,3 and 3.5 and the comparison of MNSI physical assessment score of right foot between Ca+B12 group and B12 group is shown in Fig. 4.25. This Fig. shows how the proportion of various right foot scores changed after supplementation in Ca+B12 group and B12 group. However, the difference between the two groups cannot be tested statistically by chi square because several cells have count less than 5.

MNSI physical assessment score of left foot obtained in the study sample were 0, 0.5,1,1.5,2,2.5 and 3 and the comparison of MNSI physical assessment score of left foot between Ca+B12 group and B12 group is shown in Fig. 4.26. This Fig. shows how the proportion of various right foot scores changed after supplementation in Ca+B12 group and B12 group. However, the difference between the two groups cannot be tested statistically by chi square because several cells have count less than 5.

The impact of supplementation on mean scores of right foot and left foot are shown in Table 4.61. Each foot score was computed by a sum of individual scores of appearance of feet, ulceration, ankle reflex, vibration perception at great toe and monofilament score. There were no cases of ulceration among the 80 supplemented T2DM adults on metformin.

As regards right foot, the mean scores of appearance of foot showed a significant difference between Ca+B12 group and B12 group in post supplementation stage

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($p<0.05$). There was also a significant difference in mean scores of ankle reflexes between the two groups ($p<0.05$) in post supplementation stage. There was very significant difference in mean scores of vibration perception at great toe ($p<0.01$) in post supplementation stage. Further there was very significant difference when before and after supplementation mean scores were compared by paired t for both the groups. The mean foot score of right foot showed a very significant difference in post supplementation stage($p<0.01$) and further the pre and post mean scores when compared by paired t it showed a highly significant difference for B12 group ($p<0.001$) but not for the Ca+B12 group.

As regards left foot the mean scores of appearance of foot showed a significant difference between the two groups ($p<0.05$) in post supplementation stage. When pre and post mean scores of appearance of foot were compared by paired t test there was a significant difference in mean scores for Ca+B12 group but not for B12 group. There was a highly significant difference in pre and post supplementation mean scores of vibration perception at great toe for Ca+B12 group ($p<0.001$) as well as B12 ($p<0.01$). Further the pre and post mean left foot score showed a very significant difference for both the groups ($p<0.01$).

It was found that among the various components of MNSI physical assessment it was the vibration perception at great toe which showed significant differences in pre and post supplementation mean scores (when compared by paired t test) for both the feet. So it can be said that the vibration perception at great toe was the crucial contributor for bringing decrease in the DPN scores after supplementation.

IMPACT OF SUPPLEMENTATION ON PHYSICAL ASSESSMENT MNSI TOTAL SCORE /DPN SCORE:

It was found that in total the DPN score after supplementation and before supplementation had same range from 0-6. However, the mean of DPN score after supplementation decreased from 3.21±1.83 to 2.68 ± 1.98 for the 80 T2DM adults.

Comparison of DPN score between Ca+B12 group and B12 group in pre and post supplementation stage are shown in Table 4.62. Amongst the Ca+B12 group majority (38 %) scored 4 while in B12 group majority (26.7 %) scored 2. Chi square test was not applicable as several cells had count less than 5.

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TABLE 4.59: COMPARISON OF MNSI PHYSICAL ASSESSMENT OF RIGHT FOOT BETWEEN CA+B12 GROUP AND B12 GROUP

Physical Assessment by MNSI	Ca+B12 Group (N=50)		B12 Group (N=30)		Total (N=80)	
Pre supplementation						
	n	**%**	**n**	**%**	**N**	**%**
1. Appearance of feet						
a. Normal	40	80	28	93.3	68	85
b. Abnormal	10	20	2	6.7	12	15
P value	N.A					
-Deformities						
Yes	0	0	4	13.3	4	5
No	50	100	26	86.7	76	95
P value	N.A					
-Dry skin, callus						
Yes	12	24	3	10	15	18.75
No	38	76	27	90	65	81.25
p value	N.A					
-Infection						
Yes	7	14	0	0	7	8.8
No	43	86.0	30	100	73	91.2
p value	N.A					
-Fissure						
Yes	7	14	0	0	7	8.8
No	43	86	30	100	73	91.2
p value	N.A					
2.Ulceration						
a. Absent	50	100	30	100	80	100
b. Present	0	0	0	0	0	0
	N.A					
3.Ankle Reflexes						
a. Present	15	30	10	33.3	25	31.2
b. Present /Reinforcement	2	4	2	6.7	4	5
c. Absent	33	66	18	60	51	63.7
P value	N.A					
4. Vibration Perception at great toe						
a. Present	11	22	10	33.3	21	26.2
b. Decreased	6	12	4	13.3	10	12.5
c. Absent	33	66	16	53.3	49	61.3

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P value	N.A					
5.Monofilament						
Normal	42	84	24	80	66	82.5
Reduced	3	6	0	0	3	3.8
Absent	5	10	6	20	11	13.8
P value	N.A					
Post supplementation						
	n	**%**	**n**	**%**	**N**	**%**
1.Appearance of feet						
a. Normal	43	86	30	100	73	91.2
b. Abnormal	7	14	0	0	7	8.8
p value	N.A					
-Deformities						
No	50	100	26	86.7	76	95
Yes	0	0	4	13.3	4	5
p value	N.A					
-Dry skin, callus						
Yes	9	18	3	10	12	15
No	41	82	27	90	68	85
p value	N.A					
-Infection						
Yes	7	14	0	0	7	8.8
No	43	86	30	100	73	91.2
p value	N.A					
-Fissure						
Yes	7	14	0	0	7	8.8
No	43	86	30	100	73	91.2
	N.A					
2.Ulceration						
a. Absent	50	100	30	100	80	100
b. Present	0	0	0	0	0	0
p value	N.A					
3.Ankle Reflexes						
a. Present	15	30	14	46.7	29	36.2
b. Present /Reinforcement	5	10	6	20	11	13.8
c. Absent	30	60	10	33.3	40	50
p value	0.065					
4.Vibration Perception at great toe						
Present	15	30	17	56.7	32	40

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Decreased	9	18	6	20	15	18.8
Absent	26	52	7	23.3	33	41.2
P value	0.029*					
5.Monofilament						
Normal	37	74	24	80	61	76.2
Reduced	7	14	1	3.3	8	10
Absent	6	12	5	16.7	11	13.8
P value	N.A					

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TABLE 4.60: COMPARISON OF MNSI PHYSICAL ASSESSMENT OF LEFT FOOT BETWEEN CA+B12 GROUP AND B12 GROUP

Physical Assessment by MNSI	Ca+B12 Group (N=50)		B12 Group (N=30)		Total (N=80)	
Pre supplementation						
	n	**%**	**n**	**%**	**N**	**%**
1. Appearance of feet						
a. Normal	43	86	28	93.3	71	88.8
b. Abnormal	7	14	2	6.7	9	11.2
P value	N.A					
-Deformities						
Yes	0	0	4	13.3	4	5
No	50	100	26	86.7	76	95
p value	N.A					
-Dry skin, callus						
Yes	41	82	27	90	68	85
No	9	18	3	10	12	15
p value	N.A					
-Infection						
Yes	7	14	0	0.0	7	8.8
No	43	86	30	100	73	91.2
p value	N.A					
-Fissure						
Yes	7	14	0	0	7	8.8
No	43	86	30	100	73	91.2
P value	N.A					
2.Ulceration						
a. Absent	50	100	30	100	80	100
b. Present	0	0	0	0	0	0
P value	N.A					
3.Ankle Reflexes						
a. Present	17	34	11	36.7	28	35
b. Present /Reinforcement	2	4	2	6.7	4	5
c. Absent	31	62	17	56.7	48	60
P value	N.A					
4. Vibration Perception at great toe						
a. Present	10	20	11	36.7	21	26.2
b. Decreased	6	12	4	13.3	10	12.5

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c.Absent	34	68	15	50	49	61.3
P value	N.A					
5.Monofilament						
Normal	42	84	24	80	66	82.5
Reduced	3	6	0	0	3	3.8
Absent	5	10	6	20	11	13.8
P value	N.A					
Post supplementation						
	n	**%**	**n**	**%**	**N**	**%**
1.Appearance of feet						
a. Normal	43	86	30	100	73	91.2
b. Abnormal	7	14	0	0	7	8.8
P value	N.A					
-Deformities						
No	50	100	26	86.7	76	95
Yes	0	0	4	13.3	4	5
p value	N.A					
-Dry skin, callus						
No	41	82	27	90	68	85
Yes	9	18	3	10	12	15
p value	N.A					
-Infection						
No	43	86	30	100	73	91.2
Yes	7	14	0	0	7	8.8
p value	N.A					
-Fissure						
No	43	86	30	100	73	91.2
Yes	7	14	0	0	7	8.8
2.Ulceration						
a. Absent	50	100	30	100	80	100
b. Present	0	0	0	0	0	0
p value	N.A					
3.Ankle Reflexes						
a. Present	18	36	14	46.7	32	40
b.Present /Reinforcement	4	8	4	13.3	8	10
c. Absent	28	56	12	40	40	50
p value	N.A					

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4.Vibration Perception at great toe						
Present	15	30	17	56.7	32	40
Decreased	16	32	3	10	19	23.8
Absent	19	38	10	33.3	29	36.2
p value	N.A					
5.Monofilament						
Normal	38	76	24	80	62	77.5
Reduced	8	16	1	3.3	9	11.2
Absent	4	8	5	16.7	9	11.2
P value	N.A					

FIG. 4.25:COMPARISON OF PRE AND POST SUPPLEMENTATION MNSI PHYSICAL ASSESSMENT SCORE OF RIGHT FOOT

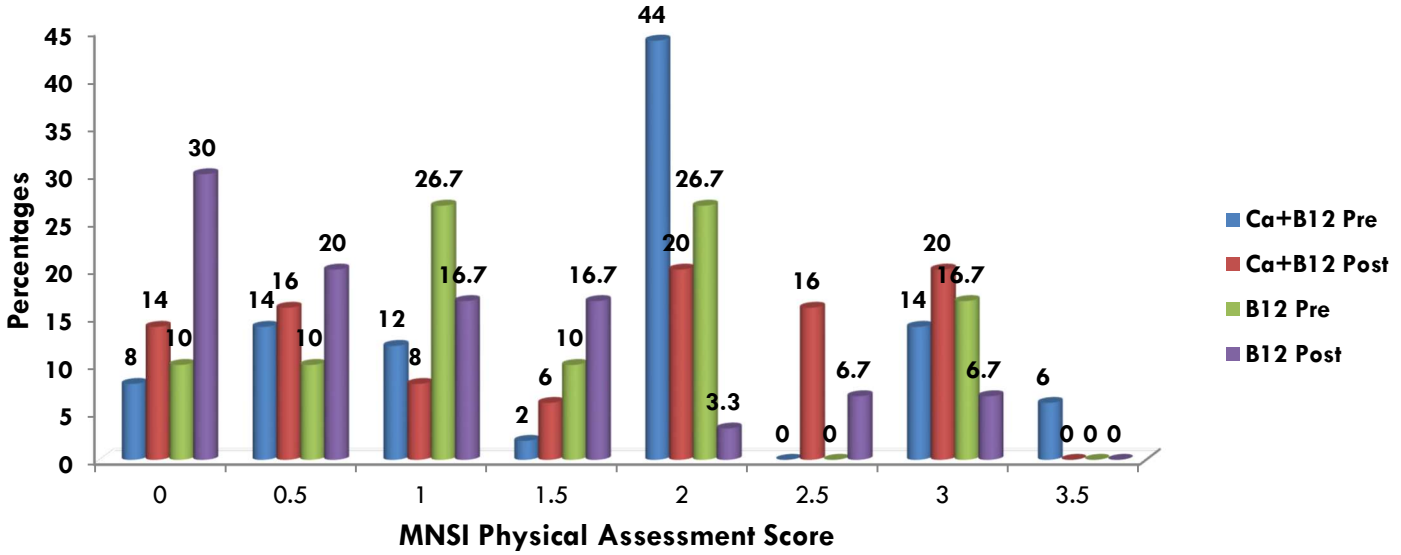

FIG.4.26: COMPARISON OF PRE AND POST SUPPLEMENTATION MNSI SCORES OF PHYSICAL ASSESMENT OF LEFT FOOT

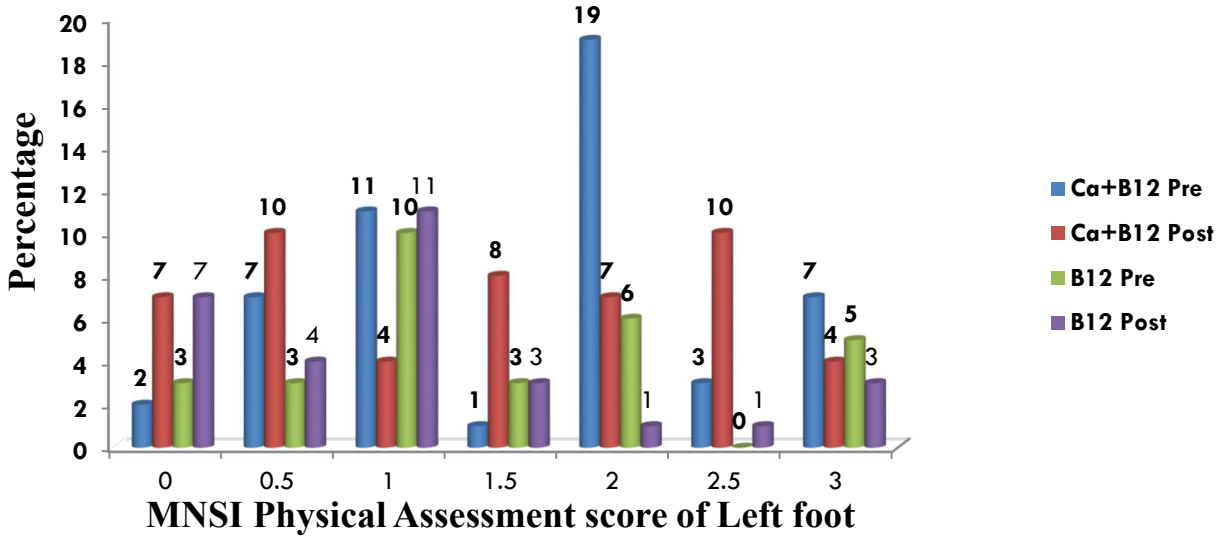

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TABLE 4.61: IMPACT OF SUPPLEMENTATION ON MNSI PHYSICALASSESSMENT MEAN SCORES OF RIGHT FOOT AND LEFT FOOT (MEAN±S.D)

Physical Assessment	Stage	Right Foot. Ca+B12 group	Right Foot B12 group	P value	Left Foot Ca+B12 group	Left Foot B12 group	P value
1.Appearance of feet	Pre	.204±.404	.07±.254	.109	.14±.351	.07±.254	.321
	Post	.20±.404	.00±.000	.032*	.14±.351	.00±.000	.032*
	Paired t	.161	-		.046*	.161	
2.Ulceration	Pre	.00	.00	.00	.00	.00	.00
	Post	.00	.00	.00	.00	.00	.00
	Paired t	-	-		-	-	
3.Ankle Reflex	Pre	.68±.460	.63±.472	.665	.64±.474	.60±.481	.717
	Post	.65±0.455	.43±.450	.023*	.60±.474	.47±.472	.226
	Paired t	.083	.001		.103	.009	
4.Vibration Perception at great toe	Pre	.72±.418	.60±.462	.236	.74±.407	.57±.469	.086
	Post	.61±.444	.33±.422	.007**	.54±.415	.38±.468	.123
	Paired t	0.003**	.001**		.000***	.009**	
5.Monofilament	Pre	.13±.316	.20±.407	.393	.13±.316	.20±.407	.393
	Post	.19±.348	.18±.382	.937	.16±.310	.18±.382	.766
	Paired t	0.278	0.326		.371	.326	
6.Foot Score	Pre	1.73±.991	1.50±.928	.307	1.65±.865	1.43±.926	.294
	Post	1.65±1.075	.95±.932	.004**	1.44±.983	1.03±.909	.069
	Paired t	.262	.000***		.001**	.001**	

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TABLE 4.62: COMPARISON OF DPN SCORE BETWEEN CA+B12 GROUP AND B12 GROUP IN PRE AND POST SUPPLEMENTATION STAGE

Pre Supplementation Stage						
DPN score	**Ca+B12 group (N=50)**		**B12 group (N=30)**		**Total (N=80)**	
	n	**%**	**n**	**%**	**N**	**%**
0	2	4	3	10	5	6.2
1	9	18	3	10	12	15
2	6	12	8	26.7	14	17.5
3	4	8	5	16.7	9	11.2
4	19	38	6	20	25	31.2
6	10	20	5	16.7	15	18.8
Post Supplementation Stage						
DPN Score	**Ca+B12 group (N=50)**		**B12 group (N=30)**		**Total (N=80)**	
	n	**%**	**n**	**%**	**N**	**%**
0	2	4	7	23.3	9	11.2
0.5	7	14	1	3.3	8	10
1	6	12	4	13.3	10	12.5
1.5	2	4	3	10	5	6.2
2	2	4	5	16.7	7	8.8
2.5	4	8	2	6.7	6	7.5
3	0	0	3	10	3	3.8
3.5	4	8	0	0	4	5
4	4	8	1	3.3	5	6.2
4.5	4	8	0	0	4	5
5	8	16	0	0	8	10
5.5	4	8	3	10	7	8.8
6	3	6	1	3.3	4	5

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IMPACT OF SUPPLEMENTATION ON QOL

In Table 4.63 the mean scores of each domain indicates the individual's perception of their satisfaction with each aspect of their life, relating it with quality of life. The higher the score, the better this is perceived to be.

Total Scores of the 4 domains: The pre-post results of the supplementation of overall scores of the 4 domains are given in Table 4.63. As can be seen from the table, there was significant improvement in both the groups after supplementation in two domains i.e. physical health ($p<0.001$) & psychological health ($p<0.01$) with no changes in social relationship and environmental domains.

The Pre & Post supplementation scores for the 26 facets of QoL between the two groups are depicted in Table 4.64 & 4.65 respectively. There was significant difference ($p<0.05$) in post supplementation mean QoL scores in between two groups for several facets of QoL like overall QoL, physical pain and discomfort, need of medical treatment in daily life, accept bodily appearance, activities of daily living, sex life and negative feelings in life.

Further there was very significant difference($p<0.01$) in post supplementation mean QoL scores in between the two groups for several facets of QLI like safety in daily life, how healthy is physical environment, financial resources, opportunity for acquiring new information, leisure activities, work capacity, personal relationship, condition of living place, satisfaction with access to health services, satisfaction with transport.

IMPACT OF SUPPLEMENTATION ON PHYSICAL HEALTH DOMAIN OF QOL

Impact of supplementation on various facets of quality of life of physical Health domain are depicted in Table 4.66 to Table 4.71. Among the various facets of quality of life in physical health domain the difference between the two groups cannot be compared because Chi square cannot be applied as cells have count less than five.

IMPACT OF SUPPLEMENTATION ON PSYCHOLOGICAL HEALTH DOMAIN

Impact of supplementation on various facets of quality of life of Psychological Health domain are depicted in Table 4.73 to Table 4.78. Among the various facets of quality of

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TABLE 4.63: IMPACT OF SUPPLEMENTATION ON MEAN SCORES OF VARIOUS DOMAINS OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHO QOL BREF

Domains of WHO QOL BREF	Stage	Ca+ B12group (N= 50) Mean ± SD	B12group (N= 30) Mean ± SD	Student t test p value
Physical Health	Pre Supplementation	20.64 ± 3.10	22.10 ± 2.98	0.042*
	Post Supplementation	23.42 ± 1.93	23.47 ± 1.91	0.916
	Paired t test p value	0.001***	0.008**	
Psychological Health	Pre Supplementation	16.78 ± 3.07	17.83 ± 3.05	0.140
	Post Supplementation	17.62 ± 2.54	18.60 ± 2.75	0.109
	Paired t test p value	0.003**	0.031**	
Social Relationship	Pre Supplementation	8.12 ± 2.50	10.03 ± 1.99	0.001**
	Post Supplementation	8.66 ± 2.34	10.03 ± 1.83	0.005**
	Paired t test p Value	0.015	1.000	
Environment	Pre Supplementation	21.04 ± 6.35	25.57 ± 5.98	0.002**
	Post Supplementation	21.30 ± 5.95	26.13 ± 5.11	0.001**
	Paired t test p value	0.427	0.173	

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TABLE 4.64: PRE SUPPLEMENTATION MEAN SCORES OF 26 FACETS OF QOL IN WHOQOL BREF IN CA+B12 GROUP AND B12 GROUP

26 FACETS OF QOL	Pre Supplementation stage		P Value
	Ca+B12 Group (N=50)	B12 Group (N=30)	
	Mean ± SD	Mean ± SD	
How would you rate your quality of life?	2.7 ± 0.84	3.17 ± 0.79	.016*
How satisfied are you with your health?	2.68 ± 0.79	2.73 ± 0.58	0.731
To what extent do you feel that physical pain prevents you from doing what you need to do?	2.56 ± 0.76	3.03 ± 0.89	0.014*
How much do you need any medical treatment to function in your daily life?	2.52 ± 0.79	2.87 ± 0.68	0.049
How much do you enjoy life?	2.62 ± 0.83	2.87 ± 0.78	0.191
To what extent do you feel your life to be meaningful?	2.60 ± 0.90	2.97 ± 1.03	0.100
How well are you able to concentrate?	2.68 ± 0.79	2.87 ± 0.86	0.327
How safe do you feel in your daily life?	2.60 ± 0.76	3.10 ± 0.80	0.006*
How healthy is your physical environment?	2.56 ± 0.84	3.00 ± 0.87	0.028*
Do you have enough energy for everyday life?	2.72 ± 0.90	3.03 ± 0.89	0.135
Are you able to accept your bodily appearance?	2.78 ± 0.89	3.13 ± 0.86	0.085
Have you enough money to meet your needs?	2.60 ± 0.86	3.20 ± 0.85	0.003*
How available to you is the information that you need in your day-to-day life?	2.60 ± 0.93	3.17 ± 0.91	0.009*
To what extent do you have the opportunity for leisure	2.66 ± 0.98	3.20 ± 0.93	0.017*

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activities?			
How well are you able to get around?	2.70 ± 0.86	3.20 ± 0.76	0.011*
How satisfied are you with your sleep?	2.74 ± 0.94	3.30 ± 0.88	0.010*
How satisfied are you with your ability to perform your daily living activities?	2.80 ± 0.83	3.20 ± 0.85	0.042*
How satisfied are you with your capacity for work?	2.76 ± 0.92	3.27 ± 0.79	0.014*
How satisfied are you with yourself?	2.78 ± 0.89	3.23 ± 0.77	0.023*
How satisfied are you with your personal relationships?	2.64 ± 0.90	3.33 ± 0.71	0.001**
How satisfied are you with your sex life?	2.76 ± 0.87	3.43 ± 0.63	0.001**
How satisfied are you with the support you get from your friends?	2.72 ± 0.86	3.27 ± 0.74	0.005*
How satisfied are you with the conditions of your living place?	2.66 ± 0.94	3.37 ± 0.77	0.001**
How satisfied are you with your access to health services?	2.66 ± 1.00	3.2 ± 0.93	0.019*
How satisfied are you with your transport?	2.70 ± 0.89	3.33 ± 0.80	0.002**
How often do you have negative feelings such as blue mood, despair, anxiety, depression?	2.68 ± 0.94	3.23 ± 0.90	0.011*

*p<0.05, **p<0.005

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TABLE 4.65: POST SUPPLEMENTATION MEAN SCORES OF 26 FACETS OF QOL IN WHOQOL BREF BY SUPPLEMENTATION GROUPS OF T2DM ADULTS

26 FACETS OF QOL	Post Supplementation stage		P Value
	Ca+B12Group (N=50)	B12 Group (N=30)	
	Mean ± SD	Mean ± SD	
How would you rate your quality of life?	2.7±0.84	3.13±.82	0.027*
How satisfied are you with your health?	2.68±.79	2.77±.57	0.603
To what extent do you feel that physical pain prevents you from doing what you need to do?	2.60±083	3.17±1.08	0.010*
How much do you need any medical treatment to function in your daily life?	2.56±.81	3±.91	0.028*
How much do you enjoy life?	2.76±.77	2.87±.78	0.552
To what extent do you feel your life to be meaningful?	2.82±0.80	3.07±.94	0.216
How well are you able to concentrate?	2.94±0.65	3.20±.80	0.118
How safe do you feel in your daily life?	2.56±.76	2.64±.75	0.004**
How healthy is your physical environment?	3.34±.63	3.27±.69	0.037**
Do you have enough energy for everyday life?	3.34±.63	3.27±.69	0.627
Are you able to accept your bodily appearance?	2.98±.71	3.37±.72	0.022*
Have you enough money to meet your needs?	2.66±.82	3.33±.80	0.001**
How available to you is the information that you need in your day-to-day life?	2.62±.86	3.20±.89	.005**

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To what extent do you have the opportunity for leisure activities?	2.64±.94	3.33±0.76	.001**
How well are you able to get around?	3.44±.66	3.67±.711	0.158
How satisfied are you with your sleep?	3.44±.70	3.6±.67	0.321
How satisfied are you with your ability to perform your daily living activities?	3.30±.65	3.63±.56	0.021*
How satisfied are you with your capacity for work?	3.06±.71	3.57±.57	0.001**
How satisfied are you with yourself?	2.96±.90	3.37±.72	0.015
How satisfied are you with your personal relationships?	2.72±.88	3.37±.76	0.001**
How satisfied are you with your sex life?	3.02±.87	3.37±.81	0.080*
How satisfied are you with the support you get from your friends?	2.92±.88	3.23±.73	0.104
How satisfied are you with the conditions of your living place?	2.74±.97	3.37±.56	0.002**
How satisfied are you with your access to health services?	2.68±.99	3.27±87	0.009**
How satisfied are you with your transport?	2.72±.90	3.33±.80	0.003**
How often do you have negative feelings such as blue mood, despair, anxiety, depression?	2.84±1	3.30±1	0.048*

*p<0.01,**p<0.05

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TABLE 4.66: DIFFERENCES IN THE POST SUPPLEMENTATION MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION ON '*PHYSICAL PAIN AND DISCOMFORT'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

To what extent do you feel that physical pain prevents you from doing what you need to do?	Post Supplementation Stage				P Value
	Ca+B12Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
An extreme amount	2	4	0	0	N.A
Very much	24	10	10	33.3	
A moderate amount	17	10	10	33.3	
A little	6	5	5	16.7	
Not at all	1	5	5	16.7	
Total	50	100	30	100	

TABLE 4.67 DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON *'DEPENDENCE ON MEDICINAL SUBSTANCES AND MEDICAL AIDS'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How much do you need any medical treatment to function in your daily life?	Post Supplementation Stage				P Value
	Ca+B12 Group (N= 50)		B12 Group (N=30)		
	n	%	n	%	
An extreme amount	3	6	0	0	N.A
Very much	23	46	10	33.3	
A moderate amount	17	34	12	40	
A little	7	14	6	20	
Not at all	0	0	2	6.7	
Total	50	100	30	100	

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TABLE 4.68: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN TWO GROUPS IN POST SUPPLEMENTATION STAGE ON *'ENERGY AND FATIGUE'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

Do you have enough energy for everyday life?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	0	0	0	0	N.A
A little	2	4	2	6.7	
Moderately	31	62	20	66.7	
Mostly	15	30	6	20	
Completely	2	4	2	6.7	
Total	50	100	30	100	

TABLE 4.69: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*MOBILITY'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How well are you able to get around?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Poor	0	0	0	0	N.A
Poor	3	6	1	3.3	
Neither Poor nor Good	24	48	11	36.7	
Good	21	42	15	50	
Very good	2	4	3	10	
Total	50	100	30	100	

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TABLE 4.70: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*SLEEP AND REST'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your sleep?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	0	0	0	0	N.A
Dissatisfied	4	8	1	3.3	
Neither Satisfied nor Dissatisfied	22	44	12	40	
Satisfied	22	44	15	50	
Very Satisfied	2	4	2	6.7	
Total	50	100	30	100	

TABLE 4.71: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON *'ACTIVITIES OF DAILY LIVING'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your ability to perform your daily living activities?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	0	0	0	0	N.A
Dissatisfied	5	10	0	0	
Neither Satisfied nor Dissatisfied	25	50	12	40	
Satisfied	20	40	17	56.7	
Very satisfied	0	0	1	3.3	
Total	50	100	30	100	

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TABLE 4.72: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON *'WORK CAPACITY'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your capacity for work?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	0	0	0	0	N.A
Dissatisfied	10	20	1	3.3	
Neither Satisfied nor Dissatisfied	28	56	11	36.7	
Satisfied	11	22	18	60	
Very satisfied	1	2	0	0	
Total	50	100	30	100	

TABLE 4.73: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*POSITIVE FEELING'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How much do you enjoy life?	Post Supplementation stage				P Value
	Ca+B12Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
A little	0	0	0	0	N.A
A moderate amount	22	44	11	36.7	
Very much	18	36	12	40	
An extreme amount	10	20	7	23.3	
Total	50	100	30	100	

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TABLE 4.74: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*PERSONAL BELIEFS'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

To what extent do you feel your life to be meaningful?	Post Supplementation Stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	1	2	0	0	N.A
A little	16	32	8	26.7	
A moderate amount	26	52	16	53.3	
Very much	5	10	2	6.7	
An extreme amount	2	4	4	13.3	
Total	50	100	30	100	

TABLE 4.75: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION ON '*CONCENTRATION'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How well are you able to concentrate?	Post Supplementation Stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	1	2	0	0	N.A
A little	9	18	6	20	
A moderate amount	32	64	13	43.3	
Very much	8	16	10	33.3	
An extreme amount	0	0	1	3.3	
Total	50	100	30	100	

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TABLE 4.76: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*BODILY IMAGE AND APPEARANCE'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

Are you able to accept your bodily appearance?	Post Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	1	2	0	0	N.A
A little	10	20	3	10	
Moderately	28	56	14	46.7	
Mostly	11	22	12	40	
Completely	0	0	1	3.3	
Total	50	100	30	100	

TABLE 4.77: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN POST SUPPLEMENTATION STAGE ON '*SELF ESTEEM'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with yourself?	Post Supplementation Stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	0	0	0	0	N.A
Dissatisfied	13	26	4	13.3	
Neither Satisfied nor Dissatisfied	26	52	11	36.7	
Satisfied	11	22	15	50	
Total	50	100	30	100	

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TABLE 4.78: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS OF SUPPLEMENTATION ON *'NEGATIVE FEELINGS'* FACET OF 'PSYCHOLOGICAL' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How often do you have negative feelings such as blue mood, despair, anxiety, depression?	Post Supplementation Stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Always	3	6	1	3.3	N.A
Very Often	18	36	5	16.7	
Quiet Often	15	30	11	36.7	
Seldom	12	24	10	33.3	
Never	2	4	3	10	
Total	50	100	3	100	

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psychological domain the difference between the two groups cannot be compared because Chi square cannot be applied as cells have count less than five.

COMPARING THE PRE SUPPLEMENTATION OR BASELINE QOL CHARACTERISTICS OF THE TWO GROUPS OF SUPPLEMENTATION FOR SEVERAL RESPONSES TO 26 QUESTIONS OF WHO QOL BREF

The baseline values for the responses to two individual questions of QoL - 'Over all quality of life' and 'satisfaction with health' for the two groups are depicted in Table 4.79 and Table 4.80 respectively. But responses to these questions cannot be compared between two groups as Chi square cannot be applied because several cells have count less than five.

The baseline values for the responses to the various facets of Physical Health domain of QoL for the two groups are depicted from Table 4.81 to 4.87. But responses to these several facets cannot be compared between the two groups as Chi square cannot be applied because several cells have count less than five.

The baseline values for the responses to the various facets of Psychological Health domain of QoL for the two groups are depicted from Table 4.88 to 4.93. But responses to these several facets cannot be compared between the two groups as Chi square cannot be applied because several cells have count less than five.

The baseline values for the responses to the various facets of Social Relationship domain of QoL for the two groups are depicted from Table 4.94 to 4.96. But responses to these several facets cannot be compared between the two groups as Chi square cannot be applied because several cells have count less than five.

The baseline values for the responses to the various facets of Environment Health domain of QoL for the two groups are depicted from Table 4.97 to 4.104. But responses to these several facets cannot be compared between the two groups as Chi square cannot be applied because several cells have count less than five.

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TABLE 4:79: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON OVERALL QUALITY FACET OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How would you rate your quality of life?	Pre Supplementation stage				P Value
	Ca+ B12 group (N=50)		B12 group (N=30)		
	n	%	n	%	
Very Poor	2	4.0%	0	0.0%	N.A
Poor	20	40.0%	5	16.7%	
Neither Poor nor Good	20	40.0%	17	56.7%	
Good	7	14.0%	6	20.0%	
Very Good	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4:80: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON GENERAL HEALTH FACET OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your health?	Pre Supplementation stage				P Value
	Ca+B12 Group N=(50)		Ca+B12 Group N=(50)		
	n	%	n	%	
Very Dissatisfied	2	4.0%	0	0.0%	N.A
Dissatisfied	20	40.0%	10	33.3%	
Neither Satisfied nor Dissatisfied	20	40.0%	18	60.0%	
Satisfied	8	16.0%	2	6.7%	
Very Satisfied	0	0%	0%	0%	
Total	50	100%	30	100%	

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PHYSICAL HEALTH DOMAIN:

TABLE 4.81: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*PHYSICAL PAIN AND DISCOMFORT'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

To what extent do you feel that physical pain prevents you from doing what you need to do?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
An extreme amount	1	2.0%	0	0.0%	N.A
Very much	26	52.0%	9	30.0%	
A moderate amount	18	36.0%	13	43.3%	
A little	4	8.0%	6	20.0%	
Not at all	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.82: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON *'DEPENDENCE ON MEDICINAL SUBSTANCES AND MEDICAL AIDS'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How much do you need any medical treatment to function in your daily life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N= 50)		B12 Group (N=30)		
	n	%	n	%	
An extreme amount	3	6.0%	0	0.0%	N.A
Very much	24	48.0%	9	30.0%	
A moderate amount	17	34.0%	16	53.3%	
A little	6	12.0%	5	16.7%	
Total	50	100%	30	100%	

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TABLE 4.83: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN TWO GROUPS OF SUPPLEMENTATION ON *'ENERGY AND FATIGUE'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

Do you have enough energy for everyday life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	2	4.0%	0	0.0%	N.A
A little	22	44.0%	9	30.0%	
Moderately	15	30.0%	13	43.3%	
Mostly	10	20.0%	6	20.0%	
Completely	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.84: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*MOBILITY'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How well are you able to get around?	Pre Supplementation stage				P Value
	Ca+ B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Poor	3	6.0%	0	0.0%	N.A
Poor	19	38.0%	6	20.0%	
Neither Poor nor Good	18	36.0%	12	40.0%	
Good	10	20.0%	12	40.0%	
Total	50	100%	30	100%	

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TABLE 4.85: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*SLEEP AND REST'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your sleep?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	5	10.0%	0	0.0%	N.A
Dissatisfied	14	28.0%	6	20.0%	
Neither Satisfied nor Dissatisfied	21	42.0%	11	36.7%	
Satisfied	9	18.0%	11	36.7%	
Very Satisfied	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.86: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON *'ACTIVITIES OF DAILY LIVING'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your ability to perform your daily living activities?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	2	4.0%	0	0.0%	N.A
Dissatisfied	17	34.0%	8	26.7%	
Neither Satisfied nor Dissatisfied	20	40.0%	8	26.7%	
Satisfied	11	22.0%	14	46.7%	
Total	50	100%	30	100%	

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TABLE 4.87: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON *'WORK CAPACITY'* FACET OF 'PHYSICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your capacity for work?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	4	8.0%	0	0.0%	N.A
Dissatisfied	16	32.0%	6	20.0%	
Neither Satisfied nor Dissatisfied	18	36.0%	10	33.3%	
Satisfied	12	24.0%	14	46.7%	
Total	50	100%	30	100%	

PSYCHOLOGICAL HEALTH DOMAIN:

TABLE 4.88: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION ON '*POSITIVE FEELING*' FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How much do you enjoy life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
A little	2	4.0%	0	0.0%	N.A
A moderate amount	24	48.0%	11	36.7%	
Very much	15	30.0%	12	40.0%	
An extreme amount	9	18.0%	7	23.3%	
Total	50	100%	30	100%	

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TABLE 4.89: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*PERSONAL BELIEFS'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

To what extent do you feel your life to be meaningful?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	3	6.0%	0	0.0%	N.A
A little	23	46.0%	11	36.7%	
A moderate amount	17	34.0%	14	46.7%	
Very much	5	10.0%	0	0.0%	
An extreme amount	2	4.0%	5	16.7%	
Total	50	100%	30	100%	

TABLE 4.90: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*CONCENTRATION'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How well are you able to concentrate?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	3	6.0%	0	0.0%	N.A
A little	17	34.0%	12	40.0%	
A moderate amount	23	46.0%	11	36.7%	
Very much	7	14.0%	6	20.0%	
An extreme amount	0	0.0%	1	3.3%	
Total	50	100%	30	100%	

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TABLE 4.91: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*SELF ESTEEM'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with yourself?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	3	6.0%	0	0.0%	N.A.
Dissatisfied	17	34.0%	6	20.0%	
Neither Satisfied nor Dissatisfied	18	36.0%	11	36.7%	
Satisfied	12	24.0%	13	43.3%	
Total	50	100%	30	100%	

TABLE 4.92: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*BODILY IMAGE AND APPEARANCE'* FACET OF 'PSYCHOLOGICAL HEALTH' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

Are you able to accept your bodily appearance?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	1	2.0%	0	0.0%	N.A
A little	22	44.0%	7	23.3%	
Moderately	15	30.0%	14	46.7%	
Mostly	11	22.0%	7	23.3%	
Completely	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

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TABLE 4.93: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION ON *'NEGATIVE FEELINGS'* FACET OF 'PSYCHOLOGICAL' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How often do you have negative feelings such as blue mood, despair, anxiety, depression?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		Ca+B12 Group (N=30)		
	n	%	n	%	
Always	3	6.0%	0	0.0%	N.A
Very Often	22	44.0%	7	23.3%	
Quiet Often	14	28.0%	11	36.7%	
Seldom	10	20.0%	10	33.3%	
Never	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

SOCIAL RELATIONSHIP DOMAIN:

TABLE 4.94: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*PERSONAL RELATIONSHIP'* FACET OF 'SOCIAL RELATIONSHIP' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your personal relationships?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	4	8.0%	0	0.0%	N.A
Dissatisfied	20	40.0%	4	13.3%	
Neither Satisfied nor Dissatisfied	16	32.0%	12	40.0%	
Satisfied	10	20.0%	14	46.7%	
Total	50	100%	30	100%	

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TABLE 4.95: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*SEXUAL ACTIVITY'* FACET OF 'SOCIAL RELATIONSHIP' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your sex life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		Ca+B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	4	8.0%	0	0.0%	N.A
Dissatisfied	14	28.0%	2	6.7%	
Neither Satisfied nor Dissatisfied	22	44.0%	13	43.3%	
Satisfied	10	20.0%	15	50.0%	
Total	50	100%	30	100%	

TABLE 4.96 DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*SOCIAL SUPPORT'* FACET OF 'SOCIAL RELATIONSHIP' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with the support you get from your friends?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	2	4.0%	0	0.0%	N.A
Dissatisfied	21	42.0%	5	16.7%	
Neither Satisfied nor Dissatisfied	16	32.0%	12	40.0%	
Satisfied	11	22.0%	13	43.3%	
Total	50	100%	30	100%	

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TABLE 4.97: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION ON *'FREEDOM, PHYSICAL SAFETY AND PERSONAL BELIEFS'* FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How safe do you feel in your daily life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	2	4.0%	0	0.0%	N.A
A little	21	42.0%	7	23.3%	
A moderate amount	23	46.0%	14	46.7%	
Very much	3	6.0%	8	26.7%	
An extreme amount	1	2.0%	1	3.3%	
Total	50	100%	30	100%	

TABLE 4.98: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*PHYSICAL ENVIRONMENT'* FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How healthy is your physical environment?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	3	6.0%	0	0.0%	N.A
A little	24	48.0%	9	30.0%	
A moderate amount	15	30.0%	14	46.7%	
Very much	8	16.0%	5	16.7%	
An extreme amount	0	0.0%	2	6.7%	
Total	50	100%	30	100%	

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TABLE 4.99: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION ON '*FINANCIAL RESOURCES'* FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

Have you enough money to meet your needs?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	3	6.0%	0	0.0%	N.A
A little	23	46.0%	6	20.0%	
Moderately	15	30.0%	14	46.7%	
Mostly	9	18.0%	8	26.7%	
Completely	0	0.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.100: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON *'OPPORTUNITIES FOR ACQUIRING NEW INFORMATION AND SKILL'* FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How available to you is the information that you need in your day-to-day life?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	5	10.0%	0	0.0%	N.A
A little	20	40.0%	8	26.7%	
Moderately	15	30.0%	11	36.7%	
Mostly	10	20.0%	9	30.0%	
Completely	0	0.0%	2	6.7%	
Total	50	100%	30	100%	

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TABLE 4.101: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*LEISURE ACTIVITIES*' FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

To what extent do you have the opportunity for leisure activities?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Not at all	6	12.0%	0	0.0%	N.A
A little	16	32.0%	8	26.7%	
Moderately	18	36.0%	10	33.3%	
Mostly	9	18.0%	10	33.3%	
Completely	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.102: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON *'HOME ENVIRONMENT'* FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with the conditions of your living place?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	5	10.0%	0	0.0%	N.A
Dissatisfied	18	36.0%	4	13.3%	
Neither Satisfied nor Dissatisfied	16	32.0%	12	40.0%	
Satisfied	11	22.0%	13	43.3%	
Very Satisfied	0	0.0%	1	3.3%	
Total	50	100%	30	100%	

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TABLE 4.103: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*HEALTH AND SOCIAL CARE: ACCESSIBILITY AND QUALITY*' FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your access to health services?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	5	10.0%	0	0.0%	N.A
Dissatisfied	20	40.0%	8	26.7%	
Neither Satisfied nor Dissatisfied	13	26.0%	10	33.3%	
Satisfied	11	22.0%	10	33.3%	
Very Satisfied	1	2.0%	2	6.7%	
Total	50	100%	30	100%	

TABLE 4.104: DIFFERENCES IN THE MEASURE OF PERCEPTION BETWEEN THE TWO GROUPS IN PRE SUPPLEMENTATION STAGE ON '*TRANSPORT*' FACET OF 'ENVIRONMENT' DOMAIN OF QUALITY OF LIFE OF T2DM ADULTS AS MEASURED BY WHOQOL BREF

How satisfied are you with your transport?	Pre Supplementation stage				P Value
	Ca+B12 Group (N=50)		B12 Group (N=30)		
	n	%	n	%	
Very Dissatisfied	3	6.0%	0	0.0%	N.A
Dissatisfied	20	40.0%	5	16.7%	
Neither Satisfied nor Dissatisfied	16	32.0%	11	36.7%	
Satisfied	11	22.0%	13	43.3%	
Very Satisfied	0	0.0%	1	3.3%	
Total	50	100%	30	100%	

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COMPARISON OF IMPACT OF SUPPLEMENTATION IN MEAN DIFFERENCE (POST-PRE) OF SERUM B12, HbA1c, DPN SCORE, MNSI HISTORY SCORE AND FOUR DOMAINS OF QOL IN BETWEEN THE TWO SUPPLEMENTATION GROUPS

The mean difference of several parameters assessed for supplementation are depicted in Table 4.105 .The mean difference of each parameter was calculated as the mean of the difference of post and pre supplementation values of each individual subject with respect to each parameter. The mean difference with negative values (Fig 4.27) simply indicate that the supplementation caused a decrease in that particular parameter. The relative magnitude of the mean difference between the two groups indicates the efficacy of supplementation of that particular group. Then mean difference of each parameter was compared between the two supplementation groups by Student's t test as shown in Table 4.105 and these are depicted in Fig 4.27.

As regards serum B12 the mean difference was significantly more in Ca+B12 group than B12 group ($P<0.001$) indicating that the 500mg calcium along with 1000µg B12 supplementation was better than 1000µg B12 supplementation alone.

As regards HbA1c the mean difference was significantly more in B12 group than Ca+B12 group ($p<0.001$) indicating that the 1000µg B12 supplementation alone was better than 500mg calcium with 1000µg B12 supplementation in decreasing HbA1c.

As regards DPN scores the mean difference was significantly more in B12 group than Ca+B12 group ($p<0.01$) indicating that the 1000µg B12 supplementation alone was better than 500mg calcium with 1000µg B12 supplementation in decreasing DPN scores.

As regards QoL the mean difference of physical health was significantly more in Ca+B12 group than in B12 group ($p<0.01$) indicating that the 500mg Calcium with 1000 µg B12 supplementation was better than 1000µg B12 alone in increasing the physical health domain scores of QoL.

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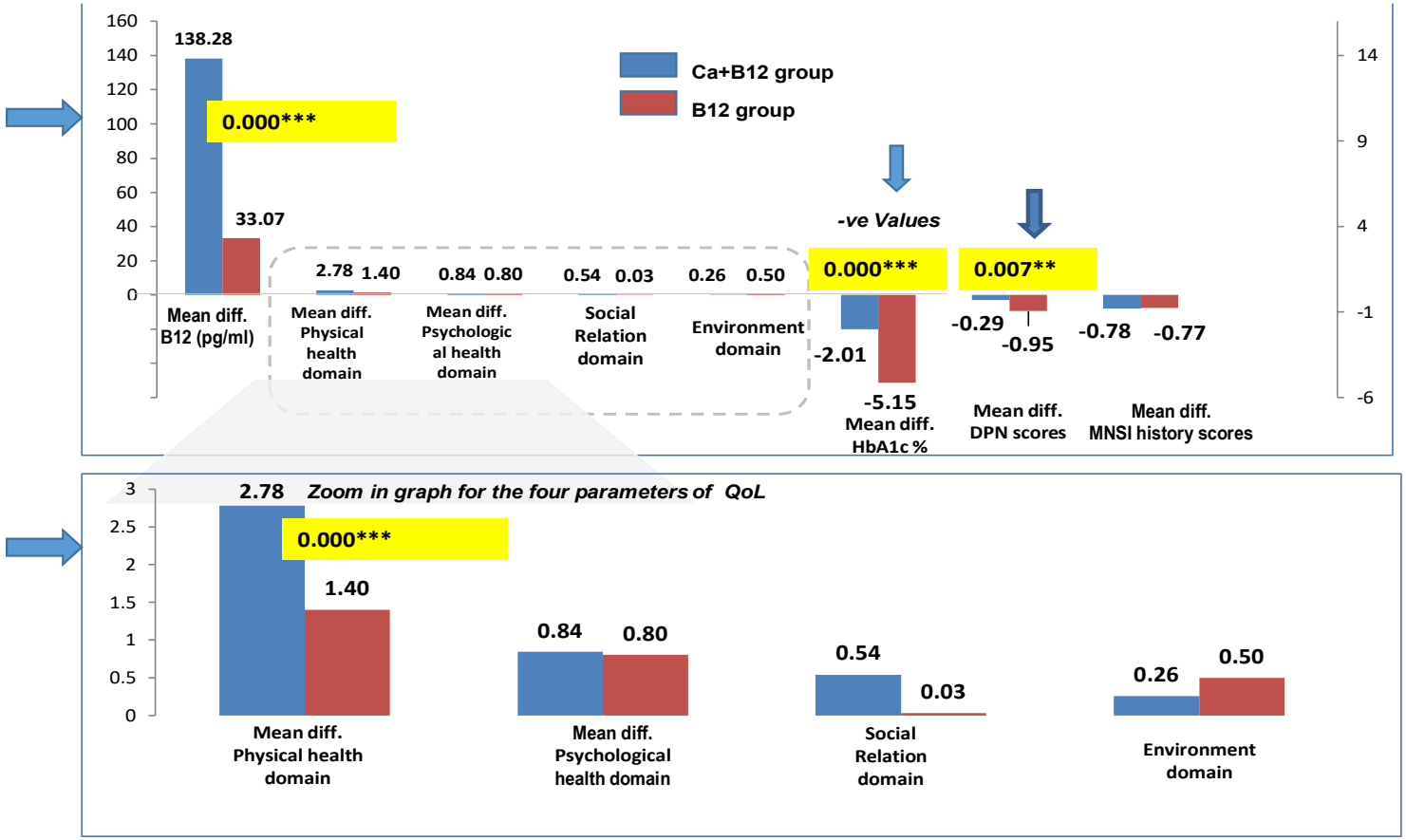
TABLE 4.105: COMPARISON OF IMPACT OF SUPPLEMENTATION IN MEAN DIFFERENCE (POST-PRE) OF SERUM B12, HbA1c, DPN SCORE, MNSI HISTORY SCORE AND FOUR DOMAINS OF QOL IN BETWEEN THE TWO SUPPLEMENTATION GROUPS

Parameter	Ca+B12 group (Mean difference =Post- Pre) Mean ±S.D	B12 group (Mean difference =Post-Pre) Mean ±S.D	P value (Student's t test)
Serum B12	138.28±53.20	38.07±37.72	0.000***
HbA1c	-2.01±2.80	-5.15±3.69	0.000***
DPN scores/MNSI Physical assessment score	-0.29±0.83	-0.95±1.12	0.007**
MNSI History Score	-0.78±0.76	-0.77±0.86	0.944
Physical Health domain of QOL	2.78±2.41	1.40±2.67	0.024*
Psychological Health domain of QOL	0.84±1.87	0.80±1.86	0.926
Social Relationship domain of QoL	0.54± 1.51	0.03±1.30	0.118
Environment domain of QoL	0.26±2.29	0.50±2.15	0.639

Fig:4.27 COMPARISON OF IMPACT OF SUPPLEMENTATION IN MEAN DIFFERENCE (POST-PRE) OF SERUM B12, HbA1c, DPN SCORE, MNSI HISTORY SCORE AND FOUR DOMAINS OF QOL IN BETWEEN THE TWO SUPPLEMENTATION GROUPS



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CONCLUSION ABOUT SUPPLEMENTATION IN RELATION TO NUTRIENT-DRUG INTERACTION (B12-METFORMIN):

To conclude as regards supplementation it can be said that 500mg calcium supplementation along with 1000µg B12 supplementation showed better response in improving B12 levels and physical domain of QoL than 1000µg B12 supplementation alone (Table 4.105 and Fig 4.27).

However, the rise in serum B12 and better improvement in physical domain of QoL cannot be attributed solely due to the calcium supplementation as the mean values of serum B12 and mean physical domain QoL scores before supplementation were higher in B12+Ca group than B12 group ($p<0.05$) (Table 4.52 and Table 4.63 respectively).

Thus cause and effect relationship between 500 mg Calcium supplementation and serum B12 cannot be established from this randomized control trial.

The calcium supplementation at 500mg dosage, used in study keeping in mind the RDA for Indians in adult population was not enough to overcome the metformin induced low B12 levels over and above 1000µg B12 supplementation alone. There is a need to study this metformin- induced low B12 levels (nutrient- drug interaction) by planning randomized control trials at higher doses of calcium, above its RDA amongst the Indian population of T2DM adults on metformin.

We would like to mention the fact that the impact of 500mg calcium supplementation studied here was in presence of 400IU vitamin D3 as the calcium supplementation used in the study was in combination with vitamin D3 because in India calcium supplements cannot be manufactured without D3 as vitamin D is required for calcium absorption. Moreover, there was 100% compliance of supplementation by the study subjects in both the groups.

Highlights of Phase III:

- The T2DM adults in intervention trial belonged to a mean age of 59.26. The two supplementation groups were comparable for sex, age, duration of diabetes and risk factors like BMI>23, abnormal W.C. and abnormal BP.

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- By student t test it was found that before supplementation there was no difference in the mean glycated Hb , mean DPN score and mean MNSI history total score of the T2DM adults between the two groups. However, as regards mean serum B12 levels before supplementation there was significant difference in between two groups ($p<0.05$).
- After supplementation there was a significant decrease in the mean glycated hemoglobin levels and DPN scores while a significant increase in the mean serum B12 levels of both the groups by paired t test ($p<0.05$). However, the increase in serum B12 was more in Ca+B12 group than that in B12 group indicating that calcium supplementation in addition to B12 gave better response in improving serum B12. However, this increase in serum B12 cannot be solely attributed to calcium supplementation because the mean serum B12 levels in Ca+B12 group was higher than the B12 group($p<0.05$).
- After supplementation overall 38.8% attained normal glycemic control. The prevalence of poor glycemic control decreased from 73.8% to 48.8%.
- After supplementation overall total 52 (65%) attained normal B12 levels (200pg/ml). In Ca+B12 group 92% (46 out of 50) attained normal serum B12 levels while only 20% (6 out of 30) attained normal B12 levels in control group and these results were highly significant ($p<0.001$).
- Overall after supplementation the prevalence of high DPN fell by 17.5% (61.25 to 43.75) and the prevalence of No DPN increased by 5% (6.25 to 11.2). But when the differences in prevalence of low grade ($>0\leq2.5$) and high grade DPN (>2.5) were compared were compared in between the two groups after supplementation then there was no significant difference by Chi square. It can be said that Ca+B12 supplementation was not better than B12 supplementation alone in bringing significant difference in DPN prevalence of low and high grade.
- Impact of supplementation on MNSI Physical assessment of feet showed that there was significant difference in both the groups for vibration perception at great toe for both legs($p<0.05$). This indicates that both supplementations helped in improving vibration perception at great toe. Improvement in vibration

perception at great toe was the crucial contributor in decreasing the DPN scores after supplementation.

- As regards QoL there was significant improvement in both the groups after supplementation in two domains i.e. physical health ($p<0.001$) & psychological health ($p<0.01$) with no changes in social relationship and environmental domains.
- By students t test the mean difference of serum B12 and Physical domain scores of QoL was significantly more in Ca+B12 group than B12 group ($P<0.001$) indicating that Ca+ B12 supplementation was better than B12 supplementation alone in these two parameters. However by mean difference the fall in HbA1c and DPN scores was significantly more in B12 group than Ca+B12 group ($p<0.001$) indicating that the 1000µg B12 supplementation alone was better than calcium with B12 supplementation in decreasing HbA1c.
- 500mg calcium supplementation along with 1000µg B12 supplementation showed better response in improving B12 levels and physical domain of QoL than 1000µg B12 supplementation alone. However, the rise in serum B12 and better improvement in physical domain of QoL cannot be attributed solely due to the calcium supplementation as the mean values of serum B12 and mean physical domain QoL scores before supplementation were higher in B12+Ca group than B12 group ($p<0.05$).
- 500mg Calcium dosage, used in study keeping in mind the RDA for Indians in adult population, was not enough to overcome the metformin induced low B12 levels over and above 1000µg B12 supplementation alone. There is a need to study this metformin- induced low B12 levels (nutrient- drug interaction) by planning randomized control trials at higher doses of calcium, above its RDA amongst the Indian population of T2DM adults on metformin.

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DISCUSSION

This study has been an attempt to study the implications of nutrient- drug interaction in T2DM adults in an Indian hospital based population setting and suggests how this issue can be rectified.

Metformin is representative of the biguanide category of drug which have been most commonly used hypoglycemic agent in the treatment of diabetes mellitus since 1957 and continues till date in WHO essential list of medicine as well as first line of therapies in T2DM by ADA as stated in historical overview on metformin by Bailey 2017. The glucose lowering property of metformin was pursued by the French physician Jean Sterne, who first reported the use of metformin to treat diabetes in humans in 1957.

The B12 lowering mechanism of metformin is not yet clear though there have been immense research in this area which is not discussed here as it is not the domain of this study. There is intestinal malabsorption of B12 in T2DM adults which makes them potent to B12 deficiency as evident from several studies since four to five decades as explained in the following text.

The intestinal vitamin B12 malabsorption in T2DM adults on metformin has been reported in as early as 1969 by Berchtold et al in short term duration of 3 months of metformin administration. These were the observations on the mode of biguanide drug metformin which paved way to the study taken up by Tomkin et al in 1971 where he studied long term effect of metformin on type 2 diabetes patients taking metformin for 2 years. In 2003 it was found that short term metformin treatment of 16 weeks in patients with type 2 diabetes was associated with a decrease in vitamin B12 by Wulffelé et. al 2003 who carried out the first randomized, placebo-controlled study that reports on the effects of treatment with metformin on serum concentrations of B12.

Thereafter there have been several observational studies including cross sectional as well as case reports (Bell, 2010; Sparre Hermann et al, 2004; Kumthekar et al, 2012; Liu et al,

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2011; Nervo et al, 2011; Pflipsen et al, 2009;) to share their experience of the use of metformin in context of B12 deficiency among T2DM adults from several countries in past four-five decades but the data regarding the same is sparse in Indian context and it has gained impetus since last decade.

However, there are only few RCTs addressing this issue across the globe (Wulffele et al, 2003, de Jager et al, 2010).

As vitamin B12 plays crucial role in optimal hemopoetic and neuro-cognitive function so intervening in this issue was of priority. In olden days the traditional treatment for B12 deficiency has been with parenteral therapy due to poor oral absorption. However, it is currently recognized that high dose oral B12 (1000 μg) appears to rectify the levels in most patients being as effective as parenteral therapy. Regular oral B12 or annual B12 injections have been recommended as safe ways to maintain levels in B12 deficient patients. Therefore, effective treatments are available, but it is unclear what the vitamin B12 monitoring strategy should be for patients being prescribed metformin. (Upadhyay, 2016).

There have been good amount of evidence addressing low serum B12 levels on metformin therapy in T2DM along with folate and homocystiene in relationship to cardiovascular risk among T2DM adults on metformin (Pongchaidecha et al, 2004; Sahin et al, 2007, Upadhyay, 2016). But, because peripheral neuropathy of diabetes may present with symptoms that may be indistinguishable from that of vitamin B12 deficiency, the condition of metformin-associated low serum vitamin B12 is of great concern. So it is necessary to study neuropathy in T2DM adults so as to take care of deterioration caused by it in quality of life of T2DM adults.

Further B12 deficiency was studied in association with DPN, a very common micro vascular complication of diabetes which has gained attention in recent past where physician, diabetologist, endocrinologist and others in allied field has gained impetus in foot work.

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There is scanty literature to bridge the link between the two diseased conditions:B12 deficiency and DPN, in T2DM adults on metformin in Indian setting. Further it was thought that the coexistence of diabetes, B12 deficiency and DPN would deteriorate quality of life in T2DM adults so the QLI was studied to draw a holistic picture of the wellbeing of T2DM adults on metformin.

SERUM B12 AND PREVALENCE OF B12 DEFICIENCY IN T2DM ADULTS ON METFORMIN: WORLD SCENARIO

The concentrations suggested for defining **B12 deficiency by de Benoist, (2008) in WHO technical consultaion on folate and vitamin B12 deficiency are: < 150 pmol/L (203 pg/mL)** for serum vitamin B12. Conversion factor used for vitamin B12 is 0.737 which means 1pg/ml is equal to 0.737 pmol/L. So it means ~ 200pg/ml serum B12 is equal to 150pmol/L.

In **our study the cut off used to define B12 deficiency was 200pg/ml which can be considered as aprox.150pmol/L** while comparing it with other studies .

SERUM B12 AMONG T2DM ADULTS

Our study showed that serum B12 levels of the sample ranged from 94 to 1746 pg/ml among 155 T2DM adults with mean age of 58 y and mean age of duration of diabetes of 7.58 y. This result is consistent with the study of Pflipsen et al 2009 who reported that serum B12 levels ranged from 91 to 2818 pg/mL among 195 T2DM adults with mean age of 61y and mean duration of diabetes as 8.3 y.

Sparre Hermann et al, 2004 conducted a study to assess the serum B12 status of patients with type 2diabetes who had been receiving metformin treatment for at least one year. Patients exposed to metformin for more than one year (n=53) were compared with a non-exposed control group (n=31). Patients on metformin had lower cobalamin (**289±137** vs. 395±162 pmol/L;$p<0.05$); these changes were correlated. Eight metformin patients, but

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no controls, had holotranscobalamin below the normal range ($p<0.05$). Methylmalonic acid and folate were similar in both groups.

In **our study** the serum B12 ranged from 94 to 1746pg/ml which was similar to the study of Pflipsen which reported that the Serum B_{12} levels ranged from 91 to 2818 pg/mL. In our study the mean serum B12 levels were **287.31±249.6** pg/ml among T2DM adults on metformin while that reported by Pflipsen et al 2009 showed the mean serum B12 to be 470±311.4pg/ml among all the T2DM adults. Patients on metformin had lower serum B12 levels (**425.99 pg/mL** vs 527.49 pg/mL; $P = .012$) and were at increased risk for B12 deficiency ($P = .04$), as defined by a serum B12 level <350 pg/mL.

In the large National Health and Nutrition Examination survey (NHANES 1999-2006) (Reinstatler et al, 2012) on U.S adults ≥ 50 y of age the geometric mean serum B12 (with SE) of metformin users were 317.5±9.6 pmol/l. This mean was on log scale so it is presented as geometric mean. This large survey was conducted to study serum B12 on adults with(n=1,621) and without diabetes (n=6867) and among those who had diabetes there were 575 metformin users and 1046 were non metformin users. The survey result showed a significant difference in mean serum B12 of metformin (**317.5±9.6 pmol/l**) and non metformin users (386.7±7.8pmol/l) (P=0.0116).

Ahmed et al, 2016 conducted a cross sectional study in diabetes clinic at Steve Biko Academic hospital and Kalafong hospital in Pretoria, South Africa. The study reported that among 121 T2DM adults with mean age of 58.5±10.5 y and diabetes duration of 11.6± 7.5 y the serum B12 was 260±163.86 pmol/l.

In 2016 Diabetes Prevention Program (DPP) and Diabetes Prevention Program Output Study (DPPOS) on 1800 participants whose average age was >51 y at baseline, Aroda et al 2016 found that baseline serum B12 were 546±337.2 pg/ml in metformin group and 606.6±352.7 pg/ml in placebo group ($p<0.01$) after mean 5y follow up. However after mean 13y follow up the serum B12 were 615.9±503.8 pg/ml in metformin group and 650±503.8 pg/ml in placebo group (p=0.19).

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Our study showed a lower mean serum B12 levels in comparison to the mean serum B12 levels showed by Pflipsen et al, 2009, Reinstatler et al, 2012 and Aroda et al, 2016. This could be because of the following possible reason. Our data is from Indian population (Asian race) whose predominant diet is vegetarian while the data from Pflipsen, 2009 is from a mix of 110 whites, 38 asians,24 black who are said to be on a non-vegetarian diet usually though the study has no mention of their dietary intake pattern.

It is also thought that racial differences can contribute to the above stated differences in vitamin B12 levels. As cited in review by Ahmed et al, 2017, several studies reported higher concentrations of vitamin B12 in black individuals when compared to white individuals (Stabler et al, 1999 ; Saxena and Carmel, 1987). This is attributed to higher levels of vitamin B12-binding proteins in the black populations (Fernandes-Costa, & Metz, 1982). To our knowledge, the impact of ethnicity on the cellular status of the vitamin is not yet discovered. We would like to state that ethnicity should be taken into consideration as a contributing factor when discussing B12 deficiency induced by metformin.

PREVALENCE OF B12 DEFICIENCY:

In our study it was found that 81 (52%) of 155 among T2DM adults on metformin for more than three months duration were B12 deficient(B12≤ 200pg/ml). Our data demonstrates clear association between metformin and biochemical B12 deficiency among T2DM adults.

As regards various grades of B12 deficiency our study shows that majority (47 out of 155 ,30.3%) of them had mild B12 deficiency(150-200pg/ml).32 of 155 (20.6%) had moderate B12 deficiency(101-149pg/ml).Only 2(1.3%) of 155 had severe B12 deficiency (B12≤ 100pg/ml).

In 2004 Sparre Hermann L. et al stated that patients exposed to long-term metformin therapy had 26.7% lower cobalamin, 21.6% lower holotranscobalamin and 9.7% higher

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Hcy serum concentrations than control subjects. These changes indicated a potential risk for development of vitamin B12 deficiency.

In 2009 Pflipsen et al showed that 79 had intermediate B12 levels between 100 and 350 pg/mL and required confirmatory testing to assess for deficiency. Only one individual had a B_{12} level <100 pg/mL.

Reinstatler 2012 defined biochemical deficiency as serum B12 concentrations ≤ 148 pmol/l and borderline deficiency as >148pmol/l in NHANES 1999-2006 where the weighted biochemical B12 deficiency adjusted for age, sex, and 5.8% for those using metformin compared with 2.4% of those not using metformin (p=0.0026) and 3.3% of those without diabetes(p=0.0002).

The B12 deficiency prevalence in our study is more than the prevalence shown in similar studies from western countries across the World. Several studies across the globe, addressing B12 malabsorption and low serum B12 levels in the chronological order of the year in which they have been studied are discussed as follows.

B12 malabsorption due to metformin was studied by Berchtold et al (1969) for short duration of 3months but we could not access more details on his study because his article is in German. This paved ways to study on B12 malabsorption for long term metformin use. Vitamin B12 malabsorption was found in 21 (30%) of 71 diabetic patients taking long-term metformin therapy for 2 years in addition to dietary management by Tomkin et al, 1971. He studied only B12 malabsorption by double isotope technique and his study did not measure the serum B12 levels.

In another study carried out in the same year for the same duration of metformin treatment, it was found that low serum levels of vitamin B12 were reported in **17.5%** of patients using 2 g of metformin daily for at least 2 years (**Stowers, & Smith, 1971**). In one early randomised controlled trial by DeFronzo et al metformin decreased the serum vitamin B12 levels by 22% and 29% compared to placebo and glyburide i.e sulphonylurea respectively (De Fronzo and Goodman, 1995).

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In HOME trial **Wulfelle,** 2001 studied the effect of short term metformin treatment on serumB12 levels in a placebo controlled trial (highest level of evidence in research). He found that 16weeks (4 months) of metformin treatment in patients with type 2 diabetes was associated with an increase in serum homocysteine of approx. 4% and with decreases in serum folate and vitamin B12 of approx.7 and approx.**14%,** respectively. In addition, further analysis of this study data indicated that the increase in serum homocysteine was mediated by the decreases in serum folate and vitamin B12.

In **2009 Plifpsen et al** conducted a cross sectional study on 203 outpatient type 2 diabetic patients at a large military primary care clinic. Patients completed a survey and had B12 levels measured. Patients with borderline B12 levels also had methylmalonic acid and homocysteine levels drawn. Serum B12 levels <100 pg/mL or serum B12 levels of 100 to 350 pg/mL with elevation of serum methylmalonic acid >243 nmol/L or homocysteine >11.9 nmol/L defined B12 deficiency. This study showed that **22%** (n = 44) of diabetic patients had metabolically confirmed B12 deficiency. Prevalence of B12 deficiency was significantly lower for patients using a multivitamin (odds ratio, 0.31; 95% CI, 0.15–0.63).

In 2010 a placebo controlled trial conducted in the outpatient clinics of three hospitals, the patients were randomly assigned by a computer program to receive either 850 mg of metformin three times a day or 850 mg of placebo thrice daily, which were provided in identical looking boxes. The trial consisted of three phases: the 12 week pre-randomization phase, in which patients were treated with insulin only; the 16 week short term treatment phase, at the beginning of which patients were randomized to receive either metformin or placebo in addition to insulin therapy; and the four year long term treatment phase. At baseline, three patients (1.6%) in the metformin group and four (2.2%) in the placebo group had vitamin B-12 deficiency (serum B-12 <150 pmol/l), whereas 14 patients (7.3%) and 14 patients (7.5%), respectively, had a low serum B-12 (150-220 pmol/l). At the end of the study period, 19 patients (9.9%) in the metformin group and five (2.7%) in the placebo group had vitamin B-12 deficiency, whereas 35

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patients (18.2%) and 13 patients (7.0%), respectively, had a low serum B-12 . Low B12 (≤ 203 pg/mL) occurred more often in metformin group than placebo group at 5 years (4.3 vs 2.3%; *P*≤.02) but not at13years (7.4 vs5.4%;*P*≤.12).Combined low and borderline-low B12(≤ 298pg/mL) was more common in metformin at 5 years (19.1 vs 9.5%; *P* ≤ .01) and 13 years (20.3 vs 15.6%; *P* ≤.02). (de Jager J et al. 2010)

In 2011 in a study by Qureshi et al, serum vitamin B_{12} levels were measured in type 2 diabetes patients with high dose (>2g/day) and long-term (four years) metformin treatment. They also evaluated the effectiveness of vitamin B_{12} replacement when levels were low. Of 283 patients on high dose metformin for more than four years only, 70 (25%) had vitamin B_{12} levels checked. All of these 70 cases had peripheral neuropathy. Vitamin B_{12} deficiency (<150 pg/ml) was recorded in 23 (33%).

In 2011 in a study from Brazil, Nervo et al. estimated the prevalence of B12 deficiency among southern Brazilian T2DM patients using metformin and found that B12 deficiency occurred in 6.9% of the patients.

In 2015 Akinalade et al carried out a study to determine the serum level of vitamin B12 in Nigerian patients with T2DM on metformin. Serum vitamin B12 level in 81 T2DM patients who have been on metformin for 5 years or more was determined. Vitamin B12 deficiency was defined as serum concentration of <200 pg/dl and borderline deficiency as 200–300 pg/dl. Serum B12 >300 pg/dl were considered as normal. B12 deficiency and borderline deficiency were recorded in 8.6% and 26.0% of the patients respectively.

In 2016 Diabetes Prevention Program Outcomes Study (DPPOS) represents one of the largest and longest studies of metformin treatment, persons at high risk for type 2 diabetes were continued on randomized metformin treatment for 13 years (Aroda et al, 2016). Mean follow-up at the end of the Diabetes Prevention Program (DPP) was 3.2 years. At the end of the DPP, all participants were offered a group-implemented lifestyle intervention and invited to enroll in the follow- up study, DPPOS. During the DPPOS, those originally assigned to metformin received open-label metformin at the same prior dose of 850 mg twice daily, which was continued until such time as diabetes developed and HbA1c value reached ≤ 7%. At this point, study metformin was discontinued, and the

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participant referred to his or her own physician for treatment of diabetes, which may have included metformin. Samples used for measurement of vitamin B12 and homocysteine, as a corroborating indicator of vitamin B12 deficiency, were collected an average of 5 years (DPPOS year 1) and 13 years (DPPOS year 9).This study showed low B12 (≤203 pg/mL) occurred more often in metformin than placebo at 5 years (4.3 vs 2.3%; *P*=.02).But not at13years (7.4 vs 5.4%;*P*=.12).Combined low and borderline-low B12 (≤298pg/mL) was more common in metformin at 5 years (19.1 vs 9.5%; *P*=.01) and 13 years (20.3 vs 15.6%; *P* =.02). (Aroda et al, 2016).

In 2016 Ahmed et al conducted a cross sectional study among T2DM adults with mean age of 58.5±10.5 y in diabetes clinic of two hospitals of Africa where they reported that 34 of 121 (28%) were B12 deficient defined for serum B12≤150pmol/l.

In 2016 another cross sectional study from Brazil by Damião et al. found that the prevalence of B12 deficiency among T2DM-met patients was significantly higher than in the control group matched for sex and age (22.5% versus 7.4%). He further stated that the factors that interfered with serum B12 levels were PPI/H2-antagonist use and duration of metformin use ≥ 10years. Use of PPI/H2-antagonists was associated with B12 deficiency, with an odds ratio of 2.60 (95% C.I, 1.34-5.04).

Another cross sectional study from Oman by Al-Hamdi et al, 2020 recruited 248 subjects who had mean age of 55.3 ± 10.0 y and the mean duration of T2DM was 6.5 ± 4.5 years. The participants were classified into three categories based on their serum vitamin B12 measurements: a deficient group (<133 pmol/L), a borderline deficient group (133–200 pmol/L) and a normal group (>200 pmol/L). Vitamin B12 deficiency in metformin treated T2DM patients was found in 26 (10.5%) while borderline deficiency was found in 53 (21.4%).

Studies assessing type 2 diabetic patients on metformin have reported the prevalence of vitamin B12 deficiency to range from 5.8% to 33% (Kibirige & Mwebaze, 2013). However in our study the prevalence of B12 deficiency among T2DM adults on metformin was 52% (81 of 155; B12 ≤ 200pg/ml) which was higher than the other

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studies. This wide variation in the reported prevalence could probably be explained by the varied study definitions of vitamin B12 deficiency. The low prevalence of B12 deficiency in countries from west may be because of the fact that in western countries, the intake of vitamin B12 in the general population appears to be above the estimated requirements (Rizzo et al, 2016).

A recently published meta-analysis found that the use of metformin is a risk factor for vitamin B12 deficiency in diabetic patients.(Yang et al, 2019)

Another meta-analysis showed evidence from this review demonstrates an association between metformin usage and lower levels of vitamin B by 57 pmol/L, which leads to frank deficiency or borderline status in some patients with type 2 diabetes. (Chapmana et al, 2016).

To conclude it can be said that there is a wide range of serum B12 deficiency among T2DM adults on metformin. It may be attributed to differences in cut-points chosen to define the deficiency, participants mean age, study settings, and metformin dose and duration of use.

A COMPARISON OF SERUM B12 PREVALENCE OF B12 DEFICIENCY IN T2DM ADULTS ON METFORMIN AND OTHER POPULATION: INDIAN SCENARIO

The area of research on B12 deficiency across various population and several disease conditions in which metformin(the drug potent to induce low serum B12) is used is sparse. In India Vitamin B12 status has been studied in men in relation to homocysteine, adolescents in relation to nutritional anemia and elderly in relation to neuro-cognition however there is limited evidence for vitamin B12 status in diabetes.

In India serum B12 levels have been studied in males by **Yajnik** et. al, 2006 where he showed that overall, **67%** of men had low vitamin B12 (<150 pmol/L) and 58% had hyperhomocysteinemia (>15 μmol/L). Vegetarians had 4.4 times (95%CI 2.1, 9.4) higher risk of low vitamin B12 concentrations and 3.0 times (95%CI 1.4, 6.5) higher risk of

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hyperhomocysteinemia compared to those who consumed non-vegetarian foods frequently. This high prevalence of B12 deficiency can be due to the fact that larger proportions of Indians are vegetarians owing to their cultural and religious beliefs.

In **our study** the prevalence of B12 deficiency among T2DM adults on metformin in outpatient department of multidisciplinary hospital was **52%** (81 of 155, B12≤200 pg/ml) which is higher than that shown in western studies but it is lesser than that documented by Yajnik et al. 2006 in general population.

However B12 levels in **T1DM** has been reported in one cross sectional study done in South India among 90 patients where low vitamin B12 levels were noted among **45.5%** as studied by the cutoff point of <180 pg/ml and among 54% using the published cut off point of <200 pg/ml (Shobha et al, 2011).

In **our study** the serum B12 ranged from 94 to 1746pg/ml and mean serum B12 levels were **287.31±249.6** pg/ml among T2DM adults on metformin. The mean serum B12 levels in our study were lower than the other Indian studies as discussed in the following text.

In our study it was found that **52%** (81 of 155) among T2DM adults on metformin for more than equal to four months duration were B12 deficient (**B12≤ 200pg/ml**). As regards various grades of B12 deficiency our study shows that majority (47 out of 155 ,**30.3%)** of them had mild B12 deficiency(**150-200pg/ml**).32 of 155 (20.6%) had moderate B12 deficiency(101-149pg/ml).Only 2(1.3%) of 155 had severe B12 deficiency (B12≤ 100pg/ml). The prevalence of B12 deficiency reported in our study is higher than that reported in other Indian studies among T2DM adults as described in following text.

In one study by **Kumar et al, 2017** where 161 **T2DM** subjects were studied over a period of 6 months at Karnataka institute of endocrinology and research Bangalore reported a prevalence of definite vitamin B12 deficiency(**B12<200pg/ml**) in **27.3%** and biochemical B12 deficiency (200-300pg/ml) in 26.3%. Further analysis showed that 23.2% on 1000 mg metformin and 36.73% on 2000 mg metformin were deficient in

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vitamin B12 respectively. There was no correlation between vitamin B12 deficiency and duration of metformin therapy.

In one study by **Raizada et al, 2017** a total of 183 T2DM patients were recruited from the endocrinology outpatient department of a tertiary care hospital(AIIMS) where 121 were in metformin group while 62 were in no metformin group. The mean age was 49.8 ± 10.2 y. and duration of metformin use was 27.3 ± 35.8 months (range 3–180 months). Maximum daily dose of metformin was 834.1 ± 754.2 mg (range 500–2550 mg). The cumulative dose of metformin was 980.6 ± 1576.1 g (range 75–10,950 g). Vitamin B12 deficiency prevalence was **35.5%** (**B12<150 pmol/L**) and **borderline deficiency** was **22.3%** levels between **150 and 221 pmol/L.**

Singh et al, 2013 conducted a study on 136 T2DM patients where 84 were in metformin group while 52 were in no metformin group. The prevalence of B12 deficiency (150-220 pg/ml) in metformin exposed group was significantly higher than that in non-metformin exposed group [18/84 (**21.4%)** versus 3/52 (5.7%), mean difference=15.7% 95% CI 4.9-26.5%, *P*=0.026]. While there were six patients with definite B12 deficiency (<150 pg/ml) in metformin exposed group there was none in non-metformin group. **Mean serum B12** levels was significantly lower in metformin exposed group (*n*=84) compared with non-metformin exposed group (*n*=52) (**410±230.7** versus 549.2±244.7, *P*=0.0011). Serum B12 level (in pg/ml) was significantly higher in non-metformin group as compared with metformin group. (549.2±244.7 versus 410±230.7, mean difference=139.6 95% CI 56.86-221.54, *P*=0.0011).Vitamin B12 level had significant negative correlation with cumulative metformin dose (r=−0.688, 95% CI −055 to −0.78, *P*<0.0001) and duration of metformin treatment (r=−0.74, 95% CI −0.625 to −0.824, *P*<0.001). However in our study we could not calculate cumulative dose of metformin because data on metformin duration could not be retrieved through the case papers.

In the study by Kapil, U., & Bhadoria, A. 2014, a cross-sectional, school-based study conducted in NCT of Delhi, India on 347 adolescent belonging to low- (LIG), middle-(MIG), and high-income groups (HIG) reported that 73.5% of the adolescents had

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deficiency of cobalamin. There are limited studies from India on the cobalamin status amongst adolescents. The study by Kapoor et al 2002, (as cited in Kapil & Bhadoria, 2014) conducted amongst children aged 6-30 months in an urban community documented cobalamin deficiency among 48% children. An earlier study conducted by Chandra et al, 2002, reported a prevalence of cobalamin deficiency in 62% of children suffering from megaloblastic anemia.

In a recent study by Singla et al, 2019 conducted in Delhi, North India, the prevalence of vitamin B12 deficiency (B12 <200 pg/ml) in tier 3 city was 47.19% (*n* = 267). From an urban endocrine practice, database of 11913 patients was searched for reports of vitamin B12 levels. Prevalence of vitamin B12 deficiency was 37.76% in people with pre-diabetes (*n* = 92), 31.23% in people with endocrine problems other than diabetes and pre-diabetes (*n* = 285) and 18.25% in people with diabetes (*n* = 378).This study concluded that people with diabetes have higher vitamin B12 levels than general population though still have high prevalence of deficiency. This data showed that Vitamin B12 deficiency is widespread in Indian population.

In another study by Refsum et al, 2001 conducted on 63 subjects from an urban area reported the median value of serum B12 as 216.86 and a prevalence of B12 deficiency of 46% (B12 <150 pmol/L or 200pg/ml).

In one study by Shobha et al 2011 conducted on 175 elderly urban subjects from south India reported the median serum B12 as 414.74 and a prevalence of B12 deficiency of 16%(B12<150pmol/L or 200pg/ml).This low estimate of B12 prevalence was attributed by author to the fact that the studied population was largely non vegetarian by food.
A cross sectional study from Kolkata by Roy et al, 2016 on randomly selected ninety patients in the age group of 35 -70 y compared those who had received >6 months of metformin (Group A) (*n* = 35) with those without metformin (Group B) (*n* = 35) and patients taking metformin with other OHA (Group C) (*n* = 20). Comparisons were made on serum Cbl, fasting Hcy, and folic acid, and with electrophysiological measures (nerve conduction studies of all four limbs). It was found that there was significantly low plasma

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level of Cbl in Group A (mean 306.314 pg/ml) than in Group B (mean 627.543 pg/ml) and Group C (mean 419.920 pg/ml). There was insignificant low-level plasma folic acid in Group A (16.47 ng/ml) than in Group B (16.81 ng/ml) and Group C (22.50 ng/ml). There was significantly high level of Hcy in Group A (mean 17.35 µmol/L) and Group C (mean 16.99 µmol/L) than in Group B (mean 13.22 µmol/L).

Another study by Naik et al, 2018 which was conducted on 46 males and 73 females from urban area reported the median serum B12 to be 198.56 and 222.28 respectively. The prevalence of B12 deficiency (B12<150pmol/L or 200pg/ml) was 77% in males and 50% in females.

In a departmental study (Agarwal and Chauhan, 2016) conducted in outpatient department of non-academic hospitals and health centre of The Maharaja Sayajirao University of Baroda among elderly of age 60-75 y reported that there were 60% (72 of 120) who had serum B12 deficiency (B12 between170-240pg/ml) and 32.5%(39 of 120) who had their serum B12 <170 pg/ml.

To conclude it can be said that large population based data on B12 deficiency from India is still evolving. Indian studies defining B12 deficiency as above states that the prevalence of B12 deficiency varies from 16 to 77%. The higher prevalence of B12 deficiency in our study (52%) is not surprising considering that the prevalence of B12 deficiency in apparently healthy population in India has been reported to be as high as 33.3%- 67% (Yajnik et al, 2006).

Our study prevalence of 52% B12 deficiency among T2DM adults on metformin also lies in this range. Cobalamin is derived from the food, of animal sources like liver, kidney, egg, fish, and milk. The vegetarians in India have lower serum cobalamin levels than non vegetarians (Khanduri et al, 2005). In the present study, high prevalence of cobalamin deficiency could be due to the low dietary intake owing to vegetarian diets in addition to metformin intake.

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Due to the diverse definitions of vitamin B12 deficiency used in most studies and the cultural and religious beliefs in different regions of the world, comparison of the prevalence of vitamin B12 deficiency among T2DM patients and healthy general populations is difficult.

Despite the high prevalence of B12 deficiency there are no clear cut guidelines to recommend B12 deficiency cut offs in diabetics which has been recommended by ADA or IDF.

VITAMIN B12 DEFICIENCY AND ANEMIA IN T2DM ADULTS ON METFORMIN

Our study results state that there was no significant difference in hemoglobin levels between those with B12 deficiency (serum B12 ≤ 200pg/ml) than those with normal B12 levels (serumB12>200pg/ml). Serum vitamin B12 deficient patients did not have a higher prevalence of anemia. Moreover, cell morphology and reports from CBC showed no positive cases of macrocytic anemia. This shows the absence of any clinical vitamin B12 deficiency in the studied sample. This also indicates that though there was biochemical B12 deficiency but B12 deficiency at tissue levels was not there because there were no cases of macrocytic anemia for B12 levels as low as 94pg/ml.
This study found that T2DM adults using metformin has low serum B12 as discussed initially but we did not find metformin use to be associated with overt B12 deficiency(clinical B12 deficiency) as there were no cases of macrocytic anemia.

Similar results have been reported by Raizada et al, 2017 who found that there was no decrease in hemoglobin in the Vitamin B12-deficient patients .On comparing patients with Vitamin B12 deficiency (n=58) (serum B12<150pmol/L) to those with normal vitamin (n=125) (serum B12>150 pmol/L), it was found that mean hemoglobin was not significantly different in the Vitamin B12 deficiency and normal Vitamin B12 groups. Similar results have been reported by de Groot-Kamphuis et al 2013 where it was reported that metformin use did not predict the chance on having anaemia.

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However the study results by Aroda et al, 2016 showed different results where anemia prevalence was higher in metformin group, but did not differ by B12 status. But the presence of anemia was not different across vitamin B12 categories at DPPOS year 1. There were non-significant increases in anemia in each of the vitamin B12 level groups at DPPOS year 9 (P<0.25) in the metformin group, perhaps related to the longer exposure to metformin.

Another cross sectional study from Oman by Al-Hamdi et al, 2020 showed that 36.3% patients were found to be anemic, of which 42.3% participants were in the vitamin B12-deficient group, 28.3% were in the borderline-deficient group and 37.9% were in the normal group. These differences were not statistically significant

To conclude it can be said that evidence is present to support that anemia is not associated with low serum B12 among T2DM adults on metformin though decrease in serum B12 among metformin users is confirmed. It can be said that among metformin users B12 deficiency as assessed by serum B12 occurs but this does not ensure clinical deficiency as there was no impact seen on the hematological parameter assessed in our study

SIDE EFFECTS OF METFORMIN AMONG T2DM ADULTS

Our study showed that of the reported gastro intestinal(GI) side effects of metformin like nausea, vomiting, diarrhea, anorexia and metallic taste, the population under study reported anorexia(11%) and metallic taste(29%) as their common GI problems however greater proportion of the population(59%) reported no GI side effects (Table 4.6).

Similar observations are reported by Wang et al, 2017 who stated that gastrointestinal side effects, including diarrhea, nausea, and vomiting, are very common and typically occur in up to 30% of patients taking metformin.

Since one of the major side effects of metformin is its B12 lowering effects, further association between the serum B12 levels and GI side effects of metformin was seen as

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depicted in Table 4.33.There was a significant relationship between the GI side effects and their recent B12 status ($p<0.05$)(Table 4.33).

In our study an association of side effects of metformin with metformin dosage was seen. It was found that a significant relationship between GI side effects of metformin with their present metformin dose existed ($p<0.01$). An increasing trend of the proportion of population reporting metallic taste as side effects was seen with the increase in present dosage of metformin. Almost three-fourth (70%) of the population had reported metallic taste as the most common side effects with the metformin dosage upto 2500 pg/ml; detrimental to their food ingestion required for dietary compliance; crucial for maintaining euglycemia in order to prevent micro vascular secondary complications and neuropathy and thereby maintaining their quality of life. However no significant association was found between recent serum B12 status and past metformin dosage.

Similar side effects were observed in HOME trial (de Jager et al, 2010) where 26 experienced adverse effects of which 6 were in placebo group while 20 were in metformin group. Of these 26, 11 experienced diarrhoea, five flatulence, four fatigue, one pruritus, one headaches, one pyrosis, one nausea, one myocardial infarction and one patient died suddenly.

Though the GI side effects of metformin exist but its prevalence is low and the glucose lowering capacity of drug outweighs this, so metformin still continues to be the first line of therapies in T2DM adults. Further it can be safely used by judicious administration of the physician.

B12 DEFICIENCY AND DOSAGE AND DURATION OF METFORMIN

Our study showed that the mean daily metformin dosage among those with B12 deficiency was 1092.59± 447 mg /1.09g while that of those with normal B12 was 1054.05±410mg/1.05.This showed that dose of metformin was slightly higher in those with B12 deficiency than those with normal B12 levels but this difference was not statistically significant ($p= 0.21$) as mentioned in Table 4.28 shown before. Further a comparison of distribution of serum B12 across present metformin dosage was made by

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box plot shown in fig 4.13 which showed that mean of serum B12 falls but median remains almost similar as one moves across the increasing metformin dosage from 500mg to 1000mg to ≥1500mg. However when the mean serum B12 of three groups with different metformin dosage was compared by ANOVA as shown in Table 4.30 there was no statistically significant difference in mean serum B12 levels between the three groups of 500mg, 1000mg and 1500mg dosage.

In our study (Table 4.38) it was found that among those who were B12 deficient there were more proportion (25.9% vs 17.6%) of T2DM adults with longer duration of diabetes (>10yrs) but these results were not statistically significant. Moreover the distribution with different duration of diabetes was studied by box plot as shown in fig. 4.14 which showed that there was a decrease in median of serum B12 with the increasing duration of diabetes among T2DM adults on metformin. This indicates that there was a trend of lower serum B12 in those with longer duration of diabetes among metformin users. By median test (Table 4.39 and Fig 4.15) it was found that there was no significant difference in the frequencies of median serum B12 between the three groups with different duration of diabetes.

In a study by Reinstatler et al, 2012 there were 575 T2DM patients on metformin from NHANES sample, U.S. The patients had a mean age of 63.4 y and median metformin duration of 5y. After excluding the patients with renal efficiency the reported prevalence of B12 deficiency was 5.8% with a B12 cut point between 145-150 pmol/l.

There are three studies from Netherland (Europe) which are discussed in the following text. In a study by de Jager et al. 2010 conducted in outpatient clinic of three non academic hospital, Netherland, there were 194 metformin treated patients whose mean age was 64 y. They were on mean metformin dose of 4.3g and they reported a prevalence of 9.9% B12 deficiency with a cut point of 145-150 pg/ml. A novel finding of this study was that decrease in serum B12 levels was progressive.

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Another study by de Groot-Kamphuis et al, 2013, conducted in secondary care outpatient diabetes clinic, Netherland reported a prevalence of 14% B21 deficiency among T2DM patients on metformin. They had a mean age of 62.6y with a median value of 4.9 y metformin duration.

In another study conducted in four primary care centre, Netherland; by Beulens et al, 2015 there was a prevalence of 28% B12 deficiency (serum B12 < 148 pmol/l) among T2DM patients of mean age 61.7y . They were on mean metformin dosage of 1.3g for a mean metformin duration of 5.3 y. They also checked holo transcobalamin, a marker of cellular cobalamin deficiency and found that the prevalence of holotrancobalamin deficiency was 3.9%. In this study duration of metformin use was not associated with cobalamin after multivariate adjustment however a higher daily and cumulative doses of metformin were strongly associated with lower cobalamin and holotrancobalamin.

In another study by Sparre Hermann et al, 2004, wherein he conducted on 153 T2DM patients on metformin in outpatient clinic of a general hospital, Sweden & reported the prevalence of B12 deficiency was 8% with a cut point between 145-150 pmol/l after excluding patients with renal impairment. The patients had mean age of 58.5 y and they were on mean metformin dose of 2.2 g for a mean duration of 5.2 yrs.

A study from another European country, Spain (Calvo Romero, & Ramiro Lozano, 2012) carried out on 81 T2DM patients from Internal medicine clinic of a first level hospital reported the B12 deficiency prevalence of 8.6 % where B12 deficiency prevalence was defined between 145-150pmol/l. These patients had a mean age of 71.6 y and were on a mean metformin dosage of 1.8 g for a mean duration of 3.6 y.

Another study by **de Groot-Kamphuis et al, 2013**, conducted in secondary care outpatient diabetes clinic, Netherland reported a prevalence of **14%** B21 deficiency(cut points 145-150pmol/l) among T2DM patients on metformin. They had a mean age of 62.6y with a median value of 4.9y metformin duration.

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Another study from Japan by **Sato et al, 2013** conducted among 62 consecutive metformin-treated patients, **B12 was deficient** (<150 pmol/L) in 8 (**13%**) and **borderline-deficient** (150- 220 pmol/L) in 18 (**29%**): the larger the metformin dosage, the lower the B12 (P=0.02, Spearman's ρ=-0.30). There were independent correlations between metformin use and B12 lowering (P=0.02, r = -0.25), and B12 lowering and elevation of homocysteine (P<0.01, r=-0.34).

Another study from **Korea** by **Ko et al, 2014**,conducted among 799 T2DM patients using metformin stated that vitamin B12 deficiency was defined as vitamin B12 ≤ 300 pg/mL without folate deficiency (folate > 4 ng/mL). The prevalence of vitamin B12 deficiency in metformin-treated T2DM patients was **9.5%** (n = 76), and the mean vitamin B12 level was 662.5 ± 246.7 pg/ml. This study concluded that T2DM adults on metformin should be screened for vitamin B12 deficiency, especially at higher dosages (> 1,000 mg) and longer durations (≥ 4 yr) of treatment because B12 deficient patients had longer duration of metformin use ($p < 0.001$) and higher daily metformin dose ($p< 0.001$) than non-deficient individuals. Compared with daily metformin dose of ≤ 1,000 mg, the adjusted odds ratio for 1,000-2,000 mg, and ≥ 2,000 mg were 2.52 (95% CI, 1.27-4.99, $P = 0.008$) and 3.80 (95% CI, 1.82-7.92, $P < 0.001$). Compared with metformin use of < 4 y, the adjusted odds ratios for 4-10 y, and ≥ 10 y were 4.65 (95% CI, 2.36-9.16, $P < 0.001$) and 9.21 (95% CI, 3.38-25.11, $P < 0.001$), respectively.

In one study by **Ahmed et al, 2016** conducted in outpatient clinic of two tertiary hospital, South Africa, prevalence of B12 deficiency was **28%** defined for serum B12 levels in between 145-150 pmol/l. The mean age of T2DM patients was 58.5 y and they were on mean metformin dosage of 2.4g for a mean duration of 9.6y and they excluded renal impaired patients. The means of metformin duration of use and total daily dose were 9.6 y and 2.4 g, respectively. The mean cumulative dose of metformin was 23.7 g. The study revealed that vitamin B12-deficient participants were significantly older than those with normal vitamin levels (62.3 vs. 57 years, $P = 0.012$). They also had significantly longer metformin use duration (11 vs. 8 years, $P = 0.015$) and higher cumulative metformin dose (28.9 vs. 17 g, $P = 0.009$).

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In a recent study by Al-Hamdi, 2020 from Oman the dose of metformin was higher among the vitamin B12 deficient group compared to the normal group (1981 ± 222 versus 1695 ± 494 mg; $P = 0.004$) . A higher proportion of those receiving metformin doses of ≥2000 mg had vitamin B12 deficiency ($P = 0.004$). There was no association between the duration of metformin use and the vitamin B12 level categories. There was significant negative correlation between cumulative metformin dose and vitamin B12 level ($r=-0.68$, $P<0.000$).

However in our study we cannot comment upon cumulative metformin dosage as it could not be calculated because of the unavailability of the data on duration of metformin owing to the study being cross sectional in nature.

B12 DEFICIENCY AMONG T2DM ADULTS AND DIET

In our study we studied various factors associated with serum B12 deficiency (Table 4.49) where the diet emerged as the only significant predictor for B12 deficiency on metformin treated T2DM adults. The odds of having B12 deficiency(≤ 200pg/ml) was 2.33 times higher (CI-1.216-4.467) among T2DM adults on metformin if they were on a vegetarian diet than those on a non vegetarian diet ($p<0.05$).

In one study by Hermann et al, 2003, 174 subjects from Germany and Netherlands were recruited in three groups: 66 lactovegetarian/lactoovovegetarian, 29 vegans and 79 omnivorous group. Of these three groups, the vegans had the lowest vitamin B-12 status. In subjects who did not consume vitamins, low holotranscobalamin II (< 35 pmol/L) was found in 11% of the omnivores, 77% of the lactovegetarian/lactovegetarian group, and 92% of the vegans. Elevated methylmalonic acid (> 271 nmol/L) was found in 5% of the omnivores, 68% of the lactovegetarian/lactoovovegetarian group, and 83% of the vegans. There was Hyperhomocysteinemia (> 12 μmol/L) in 16% of the omnivores, 38% of the lactovegetarian/lacoovovegetarian group, and 67% of the vegans. There was a weak correlation between holotranscobalamin II and vitamin B-12 in the low serum vitamin B-

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12 range ($r = 0.403$) and strong in the high serum vitamin B-12 range ($r = 0.769$). Holotranscobalamin II concentration was the main determinant of total homocysteine in the vegetarians ($\beta = -0.237, P < 0.001$). Hence it was stated that vegan and lactovetarian/lactovovegetarian group had metabolic features indicating vitamin B-12 deficiency that led to a substantial increase in total homocysteine.

In one study by Yajnik et al.2006 to determine the frequency of vitamin B12 deficiency and hyperhomocysteinemia among 441 healthy middle aged Indian men, vitamin B12 deficiency as defined by vitamin B12 <150 pmol/L was reported among 67% of the study participants. Vegetarian diet was the sole significant factor associated with low vitamin B12 levels in this study on multivariate analysis (OR 4.4 95% CI 2.1-9.3).

To conclude it can be said that diet has an effect on serum B12 levels and homocysteine (crucial for preventing CVD) in an individual and the existence of two factors like vegetarianism and metformin among T2DM adults make them more prone to serum B12 deficiency.

NEUROPATHY AMONG T2DM ADULTS ON METFORMIN AND ITS RELATIONSHIP WITH B12 DEFICIENCY IN T2DM

Neurologic damage, a possible consequence of metformin- induced vitamin B12 deficiency, can present as peripheral neuropathy and may be mistaken for diabetic neuropathy in patients on metformin treatment (Bell, 2010). Low vitamin B12 levels have been reported to be associated with worse nerve conduction velocities and poorer responses to light touch by monofilament detection (Leishear et al, 2012).

As vitamin B12-associated neuropathy is a treatable and reversible condition, early detection and treatment of vitamin neuropathy along with B12 deficiency is clinically important in patients with diabetes using metformin.

In our study ROC analysis (Fig 4.18 and Table 4.44 and 4.45) between DPN and B12 deficiency showed that when B12 screening is done among diabetics on metformin then those who have B12 deficiency (defined as serum B12≤ 200 pg/ml) are probable to have DPN scores of 2.25($p<0.001$)

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Further in our study it was seen that of those suffering from DPN whether low or high majority had B12 deficiency (58.2% and 70.5% respectively) in comparison to those with no DPN where B12 deficient population was only 15.4%.(Fig 4.17 and Table 4.43). Also the mean DPN scores (3.1±1.86 vs 1.55±2.03) as well as mean MNSI History scores (2.80±2.48 vs 4.9±2.79) were significantly different between those who had B12 deficiency than those who had normal B12 levels ($p< 0.001$). Further Pearson correlation between serum B12 and DPN scores came out to be non significant ($r= - 0.127$, $p= 0.116$). Higher the DPN score lower the B12 status thus more was the occurrence of B12 deficiency. However the strength of correlation was weak.

Similar results were shown by in a study by **Qureshi et al, 2011** where vitamin B_{12} levels were measured infrequently in T2DM, in particular among those with peripheral neuropathy. Levels were frequently low when assessed among T2DM patients with peripheral neuropathy. A record that vitamin B_{12} therapy was initiated was only made in a small number of cases, so the impact on peripheral neuropathy was unclear. It was found that where vitamin B_{12} levels were deficient, replacement vitamin B_{12} was documented in only two (2.9%) patients and improvement in neuropathic symptoms post treatment were documented in only four (5.7%) patients.

Similar association was shown in one cross sectional Indian study from **Kolkata by Roy et al**, 2016 involving randomly selected ninety patients in 35 -70 y, comparing those who had received >6 months of metformin (Group A) (n = 35) with those without metformin (Group B) (n = 35) and patients taking metformin with other OHA (Group C) (n = 20). Comparisons were made on serum Cbl, fasting Hcy, and folic acid, and with electrophysiological measures (nerve conduction studies of all four limbs). **The study showed that Group A (metformin users) patients (54.28%) were prone to develop peripheral neuropathy** comparing Group B (28.57%) and Group C (35%). Metformin users even for 2 years showed evidence of neuropathy on nerve conduction velocity though their body mass index and postprandial blood sugar were maintained. Even short-term treatment with metformin causes a decrease in serum Cbl folic acid and increase in Hcy, which leads to peripheral neuropathy in Type 2 diabetes patients.

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In our study the DPN scores(1.55±2.03 vs 3.1±1.86) calculated from MNSI Physical assessment score as well as MNSI history scores(4.9±2.79 vs 2.80±2.48) were higher in those with B12 deficiency than those without B12 deficiency respectively($p<0.001$).

Similarly, association between neuropathy and B12 deficiency were seen in another study by **Singh et al, 2013** where they compared vitamin B12 levels and severity of peripheral neuropathy using **Toronto Clinical Scoring System (TCSS)** in metformin versus non metformin groups. It found that mean **neuropathy score was significantly higher in metformin exposed group** (5.72±2.04 versus 4.62±2.12, P=0.0064) as compared to non metformin group.

In one recent study from Colombia by Alverez et al, 2019 diabetic neuropathy was studied in 122 patients using clinical history, **nerve conduction study or performing MNSI**. In this group of 122 patients, 34 **(27%)** were diagnosed with diabetic neuropathy. The prevalence of altered vitamin B12 levels (low or borderline) in patients with diabetic neuropathy was 64% (95% CI: 47–78%). Low levels of vitamin B12 were found in eight patients (23%; 95% CI: 12–40%) and borderline levels were found in 14 patients (41%; 95% CI: 26–47%); 12 patients had normal levels (35%; 95% CI: 21–52%). Diagnosis of **diabetic neuropathy was associated with lower levels of vitamin B12** (Coefficient: 116.9; 95% CI: −165.8, −68.0)

On the contrary the study from diabetes clinic of two tertiary hospitals of South Africa by **Ahmed et al, 2016** showed no association between neuropathy and B12 deficiency. They found that 32.3 % of vitamin B12 deficient participants had neuropathy compared to 36.8 % of those with normal vitamin levels. Chi square test results showed a Chi square statistic value of 0.209 with an associated probability of 0.647, indicating absence of enough evidence to claim an association between vitamin B12 status and neuropathy binary variables in the population. The value of Spearman's rank correlation coefficient (rho) was 0.056 with a P value of 0.54, indicating that there was **no sufficient evidence of association between vitamin B12 levels** and NTSS-6 scores. Comparable results of no association were also obtained when the correlation between vitamin B12 levels and

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NTSS-6 scores was examined in those with deficient (rho = 0.284, $P = 0.10$) and normal (rho = 0.057, $P = 0.59$) vitamin B12.

In one Case-control study by **Wile & Toth, 2010**, conducted at Neuromuscular clinic at a university hospital, Canada among T2DM patients on metformin found 59 patients with primary diagnosis of peripheral neuropathy. Controls were T2DM patients not taking metformin with primary diagnosis of peripheral neuropathy (63 participants). The **metformin group had more severe peripheral neuropathy as assessed by TCSS and NIS**. Electrophysiological markers showed no significant difference between the two groups. Cumulative metformin dose showed a significant positive correlation with TCSS scores (rho = 0.80) and NIS scores (rho = 0.79).

In another cross sectional study at secondary care outpatient diabetes clinic Netherlands by **de Groot-Kamphuis et al, 2013** randomly selected T2DM patients who were divided into metformin users (164 participants) and non users (134 participants).**Prevalence of neuropathy as obtained from records was significantly lower in the metformin group.**

In another cross sectional study by **Chen et al, 2012** at diabetes clinic of a tertiary hospital, UK randomly selected T2DM patients were divided into metformin users (152 participants) and non users (50 participants). All peripheral neuropathy-assessing tools (monofilament, neurothesiometry, NTSS-6, and s-LANSS) showed no significant differences between the two groups.

In another cross sectional study by Biemans, E. et al. (2014) at four primary care centers in Netherlands, the metformin-treated T2DM patients were divided into the vitamin B12-deficient (126 participants) and normal (322 participants) groups. There were no significant differences in peripheral neuropathy as assessed by MNSI between the two groups.

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In another cross sectional study by Russo et al, 2016 at diabetes clinic of a university hospital, Italy the T2DM patients were divided into metformin users (124 participants) and nonusers (139 participants).There was no significant difference in prevalence of peripheral neuropathy between the two groups. Peripheral neuropathy was suspected based on abnormalities of certain evaluations and confirmed by nerve conduction velocity.

In another study by **Raizada et al, 2017** from endocrinology department of a tertiary care hospital, Delhi, India it was found that **B12-deficient patients did not have significantly higher percentage of DNE or DNS positives** on comparing patients with Vitamin B12 deficiency to those with normal Vitamin B12 levels.

To conclude we can say that the results of association of DPN with metformin induced B12 deficiency were conflicting and there were differences in designs and settings of various studies discussed above. Neuropathy was assessed by different tools with various degrees of subjectivity and most of the studies had relatively small sample sizes .

PREVALENCE OF DPN AMONG T2DM ADULTS ON METFORMIN USING DIFFERENT TOOLS FOR MEASURING DPN

Neuropathy is estimated to be present in 10%–90% of the patients with diabetes although it changes according to diagnostic criteria and patient population. Diabetic peripheral neuropathy is the most common type of diabetic neuropathy, and it is frequently used synonymously with it (Dyck et al, 1993) .

In our study the prevalence of DPN was 73.6% as assessed by MNSI physical assessment score (DPN score>0) which was alarmingly high. However when the DPN prevalence was defined at DPN scores≤2.5 then it was 39.2% and when defined at DPN scores >2.5 then it was 34.3%. Similar prevalence of DPN was reported by **Mete et al, 2013** from **Turkey** where neuropathy defined by MNSI physical assessment score ≥2.5 was found in 34 (32.1%) in type 2 diabetes patients .

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In our study the mean score of MNSI history score was 3.88± 2.79 (minimum 0,maximum 10) and the mean score of MNSI physical assessment score was 2.14±1.98 (minimum 0, maximum 7.5). The mean MNSI history score (Minimum 0 and maximum 10) in our study was less in comparison to that obtained in the study by **Mete et al, 2013** which reported 6.7 ± 2.7 (maximum 12, minimum 3 points) as its MNSI history score.

In our study no association of DPN and duration of diabetes was observed. However in study by Mete, T. et al 2013 the diabetic period was longer compared to the patients not diagnosed by MNSI, and the difference was statistically significant (p=0.04). Mean diabetic period for the 34 patients diagnosed with diabetic peripheral neuropathy by MNSI was 125.9 (0–300) months.

Our study was limited to the fact that DPN assessment by MNSI was not compared by nerve conduction velocity or neurothesiometer or biothesiometer. However in a study by Mete et al, 2013 it was found that DPN assessment by MNSI (32.1%) often present lower diabetic peripheral neuropathy prevalence than that by Neurothesimeter (74.5%) and electromyograpgh (46.2%) respectively.

Another study by Feldman et al 1994 showed that MNSI is a good screening tool for diabetic neuropathy and that the score from MNSI coupled with nerve conductions provides a simple means to confirm this diagnosis. In this study patients neuropathy was assessed in patients with type I and type II diabetes.

In our study the prevalence of various grades of DPN were as follows: low DPN(39.2%) (MNSI physical assessment score greater than 0 but less than equal to 2.5) followed by high DPN (34.3%) (MNSI physical assessment score greater than 2.5).The prevalence of DPN in our study was higher than that studied by Rani et al, 2010 where diabetic neuropathy was considered as present if the VPT value was >20 V, mild neuropathy (VPT score, 20-24.99 V), moderate neuropathy (VPT score, 25-38.99 V), and severe neuropathy (VPT score, >39 V).The study showed the prevalence of diabetic neuropathy was 18.84% (95% CI: 16.79-20.88); the prevalence of mild diabetic neuropathy was

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5.9% (95% CI: 4.68-7.15), moderate diabetic neuropathy was 7.9% (95% CI: 6.50-9.33), and severe diabetic neuropathy was 5% (95% CI: 3.86-6.14).

In our study the DPN prevalence as defined by MNSI DPN scores > 2.5 was 34.3% which was similar to 39.3% shown by Darivemula et al, 2019 where DPN prevalence was studied by a predesigned semi-structured questionnaire, Semmes-Weinstein 10-g monofilament test, ankle reflexes, and vibration perception threshold.

In our study among the several risk factors for DPN studied by O.R the significant risk factors were B12 deficiency and Glycemic control. However the study by Darivemula et al, 2019 found significant association between age, sex, BMI, duration of diabetes, and hypertension and the odds of DPN, which was observed similarly in another prevalence study by Pradeepa et al, 2008.

In our study the MNSI history questionnaire showed that 67.8% showed numbness in their feet/ legs however half of them (53.5%) had burning pain in their legs/feet and 44.1% had prickly feeling in their leg or foot. Similar results were seen in an Indian study by Darivemula S. et al 2019 which showed that 61% had a burning foot sensation, 36.9% of them had numbness of the foot, almost 50% of them had pricking sensation in the foot.

To conclude it can be said that there is evidence to state that accuracy of MNSI scoring makes it a useful screening test for diabetic neuropathy in taking a decision regarding which patients should be referred to a neurologist for electrophysiological studies. High specificity, likelihood ratios over 5 and a moderate to good post-test probability give a high diagnostic impact for MNSI scoring and it cut off point of 2 was suggested for the MNSI procedure (Moghtaderi et al, 2006). However, electrophysiological studies can be considered when the patient has signs and symptoms other than those scored by the MNSI. Thus MNSI has emerged as a good screening test for DPN.

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QUALITY OF LIFE AMONG T2DM ADULTS

B12 deficiency if remains undiagnosed or untreated it may aggravate the peripheral neuropathy symptoms, and especially neuropathic pain, can be severe, have sudden onset, and are associated with lower quality of life, limited mobility, depression, and social dysfunction (ADA, 2014).

Thus studying quality of life was important and QoL was studied using WHOQoL Bref version as it is reported that WHOQOL Bref may be useful in studies which incorporate QoL as one of the several variables or where multiple assessments over a period of time are envisaged.(Saxena et al, 1998)

Diabetic's QoL becomes worse when complications start to develop or comorbidities coexist. Dominant amongst complications, in health-related quality of life (HRQoL) lowering, but not related to risk factors (genetic, the weight of birth, or others) is coronary arterial disease followed by renal failure, blindness, and the combination of micro- and macro-vascular complications and in some studies by sexual dysfunction.(Trikkalinou et al, 2017)

Our study aimed to answer whether the quality of life was deteriorated with a co morbidity of metformin induced B12 deficiency and neuropathy microvascular complication among T2DM adults on metformin. The study showed that quality of life as assessed by WHOQOL-Bref was significantly lower for all the four domains of WHOQoL Bref (physical, psychological, social relationship and environment) among those who were B12 deficient than those with normal B12 status ($p<0.001$) (Table 4.47) .

Further the intervention trial in our study showed that after supplementation the mean scores of the two domains - Physical Health and Psychological Health increased in both the groups very significantly ($p<0.01$) however increase in the mean scores of Ca+B12 group was more than that of the B12 supplemented group (Table 4.63). This suggests that supplementation of calcium along with B12 is more effective than B12 supplementation alone in improving physical health and psychological health of the T2DM adults on meformin.

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There was significant difference ($p<0.05$)in post supplementation mean QLI scores in between two groups for several facets of QLI like overall QLI, physical pain and discomfort, need of medical treatment in daily life, accept bodily appearance, activities of daily living, sex life and negative feelings in life. However there was very significant difference ($p<0.01$) in post supplementation mean QLI scores in between two groups for several facets of QLI like safety in daily life, how healthy is physical environment, financial resources, opportunity for acquiring new information, leisure activities, work capacity, personal relationship, condition of living place, satisfaction with access to health services, satisfaction with transport.

Similar to our study the impact on various facets of QoL was studied in one cross sectional study from Argentian by Pichon-Riviere et al in 2015 conducted on 183 type 2 diabetes patients by diabetes-specific HRQoL questionnaire, the Audit of Diabetes Dependent Quality of Life (ADDQoL), the greatest negative impact on Qol was observed for domains: 'worries about the future', 'freedom to eat', 'living conditions', 'sex life', and 'family life'.

In our study it was found that those with DPN had lower domain scores for all the four domains of WHOQoL-Bref (Table 4.23).The association of DPN with QoL when seen by Pearson correlation it was found that DPN scores had a highly significant weak negative correlation with all the four domains of WHOQol Bref ($r= -0.442$, $p=0.000$ for DPN scores and Physical domain, $r= -0.435$,$p=0.000$ for DPN scores and Psychological domain,$r= -0.478$,$p=0.000$ for DPN scores and social relationship and $r= -0.484$, $p=0.000$ for DPN and Environment domain). It can be said that as the DPN scores increased the domain scores decreased among T2DM adults on metformin.

Similarly negative association between neuropathy and QOL was seen in one study from south India by Prajapati, V.B. et al 2017 which was conducted on 250 T2DM adults (from medicine wards of tertiary care hospital) to assess the quality of life using the modified diabetes quality of life (MDQoL)-17 questionnaire. Majority of the diabetic patients had the QoL score between 70 and 50. Patients without complication had a better QoL. As the number of complications increased, there was a decrease in the QoL. The presence of co morbidity also decreased the QoL showed a statistically significant

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decrease in QoL (53.99±16.39) in the patients with neuropathy (P=0.003). It was also observed that among the diabetic complications the patients with neuropathy had the least QoL score.

Similarly Benbow, 1998 reported that neuropathy impairs the QoL in his study which was conducted on 79 diabetics and 37 non diabetics. The QoL was measured using Nottingham Health profile (NHP) which consisted of six domains assessing energy, sleep, pain, physical mobility, emotional reactions and social isolation. The neuropathy patients had significantly higher scores (impaired QOL) in 5(emotional reaction, energy, pain, physical mobility and sleep) out of 6 NHP domains than either the other diabetic patients ($p<0.01$) or the non diabetics ($p<0.001$).

In our study QoL domain scores of poor glycemic control group was lower than that in the good glycemic group for all the four domains: Physical Health, Psychological health, Social relationship and Environment of WHOQOL Bref . Further correlation between HbA1c and QoL domain mean scores showed that there was significant weak correlation between HbA1c and physical domain ($r = -0.224$, $p=.044$), H1Ac psychological domain ($r=- 0.352$, $p=0.001$), HbA1c and social relationship ($r= -0.372$) and HbA1c and environment domain($r= -0.353$, $p=0.001$).

Similar association of QoL and glycemic control was shown in study by Shim et al, 2012 which reported poorer health-related quality of life to be associated with higher HbA1c values. They measured QoL by survey questionnaire which included both a generic health-related quality of life measure, the Euroqol 5-D and a diabetes-specific instrument, the Audit of Diabetes-dependent Quality of Life and patient's most recent HbA1c values were extracted from their medical records. A negative correlation was found between health-related quality of life and HbA(1c) values in both health-related quality of life measures (both $r=-0.2$, $P=0.001$).

Similar association of QoL and glycemic control was found by Svedbo Engström M. et al 2019 on type I and type 2 diabetics mixed population by using generic 36-item Short Form version 2 (SF-36v2) to assess QoL. Correlation analyses showed weak correlations between scores on the SF-36v2 and glycaemic control for both diabetes types.

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In one study by Lau et al, 2004 it was found that there was an association between better HbA1c and improved mental QoL, but not physical QoL. Here QoL was studied by Short Form 36 (SF-36), which measured health-related QOL, pre and post HbA1c data. In this study Average Mental Component Summary score increased by 8.46% and Physical Component Summary (PCS) score decreased by 2.24%. After adjustment, a 5% decrease in HbA1c values was associated with a 1% increase in Mental Component Summary(MCS). No association between changes in HbA1c and PCS was observed.

To conclude it can be said that diabetics with poor glycemic control or lower B12 levels or DPN have low quality of life. Thus proper management of co morbidities like B12 deficiency in our study and strict glycemic control is necessary to prevent progression and occurrence of complication like neuropathy to maintain a better QoL in diabetes patients. Diabetic complications, comorbidities, and cost of treatment affect the quality of life (QoL) of an individual. The QoL assessment should be considered an important measure of outcome in chronic disease management like type2 diabetes. Quality of Life is an important and measurable outcome of healthcare interventions; and the data from our study can be used by policy-makers to prioritize health resources.

INTERVENTION TO TREAT VITAMIN B12 DEFICIENCY AMONG T2DM ADULTS ON METFORMIN

Metformin induced B12 deficiency if left untreated can give rise to peripheral neuropathy as B12 has a role in myelination of nerves and it can coexist with peripheral neuropathy said to occur by uncontrolled diabetes. So it becomes crucial to plan interventions to treat B12 deficiency.

The results of meta-analysis by Liew, J et al 2019 showed that B vitamins had positive effect on neurophysiological symptoms and/or functions compared to baseline in 38 studies. RCTs evaluating the efficacy of B vitamins in standardized combination/doses are limited. Further studies are required to elucidate optimum treatment combination/doses and duration.

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Oral administration of high-dose vitamin B12 (1 to 2 mg daily) is as effective as intramuscular administration for correcting anemia and neurologic symptoms. Intramuscular therapy should be considered in patients with severe deficiency (Langan Robert and Andrew Goodbred, 2017)

The large NHANES data by Reinstatler, 2012 suggests that consumption of any supplement containing B12 was not associated with a reduction in the prevalence of biochemical B12 deficiency among those with diabetes, whereas consumption of any supplements containing B12 was associated with a two-thirds reduction among those with diabetes. The amount of B12 recommended by Institute of Medicine (2.4µg/day) and the amount present in multivitamins (6µg) may not be enough to correct biochemical B12 deficiency among type 2 diabetes adults.

In 2012 a case report (Kumthekar et al, 2012) was presented with 60pg/ml serum B12 levels and experienced tingling numbness of both hands and feet. His blood examination revealed a Hb of 12.1gm/dl and HbA1c of 8.5 %. VPT done with a Bio-Thesiometer demonstrated moderate grade peripheral neuropathy in both lower limbs (Between 21-25 Volts). Patient was asked to discontinue metformin, his anti-diabetic medications were adjusted for better glycemic control and was given injectable B12 1 week apart for 4 weeks. B12 levels after completion of injectable therapy was 888.60pg/ml and VPT testing still demonstrated moderate neuropathy. B12 level repeated after 8 months following discontinuation of metformin therapy was 424.0 pg/ml and VPT testing showed no neuropathy(< 15 volts), which was a marked improvement.

Our study showed that calcium supplementation of 500mg in combination with 1000µg of B12 supplementation for 8 weeks was efficacious than the 1000µg B12 alone in improving B12 deficiency and physical as well as psychological health of QLI. However improvement in DPN was seen in both groups, more so in B12 supplementation alone.

As depicted in Table 4.52 it was found that there was a very significant($p<0.01$) decrease in the post supplementation mean glycated Hb levels, a highly significant($p<0.001$) increase in the post supplementation mean serum B12 and a significant($p<0.05$) decrease

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in the post supplementation mean DPN scores in both the two groups. As regards QoL the mean scores of the two domains - Physical Health and Psychological Health increased significantly in the B12+Calcium group ($p<0.01$)(Table 4.63).

After supplementation of eight weeks a total of 52 out of 80 (65%) attained normal B12 levels (200pg/ml).It was seen that in experimental group 46 out of 50 (92%) attained normal serum B12 levels while only 6 out of 30 (20%) attained normal B12 levels in control group ($p<0.001$)(Table 4.54).Over all after supplementation the prevalence of high DPN (DPN scores>2.5) decreased by 17.5% (61.25% to 43.75%) whereas prevalence of no DPN increased by 5% (6.25% to 11.25%).

As shown in Table 4.55 it was seen that overall after supplementation the prevalence of high DPN (DPN scores>2.5) decreased by 17.5% (61.25% to 43.75%) whereas prevalence of no DPN increased by 5% (6.25% to 11.25%).

Efficacy of calcium supplementation in metformin users is also seen in one study by **Kocaçiftçi et al, 2013** which was conducted among T2DM adults who were randomized into two groups; one group received metformin; daily 2x1000 mg (group 1), while the other group received metformin; 2x1000 mg/d plus oral calcium supplements; 1x1000 mg/d (group 2) in a 3 month-period. It was found that vitamin B12 levels decreased less with metformin plus calcium therapy compared to only metformin therapy. This shows that additional calcium supplements may prevent B12 vitamin deficiency and associated complications in patients on metformin therapy.

In one study by Bauman et al. 2000 it was found that dietary supplements of oral calcium carbonate (Tums, 1.2 g/day) in the metformin treated group partially reversed the decreased serum holo TCII levels: the bio available form of B12. The serum holoTCII increased from 111 ± 21 to 153 ± 11 pg/ml—a 53 ± 15% increase after calcium supplementation for one month duration ($p= 0.005$)—but the serum total vitamin B12 level did not change significantly. Bauman et al 2000 concluded that individuals with type 2 diabetes receiving metformin develop low bioavailable B12 (holoTCII), which, if allowed to progress, would be expected to be followed by low serum total vitamin B12

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levels and, presumably, eventual clinical deficiency; this sequence of events was not addressed in this study.

To conclude it can be said that DPN may present with symptoms that may be indistinguishable from that of vitamin B12 deficiency, the condition of metformin-induced low serum vitamin B12 is of great concern if not recognized and treated appropriately. Ionic calcium is required for the B12-IF complex to attach to ileal cell surface receptors, and metformin competes with calcium for the mucosal cell membrane. However this form of vitamin B12 malabsorption was reversible with oral calcium supplement. T2DM adults on metformin, especially those who do not consume milk or milk products on a daily basis or do not take supplemental calcium should be encouraged to increase their intake of calcium and should be monitored for vitamin B12 deficiency.

In our study the calcium supplementation at 500mg dosage, used in study keeping in mind the RDA for Indians in adult population was not enough to overcome the metformin induced low B12 levels over and above 1000µg B12 supplementation alone. There is a need to study this metformin- induced low B12 levels (nutrient- drug interaction) by planning randomized control trials at higher doses of calcium, above its RDA amongst the Indian population of T2DM adults on metformin.

We would like to notify that the impact of 500mg calcium supplementation studied here was in presence of 400IU vitamin D3 as the calcium supplementation used in the study was in combination with vitamin D3 because in India calcium supplements cannot be manufactured without D3 as vitamin D is required for calcium absorption.

LIMITATIONS OF THE STUDY

As regards B12 status among T2DM adults on metformin the study was limited to the fact that we have not used the functional biomarkers of B12 to assess B12 deficiency at tissue level. Measurement of additional biomarkers like holotranscobalamine, methyl malonic acid, red blood cells-B12 and plasma concentration of methylation indices would provide a comprehensive assessment of B12 deficiency at tissue level. They are beyond the scope of this study.

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There is reasonable debate about the clinical significance of biochemical B12 deficiency versus the B12 tissue deficiency. As cited in Aroda et al, 2020 some studies suggested that metformin improves intracellular metabolism of B12, despite low serum B12. There are few animal studies which have suggested that metformin alters tissue distribution and metabolism of vitamin B12, rather than causing deficiency. However in 2016 Aroda et al, found that approximately 50% of participants had concurrently elevated homocystiene levels suggesting true tissue deficiency.

As regards DPN, the study was limited to the fact that we have used MNSI to assess DPN and not used Electrophysiology (Nerve conduction Velocity) , Biothesiometer or Neurothesiometer due to the unavailability of these instruments in the hospital setting .

Lastly, in our study we could not analyze an association between the duration of metformin and B12 deficiency because the exact information on metformin duration could not be collected as it was a not a cohort study. At the first point of contact with the patient we had confirmed from the patient recall that all the patients enrolled in the study were on metformin for a minimum of four months duration but the information on exactly how long had they been taking metformin since the diagnosis of diabetes could not be retrieved through case papers for valid documentation