Effect of Vitamin B12 versus Calcium with B12 supplementation for eight weeks on Neuropathy, Quality of Life and B12 levels in T2DM Adults on Metformin

Synopsis of Doctoral Research

Submitted by

Tripti Saxena

(PhD Registration No. FoFCSC/2/149)

Guide: Prof. Uma M. Iyer (Ph.D)

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 - INDIA

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INTRODUCTION

Diabetes is one of the largest global health emergencies of the 21st century.[1]
According to the 7th edition of International Diabetes Fedaration atlas, it is estimated that currently 415 million people world wide are suffering from diabetes and this number is said to increase to 642 million by the year 2040.The major proportion of this increase will occur in developing countries of the World(about 80%) like India, where the disorder predominantly affects younger adults in the economically productive age group[2]

Diabetes dramatically increases the risk for a wide variety of complications from retinopathy to peripheral neuropathy to cardiovascular disease, which if undetected can have a devastating impact on the quality of life and place a substantial burden on health care cost[3]

The problem of diabetes has been rising in leaps and bounds in India particularly type 2 diabetes(T2DM).The number of people with diabetes in India started to rise in 1980s and 1990s and from the year 2000 onwards, there has been an explosion of the number of people with diabetes in India[4]. The number of people with diabetes has increased from 19 million in 1995 to 66.8 million people in 2014[4]. There is overwhelming burden of diabetes among low and midddle income countries where India ranks second[1]. Of all forms of diabetes T2DM comprises 90% of this population around the World[2]

As per American Diabetes Association (ADA) guidelines, metformin and lifestyle modifications are first line therapies in treatment of T2DM. As of 2009, metformin is one of the only two oral antidiabetics in the WHO Model List of Essential Medicines. Due to this, metformin is either used as monotherapy or as combination therapy with other oral antidiabetic agents or insulin.
Metformin has become the most widely used antidiabetic drug despite of the fact that it causes vitamin B12 deficiency.

Vitamin B12 absorption

Vitamin B12 is ingested in body from an animal source and when it reaches mouth it combines with R proteins secreted by salivary glands. This R protein is then hydrolysed by gastric juices and B12 is released in the duodenum where it combines with the intrinsic factor(IF) secreted by gastric parietal cells and forms B12+IF complex. Thereafter it attaches to its specific receptors on the mucosa of the terminal illeum and this step is calcium mediated B12 then combines with trans cobalamine II forming Holo TC II which then comes in blood circulation.

Nutrient Drug Interaction

In the study by Bauman et al (2000) the results for calcium supplementation were significantly associated with the increase in serum holo TCII, which indicated that the transfer of B12-IF complex into holo TCII is independently facilitated by calcium despite the B12 lowering effect of metformin. This observation gave a clue that the nutrient calcium is overcoming the inhibitory effect of the drug metformin at the conversion of B12+IF complex, thereby releasing B12-IF complex in the terminal illeum for absorption. Therefore calcium seems to reverse the malabsorbtion of vitamin B12 due to metformin in T2DM males[1].

Metformin induced low B12 levels and Type 2 Diabetes Mellitus

Despite its very superior glycemic lowering effect, metformin has for long been shown to decrease vitamin B12 levels. 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 respectively [18]. This side effect of metformin has been demonstrated again in several ensuing cross sectional studies [6,19,20], case reports [21,22,23] and randomised controlled trials [24,25]. The risk of developing metformin associated vitamin B12 deficiency is greatly influenced by increasing age, metformin dose and duration of use [25,26]. Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as fourth month. Metformin induced B12 deficiency (< 200pg/ml) has been found to **be 5.8% to 33%** [5,6,7] and it is said that this wide range of B12 deficiency is due to varied study definition of B12 deficiency because there are no cut offs to define the deficiency levels. Along with oral B12 supplementation (given by physician), oral Ca supplementation was given in the form of calcium citrate melleate at a dose of 500mg per day. When calcium is given as dietary supplements in the metformin- treated group it reversed the decreased serum holoTCII levels. The serum holoTCII increased after calcium supplementation from month 3 to month 4 ($P < 0.005$) but the serum total vitamin B12 level did not change significantly[8].

Moreover the study also aims at studying neuropathy in T2DM adults on metformin because the peripheral neuropathy of diabetes 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. 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 diabetics

Rational for doing vitamin B12 screening in T2DM:

Currently, there are no published guidelines advocating for routine screening for vitamin B12 deficiency among patients with type2 DM.

Measurement of the serum vitamin B12 concentrations should be the preliminary screening step for vitamin B12 deficiency among patients with T2DM. Concentrations <200 pg/ml are usually diagnostic of vitamin B12 deficiency while concentrations >400 pg/ml confirm absence of vitamin B12 deficiency [32].

There are no guidelines to address how often patients of T2DM should be supplemented with vitamin B12. The optimal supplementation dose of vitamin B12 and the frequency with which vitamin B12 should be given is still a question mark.

Rational for Calcium Supplementation in T2DM Males with Low Vitamin B12 levels

Vitamin B12 absorption in gastrointestinal tract forms a complex with intrinsic factor after following a sequence of reaction starting its digestion from mouth. The vitamin B12–IF complex is highly resistant to proteolytic degradation. The complex attaches at its specific receptors on the mucosa of the terminal ileum, a site where its absorption occurs. This stage of vitamin B12 absorption is calcium mediated. Absorption of Ionic calcium is obligatory for the B12-IF

complex to attach to ileal cell surface receptors, and metformin competes with calcium for the mucosal cell membrane.This form of vitamin B12 malabsorption was reversible with an oral calcium supplement[1]

Metformin treated type 2 diabetic patients with low vitamin B12 levels and not on any calcium supplementation (who do not consume adequate milk or milk products on a daily basis) form a potential group to intervene with calcium supplementation along with monitoring for vitamin B12 levels because if it comes out to be effective then calcium supplements can be started as a routine line of therapy in treatment of T2DM on metformin.

Justification:
The study is aimed to assess the effect of vitamin B12 versus Calcium with B12 supplementation for eight weeks on neuropathy, quality of life and vitamin B12 status in type 2 diabetics(T2DM) adults on metformin. The study is preliminary of its kind in that it is an attempt to study nutrient - drug interaction. Vitamin B12 absorption is inhibited by metformin and it is hypothesised that calcium reverses this.
Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as fourth month. Metformin induced B12 deficiency (< 200pg/ml) has been found to be 5.8% to 33%[5,6,7,] and it is said that this wide range of B12 deficiency is due to varied study definition of B12 deficiency. Calcium reverses the metformin induced low B12 levels in T2DM [8,9]

With the above background, the study was planned with the following objectives under the mentioned phases of study:

OBJECTIVES

Phase I

1.To assess the nutritional status of T2DM adults on metformin in relation to

a) Anthropometry

b) Diabetes Peripheral neuropathy(DPN) by Michigan Neuropathy Screening Instrument(MNSI)

c) Quality of life by WHO Quality of life Index(QLI)

Phase II

1. To study the prevalence of vitamin B12 deficiency among T2DM adults on metformin
2. To study the association of vitamin B12 levels among T2DM adults in relation to DPN and Quality of Life and metformin therapy
3. To assess and study the prevalence of anemia in relation to metformin induced B12 levels

Phase III

1. To assess the effect of vitamin B12 versus Calcium with B12 supplementation for eight weeks on vitamin B12 status, neuropathy and quality of life in T2DM adults on metformin.

REVIEW OF LITERATURE

According to the ADA guidelines, metformin and lifestyle modifications are the first line therapies in the treatment of type 2 diabetes mellitus. Metformin does, however, cause vitamin B-12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency. Therefore the research was undertaken to assess the effect of vitamin B12 versus Calcium with B12 supplementation for eight weeks on neuropathy, quality of life and vitamin B12 status in T2DM adults on metformin. Thus for this, the requisite literature review will be discussed under the following heads:

- Diabetes: Definition and Diagnosis, Prevalence, Etiology, Pathogenesis and Secondary Complications
- Drugs in diabetes mellitus: Metformin in T2DM
- Metformin induced low B12 levels and Type 2 Diabetes Mellitus
- B12 metabolism
- B12 deficiencies in diabetes mellitus
- Calcium supplementation in Metformin induced low B12 levels
- Nutrient Drug Interaction: Relationship of Metformin-serum B12 and calcium
- Neuropathy in diabetes: Diabetes Peripheral Neuropathy (DPN)
- Tools available for measuring neuropathy in T2DM: Michigan Neuropathy Screening Instrument(MNSI)
- Measuring Quality of life in T2DM adults

METHODS AND MATERIALS:

Study Design

The study was a cross sectional mixed longitudnal study. It was conducted at Sir Ganga Ram Hospital, Delhi with the support of Dr. Atul Gogia.The study was approved by the Institutional Ethics Committee for Human Research (No. IECHR/2013/19).

The entire study was conducted in three phases.

Phase I: Enrollment of the T2DM adults on metformin

A cross sectional survey of previously diagnosed T2DM adults who were coming for followup was done using non invasive research methods by several non invasive tools as given in table 1.

A total of 245 T2DM adults were enrolled and data was collected over a period of nineteen months (December 2013-July 2015).The subjects were enrolled in the study only if they were on metformin therapy for a minimum of four months duration. Participants with high creatinine levels (1.7 mg/dl for men and 1.5 mg/dl for women), hypothyroidism and prescription B12 injections and proton pump inhibitors were excluded from the study. We also excluded pregnant T2DM women and those without diabetes taking metformin. Based on clinical aspects described by the American Diabetes Association participants who reported receiving a physician's diagnosis after age 30 (excluding gestational diabetes) and did not initiate insulin therapy within 1 year of diagnosis were classified as having type 2 diabetes. They were screened for nutritional status, anthropometry, socio economic status, life style factors, medical history, DPN and Quality of Life. The experimental plan of Phase I is given in Figure 1.

Sample size calculation:

Sample size was calculated for sample survey of adults (>20yrs) T2DM on metformin use to estimate the prevalence of low B12 levels in T2DM users

$N= t^2 \times p(1-p)/m^2$

n=required sample size

t=confidence level at 95% (1.96)

p= estimated prevalence of low B12 levels in T2DM on metformin = 5.8-33% (6,7,8)

m=margin of error at 5%

$$N = (1.96)^2 \times 0.20(0.8)/ (0.05)^2$$

$$N = 245$$

Phase II

In phase II all those subjects who gave consent for blood estimations were selected for the study. In all the blood could be collected on 155 T2DM adults. Out of 245 patients, vitamin B12, Hb1Ac and Hemoglobin with cell morphology were done using CLIA, HPLC and C.B.C respectively on 155 patients. The biochemical estimations were performed on fasting samples (10-12 hr without meal), and 5 ml blood was drawn by a trained technician and analysis was done at an accredited lab of Sir Ganga Ram Hospital, Delhi. The parameters monitored are given

Table 1: Parameters and Methods For Data Collection

Parameters	Tools
Medical history, Life Style habits, Anthropometry, Diet history, Biochemical measurement, Bio Physical measurement, Diet History, SES ,	B12 screening proforma (N=245)
Blood Pressure Measurement	Digital Blood Pressure Instrument by Omeron
Assessment of Quality of Life	WHO-Quality of Life Index (N=245)
Neuropathy scores	MNSI Questionnaire (N=245)
Serum B12	CLIA
Haemoglobin	HPLC
C.B.C & cell morphology	C.B.C

Experimental Design

Figure 1

Phase I

(N=245)

Out Patient Department (OPD),Sir Ganga Ram Hospital,Delhi

↓

T2DM on Metformin use≥4m

Anthropometric status

BP

DPN

QoL

Collect Data:

Medical History

Dietary History

Life Style Habits

↓

Major outcome measure :

- QoL
- DPN

in fig. 2.The primary outcome was biochemical B12 deficiency determined by serum B12 concentrations. Serum B12 levels were quantified using vitamin B12 radioassay kit from Sir Ganga Ram hospital laboratories, New Delhi. We defined biochemical B12 deficiency at serum levels < 200pg/ml, very low as serum B12 between 149 to 200 pg/ml, and normal as above 200 pg/ml.

Phase II was followed by Phase III which was an Intervention Trial on T2DM adults on metformin.

Phase III: Intervention Trial

In the intervention trial 93 T2DM adults who fulfilled the inclusion and exclusion criteria listed below were included in the study.

Inclusion criteria	**Exclusion criteria**
- Ethnicity: Indo Asians	-Alcoholism
-Serum B12<200 pg/ml	-Pernicious anemia
-Long term metformin use(> 4months)	-Drugs affecting GI motility(Proton Pump Inhibitors)
	-Medical history of the following (CRF, liver disease, Cardiopulmonary disease, Bowel disease/surgery, Cancer, Acid Base disturbance)

The physician randomly alloted the T2DM adult to control or experimental group. The control group received oral 1000µg of B12 daily for eight weeks. The experimental group received oral 1000 µg of B12 daily along with oral 500mg calcium as calcium citrate melleate for eight weeks. Thirteen subjects could not complete the trial due to their personal reasons , however no side effects were reported by them. This drop out resulted in a sample size of 50 in experimental group and 30 in control group at the end of the study. Serum B12, MNSI scores and QLI were assessed before and after intervention. The detailed experimental plan is given in fig 3.

Figure 2

Phase II(Screening of Serum B12 status)

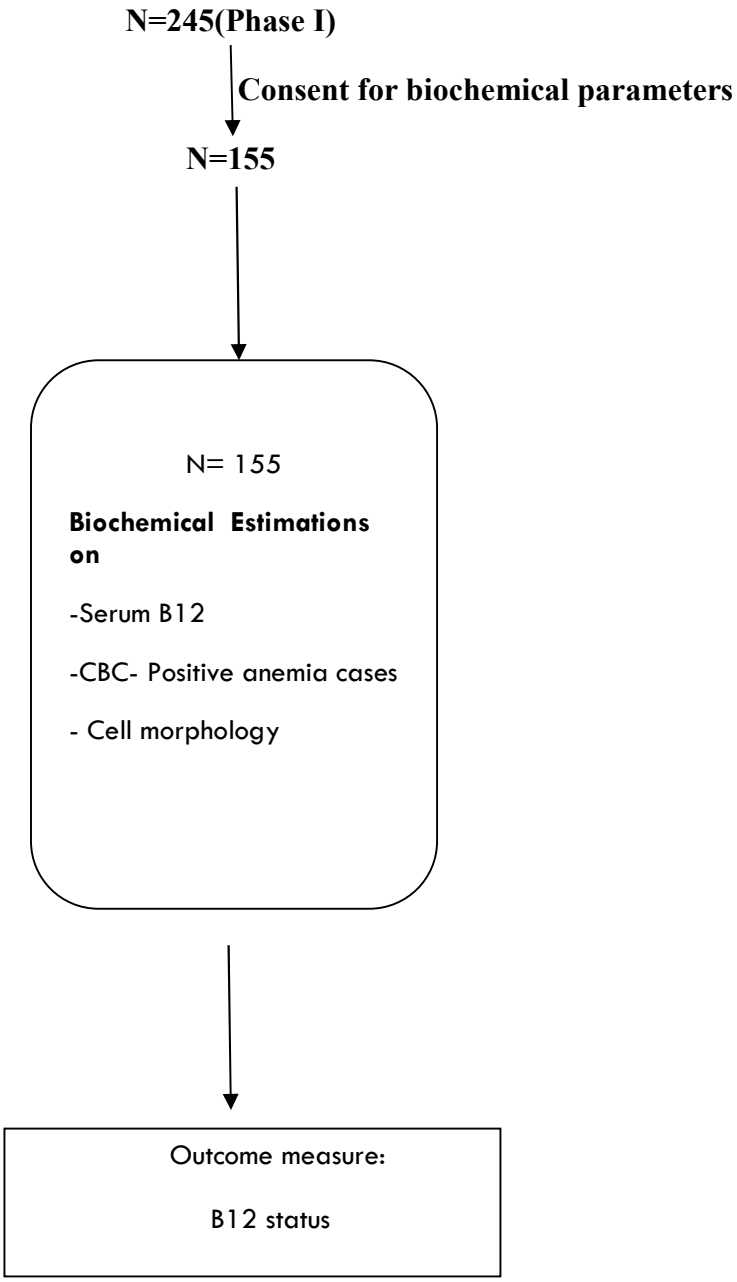
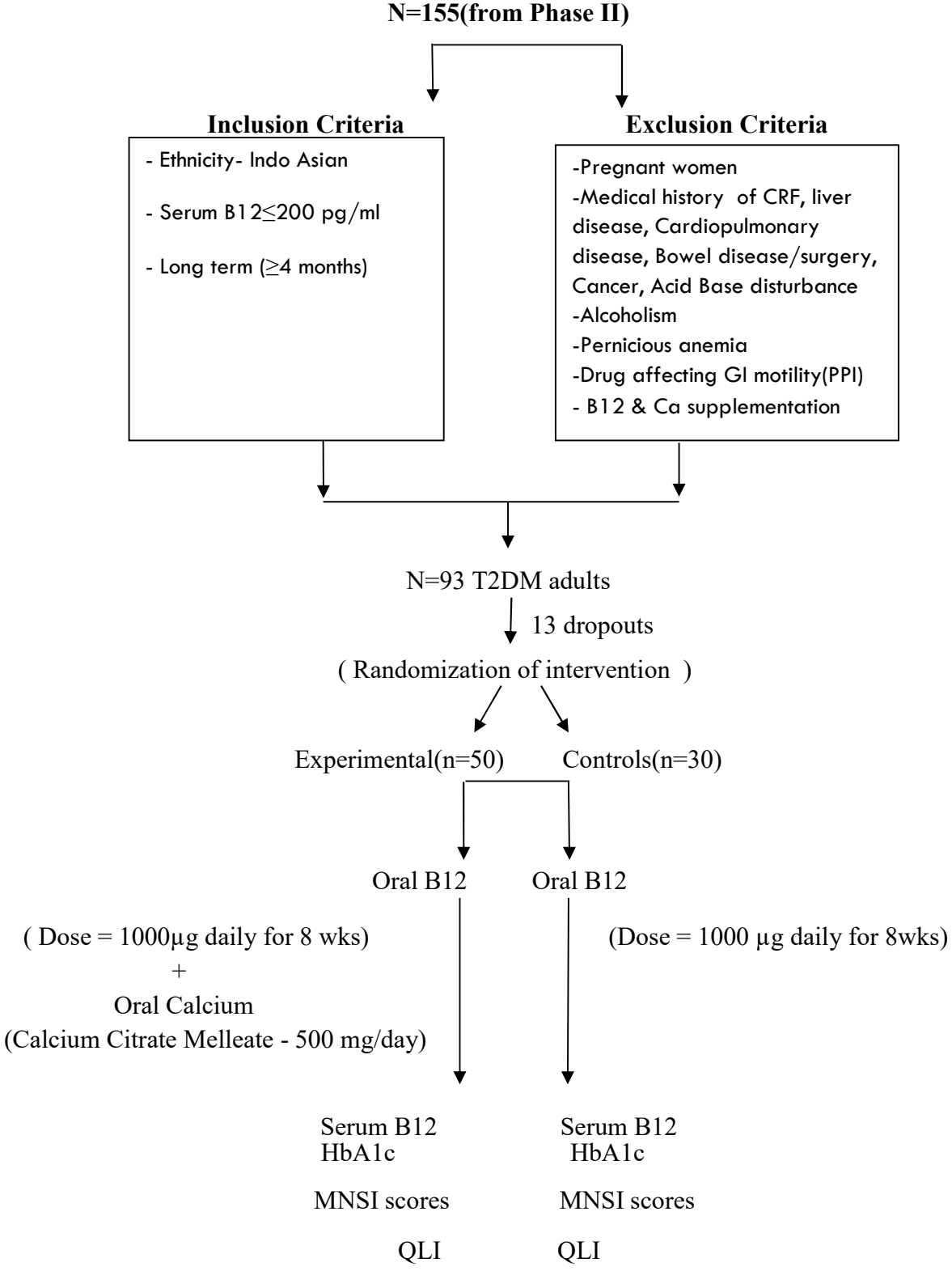


Figure 3

Phase III: Intervention Trial



STATISTICAL ANALYSIS

The data was entered in Microsoft Excel 2007 analysed IN SPSS 16.0 version (SPSS, Chicago,IL,USA) and Epi Info 7

- The quantitative(continuous) variables would be presented as mean±standard deviation.
- Frequencies and percentages would be used to quantify categorical variables.
- Analysis of variance would be performed to obtain F value for continuous variables where more than two groups are compared.
- Pearson correlation would be used to define the strength of the association between variables (+1=perfect positive correlation, -1=perfect negative correlation).
- A confidence interval of 95% would be taken, based on sample observation.
- Bar charts would be used to depict frequency distribution for nominal and ordinal data.
- Histograms would be used to depict frequency distribution of continuous data.
- Chi-square test would be performed to determine association between two categorical variables.
- Students' t test would be used to compare continuous variables in two groups.
- Paired t test would be used to compare the difference between the pre and post data for the outcome variables in the intervention study
- ODDs ratio would be calculated to estimate the strength of association between two paired dichotomous random variables.
- Results would be considered ststistically significant with a 2 tailed $p < 0.05$*,$p<0.01$**, and $p<0.001$***

RESULTS

Phase I: Cross sectional survey of T2DM adults (N=245) on metformin

1. Socio Demographic Status

- The population under study represented a heterogeneous socio economic group with a minimum per capita income (PCI) of Rs. 4000 and a maximum of Rs. 1,00,000.
- Approx three-fourth(73%) of the population were above 50years of age while a quarter (26%) of the population belonged to less than upto 50years of age group.

2. Life style habits and diet history

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- As regards the life style habits approximately 90% of the population reported that they did not drink alcohol nor did they consume cigarette or tobacco.

- Around 10% of the population were ovo-vegetarians (who consumed egg but no other non- vegetarian food) while similar proportion of the population were vegetarian(~43%) and non vegetarian (~46%).No striking gender differences were found in the dietary pattern.

- Trends of milk consumption showed that only one quarter (~25%) of the T2DM adults had an adequate consumption (200-400 ml milk) while majority (~67%) had low milk consumption(less than 200 ml) and a mere 7% had good milk consumption greater than 500 ml).Milk consumption trends were similar for males and females.

3.Anthropometry

- The prevalence of obesity and overweight by BMI was 40.4%. and 23.26 respectively. Another one quarter were morbid obese (20.40). Approximately three-fourth of females were overweight(71.9%) while majority of the males (36%) were morbid obese.

- Abdominal obesity (W.C.>90 for males and W.C>80 for females)was present in 73% and more proportion of females (83%) than males(64%) had abnormal W.C significantly($P<0.01$).

- Majority (93%) of T2DM adults had abnormal WHR (>0.9 for males and >0.85 for females) and there was no significant gender difference as regards WHR.

4. B.P Measurement

- Majority (62%) of the T2DM adults were prehypertensive followed by hypertension stage I and stage II. Proportion of males (72.3%) were higher than females(57.4%) in prehypertension category whereas proportion of females were higher in stage I hypertension than males.

- The changing trends in prevalence of Hypertension by Nutritional status(BMI by Asia Pacific Classification WHO 2000) was carried out. Surprisingly it was seen that one-third of the underweight T2DM adults suffered from stage I hypertension and 66.7% of them fell in prehypertension category.

5. Medical History

- Majority(88%) of the T2DM adults had a long standing diabetes for more than 10 years.

- 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. Moreover most common starting dosage of metformin based on the patient recall also came out to be 500 mg (90%) suggesting that the T2DM adults are susceptible to long standing use of metformin making them prone to side effects of metformin

6.Drug history

- Of the reported gastro intestinal(GI) side effects of metformin like nausea,vomitting, 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

- There was a significant relationship at 5% significance level between the GI side effects and their recent B12 status (p=0.029).

7. Diabetes Peripheral Neuropathy

- Almost three fourth(73.5%) of the population were suffering from DPN which was alarmingly high. Proportion of females suffering from DPN(67.2%) was more than double the proportion of males suffering from DPN(32.8%).However this association was non significant(P 0.545).

- Most common grade of DPN was low DPN(39.2%)*(MNSI score greater than 0 but less than equal to 2.5)* followed by high DPN (34.3%) *(MNSI score greater than 2.5).* Almost one quarter (26.4%) of the population was free of DPN*(MNSI score is 0).*

- As one moves from No DPN category to High DPN category it was seen that the proportion of females increased whereas proportion of males decreased. Though the trend was clear but this relationship was non significant(P 0.741).

8. Qualtiy of Life

- The Quality of life was measured in four domains namely physical health, psychological, social relationship and environment.T2DM adults obtained a mean score of 23.15±2.93 against a maximum score of 35 for Physical health domain, 19.56±3.39 against a maximum score of 30 for psychological domain, 10.68±2.35 against a maximum score of 15 for social relationship domain and 28.41±6.57 against a maximum score of 40 for environment domain.
- There were no significant differences in QLI scores of males and females in all the four domains of QL**I**
- Percent scores showed that social relationship and environment domain had higher percent scores(71.2% and 71.02%) than the physical and psychological domain(66.14% and 65.2%) of QLI.

Phase II: Screening of B12 among T2DM adults(N=155) on metformin

- It was found that more than half (52%) of the population were B12 deficient *(vitamin B12 deficiency is defined as serum less than 200 pg/ml).* More proportion of females (58%) than males(40%) were suffering from B12 deficiency.

- Majority(71%) of the subjects had poor glycemic (HbA1C >7%) control. More females (30.6%) than males (26%) had good glycemic control but it was not significant.

- As regards haemoglobin status majority (60%) of the population was normal. This can be attributed to the fact that T2DM adults were prescribed iron supplements. Moreover cell morphology showed no positive cases of macrocytic anemia.

- 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 by χ^2 test.

- 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.

- Correlation between glycemic control and DPN scores came out to be significant (r= - 0.381, P 0.001). Poorer the glycemic control higher the DPN score. As the glycated haemoglobin increases the DPN score also increases.

- Among the several factors associated with B12 deficiency by odds the abdominal obesity (WC),BMI, duration of diabetes, present drug therapy and diet were associated (O.R>1).

- Diet emerged as the only significant predictor for B12 deficiency on metformin treated T2DM adults. The odds of vegetarian diet was 2.33 i.e. a patient who has low B12 levels (<200pg/ml) is almost two times more likely to be on vegetarian diet than a patient who have normal B12 levels (>200pg/ml).

Phase III: Intervention Trial(N=80)

After eight weeks of supplementation

- The serum B12 levels increased significantly($p< 0.001$) in both the experimental and control groups.
- The increase in serum B12 levels was more in experimental group as compared to control group.This indicates that calcium supplementation along with B12 is efficacious as compared to B12 supplementation alone.
- DPN scores decreased significantly in both the groups.
- There was an increase in mean scores of the two domains of QLI in both the groups i.e. physical health and psychological health domains .

SUMMARY AND CONCLUSION

This chapter will summarize the major findings of each individual phase and conclusion will be discussed in relation to:

- Incorporation of B12 screening amongst T2DM adults on metformin in routine clinical practice and thereafter prescribing B12 and Calcium supplements
- Enriching diet of T2DM adults with sources of B12 and calcium
- Adapt DPN screening using MNSI tool in Indian context.
- Laying emphasis on social health of T2DM adults by measuring Quality of Life

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