CHAPTER 5

SUMMARY AND CONCLUSIONS

As per IDF atlas 2019 it is said that in 2019, the estimated number of people with diabetes aged 65–99 years is 135.6 million (19.3%). If this trend continues, the number of people above 65 years (65–99 years) with diabetes will be 195.2 million in 2030 and 276.2 million in 2045. IDF estimates of South East Asia prevalence state that in the year 2019 the Number of people with diabetes are 87.6 million (70.9–110.9 million) and it is projected that it will be 115.1 million (92.9–144.5 million) by 2030 and 152.8 million (123.4–190.1 million) by 2045.

These data point to a significant increase in the diabetes population of the aging societies in the next 25 years and the inevitable public health and economic challenges this will bring.

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. (Powers, 2012). Last twenty years have shown a rising trend of diabetes in India. The number of people with diabetes in India has increased from 19 million in 1995 to 66.8 million people in 2014 (Mohan et al, 2015).

This rising burden of diabetes is complicated by several co morbidities as well as long term and short term complications which are deleterious. However, this study addressed the vitamin B12 deficiency and micro vascular complication-DPN.

Metformin-a biguanide drug is said to be the first line therapies in treating type 2 diabetes by ADA 2017 and WHO 2017 essential medicine list since 1957 when a French physician Jean Sterne, who first reported the use of metformin to treat diabetes in humans in 1957. (Bailey, 2017). Several cross sectional studies (Bell, 2010; Sparre Hermann et al,

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2004; Liu et al 2011; Nervo et al 2011; Pflipsen et al 2009), case reports (Liu et al 2006, Tung & Tan, 2014, Kumthekar et al 2012) and few RCTs (Wulffele et al 2003, de Jager et al 2010) provide evidence for the metformin induced lowering of serum B12 among T2DM adults.

However, there is limited evidence regarding metformin induced lowering of serum B12 among type two diabetics in the Indian context and it has gained attention only in last decade. Till date no RCT in this context has been reported from India though there are few observational studies (Kumar et al 2017, Singh et al 2013**,** Raizada et al 2017).

Our study is novel in this that it is a RCT in this context and thus provides a higher degree of evidence in this area of research. This study was an attempt to study implications of nutrient- drug interaction (B12-metformin). The mechanism of metformin in lowering B12 status is not yet clear though several mechanisms have been proposed and is an entirely different area of metabolic research.

However, literature provides evidence of intestinal B12 malabsorption since 1969 by Berchtold et al who showed intestinal malabsorption of B12 by metformin in a short term duration of three months. Then came Tomkin et al 1971 who showed that metformin lowers B12 status among T2DM patients for a long term duration of two years. These were observational studies in context to metformin-B12 interaction and thereafter came the intervention trial by Bauman et al 2000 who determined the magnitude and mechanism of the reduction in serum vitamin B12 after metformin administration for three months and his study showed that oral calcium supplementation reversed the metformin-induced serum holo TCII depression (functional marker of B12). Thereafter Wulffele et al 2003 carried out first placebo controlled RCT where he showed that metformin lowers B12 status for short term duration of four months.

To conclude it can be said that reviewed literature has evidence of biochemical B12 deficiency associated with metformin users however, its implication on clinical manifestation was still not clear. Further from the biochemistry and physiology of B12 it

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was known that B12 has an important role in formation of RBCs and myelin sheath formation of nerves so it was thought that it is imperative to study B12 deficiency in association with anemia and neuropathy among metformin users.

Also it is known that B12-intrinsic factor complex uptake by ileal cell surface receptors is known to be a process dependent on calcium availability and metformin affects calcium-dependent membrane action. So it was thought that calcium supplementation of 500mg (dosage close to RDA for Indians) should be studied comparative to standard B12 supplementation of 1000µg usually given to treat B12 deficiency in general population. 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 (Chapmana et al 2016).

Since type 2 diabetes is a chronic disease so the patient has to live with metformin with or without combination of insulin or other OHAs, it is crucial to manage the associated co-morbidity and secondary complications which may further deteriorate quality of life among individuals. So it was thought that quality of life among T2DM adults on metformin should also be studied along with B12 deficiency and neuropathy.

In view of the above literature and the gaps identified in the area of B12-metfromin interaction research, the following questions were framed:

1. What will be the prevalence of B12 deficiency among T2DM adults on metformin in an Indian Setting?
2. Will the dosage and duration of metformin affect B12 deficiency?
3. What will be the prevalence of anemia among T2DM adults on metformin and what will be its association with B12 deficiency?
4. What will be the prevalence of neuropathy among T2DM adults on metformin and what will be its association with B12 deficiency?
5. What will be the quality of life among T2DM adults on metformin and how will it

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be associated with neuropathy and B12 deficiency?

6. What will be the impact of 500mg calcium along with 1000µg B12 supplementation in comparison to 1000µg B12 Supplementation alone among T2DM adults on metformin?

In order to answer the above mentioned questions the following objectives were planned which were distributed in three phases of our study as described in the following text.

PHASE I CROSS SECTIONAL SURVEY OF THE T2DM ADULTS ON METFORMIN

METHOD:

Two hundred and forty-five T2DM adults from outpatient department of a multi-specialty hospital setting were enrolled in the study if they were on metformin for a minimum of four months. We also excluded pregnant T2DM women and those without diabetes taking metformin and those who had any gastro intestinal disorder because it would affect B12 absorption.

SPECIFIC OBJECTIVES OF PHASE I:

1. To assess the socio demographic and socio-economic status of T2DM adults
2. To assess the lifestyle and dietary patterns of T2DM adults on metformin
3. To assess the nutritional status of T2DM adults on metformin using anthropometry and several nutritional indices
4. To assess DPN by using MNSI among T2DM adults on metformin
5. To assess QoL by using WHOQoL Bref among T2DM adults on metformin
6. To study the association between DPN and Nutritional status
7. To study the association between QoL and Nutritional status
8. To study the association between QoL and DPN

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RESULTS:

- The study population had more females (162 of 245 , 66.12%) than males (83 of 245 , 33.87%) where majority (54%) were housewives. The age of T2DM adults on metformin varied from 26-96 y with mean age of 58.2y. Approx three-fourth (74%) of the population were above 50y of age while a quarter (26%) belonged to less than upto 50 y of age group.
- The mean duration of diabetes was ~8 y and majority (192 of 245) had long standing diabetes of less than upto 10y while remaining 53 of 245 had more longer duration of diabetes of more than10y.
- The study population constituted heterogeneous socio economic group with a mean PCI of Rs. 21,700.82±18541.32 with a minimum PCI of Rs. 4000 and a maximum of Rs. 1,00,000. Similar proportion of T2DM adults 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 a very few (9.8%) belonged to PCI of more than Rs 40,000 but less than equal to Rs.1,00,000.
- 90% reported that they do not drink alcohol nor did they consume tobacco or cigarette
- 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 (~ 47%).
- Only one quarter (26%) of the population had an 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.
- No striking gender differences were found in the above stated dietary pattern.
- By BMI, majority (40.4%) of T2DM adults were obese whereas nearly one-quarter (20.40%) were morbid obese. Amongst the overweight T2DM adults approximately three-fourth of the females were overweight (71.9%).
- Majority (~93%) of the T2DM adults had abnormal WHR (>0.9 for males and >0.85 for females). There was no significant difference in WHR. between males and females.

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- Abdominal obesity by waist circumference (W.C.>90 for males and W.C>80 for females) was present in ~76%. There was very significant difference between males and females in that there were more proportion of females (83%) than males (~64%) who had abnormal WC($p<0.01$)
- Majority (62%) of the T2DM adults were pre hypertensive followed by hypertension stage I and stage II. Proportion of males (72.3%) were higher than females (57.4%) in pre hypertension category whereas proportion of females (33.3%) were higher in stage I hypertension than males(18.1%) but these were not statistically significant.
- 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±467.99mg.
- 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.
- The mean of MNSI history score was 3.88± 2.79(range 0-10) and there was no significant gender difference in MNSI history score.
- MNSI history/DPN history: As high as 67.8% showed numbness in their feet/ legs however 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. Approx. 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 there were no significant gender differences as regards MNSI history.

- 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.
- The mean DPN scores of the T2DM adults was 2.14 (range 0-7.5)
- Almost three fourth (180 of 245 ,73.5%) of the population were suffering from DPN (DPN score>0). There was no significant gender difference as regards the prevalence of DPN.
- Most common grade of DPN was low DPN (39.2%) *(DPN score >0 ≤ 2.5)* followed by high DPN (34.3%) *(DPN score >2.5).* Almost one quarter (26.4%) of the population was free of DPN*(DPN score=0).* There was no significant gender difference in various grades of DPN.
- No significant association of DPN with age, duration of diabetes as well as nutritional status. Long standing diabetes alone had no affect on DPN. Other factors like glycemic control has crucial role in manifestation of DPN which is discussed in Phase II.
- No significant difference between the present as well as past metformin dosage and DPN status was observed. Further there was no significant association between various grades of DPN and metformin dosage.
- There was no significant association between hypertension and DPN grades.
- Overall quality of life of T2DM adults showed that more than half (54%) reported that their quality of life was 'good'. But when asked specific questions from other facets of WHOQoL Bref depicted poor state of quality of life. As regards overall quality of health there were no significant gender differences
- Physical domain of QLI: Majority (41%) felt that physical pain prevents them from doing what they need to do to a moderate amount. Majority (60%) felt that they need some medical treatment to function in their daily life to a moderate amount. Majority felt that they have enough energy for everyday life to a moderate extent (36%) and most extent (33%). More than half of the adults

(62%) felt that they are able to get around to neither poor nor good amount. Majority (39.6%) felt that they were satisfied with their sleep. More than half (52 %) felt satisfied with their ability to perform daily living activities and their capacity to do work was also satisfactory among 53.5%. No significant gender differences were found as regards the various facets of physical domain.

- Psychological domain of QLI: Majority (48.6%) enjoyed their life very much. Majority (36%) felt their life to be meaningful only to a moderate amount. Majority (44.5%) felt that they were able to concentrate very much however as high as approximately 32% concentrated only to a moderate amount. Majority (39.6%) felt that they were able to accept their bodily appearance mostly. Majority (54.3%) 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. There were no significant gender differences for any of the facets of psychological domain.
- Social relationship domain of QLI: More than half (53.9%) of the adults were satisfied with their personal relationship. Majority (62%) felt that they were satisfied with their sex life while only a few (2%) were very dissatisfied. More than half (54.3%) were satisfied with the fact that they got support from their friend. No significant gender differences were seen for any of the facets of social relationship domain.
- Environment domain of QLI: Majority felt that they feel safe in their daily life to a moderate amount(37.5%) 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 do 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 to a moderate extent. 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. Majority (63.3%) felt satisfied with the conditions of their living place. Majority (45%) felt that they were satisfied to their access to health services. Majority (62.4%) felt that they were satisfied with their transport to a little amount. No significant gender difference was seen for any facet of environment domain.

- Among the four domains of WHOQoL Bref, the highest % mean scores was 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. 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.
- No association of QoL with sex and QoL with duration of diabetes were seen.
- QoL and age: The QoL facet 'satisfaction with health' obtained significantly lower mean scores for the older (age>50yrs) than in the younger T2DM adults($p<0.05$). There were significantly lower mean domain scores in three domains: physical health, psychological health and environment for older (>50 y) T2DM adults ($p<0.05$)
- QoL and PCI: The overall quality of life obtained lower mean scores for T2DM adults with lower per capita income (PCI≤20,000) 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.
- 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). 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

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WHOQoL -Bref.($p<0.001$). By Pearson correlation there was a highly significant weak negative correlation between DPN and 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).

- There was no significant association between QoL and nutritional status (BMI) by ANOVA and correlation

CONCLUSION:

Over nutrition and pre hypertension was common among T2DM adults on metformin. Though majority had no side effects of metformin indicating that it was administered well by the physician. Of the reported GI side effects, metallic taste was a common GI side effects among subjects. Almost three fourth of the T2DM adults were suffering from DPN as assessed by MNSI though it was of low grade which was under diagnosed by physician. Most common complaints of DPN were numbness in feet/legs, burning pain in feet, feet too sensitive to touch & muscle cramps in legs and very few reported open sore foot while amputation cases were rare. In all the four domains of QoL it was the psychological health which was the poorest while physical health was poorer and QoL was associated with age, PCI and DPN but not with the nutritional status.

PHASE II SCREENING OF SERUM B12 AMONG T2DM ADULTS ON METFORMIN

METHODS:

T2DM adults who gave consent for biochemical estimation were selected for blood estimations. Out of 245 patients from phase I, vitamin B12, HbA1c 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.

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SPECIFIC OBJECTIVES OF PHASE II:

1. To study the distribution of serum B12 and 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 the prevalence of anemia among T2DM adults on metformin and study the relationship between anemia and B12 deficiency.
4. To assess the relationship between serum B12 levels and metformin dosage and duration of diabetes.

RESULTS

- Box plot of serum B12 showed that the distribution of serum B12 had several outliers and the range of serum B12 was from 94-512 pg/ml with a median of 190pg/ml and mean of 287.31 pg/ml. 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. 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 patients were asked about the use of Vitamin B12-containing supplements), but these preparations are available over the counter, and it cannot be surely said that patients had never taken these medications earlier.
- Females had lower serum B12 levels than males (250.83±170.62 vs 363.92±353.88) ($p<0.05$).
- Hemoglobin of T2DM adults on metformin varied from 7.6-14.6 g/dl with a mean of 12.25±1.41g/dl and there was significant gender difference in mean hemoglobin.
- No cases of macrocytic anemia were seen by cell morphology and CBC.

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- Majority (60%) of the population had normal hemoglobin and there were significantly greater proportion of males than females (64%vs 28.6%, p<0.001) who were anemic. This could be attributed to the practice of prescribing iron supplements more commonly to females than males. A total of 75 T2DM adults were on iron supplements of which 46 were females and 29 were males.
- Mean Hb 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 of anemia in subjects taking iron supplementation (21.3%) versus non iron supplemented group (78.7%) (p<0.001).
- 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±1.64%. There was no significant gender difference in HbA1c .
- 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. 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.
- More than half (81 of 155, 52%) of the population were B12 deficient (vitamin B12 deficiency was defined as serum B12 less than equal to 200 pg/ml) [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~200 pg/ml]
- More proportion of females (58%) than males (40%) were suffering from B12 deficiency significantly (p<.05).
- Majority (47 out of 155, 30.3%) 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 significant difference between various grades of B12 deficiency across gender(p=0.125).

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- 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 (hemoglobin) assessed in our study. There was no significant difference in the prevalence of anemia among B12 deficient and those with normal B12 levels ($p=0.264$). Serum B12 deficient patients did not have a higher prevalence of anemia.
- Among those who were B12 deficient there was significantly higher proportion of vegetarians (54.3%) in comparison to those who had normal B12 levels ($p<0.05$). On the contrary there were significantly higher proportion of non vegetarians (66.2%) among those who had normal B12 levels($p<0.05$).
- There were no significant differences in milk consumption patterns in between the B12 deficient group and those with normal B12 levels.
- Comparison of distribution of serum B12 across present metformin dosage by box plot showed 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. By ANOVA 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 there was no fall in mean serum B12 levels between the three groups of 500mg, 1000mg and 1500mg dosage however the result was not statistically significant different between groups.
- Greater proportion of the population (59.6%) reported no GI side effects. 29% reported metallic taste as their most common GI problems followed by anorexia (11%).
- 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). However, no significant association was found between recent serum B12 status and past metformin dosage.

- There was no significant difference in the serum B12 levels between the various categories of underweight, normal, overweight, obese and morbid obese (classified by BMI) for males as well as females.
- 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
- There was no significant difference in prevalence of hypertension between those who had B12 deficiency and those who had normal B12 levels.
- There was no significant association between B12 deficiency and nutritional status by BMI or WHR or WC
- The distribution of serum B12 in various groups with different duration of diabetes was studied by box plot which showed that there was a decrease in median of serum B12 with the increasing duration of diabetes among T2DM adults on metformin. By median test 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 showed no change in the results.
- There was significant difference in the glycemic control of those who were B12 deficient than those who had normal B12 levels (p<0.05).
- B12 status had no significant association with present combination of metformin therapy
- By chi square there was a significant association between DPN and B12 deficiency (p = 0.000).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%.
- 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 negative.

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- 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.
- ROC analysis was conducted taking DPN score as 'test variable' and B12 deficiency as 'state variable' or binary variable. 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%. 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 ($p< 0.001$)
- By chi square 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. 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.
- Pearson correlation between HbA1c and DPN scores was significant ($r=0.381$, $p=0.000$). As the glycated haemoglobin increases the DPN score also increases. Poorer the glycemic control higher the DPN score
- 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 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($r= 0.313$, $p=0.000$).
- QoL domain scores of poor glycemic control 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 and this result was significant ($p<0.05$) for Physical health domain while it was very significant for Psychological health, social relationship and environment domain of QoL.

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- 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**).
- 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($p<0.05$).
- 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) ($p<0.001$).

CONCLUSIONS:

Low serum B12 levels were associated with metformin use among type 2 diabetics but we did not find metformin use to be associated with overt B12 deficiency or clinical B12 deficiency. Anemia was not associated with B12 deficiency among diabetics on metformin. Majority population were non anemic and anemia was more amongst males than females attributed to the practice of prescribing iron supplements to females ($p<0.001$). Anemia was less prevalent among iron users ($p<0.001$). More than half of the T2DM adults on metformin had B12 deficiency and B12 deficiency was more common in females than males ($p<0.05$). Those on vegetarian diet and those who were DPN positive were more likely to have B12 deficiency ($p<0.05$ and $p<0.001$). When B12 screening was done then T2DM adults who have B12 deficiency defined as <200pg/ml were probable to have moderate DPN with DPN scores of 2.25($p<0.001$). Among those T2DM adults on metformin whose glycemic control was poor then the prevalence of

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DPN was more (p< 0.001). DPN coexisted with poor glycemic control and poor B12 status and glycemic control and B12 deficiency were the risk factors for DPN (DPN score> 2.5)(p<0.001) .The QoL was poor for all the four domains among those with B12 deficiency(p<0.001) and poor glycemic control(p<0.01). **Thus early detection of B12 deficiency by timely B12 screening and DPN assessment remains cornerstone for maintaining quality of life of T2DM adults.**

PHASE III-INTERVENTION TRIAL (RCT): IMPACT OF B12 VERSUS CALCIUM ALONG WITH B12 SUPPLEMENTATION

METHODS:

80 T2DM adults from the phase II of the study who were B12 deficient (B12<200pg/ml) and met all the inclusion as well as exclusion criteria were randomized to the experimental group and control group. Experimental group also called Ca+B12 group received 500mg calcium in form of calcium citrate malate along with 1000µg B12. While control group also called B12 group received only 1000µg B12 for a period of eight weeks. Pre and post supplementation data was collected for serum B12, HbA1c, DPN assessment and QoL.

The impact of calcium supplementation studied here was in presence of 400IU vitamin D3 as in India calcium supplements cannot be manufactured without D3 as vitamin D is required for calcium absorption

SPECIFIC OBJECTIVE OF PHASE III:

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.

RESULTS:

- The T2DM adults in intervention trial belonged to the age group of 42-77y with a mean age of 59.26±8.48. The two supplementation groups were similar for

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sex, age, duration of diabetes and risk factors like BMI>23, abnormal WC and abnormal BP.

- The two supplementation groups were matched for sex, proportion of veg and non veg (including ovo veg) and proportion of various serum B12 levels by applying chi square test and it was found that the two supplementation groups were similar for the proportion of males and females
- 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 highly significant ($p<0.001$) decrease in the mean glycated hemoglobin levels in both the groups however the decrease was more in B12 group by paired t test.
- After supplementation there was a highly significant ($p<0.001$) increase in the mean serum B12 levels in both the groups however the increase was more in Ca+B12 group by paired t test. However, the increase in Ca+B12 group was more 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.
- After supplementation there was a significant decrease in the mean DPN scores of both the groups by paired t test ($p<0.05$) indicating that B12 intake in T2DM has improved DPN in T2DM adults.
- In post supplementation stage by student t test there was a very significant difference in mean glycated Hb levels ($p<0.01$), a highly significant difference in the post supplementation mean serum B12 ($p<0.001$) and a significant difference in the post supplementation mean DPN scores in between the two groups ($p<0.05$). However, there was no significant difference in the post supplementation mean MNSI history total score in between the two groups.

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- 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%).
- Overall after supplementation a total of 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 B12 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.
- As regards DPN history/MNSI history, by chi square there was no significant difference between the two groups in responses to the 15 questions in both pre and post supplementation stage
- It was found that the MNSI history score after supplementation and before supplementation had same range from 0-10. However there was fall in mean MNSI history score after supplementation (5.00 ± 2.78 to 4.22 ± 2.45.)
- MNSI Physical assessment of feet had five components: Physical Appearance, Ulceration, Ankle reflex, Vibration and Monofilament. After supplementation, among these five components 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 of 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.
- 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.
- There was very significant difference($p<0.01$) in post supplementation mean QoL scores in between the two groups for several facets of QoL 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.
- 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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CONCLUSION:

B12 supplementation improved the serum B12, HbA1c, DPN scores and physical and psychological domain of QoL. Ca+B12 supplementation was better in reducing prevalence of B12 deficiency(<200pg/ml) than B12 alone ($p<0.001$). 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 cannot be attributed solely due to the calcium supplementation as the mean values of serum B12 before supplementation were higher in B12+Ca group than B12 group. 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 R.D.A 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 by planning randomized control trials at higher doses of calcium, above its RDA amongst the Indian population of T2DM adults on metformin.

RECOMMENDATIONS:

- Our study adds to the sparse Indian literature in context of vitamin B12 deficiency. Vitamin B12 deficiency is a potential co-morbidity of T2DM adults on metformin which is under diagnosed and under treated. Given that significant proportion of the T2DM adults on metformin have biochemical B12 deficiency we suggest that support for further research in this area is a reasonable priority desired for the well being of T2DM adults who has to sustain their life on metformin.

- Our result suggests several findings that add to the complexity and importance of B12 research and its relation to diabetes, and offer new insights into the benefits of B12 and calcium supplements. Our study gives directions for further research. The results of this study suggest that further large cohort studies and high quality

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intervention trials addressing biochemical as well as clinical end points of vitamin B12 deficiency are now required.

One research design could be to identify those with B12 deficiency in T2DM adults on metformin and randomize them to receive **various doses of B12 supplementation** and then evaluate any improvements in biochemical serum B12 and / or clinical conditions.

Another design could be the use calcium supplementation along with other groups on varying doses of B12 supplements like 500µg and /or 1000µg and/or 1500µg- the amounts found in B12 supplements available in India.
Such research findings, especially in Indian context would be helpful to formulate evidence based guidelines for screening and treatment of vitamin B12 deficiency in metformin treated patient with type 2 diabetes.

Another design would be to identify those with B12 deficiency in T2DM adults on metformin and randomize them to receive higher doses (above RDA for Indains) of calcium supplementation with 1000µg B12 supplementation and then evaluate any improvements in biochemical serum B12 and / or clinical conditions and study efficacy of these interventions.

- Current American as well as European guidelines state that metformin is the first line of therapies in treatment of type 2 diabetes. ADA 2017 states that long term use of metformin may be associated with biochemical vitamin B12 deficiency and periodic measurement of vitamin B12 levels should be considered in metformin treated patients, especially in those with peripheral neuropathy. Our data provide a strong case for routine B12 screening as well as guidelines for treating B12 deficiency among T2DM adults on metformin.

- Despite emerging evidence of diabetic neuropathy there is lack of treatment options that effectively target the nerve being damaged. So prevention of DPN is

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the key which can be done if routine screening is practiced for DPN. Our study clearly shows that MNSI has emerged as a comprehensive robust noninvasive tool in an Indian setting which can be easily adopted in clinical practice to assess DPN in early stage and prevent its further deterioration.

- The peripheral neuropathy among T2DM patients can be due to uncontrolled sugars or / and it may present with symptoms that may be indistinguishable from that of vitamin B12 deficiency. So the condition of metformin-induced low serum vitamin B12 is of great concern if not recognized and treated appropriately. Our study suggest that B12 supplementation has improved DPN scores so further studies to confirm this finding are recommended.

- Our study suggests that Quality of life assessment can be included as a routine practice in T2DM patients as it is linked with blood sugar control, B12 deficiency as well as neuropathy , all being crucial for holistic well-being of the patient. The QoL assessment should be considered an important measure of outcome in chronic disease management like type 2 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.

- Calcium supplementation at 500mg (RDA) along with 1000µg B12 for T2DM adults on metformin was better than 1000µg B12 alone in treating low serum B12 and physical domain of QoL among metformin users. 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.

- The RDA of 500 mg calcium used in this study was not enough to overcome the effect of metformin in lowering serum B12 so there is a need to study this nutrient drug interaction at a higher dosages of calcium in a similar way as done in this RCT.