ABSTRACT

Diabetes is a global health emergency of 21st century. Metformin which forms the first line of therapies in treatment of type 2 diabetes across the World has been recognized to induce low serum B12. But there is sparse evidence in this context for Indian population. The symptoms of B12 deficiency are similar to those presented with diabetes peripheral neuropathy(DPN), a common secondary complication of type 2 diabetes. Assessment of DPN has been challenging due to various tools and varying study definitions. The magnitude of DPN and its determinants in Indian population has limited evidence. Co-existence of DPN along with metformin-induced low B12 levels would further deteriorate quality of life (QOL).

Ionic calcium is required to make B12 bioavailable and metformin competes with calcium thereby causing B12 malabsorption resulting in low serum B12. This form of B12 malabsorption was reversible with oral calcium supplement as evident from a study by Bauman et al 2000.

Thus the present study was formulated to address this nutrient-drug interaction by planning an intervention of eight weeks for calcium supplementation along with B12 with the following **broad objectives:** a) To map the magnitude of metformin-induced B12 deficiency and DPN along with assessment of quality of life among type 2 diabetes (T2DM) adults on metformin. b) To study metformin induced B12 deficiency in relation to anemia. c) To assess the impact of calcium supplementation of 500 mg along with 1000µg B12 versus 1000µg B12 alone on neuropathy, serum B12 and quality of life.

METHODS: A **cross sectional mixed longitudinal study** in a hospital setting of Delhi was conducted in three phases: **Phase I**: Cross sectional survey of **245** T2DM adults on metformin for a minimum of four months where they were studied for Nutritional status by anthropometry, Hypertension status, DPN assessment by Michigan Neuropathy Screening Instrument (MNSI) and QoL by WHO-QoL Bref- Hindi. **Phase II**: B12 screening was done on **155** subjects using predesigned B12 proforma and blood was estimated for serum B12 by C.L.I.A, HbA1c by HPLC and Hb and cell morphology by C.B.C. after fulfilling inclusion criteria (Ethnicity: Indo Asians, Serum B12<200 pg/ml,

Metformin use of ≥ 4m) and exclusion criteria (Alcoholism, Pernicious anemia, Drugs affecting GI Motility and GI absorption (Proton Pump Inhibitors, H2RAs Hydrogen blockers), history of CRF, liver disease, CKD, Cardiopulmonary disease, Bowel disease/surgery, Cancer, Acid Base disturbance, pregnancy, use of vitamin B12 , multivitamin, B12 injections and calcium supplements). **Phase III:** A RCT was conducted on **80** subjects with B12≤200pg/ml who were randomized into Calcium+B12 group (experimental) and B12 group (control). Pre and post supplementation data was collected on serum B12, HbA1c, DPN and QoL and the effect of 500mg Ca+ 1000µg B12 versus 1000µg B12 alone was studied. 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.

RESULTS: Phase I: Of 245 subjects 66.12% were females where 54% were housewives.76.8% were ≥50y with mean age of 58.2y (26-96 yrs) and mean duration of diabetes was 8 y. Mean PCI was Rs. 21,700.82 (Rs. 4000 - Rs. 1,00,000). 90% did not drink alcohol nor did they consume tobacco or cigarette. Around 43% were vegetarian and 47% were non vegetarian while majority (~ 67%) had low milk consumption (less than 200 ml). By BMI, 40.4% were obese, 23% overweight and 20% morbid obese. Also 93% had abnormal WHR.76% had abdominal obesity by WC. More females (83%) than males (64%) had abnormal WC ($p<0.01$). Majority (62%) were pre hypertensive followed by hypertension stage I and stage II. Majority (57.6%) were on 1000mg metformin dosage. Metformin dosage was associated with GI side effects ($p<0.001$). Three-fourth (70%) reported metallic taste with the metformin dosage upto 2500 pg/ml; which could be detrimental to their food ingestion affecting dietary compliance which is crucial for maintaining euglycemia in order to prevent DPN. With regard to DPN, majority showed numbness (67.8%) and burning pain ((53.5%) in their legs while few (10%) had an open sore on their foot and amputation cases were very rare (0.4%). Only 6.1% answered 'Yes' for 'if their doctor has ever told you that you have diabetic neuropathy' depicting that DPN was under diagnosed. The mean DPN scores was 2.14 (range 0-7.5). Three fourth (73.5%) had DPN (DPN score>0). 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).* By % mean scores QoL was better for Social relationship and Environment domain, moderate for physical

health and lowest for psychological health. There were lower mean domain scores in three domains: physical health, psychological health and environment for subjects >50 y ($p<0.05$). There were lower mean domain scores in three domains: psychological health, social relationship and environment ($p<0.05$) for those with PCI ≤20,000. Those with DPN had lower QOL for all the four domains ($p<0.05$).

Phase II: Serum B12 varied from 94-1746 pg/ml and 50% of the subjects were in 150-311pg/ml range. Males had higher mean serum B12 levels than females ($p<0.05$). More than half (**52%**) were B12 deficient *(≤ **200 pg/ml**),* more so in females (58%) than males (40%)($p<.05$). Majority (30.3%) had mild B12 deficiency(150-200pg/ml). Mean Hb was 12.25±1.41g/dl (7.6-14.6). Majority (60%) had normal Hb and greater males than females were anemic ($p<0.001$), attributed to the practice of prescribing iron supplements more to females than 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 ($p<0.001$). Anemia was more in non-iron users than iron users (78.7% vs.21.3%) ($p<0.001$). Metformin use was associated with biochemical B12 deficiency but not with clinical B12 deficiency as there were no positive cases of macrocytic anemia. B12 deficiency as defined by serum B12 had no impact on Hb. Serum B12 deficient patients did not have a higher prevalence of anemia. Odds Ratio of B12 deficiency and diet was 2.33(CI. 1.22-4.47) ($p<0.05$). HbA1c varied from 6.4-12% and 71% had poor glycemia (HbA1c >7%).There was no statistically significant association of B12 with metformin dosage and diabetes duration. GI side effects of metformin were more in B12 deficient ($p<0.05$). 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 (low or high) majority had B12 deficiency in comparison to those with no DPN($p<0.001$). Odds ratio between B12 deficiency and DPN was 10.0 (C.I. 3.89-26.). ROC curve suggested that when B12 screening is done among diabetics on metformin then those who have B12 deficiency (≤ 200 pg/ml) are probable to have DPN scores of 2.25 ($p< 0.001$). Amongst the good glycemic control (HbA1c ≤7%) majority (53.8%) had no DPN ($p<0.001$). As HbA1c increased the DPN score also increased ($r=0.381$, $p <0.001$). The significant risk factors for DPN were glycemic control (O.R =4.6) and B12 deficiency (O.R.=4.43) ($p<0.001$).All four QoL domain scores of B12 deficient was lower than that of normal B12

($p<0.001$).All four QoL domain scores of poor glycemia was lower than that of good glycemia ($p<0.05$).

Phase III: Before supplementation the two groups were comparable for sex, age, duration of diabetes, risk profile like BMI, WC, hypertension, HbA1c and DPN scores while they were different for B12($p<0.05$). After supplementation HbA1c decreased in both the groups but the decrease was more in B12 group ($p<0.001$) indicating that the B12 supplementation improved the glycemic control. There was increase in serum B12 in both the groups but the increase in Ca+B12 group was more than that in B12 group ($p<0.001$).Though rise in serum B12 and improvement in physical domain of QoL has shown better response in Ca+ B12 group but it cannot be attributed solely to the calcium supplementation as mean serum B12 and mean physical domain scores before supplementation were higher in Ca+B12 group than the B12 group ($p<0.05$).There was a decrease in DPN scores of both the groups but decrease was more in B12 group than Ca+B12 group ($p<0.05$), indicating that B12 supplementation improved DPN. Improvement in vibration perception at great toe was a crucial contributor of decrease in DPN scores ($p<0.01$). QoL improved in both the groups for physical health ($p<0.001$) and psychological health ($p<0.01$) with no changes in social relationship and environment domains.

CONCLUSIONS: Metformin use was associated with low serum B12 but not with clinical B12 deficiency. Type 2 diabetics on vegetarian diet and poor glycemia are at risk of B12 deficiency. Anemia was not associated with B12 deficiency among diabetics on metformin. B12 <200pg/ml were probable to have DPN scores of 2.25. B12 screening and DPN assessment remains cornerstone for maintaining quality of life of T2DM adults. B12 supplementation showed improvement in serum B12, DPN and psychological and physical facets of QoL. Since calcium supplementation showed better response for rise in serum B12 and physical health domain in T2DM adults on metformin so those who do not consume milk or milk products should be encouraged to increase their intake of calcium. However, 500mg calcium was not enough to overcome the B12 lowering effect of metformin and this study recommends future metformin studies to be carried out with higher dosage of calcium.