Accepted Manuscript

Title: An observational study of vitamin b12 levels and peripheral neuropathy profile in patients of diabetes mellitus on metformin therapy

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PII: S1871-4021(17)30287-4
DOI: http://dx.doi.org/10.1016/j.dsx.2017.08.014
Reference: DSX 860

To appear in: *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*

Please cite this article as: Gupta Kamesh, Jain Anand, Rohatgi Anurag.An observational study of vitamin b12 levels and peripheral neuropathy profile in patients of diabetes mellitus on metformin therapy.*Diabetes and Metabolic Syndrome: Clinical Research and Reviews* http://dx.doi.org/10.1016/j.dsx.2017.08.014

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AN OBSERVATIONAL STUDY OF VITAMIN B12 LEVELS AND PERIPHERAL NEUROPATHY PROFILE IN PATIENTS OF DIABETES MELLITUS ON METFORMIN THERAPY

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ABSTRACT

Objective: To investigate the status of vitamin B12 and peripheral neuropathy in diabetes mellitus patients on metformin therapy.

METHODS: A descriptive, observational study was completed in a tertiary care hospital between November 2014 and March 2016. Fifty consecutive patients of Type 2-Diabetes

Mellitus who had been on metformin therapy for at least three months were included in our study. Several Parameters were compared with vitamin B12 levels and severity of peripheral neuropathy (using Toronto Clinical Scoring System (TCSS) and Nerve Conduction Velocity). These included the duration of diabetes, duration of metformin usage, dietary history, and HbA1c levels. Definite B12 deficiency was defined as B12 <150 pg/ml and possible B12 deficiency as <220 pg/ml.

Results: In our study, we found a negative correlation between duration of metformin use and Vitamin B12 levels(r=-0.40). The mean Vitamin B12 levels seen in our study was 212.3 pg/mL. There is a positive correlation between the duration of metformin therapy and peripheral neuropathy (r=0.40). The mean TCSS score was 6.8. The percentage of patients with mild neuropathy was 28%, with moderate neuropathy was 20% and severe neuropathy in 12% of the patients. The average duration of metformin use in patients without peripheral neuropathy was 5.5yrs whereas the average length of metformin use in patients with peripheral neuropathy was 10.4 yrs.

Conclusion: Patients on long-term metformin therapy are at a high risk for Vitamin B12 deficiency and peripheral neuropathy. Interval Screening for peripheral neuropathy is recommended for patients on metformin even if Vitamin B12 levels appear to be normal.

Keywords: Diabetic neuropathy, Metformin, Vitamin B12, NCV, TCSS

DISCLOSURE: Authors have no conflict of interests, and the work was not supported or funded by any drug company.

The worldwide prevalence of Diabetes Mellitus (DM) especially Type 2 has risen dramatically over the past two decades from 108 million in 1980 to 422 million in 2014, increasing more rapidly in low and middle-income group countries[1]. Amongst the most common complications of diabetes is the involvement of peripheral and autonomic nervous system occurring in up to 50% of patients[2].

Another common type of peripheral neuropathy is that of Vitamin B12 deficiency, which rapidly improves with supplementation, especially in the initial stages but a prolonged deficiency of Vitamin B12 leads to irreversible nervous system damage[3], making it necessary to recognize and start the treatment promptly. However, it is challenging to differentiate between the two types of neuropathies clinically.

It is also well known that Vitamin B12 deficiency also causes hematological anemia [4]. It is however noteworthy that subtle neurological manifestation appears earlier than hematological changes [5].

India has more people with diabetes than any other country in the world [6]. The pre-existing cobalamine deficiency as a result of dietary habits poses an additional burden of neuropathy [7].

Metformin usage as a cause of B12 deficiency was first studied in 1971 [8] and since then it has been widely researched in various surveys and cross-sectional studies [9-22]. However, very few studies have assessed its impact on peripheral neuropathy [23,24]. It has been postulated that metformin interferes with the absorption of vitamin B12 in the gastrointestinal tract and therefore leads to a vitamin B12 deficient state [25]. Further, since absorptive mechanisms are altered, oral supplementation becomes ineffective in correcting the insult.

Whether Vitamin B12 deficiency causes additional neuropathic burden in diabetics on metformin therapy, is a question that needs to be further explored. The question of routine B12 level analysis and annual B12 supplementation in diabetics also needs to be addressed as there are no Indian guidelines available on this.

Until now, most of the studies have used symptom scoring (subjective) to assess neuropathy, but we have used more precise nerve conduction studies to evaluate peripheral neuropathy thus unmasking subclinical neuropathy as well.
The primary objective of our study is to explore Vitamin B12 levels and presence of peripheral neuropathy among diabetes mellitus patients on metformin therapy.

Methods

This Prospective, descriptive, observational, hospital-based study extended from November 2014 to March 2016. A total of 50 patients were studied who were attending Medicine OPD and inpatients in the medicine department of Lady Hardinge Medical College and asscoiated Smt. Sucheta Kriplani Hospital, New Delhi, India.

The patients included in the study group fulfilled the following inclusion criteria: Type 2 DM patients, 18 years and above who fulfilled WHO diagnostic criteria (Blood Sugar Fasting>126 or Blood Sugar >200,2h after glucose challenge) and were on metformin treatment for at least three months. The exclusion criteria included patients who were non-consenting; had type 1 diabetes mellitus; had other causes of cobalmine deficiency like malabsorption syndrome or Gastro-Intestinal surgery, and those had received vitamin supplementation in the last 3 months.
Approval for this study was received from the ethics board of the University of Delhi.
History: **D**ietary history was taken by the recall method and patients were grouped into vegetarian, non-vegetarian and vegan. Duration and doses of metformin taken by the patients were also recorded.
Lab tests: The study population was divided into controlled and uncontrolled DM based on their HbA1C values by the immunoassay technique. A value of less than 7 was considered as controlled whereas a value of more than 7 was considered as uncontrolled. Blood Sugar (both Fasting and Post-Prandial) were recorded to diagnose the patients.
Vitamin B12 assay was done using the electrochemiluminescence immunoassay method (on the Roche Elecsys 2010 analyzer). Serum samples were stored at room temperature (15-30°C) for not longer than 8 hours. From the results of B12 levels, patients were classified into sufficient (>220 pg/ml), possible B12 deficiency (150-220 pg/ml), and definite deficiency (<150 pg/ml)[27].
Clinical examination: Peripheral neuropathy was evaluated in all the patients in our study population by clinical examination (Toronto Clinical Scoring System) as well as by electrophysiological study (Nerve Conduction Study). *TCSS (Toronto Clinical Scoring System)* is a well-validated tool for the detection of peripheral neuropathy[29]. Peripheral Neuropathy was divided into normal, mild, moderate or severe based on the values obtained from TCSS evaluation.

Electrophysiological study: The data obtained was further evaluated by studyng Nerve conduction velocity(NCV). NCV of one upper limb and lower limb was done using the Cadwell Sierra Wave machine by a trained technician under the supervision of a neurologist. Motor conduction studies were done on the right median, ulnar, common peroneal and posterior tibial nerves. The sensory potentials were recorded by averaging responses recorded antidromically at the right median and sural nerves. Amplitudes, velocities and distal latencies were considered abnormal as per standard cut offs[28].

Statistical Analysis: All data was entered into an excel sheet. Appropriate statistical analysis for observational study such as percentages, means, p-value of unpaired t-test and p-value of Fischer's exact test will be used to further evaluate all data during final analysis. Statistical significance was set at $p<0.05$. Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 17.1. software will be used for statistical analysis.

Results

The age of patients ranged from 38 to 80 years. The mean age was 57.8 years(SD=10.2), and the median was 59 years. Out of the 50 patients included in the study, 22 were female and 28 were male.

Duration of diabetes, HbA1c levels, and peripheral neuropathy: The average length of diabetes in the patients studied was 10.2 years. The mean duration of diabetes in patients with and without neuropathies was 5.6 and 12.2 years respectively.

The average HbA1C level in the study population was 7.78. However, average HbA1C levels in patients without neuropathy was 6.78, and the average HbA1C level in patients with neuropathy was 8.21. We can also compare the severity of neuropathy with the HbA1C levels by looking at the TCSS scores (Figure 1). As the duration of diabetes or HbA1C levels increase, the severity of neuropathy also tends to increases (Figure 2)(Table 1&2).

Vitamin B12 levels and peripheral neuropathy: Vitamin B12 levels have been divided into normal, possible and definite deficiency. Out of the total 50 patients; 28 patients had definite deficiency, 11 patients had possible deficiency and the remaining 11 patients had normal levels of Vitamin B12. When the presence or absence of peripheral neuropathy in each subgroup was studied, we saw that patients with definite Vitamin B12 deficiency have a higher incidence of peripheral neuropathy as compared to the patient group with possible or normal Vitamin B12 levels (Figure 3)(Table 3 & 4).

Duration of metformin therapy and Vitamin B12 levels: The average length of metformin use in the studied population was 8.9 yrs. When we look at the duration of metformin use vis a vis Vitamin B12 deficiency we see that as the duration of metformin use increases, the deficiency of Vitamin B12 tends to intensify, as depicted in Figure 4. The metformin duration in patients with normal B12 levels was 5.6y; possible B12 deficiency was 11y, and definite deficiency was 9.4y. Now if we further subgroup the patients by their duration of metformin use it reveals a significant finding that as the duration of metformin use increases the average B12 levels tend to fall precipitously. This finding is statistically significant (p=0.01).

Peripheral neuropathy detected by NCS vs. By TCSS, 30 patients were found to have some degree of neuropathy whereas by NCV study, 35 patients had neuropathy present. Therefore, five patients had no signs and symptoms of neuropathy(TCSS normal) but showed electrophysiological evidence of neuropathy, as shown in red in table 5.

Duration of metformin & Vitamin b12 levels & peripheral neuropathy Table 6,7 & 8 elicits three things: patients with normal B12 levels depicts that longer duration of metformin use is associated with long-standing diabetes which is in itself is a cause for peripheral neuropathy. In patients with possible deficiency onset of peripheral neuropathy is slightly earlier than the subgroup of normal Vitamin B12 levels which gives credence to the thought that Vitamin B12 deficiency may have some contributory role to play in its more rapid onset. In the subgroup of definite deficiency, we can see that the start of peripheral neuropathy is even earlier than the previous group suggesting that Vitamin B12 deficiency in itself may have some contributory role to play. This data was found out to be statistically significant (p value<0.05).

Dietary profile & Vitamin B12 levels & Neuropathy Table 9 shows that Vitamin B12 deficiency is a more prominent finding in the vegetarian subgroup as compared to the nonvegetarian subset and this has been associated with a higher incidence of peripheral neuropathy in this group.

VITAMIN B12 AND PROFILE OF ANEMIA We have compared the subgroups of Vitamin B12 levels with the pattern of anemia seen in our patients (presence/absence of anemia; hemoglobin levels (Hb) and Mean Corpuscular Volume(MCV)). The data obtained in our patients shows that in the subgroup of patients with normal Vitamin B12 levels, the number of anemics is just 1 out of 11 and the mean Hb levels are 13.2 g/dl and the mean MCV is within normal limits. However, as the deficiency of Vitamin B12 increases, the proportion of anemics also increases (3 out of 11 in the possible deficiency subgroup and 14 out of 28 in the definite deficiency subgroup). Along with this, the mean Hb levels falls (10.6 g/dl in the possible deficiency subgroup and 8.4 g/dl in the definite deficiency subgroup) and the MCV rises (98.4 fL/red cell in the possible deficiency subgroup and 104.2 fL/red cell in the definite deficiency subgroup).

DISCUSSION. This study attempts to analyze the status of Vitamin B12 and peripheral neuropathy in patients of diabetes mellitus on metformin therapy.

Comparison of key parameters of our study with other similar studies is shown in table 10.

The mean HbA1C levels seen in our study is comparable to the Indian study, but it is higher than the mean HbA1C seen in the western study. This is consistent with the findings of other authors who have shown that mean HbA1C levels in Indian patients are slightly higher than their western counterparts, which highlights the poor rate of diagnosis and control of the disease in our country [30,31].

The prevalence of certain Vitamin B12 deficiency in our study was 56% which is much higher as compared to the baseline rate of Vitamin B12 deficiency in the general population of India, thus pointing to other mechanisms such as metformin usage which could be responsible for this unexplained Vitamin B12 deficiency in our study population[32]. The mean Vitamin B12 levels in metformin exposed individuals in many of the studies worldwide [22,33,34] was comparable to that seen in our study which further lends credence to the possible role of metformin leading to Vitamin B12 deficiency. In the NHANES Survey 1999 to 2006 by it was seen that amongst 5.8% diabetic patients on metformin had definite Vitamin B12 deficiency as compared to only 2.2% diabetics without metformin exposure, and 16.2% diabetics on metformin had borderline Vitamin B12 deficiency whereas only 5.5% patients had borderline deficiency in the diabetic group without metformin [21].

In a study by Braza et al on 76 patients using metformin 14(18.6%) patients had B12 deficiency and 17(22.3%) patients had low normal Vitamin B12 levels [35]. Overall around 40% of the patients had low normal or deficient Vitamin B12 levels. However there was no correlation between the duration of metformin use and the Vitamin B12 levels. In our study we found a negative correlation between duration of metformin use and Vitamin B12 levels (r=-0.40) which was also comparable to the negative correlation found in other studies (Table 10; column 2).

In our study, the prevalence of Vitamin B12 deficiency in the vegetarian population in our study was 27 patients out of the 31 vegetarians. This is much higher as compared to the baseline rate of Vitamin B12 deficiency in the general population [36-38]. Also among the non vegetarians the prevalence of Vitamin B12 deficiency was 28% which is much higher than the baseline rate of around 5% seen in the general population [36-38]. This goes to show that metformin use in the study population could be responsible for this additional burden of Vitamin B12 deficiency.

The percentage of patients with mild neuropathy was 28%, with moderate neuropathy was 20% and severe neuropathy in 12% of the patients. In the study by Singh et al, mild neuropathy was present in 48% of the patients studied and moderate neuropathy in 21% of the sample under study [26].

In our study, we have used both clinical examination (TCSS) and electrophysiology (NCV) as tools to detect peripheral neuropathy in our study patients. TCSS is a well validated scoring system for evaluation of peripheral neuropathy and has been used worldwide as an initial diagnostic tool [29,39,40]. However electrophysiological testing with NCV and EMG when available are the investigations of choice for detection of peripheral neuropathy.

Out of the 20 patients who had no signs and symptoms of neuropathy (TCSS normal), 6 of them had electrophysiological evidence of neuropathy. Thus we were able to unmask subclinical neuropathy in this subset of patients.

This has been one of the highlights of our study as previous Indian studies have used clinical scoring systems but even on extensive search we could not find any Indian study that has used electrophysiology as a diagnostic tool for detection of peripheral neuropathy [26]. However we could not perform EMG in our patients due to resource constraints.

As already discussed, metformin indirectly via Vitamin B12 deficiency could lead to an increase in the neuropathic burden in diabetes mellitus patients [41,42]. In our study we have seen that out of the 28 patients having definite Vitamin B12 deficiency, 25 had electrophysiological evidence of neuropathy, and out of the 11 patients with possible Vitamin B12 deficiency, 7 patients had evidence of neuropathy whereas only 3 out of the 11 patients had neuropathy with normal Vitamin

B12 levels. There is a strong negative correlation between Vitamin B12 levels and peripheral neuropathy(r=-0.50).

There is a positive correlation between the duration of metformin therapy and peripheral neuropathy in our study (r=0.40), similar to other studies(table 10; column 5).
However in a study by Rees et al [39] on 202 patients, it was concluded that although metformin therapy is associated with lower vitamin B12 status, there does not appear to be any significant effect on peripheral neuropathy in those receiving metformin. However, this study used NTSS scoring system for detection of peripheral neuropathy which is based on the history of sensory symptoms alone, rather than reliable and well validated TCSS and NCV used in our study, which makes our data on peripheral neuropathy highly reproducible and based on elements of history, examination as well as electrophysiological evidence [29].

In our study, we looked at the prevalence of anemia, Hb levels and MCV values for our study population and compared it with the Vitamin B12 levels. We saw that the prevalence of anemia and low Hb levels with higher MCV values were seen in the study population with definite Vitamin B12 deficiency. This finding is consistent with our existing knowledge of Vitamin B12 deficiency being an important cause of macrocytic anemia [43-45].
The mean MCV of our study is slightly higher than the other Indian study which is accountable by the greater number of Vitamin B12 deficient patients found in our study [26].
Another significant finding seen was that not all the patients with certain Vitamin B12 deficiency had evidence of anemia, increased MCV and decreased Hb. This finding has also been highlighted in a study by Krishnamurthy et al [46] which clearly says that “it’s important to know for day to day practice regarding the screening of the patients with Serum B12 levels as the Hb and MCV do not always have an anemic and macrocytic or dimorphic picture.”
It is well known that Vitamin B12 deficiency is responsible for both hematological as well as neurological complications [47]. In our study we have compared the prevalence of hematological and neurological complications in our patients on their Vitamin B12 levels. Out of the 50 patients studied, 35(70%) patients had neurological complications whereas 18(36%) patients had hematological complications. In a study by Wile et al out of the 59 patients who had metformin exposure neurological complications were present in 17% of the patients whereas hematological complications were present in 8% of the patients [23]. The lower percentage of patients afflicted in the western study could be attributable to the lower prevalence of Vitamin B12 deficiency in their research group which could be accounted for by the geographical, dietary and socioeconomic factors. However, the pertinent fact as highlighted by our research and several researches

worldwide being that the prevalence of neurological complications is higher than the hematological [46-49].

In a study by Ralapanawa et al the author writes that Vitamin B12 deficiency could rarely present with neurological manifestations in the absence of anemia [48]. Therefore a high index of suspicion is necessary for the early diagnosis, and prompt treatment to reverse the neurological manifestations, as the response to treatment is inversely proportionate to the severity and duration of the disease.

Also it has been suggested by Stabler et al that neurological manifestations of B12 deficiency appear much earlier than hematological changes [49].

Study limitations Larger randomized controlled trials are needed to confirm further the findings illustrated in our study. One limitation of our study was a control group of diabetics not on metformin was not studied. This was partly due to ethical reasons as metformin being the first line therapy in Type 2 diabetes could not be withheld. Future studies could also look at other confounding variables that could lead to peripheral neuropathy in diabetics, other micronutrient deficiencies could be accounted for, and better investigations like homocysteine and methylmalonic acid levels could be used. A longer duration of follow up could also be envisaged.

Recommendations Metformin is the cornerstone of type 2 diabetes therapy and has several beneficial effects attached to it. We thus recommend that patients on metformin for more than a year should have their Vitamin B12 levels checked yearly and parenteral supplementation provided if found deficient. Parenteral supplementation is to be preferred because enteral mechanisms are proposed to be compromised by metformin. We hope that as more and more evidence accumulates, new guidelines can be adopted by various diabetes societies in the world.

We would also like to recommend routine screening of diabetes patients on metformin for peripheral neuropathy by electrophysiological study even in the absence of signs and symptoms of neuropathy because sometimes subclinical neuropathy may be present and its progress can be halted by timely interventions.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Pimentel M, Park S, Mirocha J, Kane SV, Kong Y. The Effect of a Nonabsorbed Oral Antibiotic (Rifaximin) on the Symptoms of the Irritable Bowel Syndrome: A Randomized Trial. Ann Intern Med [Internet]. 2006 Oct 17 [cited 2016 Sep 26];145(8):557–63. Available from: http://annals.org/article.aspx?articleid=729511

2. Tracy JA, Dyck PJB. The spectrum of diabetic neuropathies. Phys Med Rehabil Clin N Am [Internet]. 2008 Feb;19(1):1–26, v. Available from: http://dx.doi.org/10.1016/j.pmr.2007.10.010

3. Gröber U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12-underestimated neurological significance. Nutrients [Internet]. 2013 Dec;5(12):5031–45. Available from: http://dx.doi.org/10.3390/nu5125031

4. Ismail II. Neurological, Hematological and Dermatological Manifestations of Vitamin B12 Deficiency Secondary to Pernicious Anemia; A Case Report and Literature Review. JNSK [Internet]. 2015 Nov 19;3(3). Available from: http://medcraveonline.com/JNSK/JNSK-03-00095.php

5. Oh R, Brown DL. Vitamin B12 deficiency. Am Fam Physician [Internet]. 2003 Mar 1;67(5):979–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12643357

6. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J [Internet]. 2014 Jan 31;7(1):45–8. Available from: http://dx.doi.org/10.4066/AMJ.2013.1979

7. Pawlak R, Parrott SJ, Raj S, Cullum-Dugan D, Lucus D. How prevalent is vitamin B(12) deficiency among vegetarians? Nutr Rev [Internet]. 2013 Feb;71(2):110–7. Available from: http://dx.doi.org/10.1111/nure.12001

8. Tomkin GH, Hadden DR, Weaver JA, Montgomery DA. Vitamin-B12 status of patients on long-term metformin therapy. Br Med J [Internet]. 1971 Jun 19;2(5763):685–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5556053

9. Ting RZ-W, Szeto CC, Chan MH-M, Ma KK, Chow KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. Arch Intern Med [Internet]. 2006 Oct 9;166(18):1975–9. Available from: http://dx.doi.org/10.1001/archinte.166.18.1975

10. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. J Med Assoc Thai [Internet]. 2004 Jul;87(7):780–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15521233

11. Sahin M, Tutuncu NB, Ertugrul D, Tanaci N, Guvener ND. Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2

diabetes mellitus. J Diabetes Complications [Internet]. 2007 Mar;21(2):118–23. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2005.10.005

12. de Jager J, Kooy A, Lehert P, Wulffelé MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ [Internet]. 2010 May 20;340:c2181. Available from: http://dx.doi.org/10.1136/bmj.c2181

13. Qureshi SA, Ainsworth A, Winocour PH. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? Practical Diabetes [Internet]. 2011 Sep 1;28(7):302–4. Available from: http://dx.doi.org/10.1002/pdi.1619

14. Nervo M, Lubini A, Raimundo FV, Faulhaber GAM, Leite C, Fischer LM, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Rev Assoc Med Bras [Internet]. 2011 Jan;57(1):46–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21390459

15. Kos E, Liszek M, Emanuele M, Durazo-Arvizu R, Camacho P. Effect of Metformin Therapy on Vitamin D and Vitamin B12 Levels in Patients with Type 2 Diabetes Mellitus. Endocr Pract [Internet]. 2012;18(2):179–84. Available from: http://dx.doi.org/10.4158/EP11009.OR

16. Sparre Hermann L, Nilsson BO, Wettre S. Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. The British Journal of Diabetes & Vascular Disease [Internet]. 2004 Nov 1;4(6):401–6. Available from: http://dvd.sagepub.com/content/4/6/401.abstract

17. Carlsen SM, Følling I, Grill V, Bjerve KS, Schneede J, Refsum H. Metformin increases total serum homocysteine levels in non-diabetic male patients with coronary heart disease. Scand J Clin Lab Invest [Internet]. 1997 Oct;57(6):521–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9350072

18. Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med [Internet]. 2003 Nov 1;254(5):455–63. Available from: http://dx.doi.org/10.1046/j.1365-2796.2003.01213.x

19. Leung S, Mattman A, Snyder F, Kassam R, Meneilly G, Nexo E. Metformin induces reductions in plasma cobalamin and haptocorrin bound cobalamin levels in elderly diabetic patients. Clin Biochem [Internet]. 2010 Jun;43(9):759–60. Available from: http://dx.doi.org/10.1016/j.clinbiochem.2010.02.011

20. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med [Internet]. 2009 Sep;22(5):528–34. Available from: http://dx.doi.org/10.3122/jabfm.2009.05.090044

21. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP. Association of Biochemical B12 Deficiency With Metformin Therapy and Vitamin B12 Supplements The National Health and Nutrition Examination Survey, 1999--2006. Diabetes Care [Internet]. 2012;35(2):327–33. Available from: http://care.diabetesjournals.org/content/35/2/327.short

22. Iftikhar R, Kamran SM, Qadir A, Iqbal Z, bin Usman H. Prevalence of vitamin B12 deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. Pan Afr Med J [Internet]. 2013 Oct 25;16:67. Available from: http://dx.doi.org/10.11604/pamj.2013.16.67.2800

23. Wile DJ, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. Diabetes Care [Internet]. 2010 Jan;33(1):156–61. Available from: http://dx.doi.org/10.2337/dc09-0606

24. Bell DSH. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. South Med J [Internet]. 2010 Mar;103(3):265–7. Available from: http://dx.doi.org/10.1097/SMJ.0b013e3181ce0e4d

25. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care [Internet]. 2000 Sep;23(9):1227–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10977010

26. Singh AK, Kumar A, Karmakar D, Jha RK. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. J Postgrad Med [Internet]. 2013 Oct;59(4):253–7. Available from: http://dx.doi.org/10.4103/0022-3859.123143

27. Berg RL, Shaw GR. Laboratory Evaluation for Vitamin B12 Deficiency: The Case for Cascade Testing. Clinical Medicine & Research. 2013;11(1):7-15. doi:10.3121/cmr.2012.1112.

28. Kraft GH. Nerve conduction velocity. Arch Intern Med [Internet]. 1972;130(3):447b – 447. Available from: http://dx.doi.org/10.1001/archinte.130.3.447b

29. Bril V, Perkins BA. Validation of the Toronto Clinical Scoring System for Diabetic Polyneuropathy. Diabetes Care [Internet]. 2002;25(11):2048–52. Available from: http://dx.doi.org/10.2337/diacare.25.11.2048

30. RahejaBS ,Kapur A , Bhoraskar A , Sathe SR , Jorgensen LN , Moorthi SR , Pendsey S , Sahay BK. diabetes care in India--current status.The Journal of the Association of Physicians of India [2001, 49:717-722]

31. Ramachandran A, Jali MV, Mohan V, Snehalatha C, Viswanathan M. High prevalence of diabetes in an urban population in South India. Br Med J. 1988;297:587–90.

32. Mahajan, Sanket K., and Swati C. Aundhakar. “A Study of the Prevalence of Serum Vitamin B12 and Folic Acid Deficiency in Western Maharashtra.” *Journal of Family Medicine and Primary Care* 4.1 (2015): 64–68. *PMC*. Web. 18 Apr. 2016.

33. Kibirige, Davis, and Raymond Mwebaze. “Vitamin B12 Deficiency among Patients with Diabetes Mellitus: Is Routine Screening and Supplementation Justified?” *Journal of Diabetes and Metabolic Disorders* 12 (2013): 17. *PMC*. Web. 18 Apr. 2016.

34. Ko, Sun-Hye et al. “Association of Vitamin B12 Deficiency and Metformin Use in Patients with Type 2 Diabetes.” Journal of Korean Medical Science 29.7 (2014): 965–972. PMC. Web. 18 Apr. 2016.

35. Braza M, Hanley J, Bhatla A, Martinez M. Prevalence of Vitamin B12 Deficiency in Hispanic Patients with Type 2 Diabetes Mellitus (DM) on Long Term Metformin-Is It Associated with Peripheral Neuropathy (PN). InDIABETES 2009 Jun 1 (Vol. 58, pp. A153-A153). 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA: AMER DIABETES ASSOC.

36. Shridhar K, Dhillon PK, Bowen L, Kinra S, Bharathi AV, Prabhakaran D, Reddy KS, Ebrahim S. Nutritional profile of Indian vegetarian diets--the Indian Migration Study (IMS).Nutr J. 2014 Jun 4;13:55. doi: 10.1186/1475-2891-13-55.

37. Pawlak R, Lester SE, Babatunde T.)The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. Eur J ClinNutr. 2014 May;68(5):541-8. doi: 10.1038/ejcn.2014.46. Epub 2014 Mar 26. Review.

38 Javid P, Christensen E. UgeskrLaeger. [Vegetarians are at high risk of vitamin B12 deficiency].2016 Jan 4;178(1). pii: V06150484. Danish.

39. Dunstan DF, Rees JA, Chen S, Lansdown JA, Moat SJ, Ellis R, et al. An observational study of the effect ofmetformin on B12 status and peripheral neuropathy. Br J Diabetes Vascular Dis 2012;12:189-93.

40. Liu F, Mao JP, Yan X.Zhong Nan Da XueXueBao Yi Xue Ban. Toronto clinical scoring system in diabetic peripheral neuropathy]. 2008 Dec;33(12):1137-41.

41. Biemans E, Hart HE, Rutten GE, Cuellar Renteria VG, Kooijman-Buiting AM, BeulensJW.Cobalamin status and its relation with depression, cognition and neuropathy in patients with type 2 diabetes mellitus using metformin. ActaDiabetol. 2015 Apr;52(2):383-93. doi: 10.1007/s00592-014-0661-4. Epub 2014 Oct 15.

42. Lubana SS, Alfishawy M, Singh N, Atkinson S.Vitamin B12 Deficiency and Elevated Folate Levels: An Unusual Cause of Generalized Tonic-Clonic Seizure.Am J Case Rep. 2015 Jun 23;16:386-9. doi: 10.12659/AJCR.893847.

43. Andrès E, Federici L, Kaltenbach G. Hematological manifestations related to cobalamin deficiency in elderly patients. Eur J Intern Med. 2008 Mar;19(2):149-50. doi: 10.1016/j.ejim.2007.05.006. Epub 2007 Aug 9.

44. Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH.Anemia in celiac disease is multifactorial in etiology.Am J Hematol. 2007 Nov;82(11):996-1000.

45. Andrès E, Affenberger S, Zimmer J, Vinzio S, Grosu D, Pistol G, Maloisel F, Weitten T, Kaltenbach G, Blicklé JF. Current hematological findings in cobalamin deficiency. A study of 201 consecutive patients with documented cobalamin deficiency.Clin Lab Haematol. 2006 Feb;28(1):50-6.

46. Dr. Akash , Dr. L. Krishnamurthy , Dr. P. Shashikala Krishnamurthy . Hematological Parameters versus Serum Vitamin B12 Levels in the Diagnosis of Vitamin B12 Deficiency Neurological Deficits .International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064.

47. Bravo J P, Ibarra C J, Paredes M M. [Hematological and neurological compromisedue to vitamin B12 deficit in infant of a vegetarian mother: case report]. RevChil Pediatr. 2014 Jun;85(3):337-43. doi: 10.4067/S0370-41062014000300010

48. Ralapanawa DM, Jayawickreme KP, Ekanayake EM, Jayalath WA. B12 deficiency withneurological manifestations in the absence of anaemia. BMC Res Notes. 2015 Sep18;8:458. doi: 10.1186/s13104-015-1437-9.

49. Stabler SP. Clinical practice. Vitamin B12 Deficiency. N Engl J Med 2013;368:149-60.

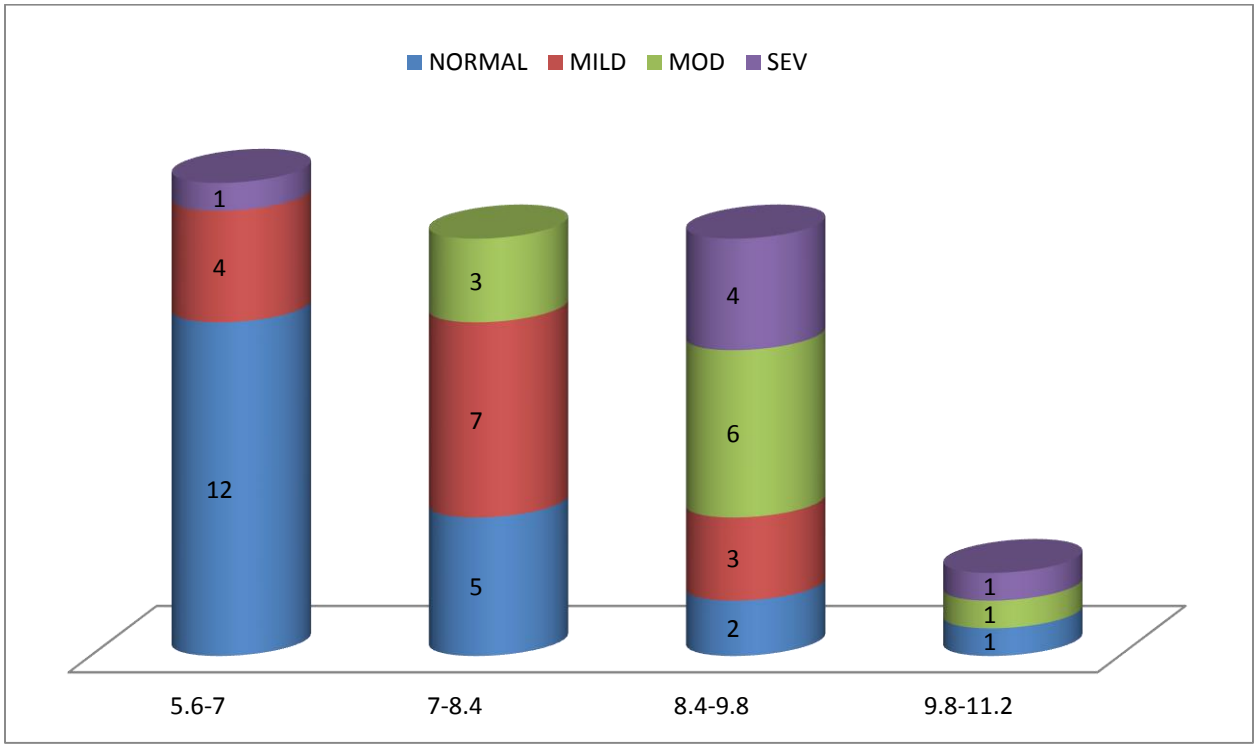

FIGURE 1 Comparison of HbA1c levels with TCSS for neuropathy.

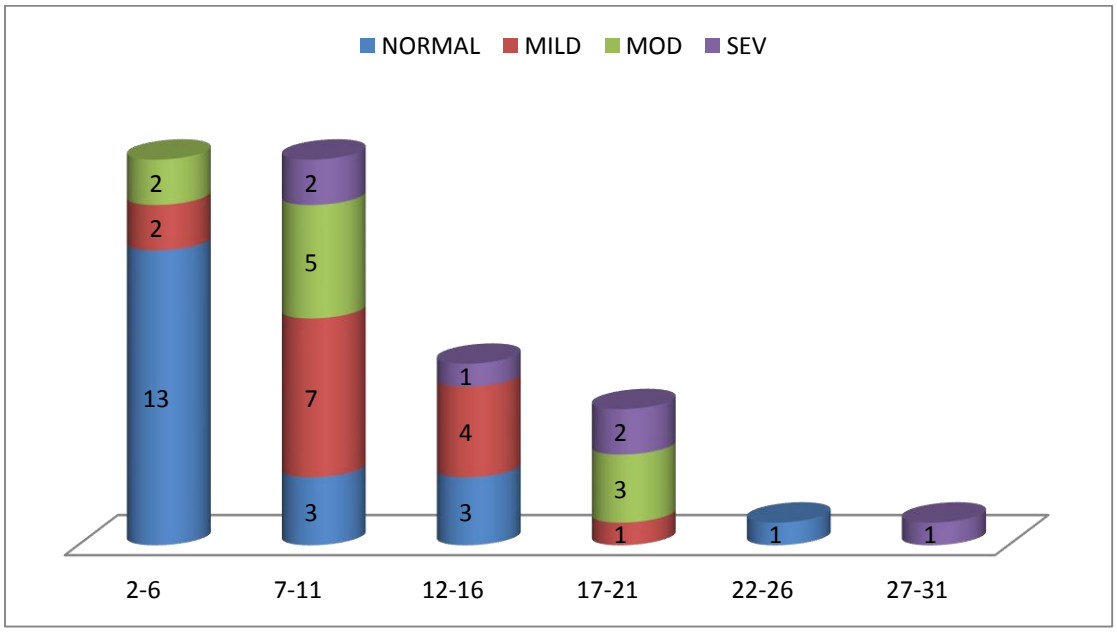

FIGURE 2 Comparison of Duration of Diabetes with TCSS for Neuropathy

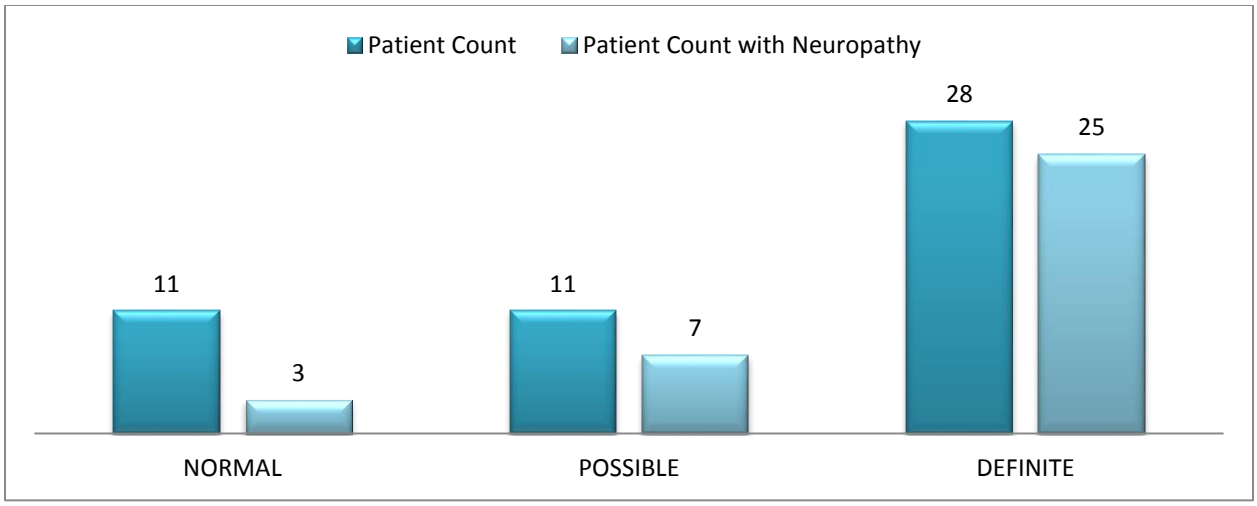

Figure 3 Comparing the subgroups of Vitamin B12 levels with the presence of neuropathy by NCV. On applying Chi Square test to the above data this finding of a higher incidence of peripheral neuropathy with increasing Vitamin B12 deficiency was found to be statistically significant (p value<0.01).

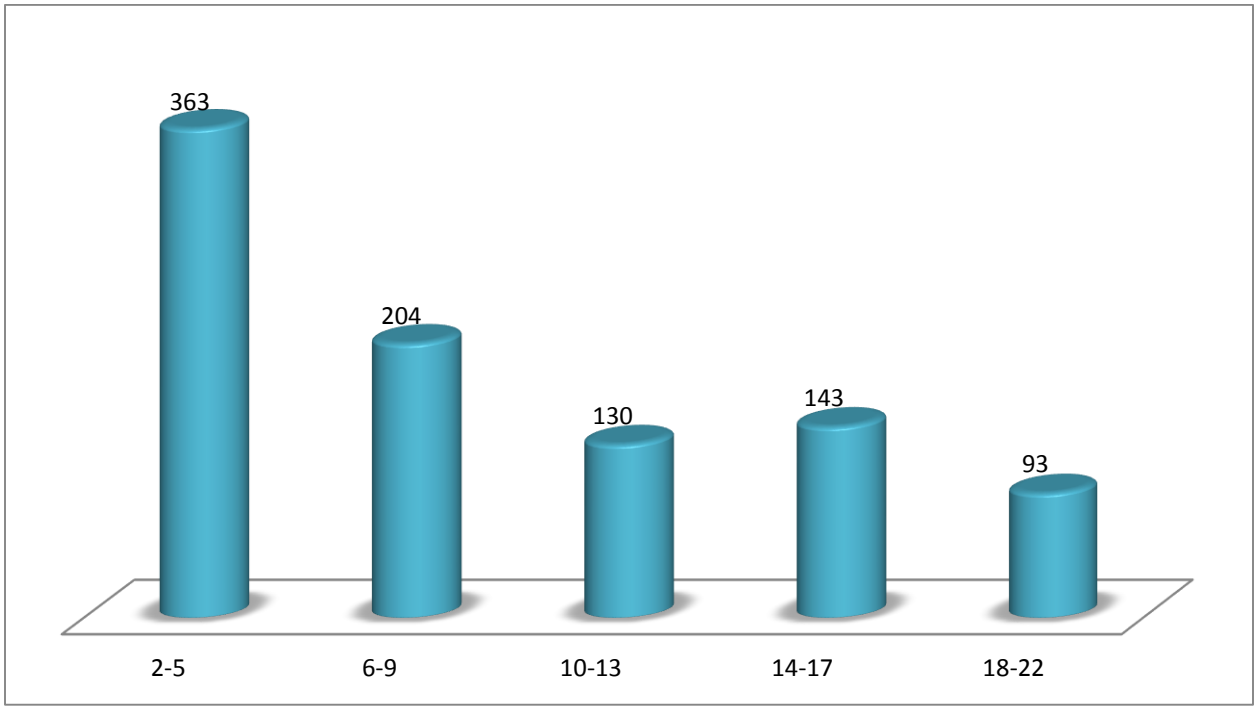

Figure 4 Absolute values of Vitamin B12 levels in pg/mL compared with the durations of metformin use divided into 5yr slots

TABLE 1 & 2: Description of Diabetes Duration in Patients with Neuropathy status detected by NCV.

DESCRIPTIVE STATISTICS	DURATION OF DM2	
	ABSENT	PRESENT
MINIMUM	2	4
MEAN	5.60	12.20
STANDARD DEVIATION	3.36	5.71
MAXIMUM	14	30
p-value of unpaired t-test	0.00(<0.05)	
95 % confidence intervals	-9.79	-3.41
STANDARD ERROR DIFFERENCE	1.59	

DURATION OF DM2	NEUROPATHY	
	ABSENT N (%)	PRESENT N (%)
0-5	8(16%)	1(2%)
5-10	5(10%)	9(18%)
10-15	2(2%)	14(28%)
15-20	0	5(10%)
20-25	0	5(10%)
25 & above	0	1(2%)
TOTAL	15(30%)	35(70%)
p-value of Fisher`s Exact test	0.000(<0.05)	

Table 3 & 4: Comparing the Vit B12 levels with Neuropathy status as detected by NCV and TCSS Values

VITAMINB12 LEVELS	NEUROPATHY	
	ABSENT N (%)	PRESENT N (%)
DIFINITE(<150)	3(20.00%)	25(71.43%)
POSSIBLE(150-220)	4(26.67%)	7(20.00%)
NORMAL(>220)	8(53.33%)	3(8.97%)
TOTAL	15(100%)	35(100%)
p-value of Fisher`s Exact test	0.000(<0.05)	

VITAMINB12 LEVELS	TCSS FOR NEUROPATHY		
	0-5 N (%)	5-10 N (%)	10-15 N (%)
DIFINITE(<150)	5(10%)	13(26%)	10(20%)
POSSIBLE(150-220)	1(2%)	5(10%)	3(6%)
NORMAL(>220)	9(18%)	4(8%)	0(0%)
TOTAL	15(30%)	22(44%)	13(26%)
p-value chi-square test of independence	0.006(<0.05)		

Table 5 Count of patients with neuropathy as detected by TCSS vs NCV study

Count of PATIENT ID	TCSS				
NCV	NORMAL	MILD	MOD	SEV	Grand Total
ABSENT	15	1	0	0	15
PRESENT	5	13	10	6	35
Grand Total	20	14	10	6	50

Table 6 compared the Vitamin B12 levels classes with the presence or absence of peripheral neuropathy (by NCS) vis a vis the duration of metformin use which has been divided into slots of 5yrs each. On applying Chi Square test to the above data this difference was found to be statistically significant (p value<0.01)

METFORMIN DURATION	COUNT OF PATIENT ID			SUM OF NEUROPATHY		
	Normal	Possible	Definite	Normal	Possible	Definite
2-5	6	2	6	0	0	4
6-9	3	2	7	1	1	7
10-13	2	3	10	2	2	10
14-17	0	3	3	0	3	2
18-22	0	1	2	0	1	2
GRAND TOTAL	11	11	28	3	7	25

Table 7 & 8: Compares and describes the duration of metformin use with Neuropathy status as found by NCV studies.

DURATION OF METFORMIN USE	NEUROPATHY	
	ABSENT N (%)	PRESENT N (%)
0-5	9(18%)	2(4%)
5-10	4(8%)	11(22%)
10-15	2(4%)	14(28%)
15-20	0	5(10%)
20-25	0	2(4%)
25 & Above	0	1(2%)
TOTAL	15(30%)	35(70%)
p-value of Fisher`s Exact test	0.001(<0.05)	

DESCRIPTIVE STATISTICS	DURATION OF METFORMIN USE	
	ABSENT	PRESENT
MINIMUM	2	4
MEAN	5.53	10.40
STANDARD DEVIATION	3.38	4.46
MAXIMUM	14	22
p-value of unpaired t-test	0.00(<0.05)	
95 % confidence intervals	-7.46	-2.28

Table 9 comparison of the subgroups of Vitamin B12 levels with the status of peripheral neuropathy (by NCS) vis a vis **of** the dietary profile of the patient. This difference is statistically significant (p value<0.05)

	Count of PATIENT ID	Sum of NEUROPATHY PRESENCE	% Patients with Neuropathy
NORMAL			
NONVEG	8	2	25.0%
VEG	2	1	**50.0%**
VEGAN	1	0	0.0%
POSSIBLE DEFICIENCY			
NONVEG	5	2	40.0%
VEG	6	5	83.3%
VEGAN	0	0	N/A
DEFINITE DEFICIENCY			
NONVEG	1	0	0.0%
VEG	23	21	91.3%
VEGAN	4	4	100%
Grand Total	**50**	**35**	

Table 10: comparison of key parameters of our study with similar international studies

	PARAMETER	OUR STUDY	WILE ET AL [23]	SINGH ET AL [26]
1	HbA1C LEVELS	7.8± 1.2	6.7± 1.0	8.2± 1.0
2	Correlation between duration of metformin use and Vitamin B12	-0.40	-0.41	-0.74
3	TCSS score(mean)	6.8	5.72	10
4	Correlation between Vitamin B12 levels and peripheral neuropathy	-0.50	N/A	-0.34
5	Correlation between duration of metformin and peripheral neuropathy	r=0.40	r=0.80	r=0.52
6	MCV Values	99.71		93.35