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Frequency of Vitamin B12 Deficiency Among Patients with Diabetes

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ABSTRACT

Background: Cobalamin, often known as vitamin B12, is an essential ingredient for DNA synthesis, brain function, and the creation of red blood cells. Metformin is a commonly used first-line treatment for diabetes mellitus (DM), especially type 2 diabetes (T2DM), which is becoming a major worldwide health concern. However, chronic metformin use has been linked to reduced absorption of vitamin B12, increasing the risk of insufficiency in diabetics. **Objective:** This study aims to determine the frequency of vitamin B12 deficiency among diabetic patients and examine its associations with metformin use, disease duration, dietary habits, and clinical symptoms. **Methods:** At tertiary care hospital Quetta, 300 patients with diabetes (type 1 and type 2) participated in a qualitative study. Structured interviews, clinical history reviews, and vitamin B12 level analysis of blood samples were all used in the data collection process. Patterns pertaining to metformin use, the length of diabetes, and dietary practices were found using thematic analysis. **Results:** Patients with long-term diabetes, long-term metformin use, and vegetarian diets were far more likely to have vitamin B12 deficiency. Deficiency rates among metformin users rose from 20% (use <5 years) to 65% (use >10 years). Neuropathy (53.3%), exhaustion (46.7%), cognitive impairment (30%), and anemia (26.7%) were among the most common symptoms. **Conclusion:** The findings highlight the need for healthcare professionals to monitor vitamin B12 levels in diabetic patients, especially those at higher risk due to prolonged metformin use and dietary restrictions. The study confirms a high prevalence of vitamin B12 deficiency among diabetic patients, especially those on long-term metformin therapy. Early intervention, including dietary modifications and supplementation, and routine screening are crucial to prevent complications like neuropathy and anemia.

INTRODUCTION

Cobalamin, or vitamin B12, is a crucial cog in the DNA synthesis, cell division, and neurological process machinery. Therefore, the most prominent clinical manifestations of vitamin B12 insufficiency are deficits in hematology and neurocognition (Oh R, Brown D: 2003). People with diabetes are at a higher risk of B12 inadequacy, however this problem is frequently overlooked. One drug that is commonly used to treat diabetes—metformin—has the unfortunate side effect of lowering serum vitamin B12. This is because metformin is associated with a vitamin B12 shortage. Ponggaidecha M. and coworkers carried out the study in 2004. Watte, Hermann, and Nilsson (2004) cite research from 1995 by DeFronzo and Goodman. In 2003, Filioussi K, Bonovas S, and Katsaros T. wrote a publication. Half of all diabetic patients and 12–23% of the general population over 60 are affected by this syndrome; this same group also has a rate of 12–23% metabolically proven B12 insufficiency. L. Pennypacker, R.H. Allen, and J.P. Kelly were the authors of the work from 1994.

A study was carried out in 2003 by Johnson, Hawthorne, Brackett, and colleagues, according to Lindenbaum J. et al. (1995). With the hope that primary care physicians will use this information to help them determine whether to test their diabetic patients for B12 insufficiency. It can be used as a screening test if it can detect a serious, treatable health problem early enough, according to the screening recommendations set out by the World Health Organization. Jones, John Michael, and Junger, Gerald (2008). These symptoms of diabetic neuropathy—paranesthesia's, decreased vibration sensitivity, and poor proprioception—are unfortunately common in patients with B12 deficiency.

A nerve injury caused by hypoglycemia might be mistaken for or exacerbate diabetic peripheral neuropathy, according to research published in 1988 by

Lindenbaum et al. Back in 1995, David Bell's While it is critical to identify the exact cause of neuropathy, hyperglycemia can alleviate certain neurologic symptoms with the help of basic vitamin B12 therapy.

Diabetes mellitus (DM) is indeed a global health concern that affects millions of people worldwide. The number of adults with diabetes is predicted to grow rapidly over the next decades, reaching 537 million [2021] (IDF, 2021). Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, and metformin is the first line pharmacological treatment for the glucemic control. Nevertheless, long term metformin usage has been implicated to reduce absorption of vitamin B12 (de Groot-Kamphuis et al., 2013) due to changes to the small intestine flora and suppression of vitamin transport systems.

Several research (Ting et al., 2017) says that vitamin B12 deficiency is more common in diabetic patients than those with non-diabetic people. For the diabetic patient, the clinical consequences of this impairment given its ability to worsen diabetic peripheral neuropathy symptoms leading to disease management becoming more difficult and lowering quality of life are especially worrisome (Kumthekar et al., 2012). Knowing the frequency and risk factors related to vitamin B12 insufficiency has become important because diabetes patients are becoming more prevalent, and metformin is widely used.

This study aims at finding out how often diabetes patients are vitamin B12 deficient and whether it is related to some of the variables including metformin use, disease duration or otherwise. Results from this study could be used to guide clinical practice with reference to routine screening and supplementation plans based on vitamin B12 insufficiency for diabetics vulnerable to the deficiency.

LITERATURE REVIEW

There is a concerning association between vitamin B12 deficiency and the use of metformin to treat type 2 diabetes mellitus (T2DM). Clinical impairments may occur or worsen due to low serum vitamin B12 levels, even if metformin is the first-line pharmaceutical treatment for type 2 diabetes. The possibility of vitamin B12 insufficiency in diabetics is the subject of this literature review.

Absorption of vitamin B12

Vitamin B12 found in diet binds to the R-protein found in salivary glands. Pancreatic proteases release vitamin B12 when they hydrolyze the R protein in the duodenum in an acidic environment. In response to newly produced vitamin B12, the parietal cells of the stomach release intrinsic factor (IF), which binds to the vitamin. As an example, see Kibirige and Mwebaze (2013). Vitamin B12 significantly increases proteolytic resistance when taken in conjunction with iron. The most straightforward route of absorption involves the complex attaching to its designated receptors on the terminal ileum mucosa. Here, calcium acts as a mediator to facilitate the absorption of vitamin B12. Clinical or biochemical vitamin B12 insufficiency can result from any of the aforementioned mechanisms, according to Andrès, Loukili, and Noel (2004). Many medical conditions and medications might hinder the body's ability to absorb certain nutrients. Chronic pancreatitis, proton pump inhibitors (PPIs), pernicious anemia, celiac disease, chronic atrophic gastritis (a condition mostly affecting the elderly), and celiac disease are all examples of such conditions. Problems with adequate nutrition might arise for some people, such as those who use excessive amounts of alcohol or who choose not to consume meat.

Vitamin B12 deficiency among patients with type 2 diabetes mellitus and the general population: a comparative review

Many case studies have shown that people with type 2 diabetes often have vitamin B12 deficiencies (Liu K, Dai L, Jean W: 2006; Bell D: 2010). This is based on multiple sources, one of which is a cross-sectional study from 2009 by Pflipsen et al. and another from 2012 by (Kumthekar et al.) (Hermann, Nilsson, and Wettre) (2004), Kos et al. (2012), De-Jager (2010), and Reinstatler et al. (2016) all found that this is the primary reason why metformin-treated type 2 diabetics have vitamin B12 deficiencies. It was discovered by Qureshi et al. (2011) that vitamin B12 insufficiency develops in 5.8% to 33% of type 2 diabetics on metformin. Vitamin B12 insufficiency has been defined differently in several studies, which may account for the seemingly contradictory prevalence statistics of this illness. Homocysteine concentrations >11.9 nanomoles per liter or serum vitamin B12 concentrations 100 to 350 pg/ml were determined by the study's authors to be indicative of a vitamin B12 insufficiency. A total of 203 outpatients with type 2 diabetes participated in the research, which took place in a sizable primary care clinic serving the United States military. The National Health and Nutrition Examination Survey of 1999–2006 in the US defines a borderline deficit as larger than 221 pmol/L, whereas a concrete biochemical vitamin B12 insufficiency is defined as less than 148 pmol/L (Reinstatler L et al., 2006). A cross-sectional study conducted by Qureshi et al. (2011) found that when blood concentrations of vitamin B12 were below 150 pg/ml, vitamin B12 deficiency was identified in 33% of persons with type 2 diabetes. This study included individuals who had been using high dosages of metformin for four years (>2g/day) since vitamin B12 insufficiency is more common in this population (Ting R et al. 2006). Vitamin B12 deficiency, defined as levels

below 150 pmol/L , affected 16% of 175 otherwise healthy senior Indian subjects (60 and higher). The serum MMA level rose in 55% of the participants, making it a more accurate indicator of vitamin B12 deficiency. In 2011, a study was conducted by Shobhaa, Tareya, Singh, and colleagues on patients at risk for vitamin B12 insufficiency who had type 1 diabetes mellitus.

Vitamin B12 deficiency among patients with type 1 diabetes mellitus

The immune system in type 1 diabetes mellitus (T1DM) aims to destroy the insulin-secreting pancreatic beta cells. Disorders affecting nondistinctive organs, autoimmune polyglandular syndromes, and endocrine ailments are always present (9 (2009) by Van-den-Driessche A. and coworkers. Pernicious anemia often occurs in type 1 diabetics due to chronic autoimmune gastritis. Around 2% of the population also deals with chronic autoimmune gastritis, which affects up to 1% of the total population. Individuals diagnosed with type 1 diabetes have a prevalence that is three to five times higher. Researchers De-Block, De-Leeuw, and Van-Gaal found that Type 1 diabetic patients often suffer from pernicious anemia, a vitamin B12 deficiency (2008). A cross-sectional study conducted by Koshy A, Kumari J, Ayyar V., and Kumar P. (2012) involving 90 patients with type 1 diabetes in southern India found that 45.5% of the participants had low vitamin B12 levels. This finding is consistent with research conducted by De-Block, De Leeuw, and Van Gaal (2008). Both the manufacturer's requirements and the published criteria agreed that this level should be below 180 pg/ml and 200 pg/ml, respectively. Age, gender, duration of diabetes mellitus, and level of glycemic control were not positively correlated with vitamin B12 insufficiency. Patients with type 1 diabetes are known to actively have autoantibodies to intrinsic factor (AIF) types 1 and 2, especially those with antibodies to glutamate decarboxylase-65 (GAD-65) and the HLA-DQA1 *0501-B1*0301 haplotype (De-Block C, De-Leeuw H, Van-Gaal L, 2008; 1999; De-Block C, Van-Gaal L, De-Leeuw I, 1999; 2000; De-Block C et al., 2000). These studies show that vitamin B12 deficiency in diabetics is complicated and involves pharmacological and dietary components. Users of Metformin, in particular, should regularly assess their vitamin B12 levels and dietary intake to reduce the risk of deficiency and its consequences.

RESEARCH OBJECTIVE

The aim of this study is to find out the prevalence of vitamin B12 deficiency in diabetic patients, especially T2DM patients. Since metformin is practically used as a first line treatment for type 2 diabetes, this study seeks to study how metformin medication can influence vitamin B12 deficiency based on dosage and usage duration on blood B12 levels. Also, this study compares vitamin B12 levels between diabetic and non-diabetic population in order to determine the degree of vitamin B12 insufficiency in people with diabetes. In addition, non-diabetic diseases as well as age and dietary practices are investigated as other inherent risk factors for reducing vitamin B12 serum concentration in diabetes patients in this study. This study aims to last but not least to assess the clinical consequences of vitamin B12 insufficiency, and therefore, if diabetic patients, and specifically those treated with long-term metformin, should undergo routine testing and supplementation in order to prevent problems and improve overall disease management.

METHODOLOGY

Frequency of Vitamin B12 deficiency in diabetic patients was determined through conducting this qualitative study at a tertiary care hospital in Quetta. The study involved 300 participants, all of them had diabetes mellitus (i.e. type 1 and type 2). Recruitment was accomplished through using purposive sampling from the hospital's outpatient and inpatient departments. All participants were enrolled in the study and after agreement to give informed consent were enrolled in the study. Data was gathered through physical tests, clinical history review and structured interviews. The main issues in the interview were demographic information, food habits, duration of diabetes, use of medications (especially metformin) and symptoms of vitamin B12 deficiency. Blood samples taken to measure vitamin B12 levels had clinical signs of vitamin B12 deficiency including neuropathy and cognitive impairment. Additionally, physician interviews were also done in order to learn more about the clinical relevance of vitamin B12 insufficiency in diabetes patients. As this study had a qualitative design, the patient experiences and medical views would be looked at in depth.

The data gathered was reviewed by a thematic analysis in order to discern patterns and trends relating to vitamin B12 deficiency. They were able to achieve a gedantic understand about the relationship between these factors and the use of metformin, diet and other risk factors for vitamin B12 insufficiency in diabetic patients via thematic analysis. Common themes including metformin use, disease duration and symptomatology were grouped with the results.

RESULTS

This study involved 300 diabetes patients in total. The results showed that vitamin B12 deficiency was significantly more common, especially in individuals who had been using metformin for a long time. The findings also showed that vitamin B12 insufficiency was

significantly influenced by age, diabetes duration, and dietary practices.

Table 1

Demographic Characteristics of Participants

Characteristic	Frequency (n=300)	Percentage (%)
Age (years)		
30-40	50	16.7
41-50	80	26.7
51-60	90	30.0
>60	80	27.6
Gender		
Male	180	60.0
Female	120	40.0

Figure 1

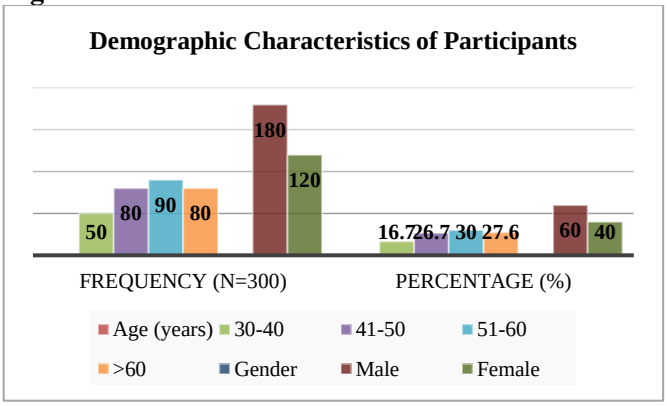

Table 2

Distribution of Participants by Type of Diabetes and Duration

Diabetes Type	Duration (Years)	Frequency (n=300)	Percentage (%)
Type 1 Diabetes	<5	10	3.3
	5-10	20	6.7
	>10	30	10.0
Type 2 Diabetes	<5	50	16.7
	5-10	80	26.7
	>10	110	36.7

Figure 2

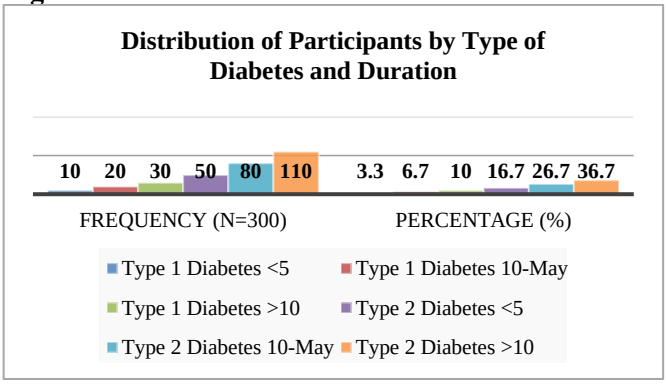

Table 3

Vitamin B12 Deficiency in Relation to Metformin Use

Metformin Use	Frequency (n=300)	Vitamin B12 Deficiency (%)
<5 years	190	20
5-10 years	120	45
>10 years	90	65

Figure 1

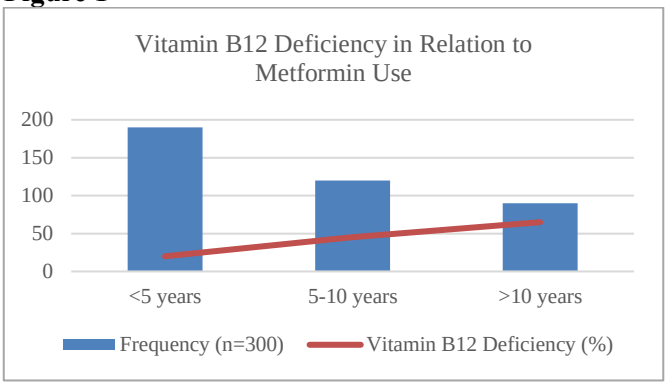

Table 4

Symptoms of Vitamin B12 Deficiency among Diabetic Patients

Symptoms	Frequency (n=300)	Percentage (%)
Neuropathy	160	53.3
Fatigue	140	46.7
Cognitive impairment	90	30
Anemia	80	26.7

Figure 3

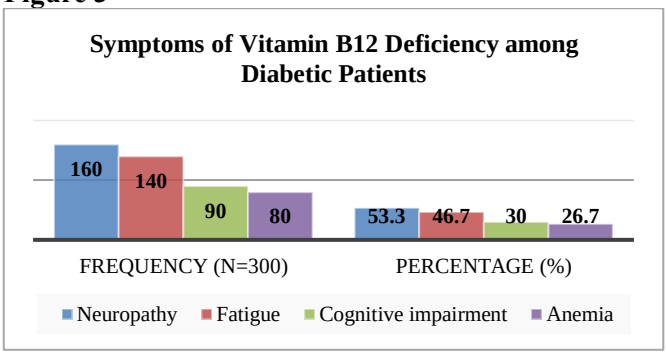

Table 5

Dietary Patterns and Vitamin B12 Deficiency

Diet Type	Frequency (n=300)	Vitamin B12 Deficiency (%)
Vegetarian	70	60
Vegetarian	230	40

DISCUSSION OF RESULTS

By determining what percentage of diabetic patients are deficient in vitamin B12, and determining the relationship, if any, to metformin use, length of diabetes and other variables, this study was pursued. The researchers found that diabetes patients are significantly more likely than controls to suffer from vitamin B12 deficiency and that this is most commonly the case in older patients, those who have been on metformin for a long time or those who have had diabetes for a long time.

Table 1 shows the demographic details of research participants: 30 per cent of participants were in the age group of 51 and 60 while 27.6% were over 60. The sample included 60% males, which might be because male diabetic patients at the study facility are more likely to take advantage of medical attention. Specifically, given the fact that older people are at higher risk of age-

related malabsorption problems — which in turn can worsen the risk due to diabetes and metformin use — of vitamin B12 insufficiency, the age distribution is extremely important.

The distribution of participants according to type and duration of diabetes is depicted in Table 2. Eighty percent of the individuals had type 2 diabetes; 36.7 percent had had type 2 diabetes for more than 10 years. The duration of diabetes was associated with vitamin B12 insufficiency. This result is consistent with other work that chronically diabetic mice suffer cumulative harmful effects on mechanisms of gastrointestinal absorption and frequently have concurrent long-term metformin use that further lower vitamin B12 levels.

Table 3 highlights the existing association between metformin use and vitamin Bli insufficiency. For example, whereas just 20 percent of people on metformin for less than five years had subnormal vitamin B12, 65 percent of people who had been taking the medication for more than 10 years had vitamin B12 deficiency. This amply supports the idea that long term metformin use is a major contributing factor in vitamin B12 depletion. It is believed that this occurs in response to metformin reducing gut microbiota and, therefore, causing vitamin B12 less to be absorbed in the ileum due to intrinsic factor binding being interfered with.

Table 4 lists the typical signs and symptoms of vitamin B12 insufficiency in participants. The most reported symptoms were neuropathy (53.3%), anemia (26.7%), cognitive impairment (30%), fatigue (46.7%), numbness (39.4%), dizziness (29.6%) and difficulty maintaining balance (29.6%). As vitamin B12 deficiency exacerbates diabetic peripheral neuropathy, which is a common manifestation of diabetes, these results are of great significance. Diabetic patients' vitamin B12 deficiency may be a major factor in the exacerbation of their diabetic problems, especially on neurological and hematological, since vitamin B12 is needed for neurons and as part of blood cells.

Table 5 shows the contribution of dietary practices based on the vitamin B12 insufficiency. Of the individuals, 60 percent of vegetarians and 40 percent of non-vegetarians were found to be vitamin B12 deficient. This is to be expected given the fact that most vitamin B12 comes from animal sources such as meat, fish, eggs and dairy products. One way that they may be at risk of vitamin B12 deficiency is if they stick closely to strict diets recommended for people with diabetes, which unintentionally reduce the amount of vitamin B12 in the diet.

CONCLUSION

The significance of this study is how widespread the shortage of vitamin B12 is among diabetic patients and especially among such diabetic patients as those taking metformin for many years. The reasons why vitamin B12 deficiency occurs were found to be: duration of the use of metformin and/or the length of the disease and the diet. Common symptoms were neuropathy, fatigue, and cognitive impairment, and such symptoms had significant clinical impact of this deficiency. Because there is an increased likelihood of diabetes and metformin use in the population, screening for vitamin B12 levels should be added to all diabetes regimens. Supplementation and dietary adjustments can prevent some of the detrimental effects associated with vitamin B12 deficiency, and timely intervention can prevent the otherwise progressive complications due to vitamin B12 deficiency.

REFERENCES

1. Andrès, E., Noel, E., & Goichot, B. (2002). Metformin-associated vitamin B12 deficiency. *Archives of internal medicine*, *162*(19), 2251-2252. https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/213629
2. Andres, E. (2004). Vitamin B12 (cobalamin) deficiency in elderly patients. *Canadian Medical Association Journal*, *171*(3), 251-259. https://doi.org/10.1503/cmaj.1031155
3. Bell, D. S. (2010). Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. *Southern Medical Journal*, *103*(3), 265-267. https://doi.org/10.1097/smj.0b013e3181ce0e4d
4. Bell, D. S. (1995). Nondiabetic neuropathy in a patient with diabetes. *Endocrine Practice*, *1*(6), 393-394. https://doi.org/10.4158/ep.1.6.393
5. Centers for Disease Control and Prevention. (2008). National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2007. *Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention*, *1*.
6. de Groot-Kamphuis, D. M., van Dijk, P. R., Groenier, K. H., Houweling, S. T., & Bilo, H. J. (2013). Metformin-associated vitamin B12 deficiency in patients with type 2 diabetes. *Diabetes Care, 36*(10), 2981–2987.
7. De Block, C. E., De Leeuw, I. H., & Van Gaal, L. F. (2008). Autoimmune gastritis in type 1 diabetes: A clinically oriented review. *The Journal of Clinical Endocrinology &*

Metabolism, *93*(2), 363-371. https://doi.org/10.1210/jc.2007-2134

8. De Block, C. E., De Leeuw, I. H., Rooman, R. P., Winnock, F., Du Caju, M. V., Van Gaal, L. F., & Registry, T. B. (2000). Gastric parietal cell antibodies are associated with glutamic acid decarboxylase-65 antibodies and the HLA dqa1*0501-dqb1*0301 haplotype in type 1 diabetes mellitus. *Diabetic Medicine*, *17*(8), 618-622. https://doi.org/10.1046/j.1464-5491.2000.00354.x
9. De Block, C. E., De Leeuw, I. H., & Van Gaal, L. F. (1999). High prevalence of manifestations of gastric autoimmunity in parietal cell-antibody positive type 1 (insulin-dependent) diabetic patients. *The Journal of Clinical Endocrinology & Metabolism*, *84*(11), 4062-4067. https://doi.org/10.1210/jcem.84.11.6095
10. DeFronzo, R. A., & Goodman, A. M. (1995). Efficacy of metformin in patients with non-insulin-Dependent diabetes mellitus. *New England Journal of Medicine*, *333*(9), 541-549. https://doi.org/10.1056/nejm199508313330902
11. De Jager, J., Kooy, A., Lehert, P., Wulffele, M. G., Van der Kolk, J., Bets, D., Verburg, J., Donker, A. J., & Stehouwer, C. D. (2010). Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: Randomised placebo controlled trial. *BMJ*, *340*(may19 4), c2181-c2181. https://doi.org/10.1136/bmj.c2181
12. Filioussi K, Bonovas S, Katsaros T. Should we screen diabetic patients using biguanides for meg aloblastic anaemia? Aust Fam Physician 2003;32: 383–4.
13. Hermann, L. S., Nilsson, B., & Wettre, S. (2004). Vitamin B12 status of patients treated with metformin: A cross-sectional cohort study. *The British Journal of Diabetes & Vascular Disease*, *4*(6), 401-406. https://doi.org/10.1177/14746514040040060701
14. Hermann, L. S., Nilsson, B., & Wettre, S. (2004). Vitamin B12 status of patients treated with metformin: A cross-sectional cohort study. *The British Journal of Diabetes & Vascular Disease*, *4*(6), 401-406. https://doi.org/10.1177/14746514040040060701
15. International Diabetes Federation. (2021). IDF Diabetes Atlas (10th ed.). https://www.diabetesatlas.org
16. Johnson, M. A., Hawthorne, N. A., Brackett, W. R., Fischer, J. G., Gunter, E. W., Allen, R. H., & Stabler, S. P. (2003). Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using title IIIc nutrition services. *The American Journal of Clinical Nutrition*, *77*(1), 211-220. https://doi.org/10.1093/ajcn/77.1.211
17. Kibirige, D., & Mwebaze, R. (2013). Vitamin B12 deficiency among patients with diabetes mellitus: Is routine screening and supplementation justified? *Journal of Diabetes & Metabolic Disorders*, *12*(1). https://doi.org/10.1186/2251-6581-12-17
18. Kos, E., Liszek, M. J., Emanuele, M. A., Durazo-Arvizu, R., & Camacho, P. (2012). Effect of metformin therapy on vitamin D and vitamin B12 levels in patients with type 2 diabetes mellitus. *Endocrine Practice*, *18*(2), 179-184. https://doi.org/10.4158/ep11009.or
19. Kumari, S., Koshy, A., Ayyar, V., & Kumar, P. (2012). Evaluation of serum vitamin B12 levels in type 1 diabetics attending a tertiary care hospital: A preliminary cross - sectional study. *Indian Journal of Endocrinology and Metabolism*, *16*(7), 79. https://doi.org/10.4103/2230-8210.94270
20. Kumthekar, A. A., Gidwani, H. V., & Kumthekar, A. B. (2012). Metformin associated B12 deficiency. *J Assoc Physicians India*, *60*(3), 58-9.
21. Kumthekar, A. A., Gidwani, H. V., & Kumthekar, A. B. (2012). Metformin-associated B12 deficiency. Journal of Clinical and Diagnostic Research, 6(4), 774–776.
22. Lindenbaum, J., Healton, E. B., Savage, D. G., Brust, J. C., Garrett, T. J., Podell, E. R., Margell, P. D., Stabler, S. P., & Allen, R. H. (1988). Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or Macrocytosis. *New England Journal of Medicine*, *318*(26), 1720-1728. https://doi.org/10.1056/nejm198806303182604
23. Liu, K. W., Dai, L. K., & Jean, W. (2006). Metformin-related vitamin B12 deficiency. *Age and Ageing*, *35*(2), 200-201. https://doi.org/10.1093/ageing/afj042
24. Nervo, M., Lubini, A., Raimundo, F. V., Faulhaber, G. A. M., Leite, C., Fischer, L. M., & Furlanetto, T. W. (2011). Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. *Revista da Associação Médica Brasileira*, *57*, 46-49.

https://doi.org/10.1590/S0104-42302011000100015

25. Oh, R. C., & Brown, D. L. (2003). Vitamin B (12) deficiency. *American family physician*, *67*(5), 979.
26. Pennypacker, L. C., Allen, R. H., Kelly, J. P., Matthews, L. M., Grigsby, J., Kaye, K., Lindenbaum, J., & Stabler, S. P. (1992). High prevalence of cobalamin deficiency in elderly outpatients. *Journal of the American Geriatrics Society*, *40*(12), 1197-1204. https://doi.org/10.1111/j.1532-5415.1992.tb03641.x
27. Pflipsen, M. C., Oh, R. C., Saguil, A., Seehusen, D. A., Seaquist, D., & Topolski, R. (2009). The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: A cross-sectional study. *The Journal of the American Board of Family Medicine*, *22*(5), 528-534. https://doi.org/10.3122/jabfm.2009.05.090044
28. 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 mel litus. J Med Assoc Thai 2004;87:780–7.
29. Qureshi, S., Ainsworth, A., & Winocour, P. (2011). Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? *Practical Diabetes*, *28*(7), 302-304. https://doi.org/10.1002/pdi.1619
30. Reinstatler, L., Qi, Y. P., Williamson, R. S., Garn, J. V., & Oakley, G. P. (2012). Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements. *Diabetes Care*, *35*(2), 327-333. https://doi.org/10.2337/dc11-1582
31. Riley, W. J., Toskes, P. P., MaClaren, N. K., & Silverstein, J. H. (1982). Predictive value of gastric parietal cell autoantibodies as a marker for gastric and hematologic abnormalities associated with insulin-dependent diabetes. *Diabetes*, *31*(12), 1051-1055. https://doi.org/10.2337/diacare.31.12.1051
32. Shobhaa V, Tareya S, Singh R, et al: Vitamin B12 deficiency and levels of metabolites in an apparently normal urban south Indian elderly population. Indian J Med Res 2011, 134:432–9.
33. Stowers, J. M., & Smith, O. A. (1971). Vitamin B 12 and metformin. BMJ, 3(5768), 246-247. https://doi.org/10.1136/bmj.3.5768.246-b
34. Ting, R. Z. (2006). Risk factors of vitamin B12 deficiency in patients receiving metformin. *Archives of Internal Medicine*, *166*(18), 1975. https://doi.org/10.1001/archinte.166.18.1975
35. Ting, R. Z., Szeto, C. C., Chan, M. H., Ma, K. K., & Chow, K. M. (2017). Risk factors of vitamin B12 deficiency in patients receiving metformin. *Archives of Internal Medicine, 176*(3), 273–279.
36. Wilson, J. M. G, & Junger, G. (2008). *Principles and practice of screening for disease*. Geneva, Switzerland: World Health Organization.
37. Lindenbaum, J., Rosenberg, I., Wilson, P., Stabler, S., & Allen, R. (1994). Prevalence of cobalamin deficiency in the Framingham elderly population. *The American Journal of Clinical Nutrition*, *60*(1), 2-11. https://doi.org/10.1093/ajcn/60.1.2