



Vitamin B₁₂ Levels in Type 2 Diabetes Patients on Metformin in Gaza Strip- Palestine

Lamia'a S. Saqer¹, Mohammed I. Al-majaidah², Youssef O. Safi³, Salem S. Shbair⁴, Tareq Z. Abu-Azab⁵

^{1,2,3,4,5}Department of Medical Science-University Collage of Science and Technology

ARTICLE INFO	ABSTRACT
Published Online: 02 October 2018	Background: Metformin is the most common oral hypoglycemic agent that used to treat Type 2 Diabetes Mellitus(T2DM) patients, which associated side effects such as Vitamin B ₁₂ (VB ₁₂) deficiency. Objective: This study was carried out to determine the serum level of vitamin B ₁₂ in Palestinian patients with T2DM lived in Gaza Strip. Materials And Methods: We conducted cross sectional study comprised 73T2DM patients on Metformin. Questionnaire interview was applied. Blood samples were collected for CBC and VB ₁₂ detection. VB ₁₂ deficiency was defined as serum concentration of <300 pg/ml. Results: There was significantly lower in mean of vitamin B ₁₂ level in participants who have used metformin for ≥5years and in patients who were on metformin at a dose of ≥1000 mg/ day. Duration of metformin use were associated with increased risk of B ₁₂ deficiency 4.9 times (OR= 4.9) which increases the risk of DM complications 2.7 times. The results showed that 100 mg increase in metformin dose was associated with a 19.3 ± 4.4 pg/ ml (P = 0.00) lowering Vitamin B ₁₂ level. Every one year increase in the duration of diabetes associated with 18.6 ± 6.7 pg/ ml (P =0.007) lowering in VB ₁₂ level. Conclusion : Low serum VB ₁₂ level is associated with longer duration and higher dose of metformin use. Therefore, routine determination of VB ₁₂ level in patients with T2DM on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from VB ₁₂ supplements.
Corresponding Author: Lamia'a S. Saqer	
KEYWORDS: Type-2 Diabetes Mellitus , Metformin, Vitamin B ₁₂ Deficiency	

I. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is an endocrine disorder and a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose that is caused by a relative or absolute deficiency in insulin (Koppad A. et al., 2017). T2DM is partly preventable by staying a normal weight, exercising regularly, and eating properly so, treatment involves exercise and dietary changes (Luo Y. et al., 2017). If blood sugar levels are not adequately lowered, the medication like metformin is typically recommended (Maruthur NM. et al., 2016). Medication is a member of Biguanides group which stay the main treatment of type 2 DM. One of documented side effect of metformin is Vitamin B₁₂ deficiency (Toh SY. et al., 2009) (Liu KW. et al., 2006). The B₁₂ intrinsic factor complex uptake by ileal cell membrane receptors is known to be calcium-dependent, and metformin affects calcium dependent membrane action, resulting in B₁₂ deficiency (Bauman WA. et al., 2000).

Vitamin B₁₂, also called cyanocobalamin, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body: it is an essential cofactor that is integral to methylation processes important in reactions related to DNA and cell metabolism, and in both fatty acid and amino acid metabolism (Sigel H. et al., 2013 and (Green R., 2013). It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin (Miller A. et al.,2005) and in the maturation of developing red blood cells in the bone marrow (Greer JP., 2014). Thus a deficiency may lead to disruption of DNA and cell metabolism and thus have serious clinical consequences (Green R., 2013). Intracellular conversion of Vitamin B₁₂ to two active coenzymes, adenosyl-cobalamin in mitochondria and methyl-cobalamin in the cytoplasm, is necessary for the homeostasis of methyl-malonic acid and homocysteine, respectively (Stabler S., 2013 and Gorska R. et al., 2014). Vitamin B₁₂ deficiency can potentially cause severe and

irreversible damage, especially to the brain and nervous system (Trijbels FJ. et al., 2001). Vitamin B₁₂ malabsorption and its levels may start declining as early as the 4th month after initiating metformin therapy (Kooy A. et al., 2003). However due to storage in liver, clinical symptoms of Vitamin B₁₂ deficiency may manifest after 5–10 years (Andres E. et al., 2004). Diabetic patient mostly type 2, may develop a deficiency of Vitamin B₁₂ (Khan A. et al., 2017), and its deficiency is another cause of neuropathy, the latter can be easily managed if the low level of Vitamin B₁₂ is detected early, but in those cases with prolonged untreated suboptimal Vitamin B₁₂ levels, irreversible damage may occur. However, consequences can be prevented if treated adequately. Therefore, this study was carried out to evaluate the serum levels of Vitamin B₁₂ in patients with T2DM on metformin in Gaza Strip-Palestine.

II. MATERIALS AND METHODS

A total of 73 patients with type 2 diabetes mellitus within the age range of 35 to 72 years were recruited into this cross-sectional study using a convenient sampling method. The patients were those attending the health care centers in Khan younis-Gaza strip from. All the patients were on metformin and have been on the drug for a minimum of 3 months.

Exclusion criteria

Patients with parasitic infections, malabsorption syndromes, Hepatocellular carcinoma, liver disease, chronic kidney disease and thyroid disease were excluded from this study. Patients with recent intake of VB₁₂ supplements and chronic atrophic gastritis and vegetarians were also excluded.

A. Ethical Considerations

Participants were recruited into this study after obtaining a written informed consent from each of them. Also, an ethical approval was obtained from the Palestinian Ministry of Health.

B. Sample collection and storage

After an overnight fast of about 8-10 hours, 5 ml of venous blood was collected from each participant and dispensed into plain and EDTA tubes to determine the VB₁₂ levels and CBC respectively for. The samples in plain tube were allowed to clot and then spun at 4000 rpm for 10 minutes to obtain serum samples which were kept at -20C until analyzed for vitamin B₁₂ level. Study population was asked to filled out a questionnaire related to their health habits by using a meeting interview for filling in a questionnaire. Serum vitamin B₁₂ levels of the patients were determined using Vitamin B₁₂ AccuBind ELISA Kit. Vitamin B₁₂ deficiency was defined as serum concentration of <300 pg/ml

C. Statistical analysis

The data was entered, stored and analyzed by personal computer using the statistical package for Social Sciences (SPSS) version 20. Independent Samples T-test and Chi square test use to compare between T2DM patients with

reference interval, linear regression and Odd's Ratio. P value < 0.05 was consider statistically significant.

III. RESULTS

The study population consisted of 73 sample treated with Metformin. The mean age of subjects was 54.48 ± 7.3. The percentage of males was 52.05% (38/73) while that of females was 47.95% (35/73). 69.9% (51/73) of the study population was physically active subjects, while 30.1% (22/83) of them were non-physically active, and about 41.1% (30/73) of population had DM complications. On the other hand, 56.2% (41/73) of the study population had a family history of DM. The duration of metformin use was 41.01 ± 26.4 months (range 3–120 months). Maximum daily dose of metformin was 1135.1 ± 476.4 mg (range 240–2000 mg). The mean Vitamin B₁₂ was 391.2 ± 200.1 pg/ml. Vitamin B₁₂ deficiency was present in 46.6% (34/73) of the patients.

Based on duration of metformin use, all the patients were divided into 2 groups: <5 years and ≥5 years. As shown in Table 1, the mean Vitamin B₁₂ level was significantly lower in participants who have used metformin for ≥5 years compared with patients who have used metformin for <5 years.

Table 1: Characteristics of the patients and serum levels of VB₁₂ based on duration of metformin use

Variables	<5 years(n=53)	≥5 years(n=20)	P-value
Age (years)	53.79±7.5	56.30±6.6	0.171
Body weight (kg)	86.68±14.89	85.2±14.97	0.71
Height (cm)	165.85±9.3	167.7±10.8	0.50
BMI (kg/m ²)	31.63±5.69	30.76±4.39	0.49
Vitamin B ₁₂ (pg/ml)	439±204	264.5±121.17	0.00
MCV	87.52±6.32	90.15±4.75	0.062

Similarly, patients were divided into 2 groups based on metformin dose. It was observed that the mean level of Vitamin B₁₂ was significantly lower in patients who were on metformin at a dose of ≥1000 mg/day compared with patients who were on metformin at a dose of <1000 mg/day. In contrast, the mean BMI was significantly lower in patients who were on metformin at a dose of ≥1000 mg/day compared with patients who were on metformin at a dose of <1000 mg/day (Table 2).

“Vitamin B₁₂ Levels in Type 2 Diabetes Patients on Metformin in Gaza Strip-Palestine”

Table 2: Characteristics of the patients and serum levels of VB₁₂ based on metformin dose

Variables	<1000mg/day (n 33=)	≥1000mg/day (n 40=)	P-value
Age (years)	53.52±8.5	55.28±6.2	0.326
Body weight (kg)	88.09±14.79	84.78±14.86	0.345
Height (cm)	163.48±9.3	168.73±9.5	0.021
BMI (kg/m ²)	33.12±6.03	29.96±4.29	0.014
Vitamin B ₁₂ (pg/ml)	468.03±214.11	327.75±164.98	0.003
MCV	87.25±6.03	89.06±5.96	0.203

By using linear regression analysis, we found that 100 mg increase in metformin dose was associated with a 19.3 ± 4.4 pg/ml ($P = 0.00$) lowering Vitamin B₁₂ level, and Vitamin B₁₂ levels were 18.6 ± 6.7 pg/ml ($P = 0.007$) lowering for every 1-year increase in the duration of diabetes. The

duration of metformin use predicted a 4.4 ± 0.73 pg/ml ($P = 0.00$) lower Vitamin B₁₂ levels for every 1 month increase in the duration of metformin use. Years of metformin use were associated with increased risk of B₁₂ deficiency (Odds Ratio(OR), VB₁₂ deficiency/year metformin use, 4.9, 95% CI= 1.7–12.4).

The mean of Vitamin B₁₂ level among patients with DM complication was 296.67 ± 157.21 , while in DM patients without complications was 457.09 ± 201.94 (95% CI=76.47-244.39, $P= 0.00$). We observed that 30 patients of 73(41.1%) had DM complications, 70% (21/30) of them were Vitamin B₁₂ deficient, which increases the risk of DM complications 2.7 times (OR =2.7, 95% CI=1.4-5.03). (Table 3) .On comparing patients with Vitamin B₁₂ deficiency to those with normal Vitamin B₁₂ levels, males have risk in deficiency of Vitamin B₁₂ levels 1.4 times than female. Mean hemoglobin, MCV and BMI were not significantly different in the Vitamin B₁₂ deficiency and normal Vitamin B₁₂ groups.

Table 3: Demographic characteristics of patient on metformin and Vitamin B₁₂

Variables	Vitamin B ₁₂ level		(OR) (95%CI)	P-value
	Normal	Deficient		
DM Complications				
No	30	13	2.7 (1.4-5.03)	0.001
Yes	9	21		
Gender				
Male	17	21	1.4 (0.91-2.2)	0.094
Female	22	13		
Metformin Dose				
<1000mg/day	26	7	2.4 (1.5-3.8)	0.000
≥1000mg/day	13	27		
Metformin Duration				
<5 years	35	18	4.6 (1.7-12.4)	0.000
≥5 years	4	16		
MCV	87.3±5.3	89.3±6.6	(-0.76-4.8)	0.46
Hb	13.2±1.4	13.5±1.4	(-0.35-0.94)	0.36
BMI	32.2±6.4	30.4±3.6	(-4.2-0.53)	0.13

IV. DISCUSSION

Majority of patients with type 2 diabetes are prescribed metformin. One of documented side effect of metformin is Vitamin B₁₂ deficiency (Toh SY. et al., 2009) (Liu KW. et al., 2006). The B₁₂ intrinsic factor complex uptake by ileal cell membrane receptors is known to be calcium-dependent, and metformin affects calcium dependent membrane action, resulting in B₁₂ deficiency (Bauman WA. et al., 2000). Only limited data is available on this topic as per literature search; no study has ever been done on Palestinian population to determine Vitamin B₁₂ deficiency in diabetic patients on metformin. The present study involving 73 patients with type 2 diabetes mellitus showed lower Vitamin B₁₂ levels with metformin use (46.6%: 34/73). the minimum duration

of metformin use for inclusion is 3 months in our study. Furthermore, the prevalence of Vitamin B₁₂ deficiency in patients on metformin in our study is higher than that reported in Western literature. In different studies, Vitamin B₁₂ deficiency was found in 5.8%, 8.6%, 6.3%, of patients with type 2 diabetes mellitus on metformin (Reinstatler L. et al., 2012) (Calvo Romero JM. & Ramiro Lozano JM., 2012) (Nervo M. et al., 2011).

A predominantly due to low dietary products and meat diet intake could be one of the causes of higher prevalence of Vitamin B₁₂ deficiency in our study. Biguanides is a group of medication which include metformin; which stay the main treatment of type 2DM. Metformin-induced Vitamin B₁₂ deficiency has been approved to the binding of the

hydrophobic tail of biguanide to the hydrocarbon core of membranes. The biguanide group being positively charged (protonated) gives a positive charge to the membrane and can displace divalent cations such as calcium. The uptake of Vitamin B₁₂ into the ileal cells is calcium dependent and can thus be impaired by metformin. During our study; we observed that the population diets have to be low in calcium. This could be another factor causing higher prevalence of Vitamin B₁₂ deficiency.

Ting et al, showed duration and dose of metformin as high risk factors for developing Vitamin B₁₂ deficiency (Ting RZ. et al., 2006). These results were very similar to our study showing significant association and inverse relation of duration and dose of metformin with B₁₂ levels. A similar study performed in United States of America didn't find any significant correlation between metformin use and B₁₂ deficiency, however there was a trend towards lower B₁₂ levels in patients on long term metformin (Filioussi K. et al., 2003). In contrast to previous study by Iftikhar R. et al (Iftikhar R. et al., 2013), we found no correlation between MCV among patients on metformin having B₁₂ deficiency as compared to those who are not deficient.

The results obtained from this current study showed that the duration of diabetes in years didn't have a statistical significance $P = 0.182$, which agree with previous studies that have revealed the same results (Florence G. et al., 2017) and (Rana R. et al., 2017).

On the other hands another studies carried out in Pakistan (Khan A. et al., 2017) and India (Verma V. et al., 2017) showed that the duration of diabetes in years was a statistical significance with $P = 0.04$ and 0.001 respectively.

The impact of Vitamin B₁₂ deficiency on hematological parameters such as MCV and hemoglobin was studied. We didn't find any increase in MCV or decrease in hemoglobin in the Vitamin B₁₂ deficient patients. Similar findings have been reported earlier (de Groot-Kamphuis DM. et al., 2013). However, serum total Vitamin B₁₂ levels may not accurately reflect Vitamin B₁₂ status of the body and therefore biochemical deficiency often does not result in clinical deficiency (Oberley MJ. and Yang DT., 2013). The limitation of our study is that we did not measure functional markers of Vitamin B₁₂ deficiency (serum homocysteine and serum methyl-malonic acid levels) which may better reflect the status of Vitamin B₁₂ levels in the body as compared to serum Vitamin B₁₂ levels. In addition, our sample size was small.

As for level of Vitamin B₁₂ based on duration of metformin use in months and Vitamin B₁₂ deficiency in our study was statistically significant $P = 0.000$. The duration of metformin use predicted a 4.4 ± 0.73 pg/ml ($P = 0.00$) lower Vitamin B₁₂ levels for every one-month increase in the duration of metformin use. Years of metformin use were associated with increased risk of B₁₂ deficiency (OD Ratio:4.9, 95% CI= 1.7–12.4). Based on metformin dose also have statistically

significant $P = 0.003$. We found that 100 mg increase in metformin dose was associated with a 19.3 ± 4.4 pg/ ml ($P = 0.00$) lowering Vitamin B₁₂ level.

Other study conducted in Pakistan clarified that the duration of metformin use in years have decreased in Vitamin B₁₂ in statistically significant with $P = 0.001$ (Khan A. et al., 2017) on other hands study in Lebanon also showed that the duration of metformin use in months lowering Vitamin B₁₂ level with statistically significant ($P = 0.004$) (Zalaket J. et al., 2018).

The deficiency worsened with the dose and duration of metformin intake and these results were consistent with Ting et al. who postulated that each 1 g/day increase in metformin dose conferred an overall risk of 2.88 of developing Vitamin B₁₂ deficiency (95% CI, 2.15-3.87). In contrast, another research study in India conflicted with our result where they concluded that the duration of metformin use in years did not had statistically significant $P = 0.4$. Also they recorded that Vitamin B₁₂ level was significantly lower in patients who have been on metformin for >10 years compared with patients with < 10-years history of metformin use. Similarly, patients who were on metformin at a dose of >1000 mg/day had significantly lower Vitamin B₁₂ level when compared with patients on <1000 mg/day (Shailendra D. et al., 2018). Ko et al; 2014 illustrated that increase in metformin dose by 1g /day increases risk of Vitamin B₁₂ deficiency by greater than two fold. Subjects consuming metformin for more than ten to twelve year and daily dosage $\geq 2g$ showed about a fourfold higher risk of Vitamin B₁₂ deficiency compared to those with metformin use of less than four years and daily dosage of $\leq 1g$ (Ko. et al., 2014) which agree with our finding.

The present study showed that the duration of metformin use and BMI did not had a statistically significant with $P = 0.49$ (when patients were divided into 2 groups: <5 years and ≥ 5 years based on duration of metformin use) and on the other hand the increase of metformin dose have lowering effect on the BMI which reached statistically significant with $P = 0.014$, but this results disagree with others in Nigeria with $P = 0.002$ and 0.512 for metformin duration and the metformin dose in relation to BMI respectively (Akinlade K.S. et al., 2015). The number of patients included in the present study was 20/73 who had ≥ 5 -years duration of metformin use with BMI 30.76 ± 4.39 , due to wide use of metformin drug the BMI didn't had statistically significant.

Based on DM complication, we found that the mean of Vitamin B₁₂ level among patients on metformin that suffered from DM complication was 296.67 ± 157.21 , in compared with DM patients without complications was 457.09 ± 201.94 (95% CI=76.47-244.39, $P = 0.00$), which increases the risk of DM complications 2.7 times (OR Ratio=2.7, 95% CI=1.4-5.03). This result with agreement with those one conducted in Lebanon (Zalaket J. et al., 2018).

V. CONCLUSIONS

Our study demonstrated significantly high prevalence of Vitamin B₁₂ deficiency in patients treated with metformin with significant effect of dose and duration of metformin use on B₁₂ levels. Physicians must recognize this important fact and screen diabetics on metformin therapy for underlying B₁₂ deficiency specially those presenting with neuropathic symptoms. Thus routine supplementation of Vitamin B₁₂ given to patients on long-term high dose metformin therapy seems to be clinically more sensible and a cost-effective method.

Acknowledgement

The authors are grateful to all participants for their cooperation and gratefully acknowledge to the University Collage of Science and Technology-Medical Department for its facilitations to achieve this research.

VI. REFERENCES

1. Koppad, A. K., Patil, G., Baligar, B. D., & Renukappa, V. B.(2017) Evaluation of serum vitamin B12 levels in type 2 diabetics patients on metformin therapy attending a tertiary care hospital. *Journal of Evidence Based Medicine and Healthcare*, 4(90), 5399-5404
2. Luo, Y., Zhang, X., Li, Y., Deng, J., Li, X., Qu, Y., ... & Lin, B. (2018). High-glucose 3D INS-1 cell model combined with a microfluidic circular concentration gradient generator for high throughput screening of drugs against type 2 diabetes. *RSC Advances*, 8(45), 25409-25416.
3. Maruthur, NM; Tseng, E; Hutfless, S; Wilson, LM; Suarez-Cuervo, C; Berger, Z; Chu, Y; Iyoha, E; Segal, JB; Bolen, S (19 April 2016). "Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis". *Annals of Internal Medicine*. 164 (11): 740–5
4. Toh SY, Zarshenas N, Jorgensen J. Prevalence of nutrient deficiencies in bariatric patients. *Nutrition*. 2009; 25:1150. PubMed
5. Liu KW, Dai LK, Jean W. Metformin related vitamin B12 deficiency. *Age and Ageing*. 2006; 35(2):200-1. PubMed
6. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care*. 2000; 23(9): 1227-1231.
7. Sigel, A., Sigel, H., & Sigel, R. K. (Eds.). (2013). *Interrelations between essential metal ions and human diseases*. Springer Netherlands.
8. Green R. Physiology, dietary sources, and requirements. In: *Encyclopedia of human nutrition* . Vol 4. Academic Press, 2013: 351-6.
9. Miller A, Korem M, Almog R, Galboiz Y (June 2005). "Vitamin B12, demyelination, remyelination and repair in multiple sclerosis". *Journal of the Neurological Sciences*. 233 (1–2): 93–7.
10. Greer JP (2014). *Wintrobe's Clinical Hematology Thirteenth Edition*. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins. ISBN 978-1-4511-7268-3.
11. Stabler S. Vitamin B12 deficiency. *N Engl J Med* 2013;368:149-60.
12. Gorska R ,Sobczyńska-Malefora A, Pelisser M, Ruwona P, Witchlow B, Harrington D. An audit of holotranscobalamin (“Active” B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. *Clin Biochem* 2014;47:82-6.
13. Trijbels FJ, van der Put NM, van Straaten HW, Blom HJ (April 2001). "Folate, homocysteine and neural tube defects: an overview". *Experimental Biology and Medicine*. 226 (4): 243–70.
14. Kooy A, Wulffele M, Lehert P, 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*. 2003; 254:455–63.
15. Andres, E., Loukili, N. H., Noel, E., Kaltenbach, G., Abdelgheni, M. B., Perrin, A. E. & Blicklé, J. F. (2004). Vitamin B12 (cobalamin) deficiency in elderly patients.
16. Khan, A., Shafiq, I., & Shah, M. H. (2017). Prevalence of Vitamin B12 Deficiency in Patients with Type II Diabetes Mellitus on Metformin: A Study from Khyber Pakhtunkhwa. *Cureus*, 9(8).
17. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr. Association of biochemical B12 deficiency with metformin therapy and Vitamin B12 supplements: The National Health and Nutrition Examination Survey, 1999-2006. *Diabetes Care* 2012;35:327-33.
18. Calvo Romero JM, Ramiro Lozano JM. Vitamin B(12) in type 2 diabetic patients treated with metformin. *Endocrinol Nutr* 2012;59:487-90.
19. 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. *Rev Assoc Med Bras* ;57(1), 46-49.
20. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Internal Med*. 2006; 166(18):1975-9.PubMed .
21. Filioussi K, Bonvoas S, Katsaros T. Should we screen diabetes patients using biguanides for

- megaloblastic anaemia. *Aust Fam Physician*. 2003; 32(5): 383-4.PubMed
22. Iftikhar R.,& kamran S., 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 African Medical Journal*. 2013,1937- 8688.
 23. Florence G., Giacomo G., Thibaud M., Ariane S., Christophe P., Jean-Paul C., Valérie M., Denis MG., Eric R., Anne-Marie D., Antoine A. (2017) Clinical Consequences of Metformin-Associated Vitamin B12 Deficiency Among Patients with Type 2 Diabetes. *JSM Nutritional Disorders* 1(1): 1002.
 24. Rana, R., Fayyaz, M., Iftikhar, T., Ullah Makki M. (2017). Metformin use and Vitamin B12 deficiency in patients with Type 2 Diabetes Mellitus. *James Madison High School*, 11(3) 1017-1020.
 25. Khan, A., Shafiq, I., & Shah, M. H. (2017). Prevalence of Vitamin B12 Deficiency in Patients with Type II Diabetes Mellitus on Metformin: A Study from Khyber Pakhtunkhwa. *Cureus*, 9(8).
 26. Verma V., Nim R., Singh P., Kumar M., Singh G., Singh AK. Vitamin B12 deficiency among vegetarian and non-vegetarian diabetic population receiving prolonged Metformin based oral hypoglycemic agents therapy *International Journal of Advances in Medicine*;4(4):1150-1154.
 27. de Groot Kamphuis DM, van Dijk PR, Groenier KH, Houweling ST, Bilo HJ, Kleefstra N. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. *Neth J Med* 2013;71:386-90.
 28. Oberley MJ, Yang DT (2013). Laboratory testing for cobalamin deficiency in megaloblastic anemia. *American Journal of Hematology*;88:522-526.
 29. Zalaket, J., Wehbe, T., & Jaoude, E. A. (2018). Vitamin B12 deficiency in diabetic subjects taking metformin: A cross sectional study in a Lebanese cohort. *Journal of Nutrition & Intermediary Metabolism*; 11(4), 9-13.
 30. Shailendra D., Kalani, R., Narayan, K. V., Prabhakaran, D., Tandon, N., & Reddy, P. S. (2018). Prevalence of vitamin B12 deficiency among individuals with type 2 diabetes mellitus in a South Indian rural community. *International Journal of Basic & Clinical Pharmacology*; 7(2), 309-314.
 31. Ko S-H, Ahn Y-B, Song K-H, Han K-D, Park Y-M, Kim H-S (2014). Association of Vitamin B12 Deficiency and Metformin Use in Patients with Type 2 Diabetes. *Journal Korean Medical Science*; 29:965–972.
 32. Akinlade, K. S., Agbebaku, S. O., Rahamon, S. K., & Balogun, W. O. (2015). Vitamin B 12 levels in patients with type 2 diabetes mellitus on metformin. *Annals of Ibadan postgraduate medicine*, 13(2), 79-83.