Adverse Events Induced by Metformin Treatment in Patients with Type 2 Diabetes Mellitus: Metaanalysis

Kabelo Mokgalaboni* and Moshoeu S Mabusela

1School of Laboratory Medicine and Medical Sciences [SLMMS], College of Health Sciences, University of KwaZulu-Natal, Westville Campus South Africa
2School of Medical Science and Pathology, Faculty of Health Care Sciences, University of Limpopo, South Africa

*Corresponding author: Kabelo Mokgalaboni, School of Laboratory Medicine and Medical Sciences [SLMMS], College of Health Sciences, University of KwaZulu-Natal, Durban 4001, Westville Campus South Africa

INTRODUCTION

Type 2 diabetes mellitus [T2DM] is a metabolic condition characterised by hyperglycaemia resulting from insulin resistant or impaired insulin function [1]. T2DM have a high risk of developing cardiovascular disease [CVD] compared to individuals without diabetes [2,3]. The pharmacological therapies currently used in the prevention of secondary complications associated with T2DM include metformin; this class of drugs improve insulin sensitivity and reduce body weight [4]. Although this drug provides therapeutic benefits in T2DM patients and associated CVD, there are other concerns it poses to individuals using it, including decreasing serum vitamin B12 levels [5]. In acinical setting, when it is not detected early or if there is an incorrect diagnosis, this deficiency remains untreated, resulting in severe deficiency. Ultimately, this causes megaloblastic anaemia, alteration of mental states and neurological damage [5-7]. In most cases, diabetic neuropathy symptoms can overlap with pricking, impaired vibration and muscle sensation [8].

Thus, peripheral neuropathy as a result of vitamin B12 deficiency may contribute to the aggravation of diabetic peripheral neuropathy [6,7]. The progression of neurologic damage due to vitamin B12 deficiency can be treated if diagnosed early with the administration of vitamin B12 [9]. However, if there is...
misdiagnosis, permanent neurological damage may not be reversed [7]. Additionally, these patients present with gastric abnormalities, including diarrhoea, nausea, and loss of appetite, others may present with a sore and reddened tongue. These abnormalities may result in an unexpected reduction in body weight. Similarly, hepatomegaly and jaundice around the eyes and skin may also be observed [10]. Although previous studies have reported vitamin B12 deficiency associated with metformin, there is contradicting outcomes presented. Moreover, some studies show no association [11]; others are showing positive association. Therefore, we aimed to conduct a first meta-analysis on the effect of metformin treatment on vitamin B12 level and associated events in patients living with type 2 diabetes mellitus. Furthermore, to assess the efficacy of this drug in ameliorating cardiovascular disease associated with type 2 diabetes mellitus.

**Material and Methods**

**Preferred Reporting Item for Systematic Reviews and Meta-Analysis [PRISMA] Guidelines [12]**

was used when preparing this meta-analysis [Appendix file 1]. The ethics approval was not needed as this study only assess data extracted from already published studies.

**Research question**

Does metformin treatment alleviate type 2 diabetes mellitus related adverse events?

**Search Strategy and Information Source**

PubMed-Medline electronic database was used to search for published literature using the Medical Subject Headings [MeSH] terms “metformin”, “vitamin B12 deficiency” and “diabetes mellitus” without language restriction. The studies meeting eligibility criteria were subjected to critical evaluation and included in the final synthesis. The search was for studies published since inception until 07 March 2020. The exact search strategy is attached in appendix 1.

**Study Selection**

The selection procedure was conducted by two authors independently [KM and MSM]. Reference manager software, Mendeley Desktop version 1.19.4 [Elsevier, Amsterdam, Netherlands] was used to store retrieved studies. Firstly, we screened studies based on the title and abstract for relevance. Subsequently, the full-text studies were retrieved and critically evaluated for eligibility in the systematic review and meta-analysis. Additionally, we screened the bibliographical lists of included studies to identify additional eligible studies that might have been missed on electronic database search.

PECO: P: patients with type 2 diabetes mellitus; E: diabetes status; C: healthy control participants; O: adverse events, including cardiovascular disease, neuropathy, nephropathy, myocardial infarction, hypertension, stroke and anaemia.

**Eligibility Criteria**

**Inclusion Criteria:** Randomised control trials, cross-sectional, prospective or retrospective observational and cohort studies. Mainly studies reporting on the impact of metformin treatment on the development of adverse events including vitamin B12 deficiency, neuropathy, nephropathy, hypertension, anaemia, myocardial infarction, retinopathy and stroke were included. Study selection was carried out by KM and MSM, and where there was disagreement, the same authors reached a conclusion through discussion and re-evaluating the study.

**Exclusion Criteria:** We excluded editorials, letters to editors, case reports, reviews, the study on other treatment other than metformin and studies with no proper control.

**Data Extraction and Data Items**

Two independent authors [KM and MSM] reviewed each abstract, retrieved full-text studies and extracted information from each study. Relevant data items extracted from each study were primary author surname and year of publication, the country, study design, population size, age and events. Where there was disagreement between the two independent authors, a resolution was reached through discussion and consensus and re-evaluation of study in question. Mendeley reference manager version (1.19.4) software [Elsevier, Amsterdam, Netherlands] was used to save collected data.

**Assessment of Risk and Quality**

The assessment of the quality of each included study was evaluated using a standard score by independent authors investigators. Cochrane tool was used to assess the risk of bias and quality of the studies. The disagreement between the two independent authors was resolved through discussion and re-evaluation of study in question.

**Data Synthesis and Analysis**

The Review Manager [RevMan] version 5.3 software [The Nordic Cochrane Centre, The Cochrane Collaboration, 2014] data analysis was used to carry out all the dichotomous data analysis. To determine the odds ratio [OR], the number of events and the total number of participants in the metformin and placebo group were computed. Effects measures were reported as OR and 95% confidence intervals [CI]. OR<1, OR=1, OR>1, classified as not associated with exposure of metformin, metformin not affecting odds of adverse events and metformin-associated with higher odds of adverse events respectively [13]. Heterogeneity was tested with Cochrane chi-square statistics and measured with the Higgins [12] statistic tests [14]. In the case where heterogeneity was observed, an attempt to find sources of heterogeneity was made through
subgroup analysis and sensitivity tests. The I² = 0%, I² = 50%, were no and substantial level of heterogeneity respectively. Considering high clinical heterogeneity, a random effects model was used for meta-analyses in case of substantial heterogeneity while the fixed-effect model was used for studies which showed no presence of heterogeneity. Subgroup analyses were performed according to different events, including CVD, anaemia, neuropathy, retinopathy, nephropathy, stroke, hypertension and vitamin B12 deficiency. Publication bias was visually assessed using the funnel plots [symmetrical shape demonstrating an absence of publication bias]. A probability values of less than 0.05 were considered significant statistically.

**Result**

**Selected Studies**

A search on PubMed database yielded 64 studies, based on our eligibility criteria 12 studies [5,15,16-23,24] were critically analysed and further included in the final synthesis (Figure 1).

![Flow diagram showing the study selection.](image)

**Overview of the Included Studies**

All included studies were published in peer-review journals from 2003 to 2018, and their characteristics are shown in Table 1. The included studies comprised of 25936 participants, 14720 [56.8%] of whom were T2DM on metformin, and 11216 [43.2%] were T2DM on placebo. The sample size of included studies ranged between 31 and 7493 participants. Among the included 11 studies, three were randomized control trials; three were cross-sectional, two retrospective cohorts, one observational retrospective cohort, one longitudinal study and one study that was a mixture of an observational, cross-sectional cohort. These studies were conducted all over the world with four studies conducted in the Netherlands, two in the United States, one in each of the following country [China, Japan, south Arabia, Sweden, and Taiwan] (Table 1S -3S).

**Table 1S: Characteristics of included studies.**

| Study   | Country       | Design               | Population size | Age (years) | CVD | Neuropathy | B12 | Anaemia | Neuropathy | Stroke | Hypertension | Retinopathy | MI |
|---------|---------------|----------------------|-----------------|-------------|-----|------------|-----|---------|------------|--------|--------------|-------------|----|
| Allarbi | South Arabia | Observational retrospective cohort | Metformin (319) | 57.8 ± 0.6 | NR  | 96         | 30  | NR      | NR         | NR     | NR           | NR          |    |
|         |               |                      | Placebo (93)    | 56.6 ± 1.4 | NR  | 18         | 2   | NR      | NR         | NR     | NR           | NR          |    |
| Aroda   | United States | longitudinal         | Metformin (n=859) | 56.7 ± 10.1 | NR  | 83         | 37  | 123     | NR         | NR     | NR           | NR          |    |
|         |               |                      | Placebo (n=856)  | 56.0 ± 9.9 | NR  | 85         | 20  | 90      | NR         | NR     | NR           | NR          |    |
De Groot-Kamphuis, 2013  Netherlands  Cross sectional  Metformin (n=164)  62.6 ± 11.9  NR  28  23  NR  30  NR  NR  41  NR  Placebo (n=134)  67.2 ± 10.8  NR  38  6  NR  29  NR  NR  29  NR

De Jager resident, 2010  Netherlands  Randomised control trial (RCT)  Metformin (n=193)  64 ±10  NR  NR  NR  NR  NR  8  NR  NR  24  NR  Placebo (n=191)  59 ±11  NR  NR  NR  NR  NR  8  NR  NR  21  NR

Wuffel, 2003  Netherlands  RCT  Metformin (n=171)  63.2 ±9.8  NR  59  NR  NR  NR  NR  NR  NR  NR  NR
Placebo (n=182)  58.9 ±11.1  53  NR  NR  NR  NR  NR  NR  NR  NR  NR

Reinstatler, 2012  United States  Cross sectional  Metformin (n=575)  63.4 ±11.99  NR  NR  NR  104  NR  NR  NR  NR  NR  NR
Placebo (n=1046)  66.4 ±16.17  NR  NR  NR  222  NR  NR  NR  NR  NR  NR

Sato, 2013  Japan  Cross sectional  Metformin (n=46)  61 ±11  NR  NR  NR  NR  NR  9  NR  NR  NR  NR
Placebo (n=38)  62 ±10  NR  NR  NR  NR  NR  7  NR  NR  NR  NR

Hermann, 2004  Sweden  Observational, cross-sectional cohort study  Metformin (n=53)  58.75 ±11  NR  NR  8  NR  NR  22  28  NR  19  NR
Placebo (n=31)  64.5 ±8.25  NR  NR  3  NR  NR  7  18  NR  13  NR

Fung, 2015  China  Retrospective cohort  Metformin (n=7493)  61.70 ±10.75  NR  NR  NR  NR  NR  5126  NR  NR  NR
Placebo (n=3800)  62.75 ±10.97  NR  NR  NR  NR  NR  2971  NR  NR  NR

Out, 2018  Netherlands  RCT  Metformin (n=196)  64 ±10  24  167  NR  NR  NR  NR  NR  NR  NR  NR  NR
Placebo (n=194)  59 ±11  21  162  NR  NR  NR  NR  NR  NR  NR  NR  NR

Kuan, 2017  Taiwan  Retrospective cohort  Metformin (n=4651)  64.7 ±9.46  NR  NR  NR  NR  NR  3406  641  NR  NR
Placebo (n=4651)  64.7 ±10.0  NR  NR  NR  NR  NR  3408  623  NR

Note: CDV: Cardiovascular Disease, B12: Vitamin B12, MI: Myocardial Infarction.

Table 25

| Section/Topic        | # | Checklist Item                                                                 | Reported on Page # |
|----------------------|---|--------------------------------------------------------------------------------|-------------------|
| **Title**            |   | **Identify the report as a systematic review, meta-analysis, or both.**          | 1                 |
| **Abstract**         |   | **Structured Summary**                                                          | 2                 |
|                      |   | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2                 |
| **Introduction**     |   | **Rationale**                                                                  | 3                 |
|                      |   | Describe the rationale for the review in the context of what is already known.   | 3                 |
| **Objectives**       |   | **Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).** | 3                 |
| **Methods**          |   | **Protocol and registration**                                                   | N/A               |
|                      |   | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | N/A               |
Eligibility Criteria

Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.

Information Sources

Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.

Search

Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.

Study Selection

State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).

Data Collection process

Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.

Data Items

List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.

Risk of bias in individual studies

Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level, and how this information is to be used in any data synthesis).

Summary Measures

State the principal summary measures (e.g., risk ratio, difference in means).

Synthesis of Results

Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I²) for each meta-analysis.

Table 3S

| Section/topic          | # | Checklist item                                                                 | Reported on page # |
|-----------------------|---|---------------------------------------------------------------------------------|--------------------|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies) | 5                  |
| Additional analyses   | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 5                  |
| Results               |   |                                                                                  |                    |
| Study selection       | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 6                  |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 6                  |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 9                  |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 8-Jul              |
| Synthesis of results  | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 8-Jul              |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 9                  |
| Additional analysis   | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 9-Aug              |
| Discussion            |   |                                                                                  |                    |
| Summary of evidence   | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 10                 |
| Limitations           | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 11                 |
| Conclusions           | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 11                 |
| Funding               |   |                                                                                  |                    |
| Funding               | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data), role of funders for the systematic review. | 11                 |
Data Synthesis

Vitamin B12 in Type 2 Diabetes Mellitus Patients on Metformin Treatment: The current meta-analysis has shown that T2DM patient on metformin has an increased risk of developing vitamin B12 deficiency when compared to healthy patient OR, 95%CI [2.41 [1.58,3.68], p<0.0001], interestingly the analysed studies showed no level of heterogeneity [Chi2=2.47, I2=0%, p=0.048] (Figure 2).

Adverse Events Induced by Metformin Treatment in Type 2 Diabetes Mellitus: According to the current study, patient with type 2 diabetes mellitus on metformin have increased risk of being attacked by stroke compared to T2DM not on metformin treatment OR, 95%CI [1.44 [0.80,2.60], Chi2=1.72, I2=0%, p=0.42], retinopathy [1.04[0.93, 1.17], Chi2=0.30, I2=0%, p=0.58], myocardial infarction [1.01[0.61,1.69], Chi2=0.50, I2=0%, p=0.96]. The overall effect estimates for these adverse events also indicated that metformin is associated with an increased risk of stroke, retinopathy and myocardial infarction [1.05[0.94,1.18]] of interest was absence of heterogeneity which was also confirmed by subgroup analysis [Chi2=1.17, I2=0%, p=0.56] (Figure 3).

Other Adverse Events Induced by Metformin Treatment: Current metaanalysis shows that metformin treatment in type 2 diabetes mellitus patients reduces the risk of developing cardiovascular disease OR, 95%CI [0.81[0.31,2.10], Chi2=5.86, I2=83%, p=0.02], including hypertension [0.78[0.50,1.12], Chi2=57.79, I2=97%, p=0.00001] however shows an increased risk of neuropathy [1.00[0.65,1.55], Chi2=9.68, I2=69%] and anaemia [1.08[0.63,1.85], Chi2=7.72, I2=87%, p=0.0005] (Figure 4). The overall pooled effect estimate revealed that metformin treatment is not associated with these adverse events in T2DM [0.92[0.73,1.16], Chi2=93.18, I2=89%, p=0.00001], as a result of moderate level of heterogeneity, subgroup analysis was performed and it revealed no heterogeneity [Chi2=1.05, I2=0%, p=0.6601] (Figure 4).
Figure 4: Cardiovascular disease and related adverse events triggered by metformin treatment in type 2 diabetes mellitus compared to placebo.

Table 1: Sensitivity analysis.

| Study weight       | OR, 95%CI | P    | p     |
|--------------------|-----------|------|-------|
| Low weight study   | 0.92(0.73, 1.18) | 90   | <0.00001 |
| High weight study  | 0.92(0.71, 1.18)  | 90   | <0.00001 |

Sensitivity Analysis for Studies That Showed A Substantial Level Of Heterogeneity: A sensitivity test was performed by removing one study at a time, and recalculating the effect measures by removing low weight study, the OR did not change; however, the direction of confidence intervals slightly changed from [0.92(0.73, 1.16)] to [0.92(0.73, 1.8)], similarly when high weight study was removed we noted a slight change in confidence interval [0.92(0.71, 1.18)] (Table 1).

Risk of Bias and Quality Assessment: Studies were scored as good quality when it has four or more positive, which showed a low risk of bias if it has three scores out of six domains. Three studies scored all six positive, thus low risk of bias in all domains. Four scored four out of possible six domains with two domains classified as a high risk this included allocation and blinding of participants and personnel. One study was rated high risk in terms of the blinding and incomplete outcome however other four domains were of low risk, and lastly, one study had a fair quality as it has scored 3 points out of possible six domains (Figure 5 & Figure 1S-4S).
Figure 1S: Symmetrical presentation using funnel plot of vitamin B12 amongst the included studies showing no publication bias.

Figure 2S: Funnel plot of adverse events showing no publication bias.

Figure 3S: Symmetrical presentation of adverse events showing the absence of publication bias.

Figure 4S: Quality assessment and risk of bias amongst the included studies.
anaemia. manifestation of other complications including neuropathy and vitamin B12 levels not to be overlooked as it might result into the patients living with T2DM; however, we suggest its impact on treatment has therapeutic benefits in terms of preventing CVD in metformin treatment. Our results are suggesting that metformin treatment have a high risk of developing vitamin B12 deficiency which further predisposes them to neuropathy, and other related adverse events including anaemia, retinopathy, nephropathy, stroke and myocardial infarction, of importance, is its beneficial effects in reducing cardiovascular disease including hypertension.

Strength and Limitation
One of the limitations includes different study design amongst the included studies. With that been said, the study has its strength which ranges from good quality of studies been included. The literature search was from inception until 07 march, to make sure we get old studies with background knowledge about metformin and its impacts on type 2 diabetes. Pooling all these studies have increased the statistical power as compared to individual study. The combined sample size was sufficient; thus, we can conclude that our meta-analysis was not statistically underpowered. The studies showed no heterogeneity in many adverse events, and the sensitivity analysis also showed a slight change in effect measures and the funnel plots showed no presence of publication bias amongst the included studies. The studies were published in different regions of the world.

Ethical Considerations
Not required as this is review and analysis of studies that are already published.

Source of Funding
The study was partially funded by the National Research Foundation of South Africa [NRF, grantno:121496].

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Author Contribution
KM and MSM contributed equally from conceptualisation, screening, analysis, first draft to the final approval for publication of this manuscript.

References
1. Kahn CR (1994) Baniting lecture: Insulin action, diabetogenes, and the cause of type II diabetes. Diabetes43(8):1066-1084.
2. Mokgalaboni K, Dhlala PV, Nyambuya TM, Yakobi SH, Mxinwa V, et al. (2020) Monocyte-mediated inflammation and cardiovascular risk factors in type 2 diabetes mellitus: A systematic review and meta-analysis of pre-clinical and clinical studies.
3. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, et al. (1999) The online version of this article, along with updated information and services, is located on the World Wide Web: 1134-1146.
4. Domécq JP, Prutsky G, Leppin A, Sonbol MB, Altayar O, et al. (2015) Drugs commonly associated with weight change: A systematic review and meta-analysis. J Clin Endocrinol Metab 100(2): 363-370.

5. De Jager J, Kooy A, Lehert P, Wulffköté MG, Van Der Kolk J, et al. (2010) Long-term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: Randomised placebo controlled trial. BMJ340 (7775): 1177.

6. Bell DSH (2010) Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. South Med J 103(3): 265-267.

7. Pierce SA, Chung AH, Black KK (2012) El seguimiento a las concentraciones de vitamina B12 en una población de veteranos que utilizandossalitas de metformín por periodos largos detiempo. Ann Pharmacother 46(11): 1470-1475.

8. Pliipsen MC, Oh RC, Sagüil A, Seehusen DA, Topolksi R (2009) The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: A cross-sectional study. J Am Board Fam Med 22(5): 528-534.

9. Lindenbaum J, Heaton EB, Savage DG, Brust JC, Garrett TJ, et al. (1988) Stabler SP AR. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia macrocytosis. N Engl J Med 318: 1720-1728.

10. (2008) Disorders, NORD: National Organization for Rare Disorders. Anemia, Megaloblastic p. 1-7.

11. Elhadd T, Ponirakis G, Dabbous Z, Siddique M, Chinmayan S, et al. (2018) Metformin use is not associated with B12 deficiency or neuropathy in patients with type 2 diabetes Mellitus in Qatar. Front Endocrinol (Lausanne) 9: 2-6.

12. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62(10): 1006-1012.

13. Sedgwick P, Marston L (2010) Statistical question: Odds ratios. BMJ 341(7769): 407.

14. Higgins JPT, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327(7414): 557-560.

15. Alharbi TJ, Tourkmani AM, Abdelhay O, Alkhashan HI, Al-Asmari AK, et al. (2018) The association of metformin use with vitamin B12 deficiency and peripheral neuropathy in Saudi individuals with type 2 diabetes mellitus. PLoS One13(10): e0204420.

16. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, et al. (2016) Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metab 101(4): 1754-1761.

17. DM De Groot-Kamphuis , PR Van Dijk, KH Groenier, ST Houweling, HJG Bilo, et al. (2013) Vitamin B12 Deficiency Among Type 2 Diabetes Patients Using Metformin. Neth J Med 71(7): 386-390.

18. Fung CSC, Wan BIL, Wong CKH, Jiao F, Chan ACK (2015) Effect of metformin monotherapy on cardiovascular diseases and mortality: A retrospective cohort study on Chinese type 2 diabetes mellitus patients. Cardiovasc Diabetol 14(1): 1-14.

19. Hermann LS, Nilsson B, Watres S (2004) Vitamin B12 status of patients treated with metformin: Across-sectional cohort study. Br J Diabetes Vasc Dis 4(6): 401-406.

20. Kuan YC, Huang KW, Lin CL, Hu CJ, Kao CH (2017) Effects of metformin exposure on neurodegenerative diseases in elderly patients with type 2 diabetes mellitus. Prog Neuro-Psychopharmacology Biol Psychiatry 79(2): 77-83.

21. Metaxas C, Zurwerra C, Rudofsky G, Hersberger KE, Walter PN (2018) Impact of type 2 Diabetes and Metformin use on Vitamin B12 Associated Biomarkers - An Observational Study. Exp Clin Endocrinol Diabetes 126(6): 394-400.

22. Out M, Kooy A, Lehert P, Schalkwijk CA, Stehouwer CDA (2018) Long-term treatment withmetformin in type 2 diabetes and methylmalonic acid: Post hoc analysis of a randomizedcontrolled 4.3-year trial. J Diabetes Complications 32(2): 171-178.

23. Reinstein L, Qi YP, Williams on RS, Garn JV, Oakley GP (2012) Association of biochemical B12 deficiency with metformin in therapy and vitamin B12 supplements: The National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care 35(2): 327-333.

24. Sato Y, Ouchi K, Funase Y, Yamauchi K, Aizawa T (2013) Relationship between metformin use, vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes. Endocr J 60(12): 1275-1280.

25. Calvo Romero JM, Ramiro Lozano JM (2012) Vitamin B12 in type 2 diabetic patients treated withmetformin. Endocrinol y Nutr English Ed 59(9): 467-490.

26. Thomas MC, Macisaac Rj, Tsalamandris C, Molynieux L, Goubinal, et al. (2004) The burden of anaemia in type 2 diabetes and the role of nephropathy: A cross-sectional audit. 19(7):1792-1797.

27. Dilov R, Schwenger V, Scho M, Ritz E (2002) How should we manage anaemia in patients with diabetes? 67–72.

28. Bosman, Winkler AS, Marsden JT, Macduugallc, Peter J Watkins (2001) Anemia with Erythropoietin Deficiency Occurs Early in Diabetic Nephropathy DEBORAH 24(3).