

## Research Article

# Prevalence of Vitamin B12 deficiency in patients with type 2 diabetes mellitus on Metformin therapy at a single centre in Johannesburg, South Africa

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### ABSTRACT

**Introduction:** Metformin is a first-line oral hypoglycaemic agent for treating patients with type 2 diabetes mellitus (T2DM). Studies suggest that metformin use is associated with B12 deficiency. There is a paucity of data regarding this association in South Africa. The current study aimed to determine the prevalence of B12 deficiency in a South African cohort of T2DM patients on metformin therapy.

**Methods:** The study was a retrospective clinical audit of patients recruited from a medical outpatient setting. A consecutive sampling technique was employed; T2DM patients who were 40 years or older and on metformin for a minimum of 6 months were recruited. B12 deficiency was defined as severe (<150 pmol/L), moderate (150–169 pmol/L) or mild (170–200 pmol/L).

**Results:** One hundred and one (n = 101) patients were enrolled, most of whom were females (65%). The prevalence of B12 deficiency was 14.9%, with most patients in the mild category. The majority of B12 deficient patients were female (67%), elderly (70.2 ± 10.7 years) and of Coloured race (67%). The median duration of metformin use in B12 deficient and non-deficient patients was 10.9 (6–13) and 7 (3–12) years respectively (p = 0.179). The median metformin dosage was 1700 mg. Older age (Adjusted odd ratio (AOR) 6.67 (1.16–38.3), p = 0.033) and Coloured race (AOR 7.8(1.78–34.2) p = 0.006) were associated with vitamin B12 deficiency.

**Conclusion:** In our setting, vitamin B12 deficiency is prevalent amongst T2DM patients on metformin therapy. Older age and the novel finding of Coloured race were associated with B12 deficiency. We recommend screening for B12 deficiency amongst T2DM patients on metformin therapy.

**Keywords:** Vitamin B12 deficiency, diabetes mellitus type 2, metformin

### INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by persistent hyperglycaemia and alterations in carbohydrate, lipid, and protein metabolism due to a defect in insulin secretion, action, or both.(1)

Metformin is an oral hypoglycaemic agent belonging to a class of drugs called biguanides. It is the most frequently prescribed oral agent for Type 2 Diabetes Mellitus (T2DM).(1) The 2017 Guidelines of the South African Society for Endocrinology, Metabolism, and Diabetes (SEMDSA) advocate metformin as first-line therapy for T2DM.(2) This guideline is informed by metformin's various clinical benefits associated with glycaemic control, a relatively minimal side effect profile, weight neutrality/modest weight reduction benefit, and affordability.(1)

Metformin use has been shown to significantly decrease diabetes-related complications, including myocardial infarction and all-cause mortality.(3) It is prescribed as an oral tablet between 500 mg and 3000 mg per day, with most of its absorption occurring in the duodenum and jejunum via the plasma membrane monoamine transporter into the enterocytes.(4)

Vitamin B12 (B12) is an essential coenzyme in synthesising deoxyribonucleic acid (DNA). It is found in animal products and fortified foods, including milk and cereals.(5) In the body, B12 is converted to its two active metabolites, methylcobalamin and 5-deoxyadenosylcobalamin, which function as cofactors for the enzymes methionine synthase and L-methyl-malonyl-CoA mutase.(6–8)

Metformin-associated B12 deficiency in high-income countries (HIC) is well-documented. Several studies report a prevalence ranging from 5% to 30%, ascribed to various factors such as the absence of a universal reference range for B12 levels, a gold-standard diagnostic test, as well as differences in study designs with varying ethnicities.(9–13) The mechanism of B12 deficiency with metformin use is unclear. Numerous hypotheses have been proposed, including intestinal dysmotility and bacterial overgrowth, alteration of bile acid metabolism, and inhibition of intrinsic factor secretion by biguanides.(14,15) The most widely accepted hypothesis is the displacement of calcium in the terminal ileum by metformin, which is thought to inhibit calcium-dependent B12 absorption.(16)

Exactly what dosage and duration of metformin results in B12 deficiency is unknown. Data suggests that the risk of B12 deficiency increases with higher doses and longer duration of metformin use.(9,10,17) Beulens *et al.* postulated an inverse association between higher metformin doses and B12 levels.(10) Similar findings were reported by de Groot-Kamphuis and colleagues.(13) In keeping with these findings, Alharbi *et al.* showed that a longer duration of metformin use and a higher dosage of more than 2000 mg/day were associated with lower B12 levels.(11)

Whether metformin-associated B12 deficiency results in adverse clinical effects remains controversial. Nevertheless, an association between peripheral neuropathy and B12 deficiency has been reported in patients with prolonged (5.2 years) metformin use.(12) A cross-sectional study in the Netherlands showed an association between metformin use and B12 deficiency. However, metformin use did not predict the development of anaemia and neuropathy.(13) This difference in opinion has led to conflicting guideline recommendations for and against routine B12 monitoring in patients on metformin. A systematic review and meta-analysis summarised evidence on the association between metformin and B12 deficiency in T2DM patients.(16) This review by Chapman *et al.* found that metformin users had lower vitamin B12 levels, consistent with previous studies.(9,18) In light of this evidence, the 2023 American Diabetes Association (ADA) Standards of Medical Care in Diabetes recommends (level B evidence) periodic B12 monitoring.(19) It is worth noting that the populations investigated in the studies mentioned above consisted of participants from high-income countries, with no study sites in Africa.

In a small ( $n = 121$ ) South African study, Ahmed *et al.* found a 28% prevalence of B12 deficiency in T2DM patients on metformin therapy.(20) The most recent SEMDSA guidelines do not recommend routine B12 monitoring in patients with T2DM. These conflicting views are influenced by a lack of large randomised controlled trials addressing B12 deficiency as a primary outcome.

Most published data on metformin-associated B12 deficiency emanates from North America, Europe and Asia.(13,21,22) There are few studies on metformin use and associated B12 deficiency conducted in Africa.(23–26)

South Africa is one of the most ethnically diverse countries in Africa. Regarding race, South Africans are classified into four groups: Blacks, Whites, Coloureds, and Indians, who constitute 81.4%, 7.3%, 8.2% and 2.7% of the population, respectively.(27) The coloured people of South Africa are a minority group of mixed African ancestry arising from a blend of native black Africans with white Europeans and Malaysians.(28,29) Given their diverse ancestral origins, coloureds have a complex and diverse genetic makeup.(28) Only one small study has been published in South Africa, investigating the relationship between metformin use and B12 deficiency.(20) Thus we aimed to expand on this relationship, and aimed to determine the prevalence of B12 in T2DM patients on metformin attending a medical outpatient department at a tertiary hospital in Johannesburg.

## METHODS

### Study design and participants

The current study was a retrospective clinical audit conducted at the Helen Joseph Hospital (HJH), a tertiary-level academic hospital in Gauteng province of South Africa, from June 2018 to September 2021. A consecutive sampling method was employed. Patients older than 40 years of age, diagnosed with T2DM, on metformin therapy for a minimum of 6 months and any other hypoglycaemic agent were eligible for the study. The Division of Endocrinology at HJH routinely monitors B12 levels in all T2DM patients on metformin therapy annually.

Patients were excluded from the study if any of the following were present: patients younger than 40 years old, absence of a B12 level record, receiving B12 therapy, a diagnosis of pernicious anaemia, previous gastrectomy, T1DM, pregnancy, malabsorption syndromes and patients who self-identify as vegetarian or vegan. No distinction was made in terms of gender, age, race, socio-economic status or ethnicity.

### Data collection

The information obtained from participants' records included age, sex, race, date of diagnosis of T2DM, most recent HbA1c (glycated haemoglobin) level, estimated glomerular filtration rate (eGFR), haemoglobin, duration of metformin use, dosage of current metformin therapy, smoking status, blood pressure and body mass index (BMI). Patients were categorised by age into the following categories: middle-aged (40–59 years) and elderly ( $\geq 60$  years).

Serum B12 was measured by the National Health Laboratory Services (NHLS) using a Cobas 8000 Analyser (Roche Indianapolis, United States). B12

deficiency was defined as severe (<150 pmol/L), moderate (150–169 pmol/L) or mild (170–200 pmol/L).<sup>(30,31)</sup>

### Statistical analysis

Data was captured on an Excel spreadsheet and then imported to STATA Version 17 (College Station, Texas) for further analysis. Data was described as means (standard deviations) for continuous data, median [interquartile range] (non-normal distribution), actual numbers and percentages for categorical data. B12 deficiency in participants was defined when B12 levels were  $\leq 200$  pmol/L.

Baseline demographic and clinical characteristics of B12-deficient and typical participants were compared using Student's t-test or Wilcoxon signed-rank test for continuous variables and chi-square or Fisher's exact test for categorical variables. Variables with a significant difference were entered into the logistic regression model to determine predictors of B12 deficiency. A p-value of less than 0.05 was considered significant.

Ethical clearance for the study was obtained from the University of the Witwatersrand Human Research Ethics Committee.

### RESULTS

A total of 133 patients were enrolled in the study. Thirty-two patients were excluded based on either not fitting the inclusion criteria or lacking B12 levels, leaving a study sample of 101 patients. The baseline demographic and clinical

characteristics are summarised in Table 1. Females constituted 65% of the participants. The racial proportions in our cohort were as follows: 53% of Black African ethnicity, 31% Coloured, 11% Indian and 5.0% Caucasian (Table 1). The mean age of participants was 60.9 years, with B12 deficient patients significantly older than those without ( $70.2 \pm 10.7$  vs  $60 \pm 11.7$ ),  $p = 0.006$  (Figure 1). The median duration of metformin use was 7 (3–12) years (Table 1).

The prevalence of B12 deficiency in the current study was 14.9%, and in most cases, the deficiency was mild (Table 2). The majority of B12 deficient patients were female (67%), elderly and of Coloured race (67%) irrespective of B12 severity (Table 2 and Figure 2). Vitamin B12 deficient patients had significantly lower mean HbA1c than those not B12 deficient (Table 1). However, in a logistic regression model, HbA1c was not a predictor of B12 deficiency (Table 3). The median duration of metformin use in B12 deficient patients was 10 (6–13) years, which was more than in non-deficient patients (7 (3–12) years), but it was not statistically significant ( $p = 0.178$ ) (Table 1). There was no demonstrable difference in metformin dosage between those who were B12 deficient and those who were not (Figure 3). Hence, no direct relationship between B12 deficiency and metformin dosage was observed.

A logistic regression model demonstrated that older age (Adjusted odds ratio (AOR) 6.67 (1.16–38.3),  $p = 0.033$ ) and Coloured race were associated with B12 deficiency (AOR 7.8 (1.78–34.2)  $p = 0.006$ ) (Table 3).

**Table 1:** Baseline demographics

Variable	Total (N = 101)	Non-Deficient (n = 86)	B12 Deficient (n = 15)	p-value
Age (years)	61.5 $\pm$ 12.1	61 $\pm$ 10.4	70.2 $\pm$ 10.7	<b>0.006*</b>
Gender N (%)	66 (65.4)	56 (65.1)	10 (67)	0.844
Race				<b>0.008*</b>
Black African Ancestry N (%)	54 (53)	51 (59)	3 (20) <sup>a</sup>	
Caucasian	5 (5)	3 (3)	2 (13)	
Coloured	31 (31)	21 (25)	10 (67)	
Indian	11 (11)	11 (13)	–	
Duration on metformin (years)	7 (3–12)	7 (3–12)	10 (6–13)	0.1788
BMI	30.6 $\pm$ 8.4	30.6 $\pm$ 9.05	30.7 $\pm$ 4.6	0.9729
SBP	131 $\pm$ 35.6	132 $\pm$ 33.8	125.3 $\pm$ 47.7	0.6101
DBP (mmHg)	84.7 $\pm$ 16.9	85.7 $\pm$ 17.1	80.1 $\pm$ 15.7	0.2561
Smoking (yes)	36 (36)	29 (34)	7 (47)	0.501
Hb	13.1 $\pm$ 2.49	13.2 $\pm$ 2.29	12.3 $\pm$ 3.5	0.3362
HbA1c	8.43 $\pm$ 2.1	8.6 $\pm$ 2.1	7.5 $\pm$ 1.5	<b>0.0153*</b>

\*Denotes Statistically significant

<sup>a</sup>Significantly different to Coloured patients ( $p = 0.004$ )

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hb, haemoglobin; HbA1c, glycated haemoglobin

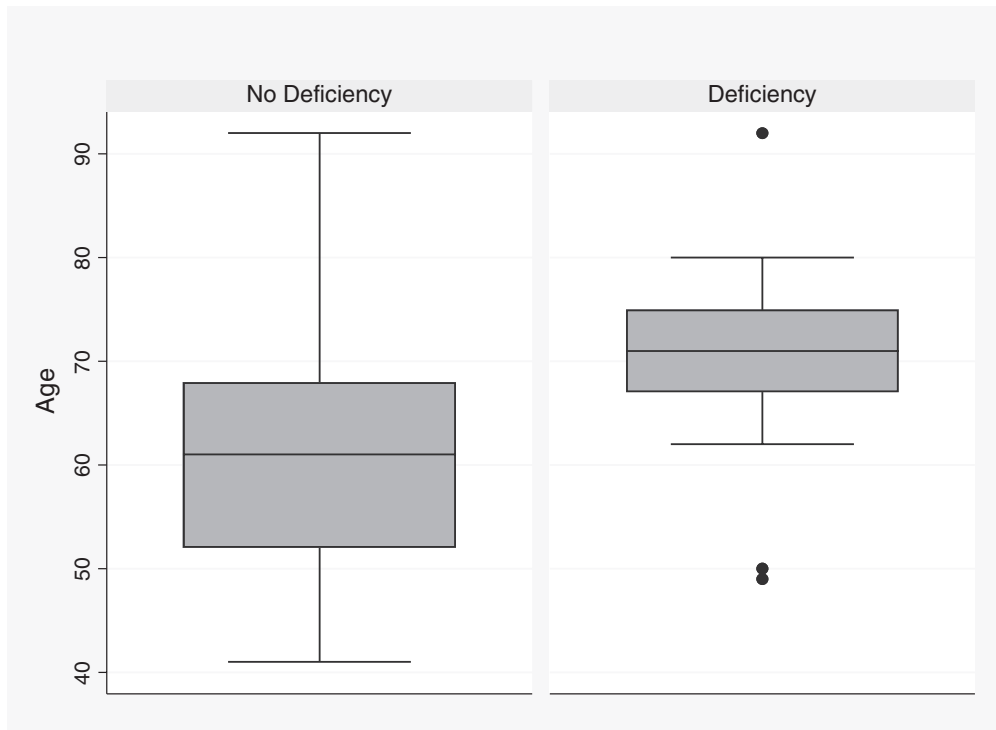
## DISCUSSION

In this retrospective study, we report a prevalence of B12 deficiency (predominantly mild) of 14.9% amongst patients with T2DM on metformin therapy. Notably, there was an association between B12 deficiency and being of a Coloured race and being older. Despite most participants in our cohort being of black African ethnicity, in a multivariate logistic regression analysis, patients of Coloured race showed a predilection for B12 deficiency. The reason

behind this association is presently unclear and not previously defined. A possible explanation could be that of ethnic-specific genetic differences. A review by S. Surendran and colleagues found that ethnic-specific genetic makeup and environmental factors may influence B12 levels.(37)

## DEMOGRAPHICS

Similar studies conducted in Pretoria (South Africa) and Botswana did not report comparable findings concerning

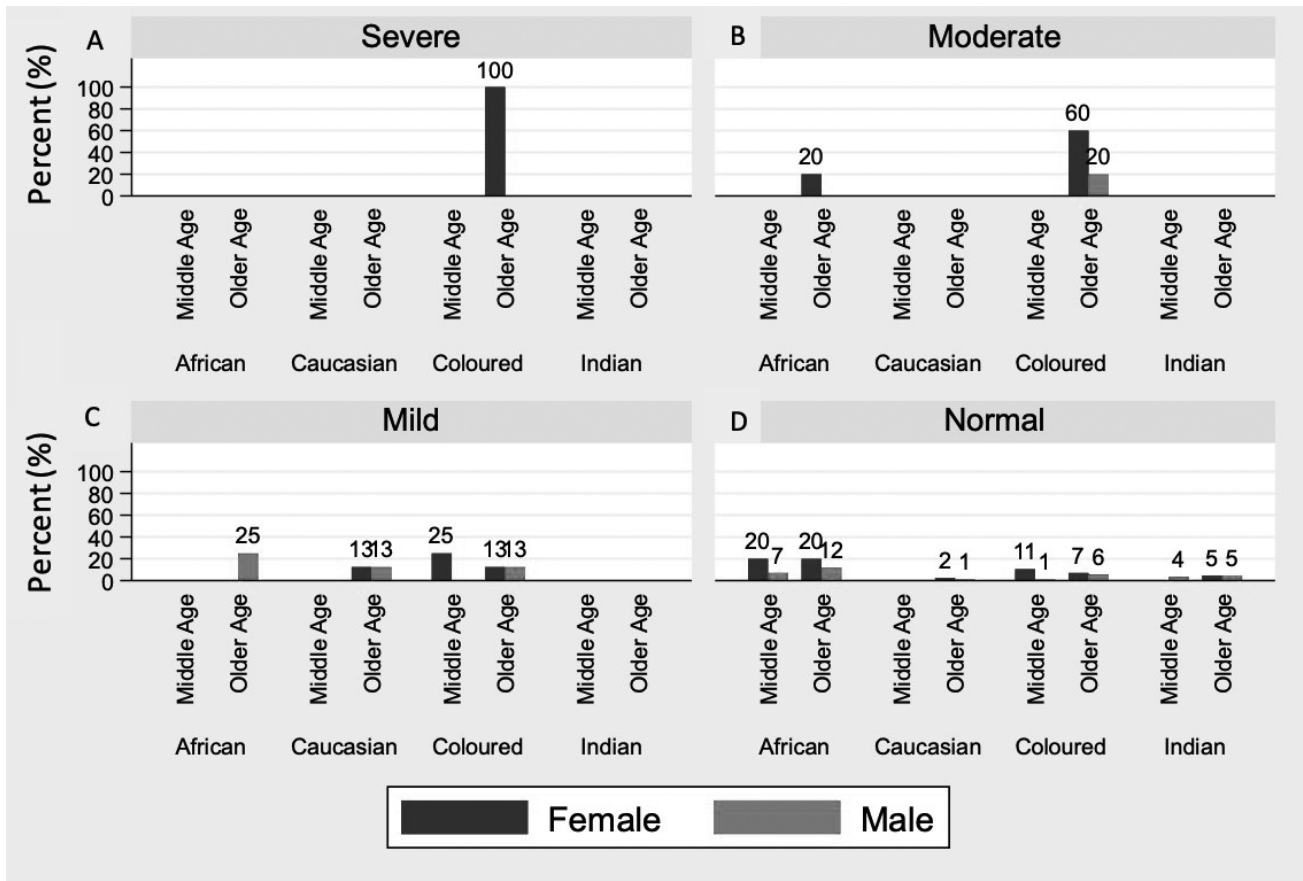


**Figure 1:** Box and whisker plot illustrating the age distribution between B12 deficient and non-deficient participants ( $p=0.006$ ).

**Table 2:** Characteristics of B12 severity

	Total N (%)	Normal (n = 86)	Severe (n = 2)	Moderate (n = 5)	Mild (n = 8)	p-value
<b>Gender n (%)</b>						0.592
Female	66 (65)	56 (65)	2 (100)	4 (80)	4 (50)	
Male	35 (35)	30 (35)	0	1 (20)	4 (50)	
<b>Race n (%)</b>						<b>0.012*</b>
African	54 (53)	51 (59)	0	1 (20)	2 (25)	
Caucasian	5 (5)	3 (3)	0	0	2 (25)	
Coloured	31 (31)	21 (25)	2 (100)	4 (80)	4 (50)	
Indian	11 (11)	11 (13)	0	0	0	
<b>eGFR n (%)</b>						0.688
20–29	5 (5)	4 (5)	0	0	1 (14)	
30–39	6 (6)	5 (6)	0	1 (20)	0	
40–49	9 (9)	8 (9)	0	0	1 (14)	
50–59	9 (9)	8 (9)	0	1 (20)	0	
60+	71 (71)	61 (71)	2 (100)	3 (60)	5 (72)	

Abbreviations: eGFR, Estimated glomerular filtration rate



**Figure 2:** Bar graphs illustrating the proportion (%) of B12 deficient and non-deficient patients categorised by age group and race. The red bars represent male participants, and the blue represents female participants. **Graph A:** Proportions of patients with severe B12 deficiency categorised by age group and race, all of whom were coloured, male and elderly. **Graph B:** The proportion of patients with moderate B12 deficiency categorised by age group and race. The majority of patients were coloured and elderly. **Graph C:** The proportion of patients with mild B12 deficiency categorised by age group and race. **Graph D:** Illustrates the proportion of patients by age category and race amongst patients without B12 deficiency. No significant differences were noted across all categories.

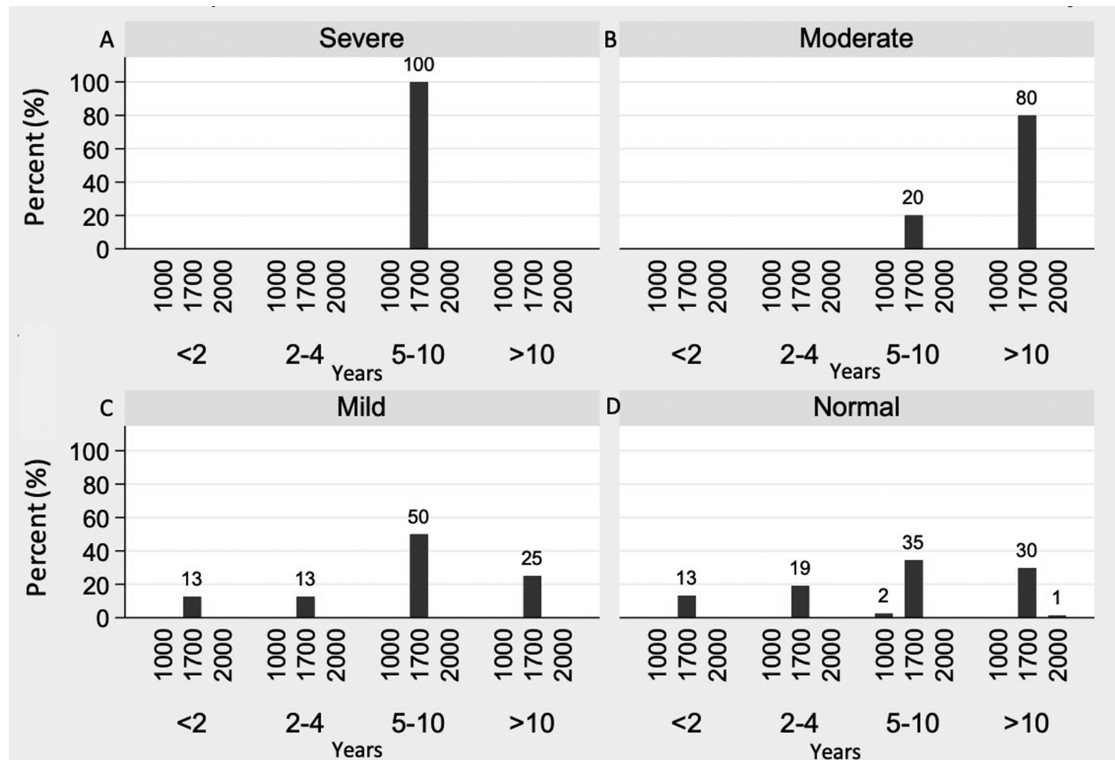
**Table 3:** Multivariate Logistic Regression Model of Variables associated with B12 deficiency

Variable	UOR	p-value	AOR	p-value
Middle Age	1 (base)	0.048	1 (base)	0.033*
Older Age	4.78 (1.01–22.5)		6.67 (1.16–38.3)	
HbA1c	0.71(0.5–1.01)	0.060	0.67 (0.43–1.05)	0.083
Race				
African	1(base)		1(base)	
Caucasian	11.8(1.39–100)	0.024	4.99(0.55–45.2)	0.152
Coloured	8.03(2.01–32)	0.003	7.8(1.78–34.2)	0.006*
Indian	1		1	

Abbreviations: AOR, Adjusted odds ratio; UOR, Unadjusted odds ratio; HbA1c, glycated haemoglobin

B12 deficiency prevalence by race.(20,25) This finding is likely attributable to a much smaller representation of the Coloured population in the Pretoria cohort (2.5%) and the absence of the Coloured community in the Botswana study.

(25) While Ahmed and colleagues found no interaction with the Coloured race, they demonstrated a significant association between black African descent and lower odds of vitamin B12 deficiency in metformin-treated patients.(20) The mean



**Figure 3:** Bar graph illustrating the proportion (%) of patients on various dosages (mg) of metformin and the duration (years) of metformin use. Graphs A, B, and C illustrate the aforementioned in patients with severe, moderate, and mild B12 deficiency. Graph D illustrates the proportion (%) of patients on various dosages (mg) of metformin and the duration (years) of metformin use in patients without B12 deficiency.

age in our study was  $61.5 \pm 12.1$  years of age, similar to both the Botswana and South African studies, where the mean ages were  $57 \pm 15$  and  $58.5 \pm 10.5$  years, respectively.(20,25)

In our study, B12-deficient patients were significantly older than those not B12-deficient. We noted the same trend in Ahmed and colleagues' report.(20) There is an established relationship between B12 deficiency and old age, which is well documented in the literature. The Framingham Study reported a B12 deficiency prevalence of 12% among the elderly.(32) The reasons for this are multifactorial; however, they are primarily attributable to a reduced capacity to absorb B12 commonly caused by atrophic gastritis.(33)

### Prevalence of B12 Deficiency

The prevalence of B12 deficiency amongst people with diabetes across Southern Africa varies, ranging from 6.6% to 28.1%.(20,25) This observation is similar to that observed in high-income countries and could be a function of many factors. The disparity (almost double) in the prevalence of B12 deficiency between our cohort and that of Ahmed et al. (28.1%) was an unexpected finding, given that both studies were conducted in tertiary hospitals in the same

province of Gauteng.(20) Ahmed et al. employed a lower cut-off value of 150 pg/ml vs 200 pg/ml used in our study to diagnose B12 deficiency, which could have contributed to the higher prevalence of B12 deficiency. In addition, Ahmed et al. reported a higher average metformin total daily dose of 2600 mg.(20) The current study found no relationship between B12 deficiency, metformin duration or dosage. Other studies, however, have demonstrated a dose-dependent relationship between metformin dose and B12 deficiency.(10,11,22,23) Total daily metformin doses greater than 2000 mg were predictors of B12 deficiency (AOR 21.67 (2.87–163.47),  $p < 0.01$ ). (11,23) In our study, the average daily metformin dose amongst B12 deficient patients was 1700 mg.

### Diagnostic Challenges

Other studies conducted in the Sub-Saharan African region also showed varying prevalence of B12 deficiency, with 6.6% in Botswana and 10.7% in Uganda.(24,25) The lack of a gold standard to diagnose B12 deficiency and the unavailability of a standardised cut-off level defining B12 deficiency may explain the varying prevalence of B12 deficiency globally.

## Social and Dietary factors

Given the disparity in B12 deficiency prevalence across studies conducted in Southern Africa, dietary confounders could help explain this disparity. In our study, patients who self-identified as vegetarian or vegan were excluded. In contrast, Ahmed et al. investigated dietary variables such as alcohol and coffee consumption but did not exclude vegan or vegetarian subjects.(20) Malnutrition and low consumption of micronutrients are not uncommon within poverty-stricken households. In South Africa, mielie meal is an affordable staple food, and incorporating animal products and other B12-enriched foods is contingent upon affordability.(36) Most participants in our study were from Johannesburg, the province's economic hub, and thus, access to better nutrition than those in rural areas.(36)

## ETHNIC DIFFERENCES AND GENETICS

The intricate relationship between genetic factors and B12 levels remains poorly described. Thus far, no direct causal genetic-B12 deficiency relationship has been demonstrated, only associations from genome-wide association studies (GWAS) in non-African populations.(37) Several polymorphisms, such as the rs28379 in the fucosyltransferase 2 (*FUT2*) gene, have been hypothesised to influence variable B12 status.(37) The role of such genetic interactions remains unexplored in Southern African populations. An underlying genetic predisposition could possibly explain our findings amongst the Coloured group. Genome-wide association studies in an Indian cohort revealed that carriers of the 'G' allele of the transcobalamin 1 (*TCN1*) gene have significantly lower B12 levels than those with the 'A' allele.(38) The same association was noted in Chinese, Icelandic, Italian and individuals residing in the United States.(39–41) Although several studies have explored the association of single nucleotide polymorphisms with B12 status, only a limited number of genetic loci have been reported to support the presence of ethnic differences in B12 status in Asian populations.(38,40)

## LIMITATIONS

The current study was a single-centre study at a tertiary hospital, and thus, results may not be generalisable to the broader South African population. We also note our small sample size and data imbalance between the two groups as significant limitations of our study. The absence of a detailed dietary history and concomitant use of proton pump inhibitors is an important limitation of the study.

## CONCLUSION

The current study demonstrated that B12 deficiency is prevalent among T2DM patients on metformin therapy. The odds of developing B12 deficiency in these patients were much higher in older patients and in Coloured patients, which is a new novel finding of this study. Whether the

latter group has a genetic predisposition to B12 deficiency whilst on metformin therapy is unknown and needs further investigation. Based on the study findings, we recommend screening for B12 deficiency amongst T2DM patients on metformin therapy.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows:

**Z.B:** Study conception and design; **B.K:** Data collection, analysis and interpretation of results, and draft manuscript preparation; **C.O:** Data collection; **Z.C:** Critical revision of the article; Authors **Z.B, B.K** and **Z.C** all contributed to the final revision and approval of the version to be published.

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