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Does Metformin in different doses cause vitamin B12 deficiency? A cross-sectional study

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ABSTRACT

The use of metformin in diabetic patients causes vitamin B12 deficiency, but there is not enough evidence about the existence of a correlation between different doses of metformin and vit B12 deficiency. Therefore, this study was conducted with the aim of investigating the correlation between different doses of metformin and vitamin B12 deficiency. This cross-sectional study was conducted on 200 patients with type 2 diabetes referred to the diabetes clinic of the central hospital of Sulaimani city in 2022. Demographic data were collected by a questionnaire and the serum level of Vit B12 data was by testing the blood samples. Data analysis was done using SPSS ver.23 and descriptive tests, chi-square, Pearson correlation and logistic regression. The results showed that 24% of patients had vitamin B12 deficiency. 45 (93.8%) patients with vitamin B12 deficiency have taken metformin. The mean vitamin B12, mean metformin consumption per year and metformin dose were significantly different between the two groups. Based on the regression model, it was shown that there was no significant relationship between the serum level of vitamin B12 and the duration of metformin medication (P=0.134). And the relationship between gender, occupation, alcohol and metformin dose (mg) was significant, so these factors have the ability to predict the serum level of vitamin B12. The results showed that vitamin B12 deficiency is common in diabetic patients who take metformin, and the vitamin deficiency will increase with the increase in the dosage.

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Introduction

Diabetes, a prolonged metabolic endocrine illness identified by unusually blood sugar levels, known as hyperglycemia, is one of the most prevalent and fastest-rising diseases globally. It is predicted that reach nearly 300 million in 2025, and 693 million in 2045, a >50% increase from 2017 (1–3).

One of the most prevalent types of diabetes and one of the most prevalent metabolic disorders in the world is type 2 diabetes (T2DM) or "adult diabetes," "obesity-related diabetes," and "non-insulin-dependent diabetes", which develops as a result of a combination of several key factors; resistance to insulin (a hormone regulate blood sugar levels), pancreatic beta cells performance decreased, and glucose production incretion by the liver because of diet and lifestyle that leads to abnormal metabolism of carbohydrate, lipid and protein (4-7). While, type 1 diabetes is an autoimmunity disease, caused by complete insufficiency in the secretion of insulin. A region in chromosome 16, which includes coding genes of 24 proteins, containing IL-27 (p28 subunit) has been identified as a powerful candidate for making type 1 diabetes (8,9). The most prevalent class of medications for people with type 2 diabetes are biguanides, such as metformin, which improve insulin sensitivity and aid in weight loss (10).

Metformin is the most popular oral insulin-sensitizing medication, being prescribed to more than 100 million inhabitants globally, including those with prediabetes, insulin resistance, and polycystic ovarian syndrome. This is due to its very well long-term safety and efficacy profile (11). In addition, metformin can enhance islet sensitivity and decrease the occurrence of cardiovascular problems (12). Chen et al. investigated the effect of using metformin with vitagliptin to treat type 2 diabetes patients which had no considerable negative reactions, and had the ability to control levels of blood glucose successfully, and help islet β-cell function recovery (13). In another study, the usefulness of Sitagliptin and metformin combination for type 2 diabetes treatment was observed. The composition improved islet function, the life quality of the patient and energy and financial costs decrease related to type 2 diabetes (14).

Chronic metformin usage has been linked to vitamin B12 insufficiency, which was initially identified by Berchtold et al, in 1969 (15), and has subsequently been supported by other research (16,17). According to reports from various nations, the frequency of vitamin B12 deficiency in T2D patients using metformin varies from 5.8 to 50% (18,19).

Cobalamin, generally known as vitamin B12, is a water-soluble vitamin essential for the healthy operation of the cardiovascular, nervous, and cognitive systems. Also, it contributes to the synthesis of DNA, the metabolism of fatty acids, and energy generation (20). Vitamin B12 insufficiency is clinically significant because it causes reversible bone marrow failure and nerve damage. Neurological damage caused by metformin-induced vitamin B12 deficiency might manifest as peripheral neuropathy and be misdiagnosed as diabetic neuropathy (21). Early identification and treatment are crucial for people with diabetes

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using metformin since vitamin B12 insufficiency and the consequences it causes are curable and perhaps reversible (17). Wenjia Yang et al (2019) concluded that when diabetic individuals use metformin, their levels of vitamin B12 were considerably decreased, increasing their chance of developing vitamin B12 deficiency. Also, analyses indicated no link between metformin usage and the incidence of anaemia or neuropathy (22). Also, Vanita R. Aroda et al. (2016) concluded that long-term use of metformin was associated with biochemical B12 deficiency and anemia (16). As mentioned, vitamin B12 deficiency can cause neurological and blood complications, and since many studies have not investigated these two complications simultaneously, and also the effect of metformin based on dose and duration can cause vitamin B12 deficiency and complications. So, it is necessary to conduct a study that, in addition to the above, examines other relevant factors such as age, sex, and race. Therefore, it is necessary to conduct a study titled Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes.

Materials and Methods

Study design and setting

This study was conducted in a cross-sectional study on 200 patients with type 2 diabetes referred to the diabetes clinic of the Central Hospital of Sulaimani, Kurdistan Region of Iraq, between January and June 2022.

Participants

200 outpatients referred to the diabetes clinic of the hospital for diabetes control who met the inclusion criteria were included in the study by the conventional method, after obtaining informed consent, and voluntarily after explaining the research process.

Inclusion criteria include definite type 2 diabetes, age 20 years and older, no history of digestive system diseases, no history of gastrectomy and colonostomy, no history of inflammatory bowel diseases, pernicious anemia, and no history of any type of cancer. Exclusion criteria included: the presence of acute cardiovascular diseases during the study, the presence of other acute diseases such as extensive infections, liver problems such as cirrhosis and acute kidney problems and kidney failure identified by laboratory tests.

Study method

After obtaining the necessary permits from the research departments and the consent of the research field, demographic information and records of drug use and diseases were collected through questionnaires, interviews and examination of patients.

In order to measure the level of vitamin B12, 5 ml of venous blood was taken from the patients after 12 hours of fasting. After blood clotting, the samples were placed in a centrifuge for 15 minutes at a speed of 1500 times per minute, and the serum was separated and frozen at -20°C until the time of measurement. Vitamin B12 was measured by ELISA using a UniCel DxI 800 device (Beckman Coulter, USA). In this study, biochemical deficiency of B12 was defined as a serum level <160 nmol/liter. While serum levels between 160-970 nmol/L were considered as normal serum levels. Based on this, the patients were divided into two groups: patients with normal serum levels of vitamin B12 and patients with deficiency of serum level of vitamin B12, and the clinical and demographic characteristics of the two groups were compared.

Data analysis

SPSS version 23 statistical software was used for data analysis. Descriptive statistical tests and mean calculations were used to analyze the demographic data. Chi-square statistical test was used to compare the intervening variables in two groups with normal and abnormal vitamin B12 serum levels. Pearson correlation test was used to check the relationship between metformin use and serum vitamin B12 level and logistic regression was also used for potential risk factors with serum vitamin B12 deficiency.

Results

In this study, the main outcome was vitamin B12 deficiency. In order to measure the level of vitamin B12, 5 ml of venous blood was taken from the patients after 12 hours of fasting. After blood clotting, the samples were placed in a centrifuge for 15 minutes at a speed of 1500 times per minute, and the serum was separated and frozen at -20°C until the time of measurement. Vitamin B12 was measured by ELISA using a UniCel DxI 800 device (Beckman Coulter, USA).

In this study, biochemical deficiency of B12 was defined as a serum level <160 nmol/liter. While serum levels between 160-970 nmol/L were considered as normal serum levels. Based on this, the patients were divided into two groups: patients with normal serum levels of vitamin B12 and patients with deficiency of serum level of vitamin B12, and the clinical and demographic characteristics of the two groups were compared.

Examination of the patients in the study who have diabetes showed that 48 (24%) patients have a vitamin B12 deficiency and 152 (76%) patients have normal levels of vitamin B12 (Figure 1).

Among patients with vitamin B12 deficiency, 22 (45.8%) patients were male, and among patients with normal vitamin B12 level, 44 (28.9%) patients were male. Most of the patients in both groups lived in the city and were married, and there was no significant difference between the two groups.

Examining the employment status, patients' smoking and alcohol consumption also showed that there was no significant difference between the two groups of patients

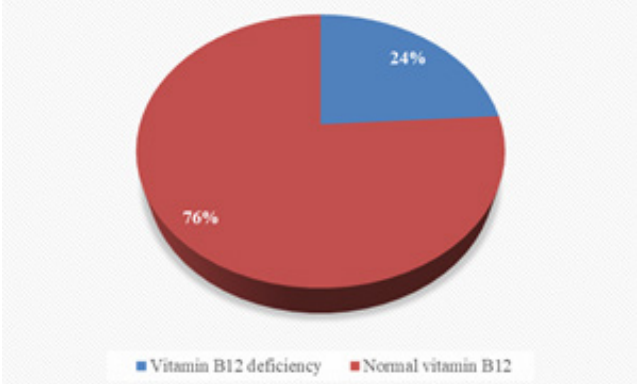

Figure 1. Prevalence of vitamin B12 deficiency among T2DM.

(patients with vitamin B12 deficiency and patients with normal vitamin B12 levels).

In addition, in terms of history of malnutrition, only one patient among those who had vitamin B12 deficiency had malnutrition.

The study of metformin drug use showed that among the patients who are facing vitamin B12 deficiency, 45 (93.8%) patients were taking metformin drug and 3 (6.2%) patients had no history of drug use. Among the patients with normal levels of vitamin B12, 77 (50.7%) patients took metformin and 75 (49.3%) patients did not take metformin, and two groups of patients had statistically significant differences in terms of metformin use ($P \leq 0.001$) (Table.1).

The mean age of patients with vitamin B12 deficiency was 58.5 (9.655) years, and in patients with normal vitamin B12 levels, the mean age of patients was 60.447 (10.367) years, and the two groups of patients had no significant difference in mean age.

The mean vitamin B12 among patients with vitamin deficiency was 129.937 (35.832) pg/mL and among patients with normal levels of vitamin B12, it was 339.3.88 (168.935) pg/mL, and these mean values were significantly different in the two groups. ($P \leq 0.001$).

The mean use of metformin in terms of years among diabetic patients with vitamin B12 deficiency was 7.333 (5.288) years and among patients with normal vitamin B12 levels, it was 3.019 (4.401) years, and this means the difference between the two groups was significant ($P \leq 0.001$).

There was a significant difference between the two groups of patients in the history of metformin use by year ($P \leq 0.001$).

The mean dose of metformin consumed among diabetic patients with vitamin B12 deficiency was 1.722 (0.625) mg and among patients with normal vitamin B12 levels, it was 0.724 (0.8111) mg, which had a significant difference ($P \leq 0.001$).

In the examination of metformin dose consumption in terms of units, it was also shown that there is a significant difference between the two groups in terms of metformin consumption ($P \leq 0.001$) (Table 2).

Based on the box plot, the results showed that the median dose was 0.5 more than the other doses. According to Pearson's correlation, the daily dose of metformin was

Table 1. Demographic characteristics of participants according to their vitamin B12 status.

Variables	Characterize	Vitamin B12 deficiency 48 (24%)	Normal vitamin B12 152 (76%)	P-value*
Gender	Male	22 (45.8%)	44 (28.9%)	0.035
	female	26 (540.2%)	108 (71.1%)	
Address	City	30 (62.5%)	110 (72.4%)	0.209
	Rural	18 (37.5)	42 (27.6%)	
Marital status	Married	38 (79.1%)	1.3 (67%)	0.196
	Single	1 (2.1%)	10 (6.6%)	
	Divorce	9 (18.8%)	39. (25.7%)	
Occupation	Yes	22 (39.2%)	40 (26.3%)	0.452
	No	26 (60.8%)	112 (73.7%)	
Smoking	Yes	7 (14.6%)	21 (13.8%)	.951
	No	41 (85.4%)	131 (86.2%)	
Alcohol	Yes	3 (6.2%)	1 (0.7%)	0.841
	No	45 (93.8%)	151 (99.3%)	
Metformin consumption	Yes	45 (93.8%)	77 (50.7%)	0.001
	No	3 (6.2%)	75 (49.3%)	

Table 2. Mean values of Age (yr.), BMI, Duration of DM (yr.), Vitamin B12 pg/mL, Duration of Met use (yr.) and Metformin Dose (mg) according to vitamin B12 status.

Variables	Vitamin B12 deficiency[&]	Normal vitamin B12[&]	P-value*
Age (yr.)	58.5 (9.655)	60.447 (10.367)	0.235
BMI	29.229 (3.302)	29.210 (3.44)	0.973
Duration of DM (yr.)	9.812 (9.621)	9.743 (7.166)	0.952
Vitamin B12, pg/mL	129.937 (35.832)	339.3.88 (168.935)	0.001
Duration of Met use (yr.)	7.333 (5.288)	3.019 (4.401)	0.001
< 10	27 (56.3%)	89 (58.6%)	0.001
10 - < 20	19 (36.5%)	52 (34.2%)	
20 ≤	2 (4.2%)	11 (7.2%)	
Metformin Dose (mg)	1.722 (0.625)	0.724 (0.8111)	0.001
≥1000 mg	9 (18.8%)	105 (69.1%)	0.001
1000–2000 mg	32 (66.7%)	46 (30.2%)	
2000 mg ≤	7 (14.6%)	1 (0.7%)	

*P-value- based on t-test and chi-square. [&]Mean (SD).

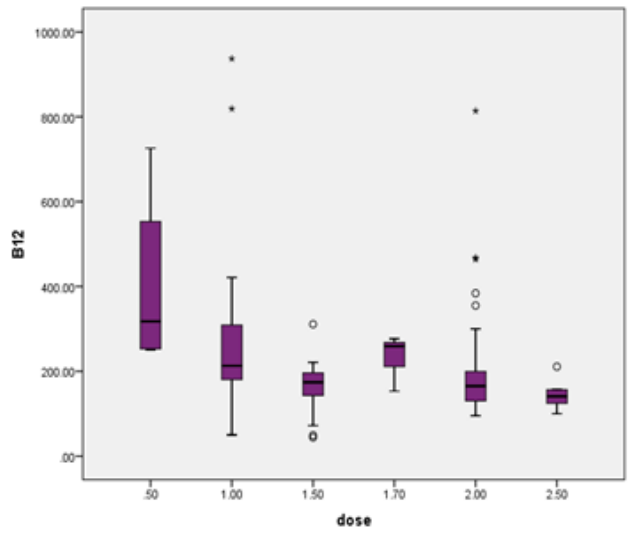

Figure 2. Box plot for serum vitamin B12 level according to different dose of metformin.

divided into 6 groups of 0.5, 1, 1.5, 1.7, 2 and 2.5, the serum level of vitamin B12 also decreased significantly with the increase of metformin dose (P < 0.001) (Figure 2).

After removing the confounding factors, based on the regression model, it was shown that there is no significant relationship between the serum level of vitamin B12 and the duration of metformin medication (P=0.134). Although the relationship between them was negative, it means that the serum level of vitamin B12 decreased with the increase in metformin use time.

However, in the examination of the serum level of vitamin B12 and the dose of metformin, it was shown that the relationship between the two variables was negative and significant (P=-0.133), so that with the increase in the dose of the drug, the serum level of the vitamin decreased more (P ≤ 0.001) (Table 3).

The effect of risk factors on the serum level of vitamin B12 was also investigated. After adjusting Age, Gender, Address, Marital status, Occupation, BMI, Smoking, Alcohol, Duration of DM (yr.), Duration of Metformin use (yr.) and Metformin Dose (mg), the relationship between Gender, Occupation, Alcohol and Metformin Dose (mg) were significant. In this way, these factors have the ability to predict the serum level of vitamin B12.

It was shown that the female gender is a risk factor in reducing the serum level of vitamin B12 in such a way that the risk of reducing the serum level of vitamin B12 in women is 2 times higher than in men OR = 2.077; 95% CI (1.066, 4.048); p = 0.032).

Also, the employment of patients in this study showed that it was a risk factor for reducing the serum level of vitamin B12, and employment increases the risk of reducing the serum level of vitamin B12 by 2% (OR = 0.241; 95% CI (0.065, 0.894); p = 0.041).

The results showed that alcohol consumption is related to the reduction of vitamin B12 serum level, so that alcohol consumption increases the risk of 10 times for the reduction of vitamin B12 serum level (OR = 10.67; 95% CI (1.022, 99.162); p = 0.048).

And by increasing the dose of metformin, the risk of decreasing the serum level of vitamin B12 will increase by 20% (OR = 1.192; 95% CI (0.109, 0.336); p = 0.001) (Table 4).

Table 3. Relationship between metformin use and serum vitamin B12 level.

	Metformin duration		Metformin dose	
	Beta (95% CI)	**P-value**	**Beta (95% CI)**	**P-value**
		vitamin B12		
Crude	0.020 (-1.034, 2.114)	0.415	-0.198 (-0.163,-0.105)	0.001>
Modeler 1	-0.634 -7.432, 5.367)	0.723	-0.142 (-0.182, -0.121)	0.001>
Model 2	4.223 (-2.301, 10.421)	0.134	-0.133 (-0.161, -0.111)	0.001>

Multivariable logistic regression analysis models were adjusted as follows: model 1: gender, age, diabetes duration; model 2: model 1 + duration of metformin, daily dose of metformin, smoking, Alcohol. CI=confidence interval.

Table.4. Logistic regression for potential risk factors with serum vitamin B12 deficiency among patients with T2DM.

Risk factor[&]	OR (95% CI)	P-value
Age	1.018 (0.987, 1.053)	0.25
Gender	2.077 (1.066, 4.048)	0.032
Address	0.636 (0.321, 1.261)	0.195
Marital status	1.311 (0.368, 1.979)	0.198
Occupation	0.241 (0.065, 0.894)	0.041
BMI	0.998 (0.907, 1.099)	0.974
Smoking	1.065 (0.423, 2.685)	0.894
Alcohol	10.67 (1.022, 99.162)	0.048
Duration of DM (yr.)	1.020 (0.602, 1.730)	0.940
Duration of Met use (yr.)	1.034 (0.965, 1.109)	0.341
Metformin Dose (mg)	1.192 (0.109, 0.336)	0.001

OR = odds ratio; CI = confidence interval. [&]the reference category is vitamin B12 deficiency. Occupation: Yes: Employee, Worker, Driver, teacher, police officer, farmer, Student, Cleaner, Peshmaraga; No: House wife, not work, Retired, Unemployed.

Discussion

Metformin is known as a safe drug in the front line of diabetes treatment. This drug is considered the most common oral drug in diabetes. There is evidence that the use of metformin is associated with a decrease in vitamin B12 levels, although there is much debate about the neuropathy side effects of the drug. In this study, it was shown that 93% of patients who took metformin had vitamin B12 deficiency, and there was a statistically significant difference between patients who had normal levels of vitamin B12 and patients who were deficient in metformin. It was shown that by increasing the dosage of the drug, the vitamin serum level decreased more.

In this study, it was shown that 24% of patients were deficient in vitamin B12, while in the study of A Khan et al. (2017) (23), it was shown that 29% of diabetic patients were deficient in vitamin B12 and this amount of deficiency was more It is from the results of our study. In the study R Jayashri et al. (2018) (24), vitamin deficiency was found to be 14.9% in the general population and 18.7% in diabetic patients, which is lower than the results obtained in our study and it is probably due to examining healthy and pre-diabetic people.

In the study, SB Almatrafi et al. (2022) (25) showed that 17.5% of patients taking metformin had vitamin B12 deficiency, while in the present study, it was shown that 93% of people taking metformin had vitamin B12 deficiency. In the study of Farooq et al. (2022) in India, it was shown that 64% of the investigated diabetic patients took metformin, which is contrary to the results of our study. It seems that this difference in the use of metformin drugs was caused by the larger sample size (26). In the study of M Shahwan et al. (2018) (27), similar to the results of our study, it was shown that most patients with vitamin B12 deficiency had taken metformin and two groups, i.e. patients who had normal levels of vitamin B12 and patients who had a vitamin deficiency, differed in the use of metformin also, vitamin B12 deficiency is more reported than in the present study.

The results of examining the mean age variable in patients showed that the mean age in patients with a normal range of vitamin B12 and patients with vitamin deficiency is higher compared to other studies. So that in the study of W Wale Tesega et al. (2021) (28). The mean age of the patients was 56 years, which is lower than the mean age of the present study. Also, the results of the mean BMI study showed that the mean obtained from our study is higher than the mean of W Wale Tesega's study.

The results of examining the gender variable of the patients showed that most of the studied patients were female, which is the same as the results of the study by Kwape et al. (2021) in Botswana, where most of the studied patients were female (29). While in other studies, unlike the present study, most of the examined patients were male (30,31).

The results of examining the variable duration of diabetes showed that the duration of the disease was almost the same in both groups and there was no difference in the duration of the disease. In the study conducted by J Kim et al. (2019) (32) no difference was seen in the duration of the disease among the studied subjects. While in the study A Badyal and S Kumar (2018) (33) there was a statistically significant difference in the duration of the disease.

There was a statistically significant difference between the duration of metformin and the dosage of the drug in patients with normal levels of vitamin B12 and patients with vitamin deficiency so both the duration of the drug and the dosage of the drug were higher in patients with vitamin deficiency. In this regard, in the study of M Yakubu et al. (2019) similar to the results of this study, there was a significant difference in the duration of metformin drug use and the amount of drug used (22). In the study of Mohammed et al. (2021) in Sudan, it was also shown that in patients with vitamin B12 deficiency, there is a significant difference in the duration of metformin and the amount of the drug used in patients with a normal range of vitamin B12 (34).

The results showed that there was no significant relationship between the serum level of vitamin B12 and the duration of taking metformin, while the serum level of vitamin B12 decreased more with the increase in the dose of metformin. A cross-sectional study of the Netherlands done by JWJ Beulens et al. (2015) (35) has shown that increasing the daily dose of the drug resulted in a significant decrease in the serum level of vitamin B12, while the serum level of the vitamin was not related to the duration of metformin drug use. In a retrospective study conducted by HX Meng et al. (2019), it was shown that the serum level of vitamin B12 decreased more with the increase in the dose of metformin, and also with the increase in the time of taking the drug, the level of vitamin B12 also decreased and these results were against the results of our study. In other studies, it has been shown that by increasing the dose of the drug, the vitamin serum level significantly decreases (36,37).

In this study, gender, occupation, alcohol consumption and drug dosage were introduced as determining factors and influencing variables on the serum level of vitamin B12. In line with these results in the study C Shivaprasad et al. (2020) (38), the amount of medicine used as an influencing factor on vitamin serum level is introduced, BMI, age, duration of diabetes and HbA1c hemoglobin level are known as influencing factors on vitamin serum level. While in the study of Kim et al. (2019) in Korea, in addition to the patient's age and vitamins, similar to our study, alcohol consumption and metformin drug dosage have been introduced as influencing factors on the serum level of vitamin B12 (32).

It seems that other factors such as genetic differences, nutrition, lack of correct use of metformin drug, as well as the difference in the drug composition of metformin can affect the serum levels of the studied vitamins. Along with the use of metformin, another effective factor in the reduction of vitamin serum levels in diabetic patients can be age, because, with increasing age, the occurrence of digestive disorders decreases the ability to absorb this vitamin (39). In our study, due to the similarity of the mean of the two age groups, it seems that the age of people did not play an important role in the effect of metformin on vitamin B12. In future studies, attention to the age factor can be paid more attention. The results of the present study showed that receiving metformin significantly decreased vitamin B12 levels in diabetic patients compared to healthy individuals. The correlation between the dose of metformin drug and the decrease in vitamin levels was shown, and there was no relationship between the duration of drug use and the decrease in vitamin levels. Therefore, the use

of vitamin supplements in parallel with the use of therapeutic methods can be effective in improving the health of diabetic patients. However, monitoring nutritional status and biochemical indicators, reducing oxidative stress and establishing a suitable nutritional pattern along with increasing physical activity are effective in improving the condition of patients and improving their health.

Limitation

One of the limitations of this study was its cross-sectional nature, and it is recommended to examine a larger volume of patients in future studies.

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Interest conflict

The author has no conflicts of interest to declare.

Author's contributions

The author has passed the criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

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None

Data Availability

The author guarantees that the data of this research will be provided at the request of other researchers.

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