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Original article

Evaluation of Vitamin B12 Deficiency among Patients administrating Metformin for Diabetes Mellitus Type 2

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Abstract

Metformin is a commonly prescribed oral anti-diabetic medications. It regulates blood glucose levels via lowering intestinal glucose absorption and enhancing muscle glucose uptake. Also, Metformin is used widely for weight loss among non-diabetics. However, the main disadvantage of Metformin is the associated vitamin B12 (vitB12) deficiency. The aim of this study was to identify and assess vitB12 insufficiency in patients with type 2 diabetes mellitus on metformin. A cross-sectional survey was applied to delve into the intricate interplay between metformin usage and vitB12 levels among diabetic (n=50) and non- diabetic (n=50) individuals. Blood analysis was also preformed to measure vitB12 serum levels. Unpaired t-test was used and $p<0.05$ was considered statistically significant. The data indicates that the majority of patients did not undergo vitB12 analysis (n= 71). On the other side only 29% of the patients underwent vitB12 analysis. 58% of the subjects are in 500-1000 mg dose of Metformin daily, while 34% of the subjects are taking 1500- 2000 mg of Metformin as a daily dose. In contrast, only 8% of subjects are taking a daily dose of 1000-1500 mg of metformin. In conclusion VitB12 deficiency was common in diabetics taking Metformin. To prevent the vitB12 deficiency associated with Metformin in diabetics patients vitB12 supplementation are recommended.

Keywords: *Diabetes mellitus, Metformin, vitamin B12, vitamin B12 deficiency, anemia.*

Introduction

Diabetes mellitus is a collection of metabolic diseases characterised by elevated blood glucose levels that are caused by either insufficient insulin secretion or an inappropriate cell response to insulin. Polyuria, polydipsia, and polyphagia are the hallmark signs of the resulting hyperglycemia. Blindness, limb loss, sexual dysfunction, nerve and renal issues, and an increased risk of heart attack or stroke are further possible consequences. The majority of patients with diabetes mellitus have type 2 diabetes mellitus (T2DM), which affects over 6% of the US population [1]. Around 120 million diabetic people are advised to use Metformin, one of the most widely used oral anti-diabetic medications [2].

A number of research were considered to determine the prevalence of T2DM in Middle Eastern men. T2DM prevalence among men was found to be low in Iran (9.90%) and Yemen (9.80%) in the Middle East, but it was high in Bahrain (33.60%), Saudi Arabia (29.10%), Kuwait (25.40%), and the United Arab Emirates (UAE; 25.83%) [3]. One medication that can reduce the chance of having high blood glucose is metformin. In order to enhance glycaemic control in persons with T2DM, metformin plus lifestyle modifications are recommended as the first line of treatment in all worldwide guidelines.It can be taken alone or in conjunction with insulin, thiazolidinedione (TZD), sodium-glucose cotransporter inhibitor (SGLT2), dipeptidyl peptidase-4 inhibitor (DP4-1), glucagon-like peptide-1 receptor agonist (GLPRA), and sulfonylurea (SU). As long as metformin medication is well tolerated and not contraindicated, it should be continued [7].

Metformin lowers blood glucose levels by reducing intestinal glucose absorption, promoting muscle glucose uptake, and decreasing liver glucose production [2]. It also triggers Adenosine Monophosphate-Activated Protein Kinase (AMPK), which suppresses glucose synthesis in primary hepatocytes through CRTC2 phosphorylation and its nuclear export by LKB1/AMPK signalling, which occurs during fasting to prevent excess gluconeogenic gene transcription [5]. Metformin indirectly affects AMPK activation by blocking mitochondrial respiratory chain complex 1, which results in lower ATP and higher AMP levels.

Metformin also improves lipid metabolism by lowering plasma triglycerides through selectively increasing the absorption of VLDL triglycerides, inhibiting lipogenesis, and oxidising fatty acids in brown adipose cells [6]. Since the great majority of worldwide guidelines advocate metformin use in prediabetes, it can be used to prevent or delay the onset of type 2 diabetes. The benefits of metformin are greater for people over 60 with a BMI of 35 kg/m or for women with polycystic ovarian syndrome. Furthermore, metformin is occasionally used to reduce the need for insulin doses in people with Type 1 diabetes mellitus (T1DM) [7]. It should be highlighted that patients with gestational diabetes mellitus (GDM) may benefit from taking metformin. However, because metformin crosses the placenta and may marginally raise the risk of preterm, the Americans Disabilities Act states that it should not be administered as a first-line medication during pregnancy [7].

In order to treat the metabolic irregularities associated with polycystic ovarian syndrome (PCOS), metformin has been used to lower insulin and testosterone levels in PCOS-afflicted women. It is important to note that population studies have demonstrated that using metformin (4-6%) increases life span in several mice breeds, with a mean life span increase of 14% and a maximum life span increase of 1 month when metformin medication is initiated early in life. Compared to other antidiabetic medications, metformin use reduced the risk of cardiovascular disease, cancer incidence, and total mortality in the United Kingdom Prospective Diabetes Study (UKPDS). According to epidemiological research, metformin has a beneficial effect on ovarian, breast, prostate, or colorectal tumours, which strengthens its anticancer effect. Furthermore, studies are demonstrating a reduced incidence of several gastroenterological cancers and a reduction in cancer mortality when using metformin [7].

Additionally, the results of meta-analyses of Recourse Centre Tiers, mostly in patients with schizoaffective disorder and schizophrenia, support the use of metformin to help people lose weight by reducing the weight gain that second-generation antipsychotics cause in adults [7].Cobalamin, another name for vitamin B12 (vitB12), is a water-soluble vitamin that is safe for human consumption. Humans must consume foods derived from animals in order to meet their vitB12 needs [10-12]. Most frequently found in supplements and some fortified foods, cyanocobalamin is the most widely used and reasonably priced form of vitB12. A highly accessible version of vitB12 called hydroxycobalamin is used to treat severe deficits.

VitB12 overdose is extremely unusual, and vitB12 supplement adverse effects are extremely uncommon; they may happen with injections of vitB12 but not with oral supplements. It is believed that vitB12 injections can prevent or reverse vitB12 deficiency by raising blood levels of the vitamin [13,14].

Notably, metformin frequently lowers vitB12 levels in patients, which can result in vitB12 insufficiency. Higher metformin dosages, longer treatment durations, and patients with risk factors for vitB12 insufficiency increase the likelihood of low vitB12 levels. Extended use of metformin is referred to as a pharmacological case of vitB12 deficiency, cobalamin deficiency), which became apparent within the first 10 to 12 years of starting the medication. It is also linked to a decrease in serum folate levels, which may cause megaloblastic anaemia, as evidenced by an increase in total serum homocysteine levels [15-16]. The study aims to identify and assess vitB12 insufficiency in patients with T2DM on metformin.

Methods

Study design and population

In this investigation, we employed a cross-sectional survey methodology to delve into the intricate interplay between metformin usage and vitB12 levels among individuals with and without diabetes. The research was conducted at Riyad Medical Lab during the months of July and August in the year 2023, demonstrating a commitment to the pursuit of scientific knowledge in a controlled and structured environment.

Our study consisted of precisely (n=100) participants, thoughtfully divided into distinct groups. The total in lab study (n= 50) the first group, consisting of (n=25) individuals diagnosed with type 2 diabetes mellitus, underwent medical assessments while simultaneously engaging in the completion of meticulously crafted structured questionnaires. In parallel, we recruited (n=25) healthy control subjects who, notably, did not partake in the questionnaire surveys but underwent medical testing.

The study population, designed to be a representative sample of a diverse demographic spectrum, encompassed individuals ranging in age from 30 to 75 years (52±13.7 years) and encompassed both genders. Mean age of patients (54±13.7 years), Mean age of control (49±13.5 years), ensuring that our research findings are reflective of a broad cross-section of the population.

The structured questionnaire employed in data collection was crafted to encompass various domains, including, but not limited to: demographic characteristics, smoking status, previous blood sugar levels, including random blood sugar (mg/dl) and Hba1c (%) values, determination of gestational diabetes among female participants, identification of any accompanying chronic diseases, comprehensive details regarding diabetes medication, encompassing type and dosage, documentation of vitb12 supplement intake, compilation of information concerning participants' vitb12 levels, assessment of symptoms related to vitb12 deficiency or anemia, and individuals with a history of other chronic diseases that could impact vitamin b12 metabolism and those under 30 years old were excluded from this study.

Table 1. Demographics

Gender		Patient	Control
Male	Count	12	13
	Percent	48.0%	52.0%
Female	Count	13	12
	Percent	52.0%	48.0%

Sample collection and analysis

Participant selection in this study was accomplished through a random sampling method, ensuring a representative sample. To collect the necessary biological material, approximately 5 mL of venous blood was carefully and hygienically obtained and placed in plain tubes designed to initiate the clotting

process (referred to as clot-activating tubes). These tubes were employed to enable the blood sample to naturally clot for an approximate duration of one hour, facilitating the separation of serum.
Subsequently, the obtained serum samples were subjected to analysis using Mindray CL 900 automated chemistry analyzer, a sophisticated piece of equipment produced by Mindray Medical International Co., Ltd., headquartered in China.
Before commencing the sample analysis, rigorous measures were taken to calibrate and validate the analyzer, following the precise instructions provided by the manufacturer.
The analysis itself was conducted using a competitive chemiluminescent immunoassay (CLIA) method, renowned for its exceptional sensitivity and specificity in accurately quantifying vitB12 levels. This technique ensures precise and reliable results in assessing vitB12 concentrations within the serum samples.

Statistical analysis
Statistical analysis was performed using SPSS version 21 program. Mean±S.D of data was calculated using descriptive analysis, Comparative analysis was done using independent sample t-test. The study involved the analysis of vitB12 values measured in milligrams per deciliter (mg/dl) in two groups of subjects: patients and controls. Various variables, including gender, age group, anemia status, neuropathy status, metformin duration, and metformin dose, were examined for their potential relationship with vitB12 levels. Descriptive statistics were used to summarize the VitB12 values in both patient and control groups. To assess the relationship between various categorical variables and vitB12 levels, chi-square tests were conducted. A p-value of less than 0.05 was Differences are considered statistically significant.

Results
In this study 100 individuals participated, of which 49% were male and 51% were female (Figure 1).

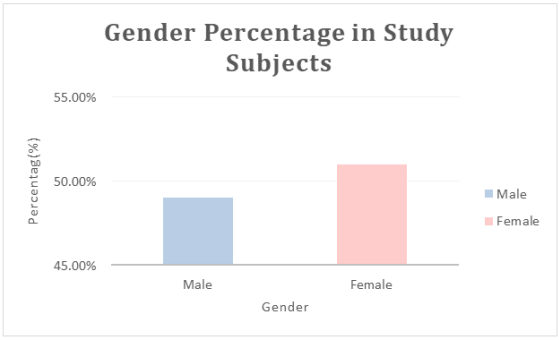

Figure 1. Distribution of male (n=49) and female (n=51) subjects

Neuropathy complication in male and female
The study evaluated to compare between male (44.26%) and female (55.73%) who suffer from neuropathy (Figure 2) shows characteristics of subjects

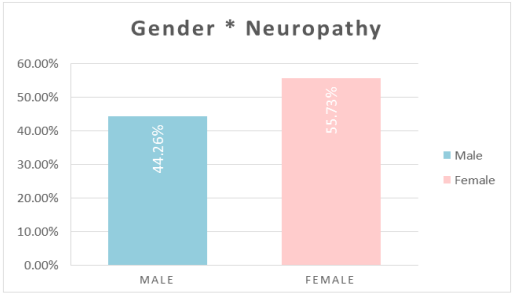

Figure 2. Neuropathy complication in male and female

Anemia complication in male and female
Also compare between male (48.78%) and female (51.21%) which suffering from anemia (Figure 3) shows characteristic subjects.

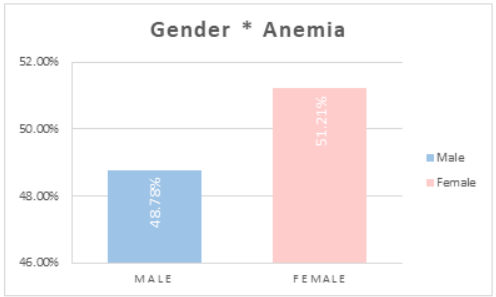

Figure 3. Anemia complication in male (n=20) and female (n=21).

Distribution of patient according to age group

Pateints where distributed according to age in to 6 groups with the youngest age group wsa < 40 years old, and the oldest age group was 70-79 years. The age group (50-59 y) had the highest count of patient the (n=31), while The age group(70-79y) had lowest patient count (n=9) (Table 2) . The age group (50-59 y) had the highest count of patients therefor (41.9%) suffering anemia and (61.2%) neuropathy (Figure 4&5) shows characteristic.

Table 2. Distribution of patient according age group.

Variable			**Total**
Age group	<40 years	Count	13
		Percent	13.00%
	40-49 years	Count	28
		Percent	28.00%
	50-59 years	Count	31
		Percent	31.00%
	60-69 years	Count	19
		Percent	19.00%
	70-79 years	Count	9
		Percent	9.00%
Total		Count	100
		Percent	100.00%

Table 3 shows the effect of adding Fenugreek on some blood characteristics, which included red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets. It was found that adding Fenugreek at all addition rates had a significant positive effect (P <0.01). As it increases at a proportional rate with increased rates of addition of Fenugreek (2g, 4g, and 8g), in general, the groups treated with Fenugreek powder showed a significant increase in all blood parameters studied.

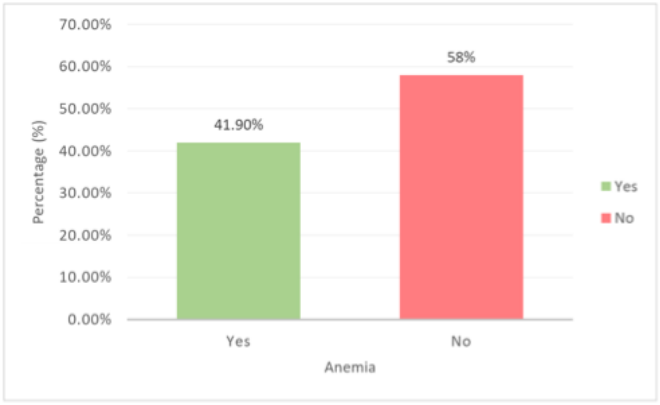

Figure 4. Percentage of subjects with anaemia in Age group 50-59 years.

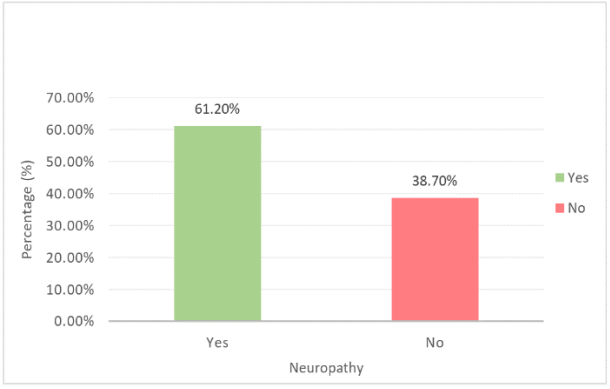

Figure 5. Percentage of subjects with neuropathy in age group 50-59 years.

The patients which advised by physician about important vitB12
The percentage of patients who were not advised much more than advised 88% and 12% respectively as seen in Table 3

Table 3. Percentage of patients which advised

Advised	No	Count	88
		Percent	88.0%
	Yes	Count	12
		Percent	12.0%
Total		Count	100
		Percent	100.0%

Patients which undergo of vitB12 analysis
The data indicates that the majority of patients did not undergo vitB12 analysis (n= 71). On the other side only 29% of the pateintes underwent vitB12 analysis (Table 4).

Table 4. Percentage and count of patients which underwent of vitB12 analysis

Analysis	No	Count	71
		Percent	71.0%
	Yes	Count	29
		Percent	29.0%
Total		Count	100
		Percent	100.0%

The patients which take vitB12 supplement
81% of the patients in this study do not take vitB12 supplement, while only the minority takes vitB12 supplement as shown in Table 5.

Table 5. Percentage of patients which take vit B12 supplement.

Variable			**Total**
Vit B12	No	Count	81
		Percent	81%
	Yes	Count	19
		Percent	19 %
Total		Count	100
		Percent	100 %

Percentage patients smoking and non-smoking
In this study the majority of the participants are non-smokers (73%), only 27%b of the participant are smokers (Figure 6). Figure 7 demonstrates that almost 52% of the smoking patients do not have anemia. On the other hand, 48% of smoking patients are suffering from anemia.

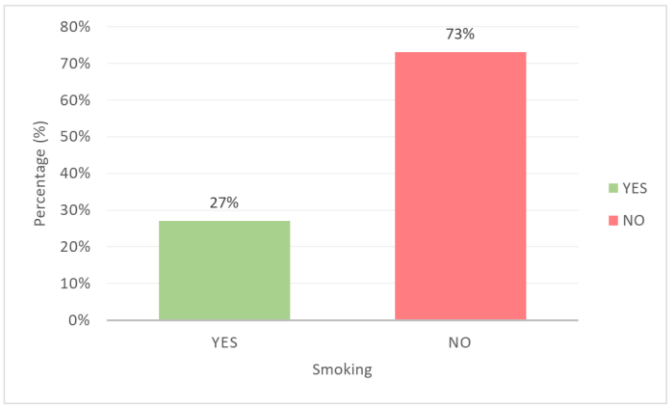

Figure 6. The percentage of patients smoking (n=27) and non-smoking (n=73).

Figure 7 demonstrates that almost 52% of the smoking patients do not have anemia. On the other hand, 48% of smoking patients are suffering from anemia.

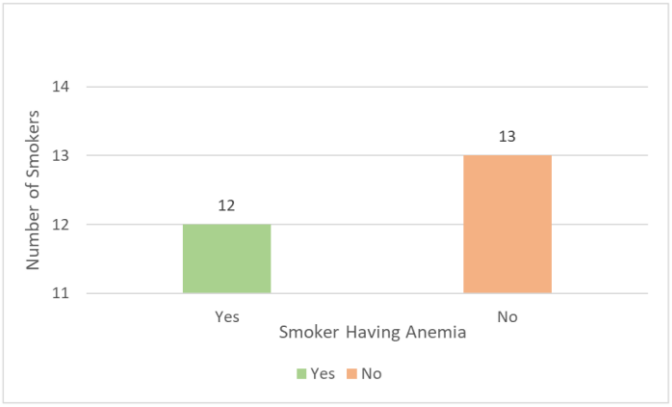

Figure 7. Number of Smokers with and without anemia.

Patients smoking suffering with neuropathy

The results demonstrated that 64% of the smoking patients are suffering from neuropathy (n=16), when approximately 36% of smoking patients do not suffer from neuropathy (Figure 8).

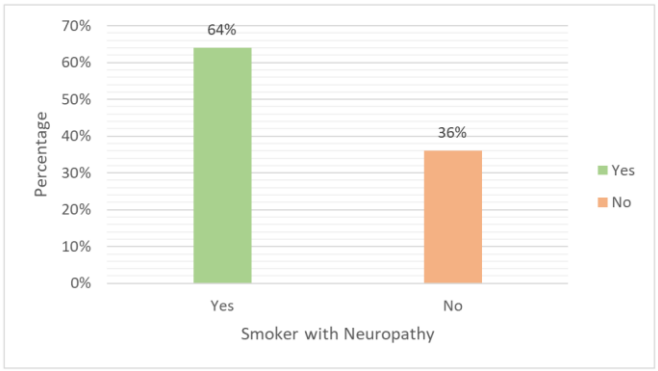

Figure 8. Percentage of smokers with Neuropathy and without Neuropathy.

Distribution patients according to dose of metformin (mg)

Figure 9 illiterates that 58% of the subjects are in 500-1000 mg dose of Metformin daily, while 34% of the subjects are taking 1500- 2000 mg of Metformin as a daily dose. In contrast, only 8% of subjects are taking a daily dose of 1000-1500 mg of metformin as shown in Figure 9.

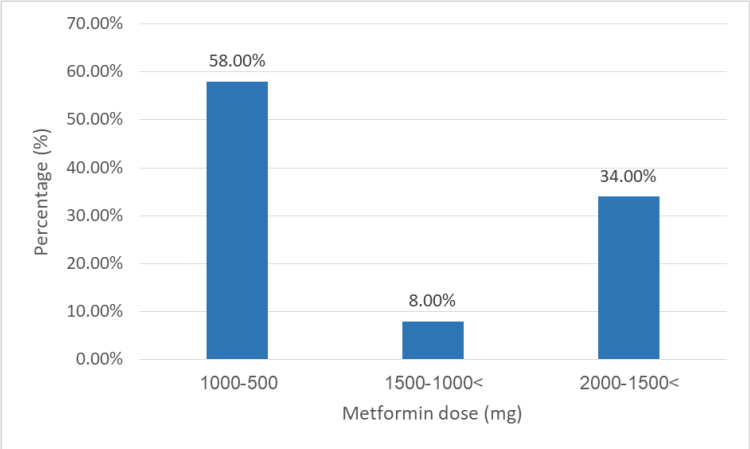

Figure 9. Metformin doses used by Subjects.

Discussion

Many patients with vitB12 deficiency experience burning, pain, and a feeling of needles in specific body parts, such as the hands and feet. The damage caused by vitB12 to the myelin sheath that surrounds and protects nerves begins six months after taking metformin. Regardless of the primary disease that was thought to be the cause of vitB12 deficiency and neuropathy (table 3), the majority of patients who suffer from neuropathy are female (55.73%) and more so than males.

This study found a correlation between serum vitamin B12 levels and anaemia caused by metformin use because vitamin B12 is a crucial micronutrient linked to DNA synthesis, cell division, and erythropoiesis. A vitamin B12 folic acid shortage can hinder DNA replication, associated cell division, and erythroblast s-phase advancement, but it has no effect on the rate of protein synthesis, including Hb. As a result, females with a vitamin B12 deficiency and anaemia (51.2%) produce more protein erythroblasts. (Table 4). Regardless of demographic characteristics, place of residence, age, type of diet, or physiological natural factors, females are more likely than males (48.78%) to develop B12 depletion and anaemia. The body can absorb vitB12 in combination with another protein produced by the stomach called intrinsic factor, but as people age, their stomach acid production changes, making it harder for them to absorb vitB12. The percentage of patients with anaemia in our study is approximately 41.9%, which is because there were only 31 percent of this group to investigate this issue. In population studies, vitB12 insufficiency is confirmed to increase with age and subsequently cause anaemia.

Neuropathies are quite common in people over 50, and their frequency rises with age because of vitB12 deficiency. In this patient with T2DM, the likelihood of neuropathy complications increases. The patients who were not informed by their doctors about the importance of vitB12 (about 88%) (table 6) may have been because they were not made aware of the problem in order to take into account their clinical condition. Additionally, the patients were not taught to analyses vitB12 (about 71%) (table 7), which means that their unknown metformin intake results in a vitB12 deficiency, and the doctors did not advise them to do so.

Additionally, take note of the 81% of patients who do not take vitB12 supplements (table 8). This is because the patients were not informed about this problem and the doctor did not prescribe them. Because tobacco smoking damages the stomach lining, making it more difficult for the body to absorb vitB12 from food, and because smokers are more likely to have GIT disorders like Crohn's disease and ulcerative colitis, which can also interfere with absorption, tobacco smoking has a negative impact on basic aspects of human health. In our study, 27% of smokers smoked (table 9), and among them, 48% suffered from anaemia (n=12; table 10). This is because the overall percentage of smokers is not very high, which may be due to the length of time they smoke and the severity of their nicotine addiction.

In our study, 64% of smokers experience neuropathy complications (table 11). Because smoking damages the body's neurological system, narrows blood vessels, and restricts blood flow, it also causes vitB12 insufficiency. Since the majority of patients (58%) take 500–1000 mg of metformin, as shown in Table 16, metformin is recommended with insulin for T2DM in addition to lifestyle modification, therefore a high dosage is not necessary.

VitB12 deficiency was considerably more common in diabetics, particularly those on metformin. This study can be repeated in the future with a larger sample size of patients who have varying metformin dosages and durations of treatment for prediabetes patients who take metformin only. Patients with T2DM who have other medical conditions and use various medications may also have an impact on the study. To avoid the development of vitB12 deficient consequences such macro-ovalocyte anaemia, peripheral neuropathy, sub-acute spinal cord degeneration, and poor immunity with hypersegmented neutrophils, we recommend vitB12 supplementation for this patient group.

In the current study found that the feed intake (FI) increases progressively with the addition of fenugreek in the diet. The highest FI is observed in the T3 group (4474.87 g), while the control group (T0) has the lowest (4342.17 g). This finding support that the intake of fenugreek powder in the diet may stimulate appetite or increase feed palatability, resulting in higher consumption. This was line with study done by Ahmad (2016) [11] supports these findings, stating that fenugreek seeds have bioactive components, which can enhance feed palatability and nutrient utilization, contributing to improved feed intake. There was no significant different between the different levels of adding Fenugreek seed

powder to the feed compared to the control group, this finding disagreement with study El-Rawi (2012) [5], reported that the addition of powdered fenugreek seeds had a significant effect ($p \leq 0.05$) on most of the productivity traits in the diet of rabbits in the third group (T3) this group, which was supplemented with 8g/kg of fenugreek seed powder in their feed, showed higher values in the measured traits compared to the control group (T1) that received a diet with 2g of Fenugreek seed powder/Kg of diet. In the present study (GW) also shows an increase with the addition of fenugreek. The T2 group (medium fenugreek level) exhibits the highest GW (1214.66 g), while the control group (T0) shows the lowest (1177.93 g) that agree with study by Ahmad et al. (2016) [11], another agreement with findings of Fadel Elseed 2013 [12] who demonstrated that fenugreek saponins enhance nitrogen retention in rabbits, leading to improved protein synthesis and tissue growth. Regarding to Hematological parameter in the present study found that differences ($P < 0.05$) in hemoglobin concentration (Hb), Red blood cells count (RBCs), (WBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets. These findings were agree with study done by Algridi, 2021[13] who reported that there was a significant increase in the levels of Hb, RBCs, and PCV after 7 days of fenugreek extract treatment. This finding consistent with study by Effraim, 2016 [14] who recorded significant findings of the hematological characteristic whose result indicated that group which received fenugreek, RBCs count remained elevated, due to the antioxidant activity of flavonoids. Another agreement with Rawi, (2012) [5] reported that adding Fenugreek seeds powder to fattening ration has significantly ($p \leq 0.05$) effect almost all production and blood traits. The total gain, total feed consumption, final weights, hot and cold carcass weights, dressing and abdominal fat percentages, red and white cell counts, hemoglobin, packet cell volume and cholesterol were increased significantly ($p \leq 0.05$).

In the current study the results showed that adding Fenugreek in all three levels of 2g, 4g, and 8g in rabbit feed has positive advantages on the weights of the internal organs of rabbits, as it has increased significantly and significantly ($P < 0.01$) compared with the control treatment without the addition of Fenugreek. The study indicated that the highest weights were with high levels of Fenugreek addition, 4 g, and 8 g, this line with study by Ahmad, (2016) [11] found the Dietary supplementations greatly influenced the percentages of carcass yield, spleen and gut weight in group case compared to control.

Conclusions

The present study concluded that the intake of fenugreek seed powder in rabbit feed has more benefits in terms of feed intake (FI), growth performance, hematological parameters, and internal organ weights. Specifically, the study found that feed intake increased progressively with the addition of fenugreek, supporting the hypothesis that fenugreek enhances appetite and feed palatability. Also, the supplementation of fenugreek had a positive effect on the weights of internal organs, further supporting its role in promoting better overall physiological conditions in rabbits.

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