Correlation between metformin use and vitamin B12 deficiency. A mini-narrative review;

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Abstract. Metformin is the most widely used oral hypoglycemic for the treatment of type 2 diabetes. However, one of the side effects of its use is vitamin B12 deficiency, which is persistent and progressive and can occur in around 5-30% of metformin users. The clinical manifestations of vitamin B12 deficiency are megaloblastic anemia (macro-ovalocytic), which affects the production and shape of red and white blood cells or manifests itself in the neurological system, through peripheral neuropathy, neurodegeneration of the spinal cord and cognitive deficits that can progress to dementia due to axonal demyelination. This study aimed to search the literature to correlate the use of metformin with vitamin B12 deficiency during the treatment of type 2 diabetes. This is a narrative review using the following databases: Google Scholar, Scielo, and Pubmed, and the descriptors: metformin, diabetes, vitamin B12 deficiency, and clinical symptoms. The term "and'' was used as a Boolean operator. According to the literature review, vitamin B12 deficiency during metformin use is associated with the fact that metformin alters the calcium channel mechanism, causing the receptor that recognizes vitamin B12 in the small intestine to be compromised, since the receptor is calcium - dependent.

Keywords. Metformin, Vitamin b12, Diabetes, Clinical symptoms.

1. Introduction

Metformin is a medication from the pharmacological class of biguanides, widely used as an oral antidiabetic in the treatment of Type 2 Diabetes Mellitus (T2DM). Metformin has gained attention as a first-line therapy for managing T2DM due to its efficacy, low toxicity, and low levels of adverse effects in patients taking this medication (1). In addition to its beneficial effects on plasma lipids, it helps prevent macro and microvascular complications and assists in weight reduction (2).

The main side effects of metformin are gastrointestinal, including symptoms such as nausea, vomiting, diarrhea, abdominal discomfort, bloating, and flatulence. In most cases, these symptoms are present in the initial phase of treatment and tend to diminish over the months. Although rare, metformin can lead to a serious condition called lactic acidosis, which occurs when there is an accumulation of lactic acid in the blood. Symptoms may include muscular weakness, difficulty breathing, abdominal pain, dizziness, and tachycardia. Another possible complication is the risk of hypoglycemia, as metformin can raise the risk when used in combination with other medications for T2DM such as insulin or sulfonylureas. Although the listed complications can occur during metformin use, this discussion will focus on vitamin B12 deficiency (cyanocobalamin) [3, 4].

Prolonged use of metformin can result in persistent and progressive vitamin B12 deficiency in approximately 5-30% of patients. The data is still limited to determine the specific prevalence. Vitamin B12 deficiency is determined when serum levels are below 180 pg/mL [5].

The mechanism of this deficiency is not yet fully understood, but it is believed that metformin alters the mechanism of calcium channels, affecting the receptor that recognizes vitamin B12 in the small intestine. This is because the receptor is calcium-dependent, and prolonged use of higher doses of metformin is associated with hypocalcemia. The difficulty of vitamin B12 absorption is closely related to calcium deficiency [6,7,8].

Further studies are needed for a more effective treatment of vitamin B12 deficiency to combat hypovitaminosis due to hypocalcemia. It is still unclear whether supplementation should be with vitamin B12 in conjunction with oral calcium

supplementation. Calcium supplementation suggests a negative reversal of the effect of metformin but is not always well-tolerated and can have negative effects such as constipation and arrhythmias. Since low calcium levels in the ileum projections are the compromising factor for B12 absorption, would parenteral supplementation be recommended? Is increasing calcium intake through diet sufficient? [9].

The objective of this narrative review was to describe the most recent publications in the literature regarding the correlation between metformin use and vitamin B12 deficiency, in patients with T2DM.

2. Research methods

The literature review was conducted through searches in electronic databases regarding publications of original articles written in English, Portuguese, and Spanish, between the years 2005 to 2023. The searches were carried out in the databases: Google Scholar, Scielo, and PubMed using the keywords metformin, vitamin B-12 deficiency, diabetes, and clinical symptoms, using the term 'and' as a boolean operator. In addition to the use of keywords, the selection of studies occurred according to inclusion criteria, which were: full original articles published between the years 2005 to 2023, with the presence of the mentioned keywords.

3. Results

Metformin is not metabolized by the human body, so it circulates freely in the blood. The highest concentrations of metformin can be found in the kidneys, liver, and salivary glands. It is excreted very quickly through the urine. The mechanisms of action of metformin are not yet fully understood, but in diabetic patients and mice, the intestine is the main site of action of metformin. Through 18F-FDG PET-CT scans, it was possible to observe an increase in glucose uptake in this region. Studies on mice and CaCo2 cells revealed that this increase was due to greater expression of GLUT1 and GLUT2, which are proteins responsible for glucose transportation in cells. This effect was mediated by two proteins, ATF4 and AMPK, which play important roles in cell metabolism [10-13].

In situations of hyperglycemia (increased blood glucose levels), metformin increases the production of lactate and acetate in the intestine. These substances affect the pH and bicarbonate levels in the portal vein, which ultimately impairs glucose production by the liver. This is because these substances modulate the activity of enzymes involved in glucose production, such as hepatic pyruvate carboxylase, MPC1/2, and FBP1. This interference ends up damaging the body's ability to produce glucose from other substances, a process known as neoglycogenesis [10].

On the other hand, in situations of normoglycemia (normal blood glucose levels), metformin causes an increase in glucose uptake by the intestine. This results in a decrease in glucose levels in the portal vein, which triggers a counter-regulatory response in the body. This response prevents excessive reductions or even an increase in glucose production by the liver. In other words, when glucose levels are normal, metformin acts to prevent glucose production from being undesirably affected. Thus, the intestine is the first site of action for metformin and new mechanisms of action may be related to communication between the intestine and the liver. Intestinal metabolites play an important role in controlling hepatic glucose production and, consequently, in regulating hyperglycemia in patients with type 2 diabetes. These findings are extremely important in the development of new therapeutic strategies for the treatment of T2DM. Understanding the mechanisms of action of metformin and identifying new therapeutic targets could contribute to better control of the disease and a better quality of life for patients [10].

The main side effects of metformin are related to the gastrointestinal system and can include symptoms such as nausea, vomiting, diarrhea, abdominal discomfort, flatulence, and a feeling of stuffiness. Generally, these symptoms manifest themselves mainly at the start of treatment and tend to diminish over the months. Although uncommon, metformin can result in a serious condition called lactic acidosis, which occurs when excessive lactic acid builds up in the bloodstream. Signs include muscle weakness, difficulty breathing, abdominal pain, dizziness, and increased heart rate. Another possible complication is the possibility of hypoglycemia. Despite the existence of these potential complications when using metformin, the focus of this study will be on vitamin B12 (cyanocobalamin) deficiency [3, 4].

The initial action of metformin occurs in the liver, and after entering the hepatocytes, it inhibits complex I of the mitochondrial respiratory chain by inhibiting the reduction of ubiquinone and independently stimulating the production of reactive oxygen species. Thus, reducing ATP synthesis and increasing the ratio of AMP/ATP (adenosine monophosphate/adenosine triphosphate) and ADP/ATP (adenosine diphosphate/adenosine triphosphate). This results in the activation of hepatic kinase B1, which will phosphorylate and activate AMPK, which will inhibit transcription factors, reducing the enzymatic activity associated with glucose synthesis (9-12). As for AMPK-independent mechanisms, the following stand out: decreased production of cAMP (cyclic adenosine monophosphate), inhibition of mitochondrial glycerol-3-phosphate dehydrogenase, redox-dependent mechanisms, and inhibition of

fructose-1,6-biphosphatase. In peripheral tissues, such as muscles and adipose tissue, metformin induces glucose entry into cells by increasing insulin sensitivity. This occurs due to the activation of the insulin receptor tyrosine kinase and also due to increased recruitment of GLUT4 transporters. This indirectly improves the Beta cell's response to glucose.
[11-13].

The exact mechanism by which metformin causes vitamin B12 deficiency is not yet fully understood, but several clinical studies have identified a significant association. Patients who take metformin for a long time may have low levels of vitamin B12 in their blood, which, if left undetected and untreated, can lead to complications [3,4].

B12 is a water-soluble vitamin (the only one that accumulates in the body), produced only by microorganisms. In the diet, it can be found in foods of animal origin such as fish, shellfish, oysters, liver, eggs, milk, and red meat. In foods of plant origin, the amount of vitamin B12 is so low that it cannot be considered significant for an adequate diet. This data draws attention to metformin users who eat a diet that excludes animal products [11].

Vitamin B12 is found in foods of animal origin, and most of it is bound to proteins. The digestion of B12 begins in the stomach, because the low pH of gastric juice facilitates proteolysis, and proteases and gastric secretions separate vitamin B12 from peptides [14].

In this way, vitamin B12 can bind to a binding protein (factor R) also known as haptocorrin, found in saliva and gastric juice. Pancreatic secretions degrade factor R so that vitamin B12 is then bound to the intrinsic factor and can make its way to the terminal ileum. The intrinsic factor (released by the parietal cells) binds to specific membrane receptors on the epithelial cells in the region of the terminal ileum. This facilitates the absorption of vitamin B12, which is then released into the plasma [15].

Vitamin B12 is absorbed in the last part of the small intestine, the ileum. The mechanism of this deficiency has not yet been fully elucidated, but it is believed to be because metformin alters the calcium channel mechanism, causing the receptor that recognizes vitamin B12 in the small intestine to be compromised, since the receptor is calcium-dependent and prolonged use of higher doses of metformin is associated with hypocalcemia. The difficulty in absorbing vitamin B12 is linked to calcium deficiency [6,7,8].

Vitamin B12 is absorbed by active mechanisms or by passive diffusion. Absorption is dependent on the uptake process, which occurs through specialized transport, as explained above. The active mechanism is responsible for the absorption of around 60% of vitamin B12. As for the passive transport absorption route, only 1-2% of the oral dose is absorbed. This route is independent of the intrinsic factor or the integrity of the ileum, but other factors such as hydrochloric acid and calcium are required [16].

There is a maximum amount of vitamin B12 that can be absorbed per meal. B12 is metabolized enterohepatic ally and it is estimated that between 0.5 and 5.0 μg of vitamin B12 is excreted through the bile per day. The vitamin that has been excreted through the bile is absorbed in the intestinal tract, continuing the cycle. The efficiency of biliary absorption is so great that a deficiency can take years to show up in tests or be symptomatic. If too much is ingested, the excess is excreted through the urine. The vitamin B12 cycle is "self-regulating" [14].

The transport of vitamin B12 is complex and depends on 30 steps. For this to occur, receptor and transporter proteins are needed. In the terminal ileum, vitamin B12 is absorbed by endocytosis linked to the intrinsic factor and reaches the enterocytes via the Cubam receptor complex, which is the union of 2 molecules (Cubilin and Amnion Less). After absorption in the ileum, the intrinsic factor is degraded by proteolysis, and vitamin B12 is released into the plasma via the cell membrane by an ATP Binding Cassette C1 (ABCC1) transporter, also known as MRP1 [15].

In plasma, vitamin B12 binds to three transcobalamin. The largest circulating percentage will be bound to transcobalamin I (TCN1). It is estimated that 10-30% of the vitamin will be transported by transcobalamin II and a small percentage will be transported by transcobalamin III. Transcobalamin II is the only transporter of B12 in its active form and is the only way for B12 to enter cells. For B12 to enter other tissues, it needs to be bound to the CD320 receptor, which will allow it to enter the cells. All of these transport routes depend on the release of B12 ingested through food, transport in the gastrointestinal tract, and absorption until it reaches the bloodstream so that it can then be transported in the circulation and then absorbed into the cells [15, 16].

Vitamin B12 is essential for two subtypes of reactions to take place. It helps in the conversion of propionyl coenzyme A into succinyl-CoA via the intermediate methylmalonyl-CoA, the other reaction is in the metabolism of nucleic acids, B12 is involved in the folate cycle and serves as an acceptor of the methyl group, which continues the synthesis of purines. In the case of vitamin B12 deficiency, these reactions are compromised, leading to an increase in plasma levels of homocysteine and methylmalonic acid [15].

After absorption, vitamin B12 is transported bound to serum proteins (globulins and transcobalamin). Transcobalamin is a plasma protein responsible for binding and transporting vitamin B12 in the plasma to the tissues, most of which is stored in the liver

and when necessary released into the bone marrow. Increased levels of transcobalamin can be found in myeloproliferative diseases, while low levels are seen in megaloblastic anemia and cases of deficiency of this plasma protein [15, 16].

Vitamin B12 is responsible for converting methylmalonic acid into succinyl-coenzyme A and converting homocysteine into methionine. Vitamin B12 deficiency can lead to an increase in methylmalonic acid and methionine. It is necessary to measure methylmalonic acid and homocysteine during treatment with metformin, as they can be indicators of B12 deficiency [17].

B12 hypovitaminosis has serious consequences for the body. It can result in neurological damage, such as cognitive and psychiatric disorders, impaired immunity, gait instability, autonomic dysfunction, and neuropathy, which can be confused with diabetic peripheral neuropathy and lead to a misdiagnosis [18].

Vitamin B-12 deficiency is clinically manifested through megaloblastic (macro-ovalocytes) anemia, affecting the production and shape of red and white blood cells, or in the neurological system, through peripheral neuropathy, neurodegeneration of the spinal cord and cognitive deficits that can progress to dementia due to axonal demyelination [15].

When vitamin B12 supplementation is recommended, oral administration can be chosen, with daily doses of 2,000 mcg for around four months, or intramuscular administration, with injections of 1,000 mcg three times a week for two weeks, followed by a monthly injection for another three months. Both are effective in replacing vitamin B12 deficiency [19].

A retrospective observational study carried out in 2018 showed that there is no significant difference in serum B12 levels when supplementation is used in cases of deficiency secondary to metformin use. Calcium supplementation is recommended for treatment, as it is at low levels, causing vitamin absorption deficiency. The treatment recommended by the Brazilian Diabetes Society is the replacement of 1.2 g of calcium per day. There is no screening protocol for vitamin B12 deficiency for patients taking metformin. However, laboratory monitoring of serum vitamin B12 levels is recommended, especially for vegans/vegetarians. The participants' serum homocysteine levels were significantly higher when taking a higher dose of metformin and for longer than groups without vitamin B12 deficiency [20, 21].

4. Conclusion:

In spite of the limitations of the present literature review, it was clear that there is a correlation between vitamin B12 deficiency and the use of the hypoglycemic drug metformin. It is believed that this is because metformin alters the calcium channel mechanism, causing the receptor that recognizes vitamin B12 in the small intestine to be compromised, since the receptor is calcium-dependent and prolonged use of higher doses of metformin is associated with hypocalcemia. However, more studies need to be carried out on the best therapeutic measure to be followed. Some studies suggest that vitamin B12 supplementation alone is not enough and that it should be associated with calcium supplementation, while others say that B12 supplementation alone minimizes low blood sugar.

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