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ABSTRACT

of the dissertation for the degree of philosophy doctor

VITAMIN B12 DEFICIENCY IN TYPE 2 DIABETES MELLITUS AND PREDIABETES

Specialty: 3216.01 – Endocrinology

Field of science: Medicine

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INTRODUCTION

Relevance and development of the topic. Vitamin B12 deficiency was first described in 1849, and was considered fatal until 1926[1], when George Richards Minot and William Parry Murphy[2] showed that the inclusion in the daily diet of patients half a pound of calf's liver, characterized by a high content of vitamin B12, significantly alleviates the course of the disease. This discovery was awarded the Nobel Prize in Physiology and Medicine in 1934[3].

In the United States, the prevalence of vitamin B12 deficiency ranges from 1.5% to 15%[4]. In general, epidemiologic data are inconsistent. In India, for example, various studies have reported vitamin B12 deficiency to be between 16% and 67%[5;6]. It was previously thought that vitamin B12 deficiency takes many years to develop and it happens only with a strict vegetarian diet or in people with pernicious anemia. The disease begins with subclinical vitamin B12 deficiency.

Some drugs affect the absorption and metabolism of vitamin B12. Of note are proton pump inhibitors, colchicine, nitrous oxide anesthesia, and drugs used in epilepsy[1].

According to the 10th edition of the International Diabetes Federation (IDF) Diabetes Atlas 2021, the number of people with diabetes mellitus (DM) in the world has increased from 151 million in 2000 to

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[1] O'Leary F., Sammman S. Vitamin B12 in Health and Disease. //Nutrients, 2010, v.2, p.299-316.

[2] Minot G.R., Murphy W.P. Treatment of pernicious anemia by a special diet. //JAMA., 1926, v.87, p.470-476.

[3] Nobelprize.org: The Official Website of the Nobel Prize. The Nobel Prize in Physiology or Medicine 1934. Nobel Media AB 2018

[4] The Guardian 2017 Carter A. Everything you need to know about vitamin B-12. Last updated Tue 28 November 2017.

[5] Yajnik C. et al. Vitamin B_{12} Deficiency and Hyperhomocysteinemia in Rural and Urban Indians. // J.A.P.I., 2006, v.54, p.775–782.

[6] Shobhaa V., et al. Vitamin B_{12} deficiency and levels of metabolites in an apparently normal urban south Indian elderly population. //Indian J. Med. Res., 2011, v.134, p.432–439.

537 million in 2023 and is expected to increase to 783 million in 2045[7]. Thus, currently 10.5% of the world's population aged 20-79 years has diabetes mellitus and this percentage will increase to 12.2% by 2045. The cost of diabetes in 2021 was 966 billion USD, in 2030 it is expected to increase to 1.028 billion and in 2045 to 1.054 billion[7]. Diabetes mellitus is also a serious medical and social problem in the Azerbaijan Republic[8;9]. According to the IDF, in Azerbaijan, there are 397.1 (95% CI 367.3-429.1) thousand patients with diabetes mellitus in 2021, which is 5.6 (95% CI 5.2-6.1) percent of the population aged 20-79 years. The cost per patient with diabetes mellitus is 482.4 USD. 7555 patients with diabetes mellitus died in 2021[7]. The high cost of DM, increased disability and mortality in this category of patients, is directly related to the presence of chronic complications of diabetes [7;9]. This includes diabetic neuropathy, a complication of diabetes that affects both the peripheral nervous system and autonomic innervation[10;11].

Diabetic neuropathy is widespread, with incidence rates ranging from 30% to 100%[12]. The presence of peripheral neuropathy not only can significantly worsen the quality of life of patients due to the development of pain syndrome, often resistant to therapy, but also represents one of the most important risk factors for the development of

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[7] International Diabetes Federation. IDF Diabetes Atlas 10th edn. Brussels, Belgium: International Diabetes Federation, 2021 p.37

[8] AEDTTA.Şəkərli diabetin diaqnostikası, profilaktikası və tibbi yardım üzrə standartları. /Bakı, 2017, "Azərdiab" nəşriyatı, 134 s.

[9] Saidova F.X., Mirzəzadə V.A. Endokrinologiya Giriş. / Bakı, «Təbib nəşriyyatı», 2016, 313 s.

[10] Juster-Switlyk K., Smith A.G. Updates in diabetic peripheral neuropathy**.** //F1000Research, 2016, v.5, p.738-745.

[11] Retinopathy, Neuropathy, and Foot Care: *Standards of Care in Diabetes—2023*. *Diabetes Care* 1 January 2023; 46 (Supplement_1): S203–S215.

[12]Kempler P., Varkonyi T. Neuropaties. A Global Clinical Guide/. Zafir Press, Budapest, Hungary, 2012, 406 p

diabetic foot syndrome and subsequent amputations[13]. Vitamin B12 deficiency may underlie the pathogenesis of the development of diabetic neuropathy[14]. B12 vitamin deficiency in type 2 diabetes mellitus (DM2) can be caused by treatment with the most widely used antihyperglycemic drug, metformin [15]. It has been shown that the use of metformin can also contribute to the occurrence (progression) of anemia[1].

Object and subject of the study: Object of the study were 206 people. Subjects were vitamin B12 levels.

The purpose of the study: to reveal the frequency rate of vitamin B12 deficiency in conditions of Azerbaijan in type 2 diabetes mellitus, prediabetes, as well as in the absence of carbohydrate metabolism disorders; to determine risk factors and protective factors in the development of vitamin B12 deficiency in patients with type 2 diabetes mellitus.

Objectives of the study:

1. To reveal the frequency of absolute and relative vitamin B12 deficiency in Azerbaijan conditions in case of type 2 diabetes mellitus, prediabetes and in the absence of carbohydrate metabolism disorders;
2. To study the significance of carbohydrate metabolism disorders and type 2 diabetes mellitus as risk factors for the development of vitamin B12 deficiency;
3. To determine the significance of sex, age, body weight, glucose control status, blood pressure, arterial hypertension as risk factors (protection from) for the development of vitamin B12 deficiency in patients with type 2 diabetes mellitus;
4. To study the influence of type 2 diabetes mellitus disease duration, glucose control status, and the effect of metformin

[13] Hicks CW, Selvin E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. Curr Diab Rep. 2019 Aug 27;19(10):86. doi: 10.1007/s11892-019-1212-8. PMID: 31456118; PMCID: PMC6755905.

[14] Pop-Busui, Rodica et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 1 January 2017; 40 (1): 136–154.

[15] Bell DSH. The Consequences of Lowering Vitamin B12 With Chronic Metformin Therapy. Endocr Pract. 2023 Nov;29(11):928-929. doi: 10.1016/j.eprac.2023.08.010. Epub 2023 Aug 23. PMID: 37625545.

treatment on the possibility of vitamin B12 deficiency in patients with type 2 diabetes mellitus;

5. If an effect of metformin on the possibility of developing vitamin B12 deficiency is identified, determine the factors contributing to this effect in patients with type 2 diabetes mellitus.

The methods of the study:

Analysis of nutrition, carbohydrate metabolism (normal, prediabetes, diabetes), height, body weight, body mass index, treatment received (presence and absence of sugar lowering therapy), including metformin with registration of its daily dose), laboratory study of glycemia level, glycohemoglobin, vitamin B12, functional state of liver and kidneys

Main points of the disseratation submitted for defence:

1. Type 2 diabetes mellitus is characterized by minimal mean vitamin B12 levels (compared with normal carbohydrate metabolism and prediabetes) and a maximum incidence of absolute and relative vitamin B12 deficiency.
2. However, the odds ratio study did not reveal the role of impaired carbohydrate metabolism and DM as a risk factor for the development of vitamin B12 deficiency.
3. No effect of gender, obesity, and arterial hypertension on the development of vitamin B12 deficiency has been found, while age over 65 years may be considered as a risk factor and age under 65 years as a protective factor against the development of this condition.
4. Taking metformin, as such, the duration of taking the drug for 5 years or more, and a daily dose of 2000 mg or more are risk factors for the development of vitamin B12 deficiency.

Scientific innovations of the study results:

- For the first time in the conditions of Azerbaijan the frequency of absolute and relative deficiency of vitamin B12 in practically healthy people, prediabetics and DM2 patients has been shown;
- The publication of data on the incidence of vitamin B12 deficiency in individuals with prediabetes was the 4th paper on this topic in the world literature;

- Statistically significant risk factors for vitamin B12 deficiency in DM2 patients have been identified;
- Statistically significant factors of protection against vitamin B12 deficiency in DM2 patients have been revealed;
- The significance of the fact of metformin intake, dose and duration of the drug use for the development of vitamin B12 deficiency has been established.

Practical significance of the study results:

- It has been shown that absolute and relative vitamin B12 deficiency can occur both in the absence of carbohydrate metabolism disorders and, to a greater extent, in the presence of prediabetes and DM2;
- Risk factors for the development of vitamin B12 deficiency in DM2 patients have been identified;
- Factors contributing to protection against development of vitamin B12 deficiency in DM2 patients identified.;

Approbation of the study: The main points of the study were reported at the V International Bariatric-Metabolic Surgical Congress (Baku, 2019), at the IV World Congress on Clinical Research in Diabetes (Amsterdam, 2019), at the II Azerbaijan Diabetes Congress (Baku, 2020), at the Scientific and Practical Conference dedicated to A.M. Aliyev (Baku, 2021).

Primary discussion of the work was conducted at the joint interdepartmental meeting (staff of the departments of "Therapy", "Family Medicine", "Cardiology" and "Central Scientific Research Laboratory") on 13.02.2024 at the Azerbaijan State Advanced Training Institute for Doctors named after A. Aliyev (protocol # 4)

Approbation of dissertation work was carried out at the approbation seminar at the Azerbaijan State Institute for Advanced Training of Doctors named after A. Aliyev on 17.04.2024 (protocol # 01).

Publications: 9 scientific papers, including 5 articles (2 abroad) and 4 abstracts were published on the subject of the study.

Implementation of the study results: The results of the study have been implemented in the work of the Therapy Department of the Azerbaijan State Institute for Advanced Training of Doctors named after A. Aliyev, the Department of Diabetes Mellitus of the

Educational-Therapeutic Clinic of the Azerbaijan Medical University, as well as in the work of the Republican Endocrinology Center, and the clinic of Azer-Turk.

The name of organization, where the dissertation work was held. Disseration work was carried out at the Azerbaijan State Advanced Training Institute for Doctors named after A.Aliyev of the Ministry of Health of the Azerbaijan Republic and on the basis of the clinic "VM Center of Endocrinology, Diabetes and Metabolism".

Structure and scope of the dissertation. The dissertation includes an Introduction-9314 symbols, Chapter 1 – "Literature review" 36378 symbols, Chapter 2 – "Materials and methods" 19237 symbols, Chapter 3 – "Results and their discussion" 85636 symbols, "Conclusion"-19610 symbols, "Findings"-1607 symbols, "Practical recommendations"-948 symbols, list of references. The work is illustrated with 24 figures and 65 tables. Bibliographic index includes 315 sources, including 12 works in Azerbaijani, 27 works in Russian and 276 works in English. Total numbers of symbols- 172730

MATERIALS AND METHODS OF THE STUDY

One-stage, single-center study.

Study location: VM Center of Endocrinology, Diabetes and Metabolism, Baku, Republic of Azerbaijan**.**

The time period of the study: was from February 01, 2012 to January 31, 2014.

The primary criteria for inclusion in the study were:

-Age over 35 years;

- availability of data allowing differentiation by groups according to the state of carbohydrate metabolism:

- group of persons without carbohydrate metabolism disorders (CMD);

- group of persons with prediabetes;

- group of persons with DM2.

For this purpose, A1c and fasting glucose values were checked. It was envisaged that, based on the history, A1c and office fasting venous blood glucose determination, the patients could be divided into 3 groups:

1. Patients with DM2:
   - DM2 history or
   - A1c ≥ 6,5% and fasting glucose ≥ 126 mg/dl
2. Persons without carbohydrate metabolism disorders:
   - No history of DM2 and
   - A1c ≤ 5,6% and fasting glucose < 100 mg/dl
3. Persons with prediabetes:
   - No history of DM2 and
   - A1c from 5,6% to 6,4% and/or fasting glucose from 100 mg/dl to 125 mg/dl.

- Availability of biochemical level tests vitamin B12 in the blood;
  - Creatinine;
  - Indicators of liver function:
  - Alaninaminotransferase - ALT;
  - Aspartataminotransferase - AST;
- availability of information on current (and past) treatment, including sugar-lowering therapy, including treatment with metformin (recording drug dose and duration of use).

The criteria for exclusion from the study were:

- Subject's non-consent to participate in the study;
- Pregnancy;
- Vegetarianism;
- Body mass index less than 18.5%;
- Consumption of grapefruit juice;
- Treatment currently or within the past 6 months with medications that affect vitamin B12 levels: Omeprazole and other proton pump inhibitors; H2 - histamine receptor blockers; colchicine; rifampicin, neomycin; barbiturates; carbamazepine or phenytoin; cimetidine; ritonavir; ketonazole; contraceptives; alpha-glucosidase inhibitors; calcium preparations; multivitamin preparations and/or vitamin B12 preparations;
- Bariatric surgery history and other gastrointestinal surgery;
- Illnesses: Presence of acute illness within 3 months preceding the study period; Chronic alcoholism with alcohol consumption of more than 21 units/week for men and more than 14

units/week for women; Crohn's disease and other chronic inflammatory bowel diseases; Presence of chronic or acute pathology on the part of kidneys with decreased CKD-EPI less than 60 ml/min/1.73m2); Tuberculosis; benign and/or malignant neoplasms on chemotherapy;

- HIV/AIDS; Presence of marked pathology on the part of internal organs, including marked disorders of the functional state of the liver (with an increase in ALT and AST over 80 "U/L"); Presence of diseases other than diabetes mellitus with endocrine system dysfunction;
- Duration of type 2 diabetes mellitus less than 1 year;
- No current treatment with metformin, but discontinuation of treatment with this drug less than 1 year before the start of the study.

Individuals who participated in the study underwent the following examinations:

1. Passport part was registered.
2. Dietary pattern was determined (presence or absence of vegetarianism).
3. Information on the treatment currently received was recorded:
   a. sugar-lowering therapy (no, yes, what kind (indicating the group of drugs used and a special note on the use of metformin and its daily dosage);
   b. Treatment for arterial hypertension (no, yes,);
   c. Pharmacotherapy that may affect vitamin B12 levels.

We determined height, body weight and calculated the body mass index (BMI) using the formula: BMI = body weight / height^2, where body weight was expressed in kilograms and height in meters. BMI values were estimated in accordance with the recommendations of the World Health Organization (WHO)[16];

4. In accordance with generally accepted rules, office measure

[16] World Health Organization. A healthy lifestyle - WHO recommendations. World Health Organization Europe. https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations

ment of blood pressure was performed, and values of SBP and DBP were recorded and expressed in mmHg. The diagnosis of arterial hypertension was made on the basis of anamnesis data and modern classification of arterial hypertension[17];

5. Laboratory examination of patients was carried out.

The tests were taken after overnight (8-12 hours of fasting). Smoking within 30 minutes before the analysis was excluded. Studies were performed on venous blood.

Vitamin B12 levels were determined on the analyzer "AxSYM System" (Abbott, USA) using appropriate reagents (closed system). According to literature recommendations, vitamin B12 levels>221 pmol/L were considered normal, vitamin B12 levels from 148 pmol/L to 221 pmol/L were considered borderline (or mild deficiency), and levels of 148 pmol/L or less were considered as severe vitamin B12 deficiency.

Fasting glycemia levels were determined using a Precision PCx Medi Sense glycemia laboratory device (Abbot, USA) and appropriate test strips.

The diagnosis of diabetes was confirmed if the anamnesis contained reliable information about previously diagnosed diabetes (and/or its treatment). The differential diagnosis of DM for selection of patients with DM2 was performed according to the recommendations of the Azerbaijan Association of Endocrinology, Diabetology and Therapeutic Education[18], American Diabetes Association[19] and WHO recommendations[20], which are fully consistent with each other. According to the standards of the Azerbaijan Association of

[17] Williams B.et al. 2018 ESC/ESH. European Heart Journal, 2018, v.39, Iss. 33, p. 3021–3104.

[18] AEDTTA / Şəkərli diabetin diaqnostikası, profilaktikası və tibbi yardım üzrə standartları./Bakı, 2017, "Azərdiab" nəşriyatı, 135 s

[19] ElSayed, Nuha A., et al. "Classification and Diagnosis of Diabetes: Standards of Care in Diabetes—2023." Diabetes Care, vol. 46, no. Supplement_1, 2023, pp. S19–S40. https://doi.org/10.2337/dc23-S002

[20] World Health Organization. "Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation." Accessed 19 November 2023, https://www.who.int/publications/i/item/definition-and-diagnosis-of-diabetes-mellitus-and-intermediate-hyperglycaemia

Endocrinology, Diabetology and Therapeutic Education, American Diabetes Association standards, prediabetes was identified when the above "diabetic" parameters were not achieved, but the "normal" parameters were exceeded. ALT (in "U/L"), AST (in "U/L"), creatinine (in "mg/dL") were determined on an automatic analyzer Cobas Mira (Roche Diagnostics Corporation, Switzerland) using reagents from Human Diagnostics Worldwide (Germany). The CKD-EPI equations were used to calculate the CKD using the online calculator called "calculator for calculating the glomerular filtration rate". During statistical analysis of the material, we determined the minimum, maximum and mean values of the sample, standard deviation and error of the mean value. In the text of the work the average value of the index is given together with the standard deviation (M±SD). Statistical analysis was carried out using a standard computer program Microsoft Excel. Using the Internet calculator "Confidence Limits for Mean Calculator" we determined on line confidence interval of mean values (CI) for a probability level of 95%, understanding the confidence interval as the range around the value of the value in which the true value of this value is located (with a certain level of confidence). The χ^2 method and the Fisher's exact test were used to calculate the significance of differences between fractions. Calculations using these methods were performed on line using the "MEDCALC" calculator.

The methodology of determining odds and odds ratio was applied. In this case, risk was understood as the probability of occurrence of a certain outcome. Chance was considered as the ratio of the probability that the event will occur to the probability that the event will not occur. The odds value was interpreted as follows:

- If Chance=1, the probability of an event occurring is equal to the probability that the event will not occur;
- If Chance >1, then the probability of the event occurring is greater than the probability that the event will not occur;
- If Odds <1, the probability of an event occurring is less than the probability that the event will not occur.

The odds ratio was understood as the ratio of the odds for the first group of objects to the odds ratio for the second group of objects. If the lower boundary of the 95% confidence interval in determining the

odds ratio is less than 1 and the upper boundary is greater than 1, it is concluded that there is no statistical significance of the factor's influence on the frequency of the outcome, regardless of the OR value ($p>0.05$).

The study analyzed the data of 206 patients, from which 3 main groups were formed:

- DM2 group (n = 123);
- Pre-diabetes group (n = 24);
- Group without carbohydrate metabolism disorders (CMD) or control group (n = 59).

The study also formed a group of individuals with CMD, which combined the examined DM2 and prediabetes groups (n = 147) and, respectively, the group without CMD (n = 59). In total, there were 109 women and 97 men among those examined (n = 206). There were 63 women and 60 men in the DM2 group. There were 13 women and 11 men in the prediabetes group. The control group included 33 women and 26 men. Analysis of gender differences within each of the 3 groups under consideration, performed on line on the basis of the χ^2 method using "Comparison of proportions calculator (MEDCALC) easy-to-use statistical software "45 showed that in all groups the differences can be considered non-significant (in all cases $p>0.05$).

The groups did not differ statistically significantly in age, height. Body mass index in the DM2 group (30.4 ± 4.01 kg/m^2) was statistically significantly higher ($p <0.001$) than in the control group (27.9 ± 2.77 kg/m^2). Body mass index in the prediabetes group (28.8 ± 3.39 kg/m^2) was not statistically significantly different from the other groups.

The mean systolic blood pressure (SBP) in the DM2 group was 137.1 ± 19.79 mmHg. It was equal to 130.2 ± 15.32 mm.Hg in the prediabetes group and 128.2 ± 15.73 mm.Hg in the control group. Only the differences between the DM2 group and the control group were statistically significant ($p <0.001$).

The mean diastolic blood pressure (DBP) in the DM2 group was 84.2 ± 13.60 mm.Hg. It was equal to 80.1 ± 13.05 mm.Hg in the prediabetes group and 78.5 ± 11.56 mm.Hg in the control group. Only the

differences between the DM2 group and the control group were statistically significant ($p < 0.01$).

Arterial hypertension occurred in 58.5% of subjects in the DM2 group, 50.0% of subjects in the prediabetes group, and 40.7% of subjects in the control group. Differences in incidence determined by the χ^2 method were statistically significant only when comparing the DM2 group and the control group ($p < 0.05$).

The mean A1c level was 8.7± 2.05% in subjects in the DM2 group, 6.0 ± 0.32% in subjects in the prediabetes group, and 4.8 ± 0.52% in subjects in the control group. Differences between all groups were statistically highly significant (<0.001). Mean glycemic levels were 8.8 ± 2.31 mmol/L in subjects in the DM2 group, 6.1 ± 0.40 mmol/L in subjects in the prediabetes group, and 4.7 ± 0.33 mmol/L in subjects in the control group.

Differences between all groups were statistically highly significant (< 0.001). The liver functional status by mean AST level was 30.2 ± 9.27 units/L in the subjects of DM2 group, 30.5 ± 5.54 units/L in the subjects of prediabetes group and 429.7 ± 8.95 units/L in the subjects of control group. Differences between groups in all cases were not statistically significant ($p > 0.05$).

The mean ALT level was 31.8 ± 10.98 units/L in subjects in the DM2 group, 33.8 ± 10.05 units/L in subjects in the prediabetes group, and 31.0 ± 10.64 units/L in subjects in the control group. The differences between the groups were not statistically significant in all cases ($p > 0.05$).

Indicators of renal functional status- the mean creatinine level was 0.74 ± 0.135 mg/dL in subjects in the DM2 group, 0.72 ± 0.156 mg/dL in subjects in the prediabetes group and 0.72 ± 0.125 mg/dL in subjects in the control group. Differences between groups in all cases were not statistically significant ($p>0.05$). The mean SCF was 92.3±15.70 ml/min/1.73 m2 in subjects in the DM2 group, 95.7 ± 13.75 ml/min/1.73 m2 in subjects in the prediabetes group and 94.6 ± 12.15 ml/min/1.73 m2 in subjects in the control group.

Differences between groups in all cases were not statistically significant ($p>0.05$).

At the first stage of the study the following results were obtained to determine the frequency of absolute and relative vitamin B12 insufficiency in Azerbaijan in diabetes mellitus type 2, prediabetes and in the absence of carbohydrate metabolism disorders:

In the control group, the minimum level of vitamin B12 was 76 pmol/L and the maximum level was 588 pmol/L. The mean level of vitamin B12 in the subjects of this group was 401.6 ± 138.06 pmol/l.

In the prediabetes group, the minimum level of vitamin B12 was 81 pmol/L and the maximum level was 600 pmol/L. The mean vitamin B12 level in the control group subjects was 364.7 ±149.68 pmol/L.

In the DM2 group, the minimum level of vitamin B12 was 82 pmol/L and the maximum level was 585 pmol/L. The mean level of vitamin B12 in the examined control group was 337.7±129.84 pmol/l.

The mean values of vitamin B12 in the control group were statistically significantly ($p < 0.01$) higher than in the DM2 group. Differences between the control group and the PD group were not statistically significant ($p > 0.05$), nor were differences between the PD group and the SD2 group ($p > 0.05$). In the control group (n=59), absolute deficiency of vitamin B12 occurred in 5.1% of cases, and relative deficiency in 8.5%, i.e., a total of 13.6%. The data obtained are close to the results of studies conducted in the USA, where the prevalence of vitamin B12 deficiency ranges from between 1.5% to 15%46 and is significantly lower than the data for Latin America (40%), Asia and Africa (up to 80%).

In the prediabetes group (n=24), absolute vitamin B12 deficiency occurred in 4.2% of cases, relative deficiency - in 12.5%, and total deficiency - in 16.7% of subjects. Only few studies of vitamin B12 in prediabetes have been conducted worldwide. A study by Aroda V.R. et al, performed as part of the Diabetes Prevention Program Outcomes Study and published in 2016, investigated the effect of metformin on vitamin B12 levels in prediabetes. According to the researchers, at the first year after the start of the study, those not taking metformin had an absolute vitamin B12 deficiency of 2.3%, and those taking metformin had an absolute deficiency of 4.3%, which was almost the same as in our study. At the 9th year of the study in the group with

metformin the absolute vitamin B12 deficiency was 7.4%, and in the group without metformin - 5.4%. Total vitamin B12 insufficiency (absolute deficiency + relative deficiency) at year 1 was 9.5% without metformin and 19.1% with metformin. At year 9, it was 20.3% in the metformin group and 15.6% in the group without metformin. The result we obtained, 16.7%, is extremely close. According to Jayashri R. et al. absolute insulin deficiency occurred in 15% of people with prediabetes - more often than in normal glucose tolerance and less often than in DM2. The results of these studies are close to those we obtained in Azerbaijan.

In the group of DM2 (n=123) absolute vitamin B12 deficiency occurred in 6.5% of cases, relative deficiency - in 16.3%, and total deficiency - in 22.8% of the examined patients. The incidence of vitamin B12 deficiency in DM2 usually ranges from 2.4% to 33%, sometimes as high as 100% in Nigeria.

The next objective of the study was to investigate the significance of carbohydrate metabolism disorders and DM2 as risk factors for the development of vitamin B12 deficiency.

The mean vitamin B12 level in the control group, i.e., in people without CMD (n = 59) was 401.6 ± and 138.06 pmol/L, and in the CMD group (n = 147), which included the pre-diabetes (n = 24) and DM2 (n = 123) groups, it was 342.1 ± 133.10 pmol/L. The differences between the groups were statistically significant ($p < 0.01$), suggesting the importance of CMD as a risk factor for vitamin B12 deficiency.

Due to the fact that the target sample may not reflect the true state of the population of patients suffering from the disease under study and healthy people, the odds ratio for the considered factors was calculated:

- the factor for the presence of CMD (risk factor)
- the factor of absence CMD (protection factor).

CMDs were present in 147 cases and absent in 59 cases. Vitamin B12 insufficiency was present in 40 cases and absent in 166 cases. Vitamin B12 insufficiency was combined with the presence of CMD in 32 (21.8%) cases. In 115 cases (78.2%) the presence of CMD was combined with the absence of vitamin B12 insufficiency. In 8 cases (13.5%) there were no CMDs but vitamin B12 insufficiency was

present. In 51 cases (86.4%), both CMD and vitamin B12 insufficiency were absent.

The odds of finding vitamin B12 deficiency in the presence of CMD was 0.278 and the odds of finding vitamin B12 deficiency in the absence of CMD was 0.157. The odds ratio (OR) was 1.774 with a standard error of the odds ratio (S) of 0.430. That is, the chance of finding vitamin B12 insufficiency in the presence of CMD was 1.774 times higher than the chance of finding it in people without CMD. The lower bound of the 95% confidence interval (CI) corresponded to 0.764, and the upper bound of the 95% confidence interval (CI) corresponded to 4.117. Thus, the odds ratio was not statistically significant ($p>0.05$).

The findings indicate that despite the presence of a statistically significant decrease in vitamin B12 levels in the CMD group (342.1 ± 133.10 pmol/L vs 401.6 ± and 138.06 pmol/L; $p<0.01$), despite the greater incidence of vitamin B12 deficiency in the CMD group (21.8% and 13.5%, respectively), the results of the study do not allow the presence of CMD to be considered a risk factor for vitamin B12 deficiency. The mean vitamin B12 level in the DM2 group (n = 123) was 337.7 ± and 129.84 pmol/L, while in the group without DM2 (n = 83), composed of subjects without NUO (n = 59) and subjects with prediabetes (n = 24) it was 390.9 ± 141.60 pmol/L. The differences between the DM2 and "without DM2" groups were statistically significant ($p < 0.01$), suggesting the importance of DM2 as a risk factor for vitamin B12 deficiency. The odds ratio (Odds ratio), that is, the ratio of the probability of finding vitamin B12 deficiency with DM2 to the probability of finding it in the absence of DM2, was 1.744 with a lower bound of the confidence interval of 0.830 and an upper bound of 3.666, that is, it was not statistically significant ($p > 0.05$).

Thus, despite the fact that our own data and numerous data from the literature indicate a lower level of vitamin B12 in DM2 and a higher incidence of vitamin B12 deficiency in this disease, the statistical analysis performed does not allow us to consider the presence of DM2 or the presence of CMD as a risk factor for the development of vitamin B12 deficiency.

The task of the next stage of the study was to determine the significance of sex, age, obesity, arterial hypertension as risk factors (protection from) the development of vitamin B12 insufficiency in the absence of IUO, in prediabetes and in patients with DM2.

In all three groups (control group, prediabetes group and DM2 group), there was no effect of sex, obesity and arterial hypertension as risk factors for (or protection against) the development of vitamin B12 deficiency (in all cases the risk ratio was not statistically significant; $p>0.05$).

In the control group, mean vitamin B12 values were 446.4 ± 95.24 pmol/L at age <65 years and 361.2 ± 158.54 pmol/L at age ≥65 years. The differences between the groups were statistically significant ($p<0.05$), which gave some grounds to suggest the role of age 65 years and older as a risk factor for vitamin B12 deficiency in individuals without CMD. However, the trend for age to influence the development of vitamin B12 deficiency in the absence of NUO was not statistically confirmed ($p>0.05$), possibly due to insufficient group size (n=59), as in prediabetes, where the sample size was even smaller (n=24).

However, Aroda V.R. et al. in their very representative Diabetes Prevention Program study also found no effect of age on vitamin B12 deficiency in prediabetes.

In the DM2 group, mean vitamin B12 values were 366.7 ± 124.26 pmol/L at age <65 years and 306.3 ± 129.46 pmol/L at age ≥65 years. Differences between groups were statistically significant ($p <0.01$).

The odds of finding vitamin B12 insufficiency in subjects aged ≥65 years was 0.143. In the same group, the chance of finding vitamin B12 insufficiency in subjects aged 65 years and older was 0.513. The Odds ratio, i.e. the odds ratio of finding vitamin B12 deficiency in those under 65 years of age compared with those aged 65 years and older, was 0.279, with a standard error of the odds ratio of 0.467, a lower bound of the 95% confidence interval (CI) of 0.111, and an upper bound of the 95% confidence interval (CI) of 0.696. Since both the lower bound of 95% confidence interval was less than 1 (0.111) and

the upper bound of 95% confidence interval was less than 1.0 (0.696) the result obtained can be considered statistically significant ($p < 0.05$).

The odds of detecting vitamin B12 deficiency in those aged 65 years and older were 3.590 times higher than the odds of detecting vitamin B12 deficiency in those under 65 years of age. Given that the odds ratio was statistically significant ($p < 0.05$), the factor "age 65 years and older" should be considered a risk factor for vitamin B12 insufficiency in DM2.

In principle, it can be considered that, although the results of scientific studies are not completely unambiguous, the opinion about the increase of vitamin B12 deficiency in older age groups both outside of DM and in DM is held by the absolute majority of researchers.

The objectives of the study were also to investigate the influence of type 2 diabetes mellitus disease duration, glucose control status, and the effect of metformin treatment on the possibility of vitamin B12 deficiency development in patients with type 2 diabetes mellitus; If the effect of metformin on the possibility of vitamin B12 deficiency development is revealed, determine the factors contributing to such an effect in patients with type 2 diabetes mellitus and in prediabetics.

In patients with DM2, there was no effect of the duration of DM2 disease and DM2 control status (as measured by A1c) on the risk of developing vitamin B12 deficiency. In both cases, the odds ratio was not statistically significant ($p > 0.05$).

Metformin is one of the main, most widely used sugar-lowering drugs used in DM2 is metformin59.

Of the 123 DM2 patients studied, 90 (73.2%) were treated with metformin, and 33 (26.8%) were not treated with this sugar-lowering drug. Vitamin B12 deficiency was detected in 25 DM2 patients or 27.8% of those treated with metformin. 65 patients out of 90 metformin-treated patients (72.2%) had no vitamin B12 insufficiency. In 3 patients out of 33 patients not treated with metformin (9,1%) there was vitamin B12 insufficiency. 30 out of 33 DM2 patients (90.9%) not treated with metformin had vitamin B12 insufficiency. When statistical analysis of differences in the incidence of vitamin B12 insufficiency in metformin treatment using "Fisher's exact method" revealed statistical significance of differences ($p < 0.01$) the chance of finding

vitamin B12 insufficiency in the DM2 group under metformin treatment was 0.385. In the same group, the chance of finding vitamin B12 deficiency in the absence of metformin therapy was lower at 0.100. Odds ratio (Odds ratio), i.e., the ratio of the probability of finding vitamin B12 deficiency in metformin treatment of DM2 to the probability of finding it in the absence of metformin among the sugar-lowering drugs taken by the patient, was equal to 3.846 with the standard error of the odds ratio (S) equal to 0.650, as well as the lower limit of the 95% confidence interval (CI) of 1.007 and the upper limit of the 95% confidence interval (CI) of 13.741.

That is, the chance of vitamin B12 insufficiency was almost four times (3.846 times) higher in metformin treatment of DM2 than in the absence of such treatment. The odds ratio was statistically significant ($p < 0.05$), i.e., according to the results of our study we obtained data that metformin treatment is a risk factor for the development of vitamin B12 deficiency in DM2 patients.

The obtained data are quite consistent with the results of numerous studies that revealed the role of metformin in the development of vitamin B12 deficiency.

At the next stage of study we analyzed the results of examination of 90 DM2 patients treated with metformin.

There were 44 women (48.9%) and 46 men (51.1%) in the group.

In 66 patients (73.3%) the serum vitamin B12 content was normal (> 221 pmol/l). Twenty-four (26.7%) had vitamin B12 deficiency with its content of 221 pmol/l or less, with absolute vitamin B12 deficiency (< 149 pmol/l) occurring in 7 cases (7.8%), and relative deficiency, also called "borderline state" and characterized by serum B12 levels from 149 pmol/l to 221 pmol/l was present in 17 DM2 patients (18.9%).

The subjects were aged between 42 and 76 years. The mean age was 62.1 ± 9.14 years, the patients' height ranged from 155 cm to 178 cm, body mass ranged from 63 kg to 110 kg, the minimum BMI was 25.3 kg/m2, while the maximum was 40.7 kg/m2.

Mean height: 166.4 ± 6.54 cm, with a mean body weight of 86.3 ± 10.95 kg and a mean BMI of 31.1 ± 3.56 kg/m2.

On assessment of BMI according to WHO guidelines, it was observed that none of the subjects had normal body weight, 40 (44.4%) were overweight with BMI ranging from 25.0 kg/m2 to 29.9 kg/m2. 50 (55.6%) had obesity.

The SBP ranged from 100 mmHg to 200 mmHg (mean 135.4 ± 19.49 mmHg). The minimum DBP was 60 mm.Hg and the maximum was 110 mm.Hg (mean 83.9 ± 13.70 mm.Hg). Arterial hypertension was present in 51 patients or 56.7%.

Diabetes control (A1c equaled 8.7± 2.13%) in the group was poor, which may have been due to the study design, since the examination was performed on patients who had to go to a private medical center, that is, a priori they were not in a very good condition at the time of referral.

In accordance with the criteria of group formation, renal functional status indicators were normal (creatinine 0.75 □ 0.14 mg/dL, CKD EPI-CKD 92.3±16.19 ml/min/1.73 m2), liver functional status indicators: AST: 29.7 ± 8.18 U/L; AST: 30.6 ± 1.14 U/L.

The daily dose of metformin ranged from 500 mg to 3000 mg. It averaged 1922.2 ± 543.2 mg.

Mean vitamin B12 values were 380.8 ± 115.27 pmol/L in the subgroup of DM2 patients treated with metformin at a dose of less than 2000 mg per day. In the subgroup of DM2 patients treated with metformin at a daily dose of 2000 or more, the mean vitamin B12 value was 251.6 ± 119.95 pmol/L. Differences between the mean were statistically significant ($p < 0.001$).

Of 55 patients treated with metformin at a dose less than 2000 mg/day, 5 (9.1%) had vitamin B12 deficiency, while 50 patients (90.9%) treated with metformin at a dose less than 2000 mg/day had no vitamin B12 deficiency. Of 35 patients with DM2 who received metformin in daily dose of 2000mg and more, 19 (34,5%) had vitamin B12 deficiency6 and 16 (45,7%) had no such deficiency. When statistical analysis of differences in the incidence of vitamin B12 insufficiency during treatment with metformin at a dose of less than 2000mg/day and at a dose of 2000mg/day or more, statistical significance of the differences was found using Fisher's exact method ($p <0.00001$).

The odds of finding vitamin B12 deficiency in the DM2 group when treated with metformin at a daily dose of 2000 mg or more, was 1.188. In the same group, the chance of finding vitamin B12 deficiency when treated with metformin at a daily dose of less than 2000 mg was lower and was 0.100. The Odds ratio, that is, the odds ratio of finding vitamin B12 deficiency when treating DM2 with metformin at a daily dose of 2000mg or more or treating with metformin at a daily dose of less than 2000mg is 11.875 with a standard error of the odds ratio (S) of 0.579 and a lower bound 95% confidence interval (CI) of 3.818 and an upper bound 95% confidence interval (CI) of 36.932. That is, the chance of vitamin B12 insufficiency during treatment of DM2 with metformin at a daily dose of 2000 mg or more is almost 12 times higher than during treatment at a dose of less than 2000 mg/day. The odds ratio was statistically significant ($p < 0.05$), that is, the results of our study provide evidence that high doses of metformin (2000 mg/day and more) is a risk factor for the development of vitamin B12 deficiency in DM2 patients.

These results are in agreement with previous data on the association between metformin dose and vitamin B12 deficiency. It should be noted that in the study of Ahmed M.A. et al. as well as in our study, a daily dose of metformin 2000 mg and more is indicated as a risk factor.

The influence of metformin treatment duration on the development of vitamin B12 insufficiency in DM2 patients was studied.

The data of 90 DM2 patients treated with metformin were analyzed again. Characterization of the group was presented above.

Among 62 patients treated with metformin for 5 years or more, 21 (33.9%) had vitamin B12 deficiency, and 41 (66.1%) had no such deficiency. Among 28 DM2 patients treated with metformin for less than 5 years, 3 (10.7%) had vitamin B12 deficiency. Twenty-five (89.3%) had no vitamin B12 deficiency. Statistical analysis of differences in the incidence of vitamin B12 insufficiency in the duration of DM2 treatment with metformin for less than 5 years and the duration of treatment with this drug for 5 years or more was carried out using "Fisher's exact method" and showed that the differences were statistically significant ($p <0.05$).

The odds of finding vitamin B12 insufficiency when the duration of treatment of DM2 with metformin for 5 years or more was 0.512. In the same group, the chance of finding vitamin B12 deficiency when the duration of DM2 treatment with metformin was less than 5 years was 0.120. The Odds ratio, that is, the ratio of the probability of finding vitamin B12 deficiency in metformin treated DM2 patients treated for 5 years or more to the probability of finding it in DM2 patients with a duration of DM2 treatment with metformin of less than 5 years, was 4.268, with a standard error of the odds ratio (S) of 0.667 and a lower bound of the 95% confidence interval (CI) of 1.154 and an upper bound of the 95% confidence interval (CI) of 15.787. The odds ratio was statistically significant ($p < 0.05$).

Thus, duration of treatment with metformin for 5 years or more increases the risk of vitamin B12 deficiency by 4.268 times. Our data on the significance of the duration of metformin treatment for the development of vitamin B12 deficiency correspond with the literature.

CONCLUSIONS

1. With mean vitamin B12 levels (M±SD) of 401.6 ± 138.06 pmol/l in the group of individuals without carbohydrate metabolism disorders, 364.7 ± 149.68 pmol/l in the prediabetes group, and statistically significantly (p<0, 01) lower level of vitamin B12 in the group of patients with type 2 diabetes mellitus (337,7±129,84 pmol/l) vitamin B12 insufficiency occurred in 13,6% of persons without carbohydrate metabolism disorders, in 16,7% of people with prediabetes and in 22,8% of patients with type 2 diabetes mellitus [3]. Thus:
   - Absolute vitamin B12 deficiency occurred in 5.1% of subjects without carbohydrate metabolism disorders (n=59), in 4.2% of subjects in the prediabetes group (n = 24), and in 6.5% of patients with type 2 diabetes mellitus [3].
   - Relative vitamin B12 deficiency occurred in 8.5% of individuals without carbohydrate metabolism disorders, 12.5% of those examined in the prediabetes group, and 16.3% of patients with type 2 diabetes mellitus [3].
2. An odds ratio study revealed no role for disorders of carbohydrate

metabolism ($p>0.05$) and no role for type 2 diabetes mellitus ($p>0.05$) as a risk factor for the development of vitamin B12 deficiency [4].

3. No effect of sex, obesity and arterial hypertension on the risk of vitamin B12 deficiency in the absence of carbohydrate metabolism disorders, in the presence of prediabetes and type 2 diabetes was found. In type 2 diabetes mellitus, the age of 65 years and older is a risk factor for the development of vitamin B12 deficiency ($p < 0.05$), while the age below 65 years protects (is a protective factor) against the development of this pathology.

4. In type 2 diabetes, taking metformin increases the risk of developing vitamin B12 deficiency ($p<0.05$) by 6.5 times compared to the group not receiving the drug [5].

5. In patients with diabetes mellitus type 2 treated with metformin, duration of taking the drug for 5 years or more, as well as the drug dose of 2000 or more mg/day are risk factors for the development of vitamin B12 deficiency ($p < 0.05$), increasing this risk by 4.9 times and 8.9 times, respectively [5].

PRACTICAL RECOMMENDATIONS

1. Due to the fact that even in the absence of carbohydrate metabolism disorders, vitamin B12 deficiency is detected in 13.6% of patients, it is necessary to conduct screening with examination of vitamin B12 content in serum in persons with risk factors for the development and symptoms of insufficiency of this vitamin;
2. The high incidence of vitamin B 12 deficiency in prediabetics (16.7%) requires screening of vitamin B 12 concentrations, especially in those treated with metformin.
3. In type 2 diabetes mellitus, periodic monitoring of vitamin B12 levels is appropriate, especially in persons 65 years of age and older.
4. In the course of treatment of patients with diabetes mellitus type 2 with metformin it is necessary to take into account mandatory control of vitamin B12 level, especially in patients taking the drug in the dose of 2000 mg and more
5. Duration of treatment with metformin for 5 years and more contributes to the development of vitamin B12 deficiency and requires periodic monitoring of its concentration in the blood.

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LIST OF ABBREVIATIONS

IDF -International Diabetes Federation
DM - diabetes mellitus
DM2- diabetes mellitus type 2
CMD- carbohydrate metabolism disorder
CKD- chronic kidney disease
BMI - Body Mass Index
WHO- World Health Organization
SBP- systolic blood pressure
DBP- diastolic blood pressure
A1C- glycohemoglobin

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