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Review article

THE IMPACT OF AGE ON THE LEVEL OF VITAMIN B12 DURING DIFFERENT PERIODS OF THE YEAR IN THE POPULATION OF POLOG REGION

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Abstract

Vitamin B12, or cobalamin, is a vitamin that is part of the water-soluble group of vitamins that are derived from animal products such as red meat, dairy, and eggs. The most used marker is total vitamin B12, which measures the level of vitamin B12 bound to transport proteins, which gives an overall assessment of the status of vitamin B12 in the blood. The purpose of this study is to determine the variations of vitamin B12 in all age groups during different periods within a year and to examine the risk of the deficit of this vitamin in the population living in the region of Polog. Data collection was carried out from January 2021 to December 2021, in the Diagnostic Biochemistry Laboratory of the Clinical Hospital of Tetovo. This study included a total of 352 subjects of both genders with female dominance. The concentration of vitamin B12 (cobalamin) was determined using the DCLIA (Direct Chemiluminescence Immunoassay) method. From the results obtained from this study, it appears that the population living in the region of Polog is not at risk of vitamin B12 deficiency, because the mean values obtained during the study in all periods are with satisfactory results within normal limits.

Keywords: vitamin B12, cobalamin, DCLIA, Polog region

Introduction

Vitamin B12, otherwise known as B12 or cobalamin, is a water-soluble vitamin derived from animal products such as red meat, dairy, and eggs. Intrinsic factor is a glycoprotein that is produced by the parietal cells in the intestine and is necessary for the absorption of vitamin B12 in the terminal ileum. The excess amount of vitamin B12 is stored in the liver, however, in cases in which it cannot be absorbed for a prolonged period (e.g. dietary insufficiency, malabsorption, lack of intrinsic factor), hepatic stores are depleted and insufficiency occurs *(Alex & Anil, 2022).* Cobalamin consists of four compounds with different biological functions, although these molecules are chemically similar. Cobalamin is a tetrapyrrolic chorine ring with a central cobalt moiety. Cyanocobalamin and hydroxocobalamin are synthetic forms of cobalamin, while adenosylcobalamin (AdoCbl) and methylcobalamin have biological activity to act as cofactors in enzymatic reactions that play a role in the synthesis of DNA, myelin and fatty acids, which are vital for the division and cell growth *(Al Amin & Gupta, 2023, González-Montaña et al., 2020).* Vitamin B12 deficiency was first described in 1849 and was considered to have a fatal outcome until 1926, when a diet of liver, rich in vitamin B12, was shown to slow the disease process. Decreased serum levels of vitamin B12 are not always associated with vitamin B12 deficiency. 15 to 40% of patients with B12 deficiency are not truly deficient, even asymptomatic patients with a normal

hematologic profile may have low vitamin B12 concentration in serum *(O'Leary& Samman, 2010, Dastidar & Sikder, 2022).* Several markers are used to evaluate the status of vitamin B12 in the blood. The most used marker is "total vitamin B12", which measures the level of vitamin B12 bound to transport proteins (haptocorrin-HCand trancobalamin-TC) and gives an overall assessment of the status of vitamin B12 in the blood. In addition, holo-TC (active B12), which represents transcobalamin-bound vitamin B12, has been suggested to be an early marker of vitamin B12 status *(Obeid et al., 2019).* In some conditions, monitoring for vitamin deficiency may improve outcomes. The establishment of reference values of their concentration including the minimum and maximum limit values, is required for the adequate screening of vitamin deficiency. Vitamin B12 deficiency should be diagnosed early and managed appropriately as lack of diagnosis can result in potentially serious complications, including spinal cord degeneration and pancytopenia. The purpose of this study is to determine the variations of vitamin B12 including all ages during different periods within a year and to examine the risk of deficiency of this vitamin in the population living in the region of Polog.

Materials and Methods

Data collection occurred in the Diagnostic Biochemistry Laboratory of the Clinical Hospital of Tetovo from January 2021 to December 2021. This study included a total of 352 subjects of both genders, with the majority of subjects being female. The subjects included in the study were divided according to gender and age groups, 0-20 years, 21-40 years, 41-60 years, and >60 years. The measurements were carried out in four time periods of the year (January-March, April-June, July-September, and October-December).
During each period when its concentration was determined, the mean of vitamin B12, standard deviation, and minimum and maximum levels were calculated and compared amongst these groups.
The statistical processing of the data was carried out using the statistical software program SPSS version 26.0 (IBM, Inc., USA), Microsoft Office Word, and Microsoft Office Excel. The value of $p<0.05$ is calculated as statistically significant.
The level of vitamin B12 (cobalamin) was determined using the DCLIA (Direct Chemiluminescence Immunoassay) method, with the ADVIA Centaur device. Serum was used as a biological material for measuring the concentration of vitamin B12. Vitamin B12 from the patient sample competes with vitamin B12 labelled with acridinum ester in the Lite Reagent for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase.
The reference values of vitamin B12 in serum are 27.24-169.62 pmol/L.

Results

The subjects included in the study belong within the age range of 1 to 83 years old, of which 97(27.55%) were male subjects and 255 (72.45%) were female subjects, in which the level of vitamin B12 was routinely measured. The average age of the patients included in the study is 44.46±18.05.

Table 1. Distribution of the subjects included in the study based on gender in the four time periods

	Male					Female					p value
Time period	**N**	**Min**	**Max**	**Mean**	**SD**	**N**	**Min**	**Max**	**Mean**	**SD**	
I-st period	32	23.29	146.0	69.39	40.10	81	2.07	146.0	79.58	39.93	0.22
II-nd period	15	40.97	146.0	101.23	41.98	59	5.92	146.0	73.88	36.72	0.01
III-rd period	16	23.35	144.88	79.45	35.21	42	19.59	146	81.88	42.35	0.83
IV-th period	34	18.52	146	70.40	35.79	73	18.70	146.0	83.32	41.82	0.12

The participation of male subjects included in the first period was 9.09% of the total number of the sample, in the second period 4.2%, in the third period 4.54%, and in the fourth period 9.69%. In comparison, the percentage of participation of the female subjects was significantly higher. In the first period the percentage of females was 23.01%, in the second period 16.76%, in the third period 11.93% and 20.73% in the fourth period. Between both genders, a significant difference was observed in the second time period, where the value of p=0.01.

Table 2. Presentation of the results obtained in the first period (January, February, March)

Age groups		**N**	**Min**	**Max**	**Mean**	**SD**	**p value**
0-20	M	4	36.67	146.00	80.74	49.61	0.87
	F	7	36.61	136.19	84.99	37.61	
21-40	M	6	26.41	146.00	64.66	45.40	0.40
	F	31	15.14	146.00	78.74	35.75	
41-60	M	13	23.29	146.00	64.82	38.74	0.42
	F	32	2.07	146.00	76.38	44.92	
>61	M	9	27.86	144.76	74.12	40.34	0.46
	F	11	35.29	146.00	87.84	41.06	

The first 3 months of the year were counted as the first period. In this period, a total of 113 subjects were tested. The above results show that in this period, the mean of vitamin B12 of all age groups and gender are with concentrations within the reference limits. The lowest value was found in the third age group of the female gender with a concentration of 2.07 pmol/L, in the second age group, also of the female gender, a patient with vitamin B12 deficiency was found with a concentration of 15.14 pmol/L. No significant difference was found between both genders in any age group and all cases the value of p>0.05.

Table 3. Presentation of the results obtained in the second period (April, May, June)

Age groups		**N**	**Min**	**Max**	**Mean**	**SD**	**p value**
0-20	M	2	146.00	146.00	146.00	0.00	/
	F	1	40.94	40.94	40.94		
21-40	M	4	44.56	146.00	89.40	42.68	0.74
	F	27	25.95	146.00	82.70	38.28	
41-60	M	5	40.97	90.72	63.74	21.46	0.88
	F	19	5.92	146.00	66.24	36.37	
>61	M	4	118.91	146.00	137.50	12.82	0.001
	F	12	24.37	140.59	68.87	32.60	

In the second period, cobalamin was tested in a total of 74 subjects, of which 15 were male and 59 were female. The mean of vitamin B12 in this period was within the normal range in all age groups and genders. The lowest value was found in patients aged 41-60 years at a concentration of 5.92 pmol/L. In this period, between male and female subjects in the age group over 61 years, a significant difference was found from the statistical processing of the data and the value of p=0.001.

Table 4. Presentation of the results obtained in the third period (July, August, September)

Age groups		N	Min	Max	Mean	SD	p value
0-20	M	2	118.17	144.88	131.52	18.88	0.59
	F	2	135.18	146.00	140.59	7.65	
21-40	M	6	29.15	117.71	82.86	34.65	0.78
	F	17	24.36	146.00	77.36	44.47	
41-60	M	4	47.09	104.24	74.38	25.90	0.98
	F	15	19.59	146.00	73.90	38.32	
>61	M	4	23.35	83.09	53.38	25.29	0.12
	F	8	48.89	146.00	91.76	41.93	

In the third period, a lower mean was found in the eldest group of over 61 years of male subjects with a value of 53.38±25.29. The other groups did not show a big difference in the mean of cobalamin concentration. The minimum value of the level of vitamin B12 was found in the 41-60 female group with a concentration of 19.59 pmol/L where a total of 15 subjects were tested. From the statistical processing of the data in the third period, no significant difference was found between the two genders and different age groups of people.

Table 5. Presentation of the results obtained in the fourth period (October, November, December)

Age groups		N	Min	Max	Mean	SD	p value
0-20	M	7	18.52	146.00	78.82	41.67	0.18
	F	3	70.90	146.00	120.96	43.35	
21-40	M	7	44.17	117.87	69.44	27.64	0.55
	F	29	20.74	146.00	77.37	32.72	
41-60	M	11	34.67	121.67	68.67	28.70	0.28
	F	22	18.70	146.00	85.02	45.66	
>61	M	9	26.58	146.00	66.71	47.94	0.37
	F	19	20.48	146.00	84.50	48.88	

Serum measurement of vitamin B12 in the fourth period was performed in a total of 107 subjects of both sexes. Compared to the previous periods, there are no changes in the mean, even in this period the mean was found within a normal range. The lowest value was found in the first male age group with a concentration of 18.52 pmol/L and the third female age group with a concentration of 18.70 pmol/L. The highest average value was found in the first age group of the female gender with an average of 120.96±43.35. A statistically significant difference between male and female genders was not found in any age group where the p-value in all comparisons is >0.05.

Table 6. Comparison of the level of vitamin B12 between time periods

	Male			Female		
	Mean	SD	p value	Mean	SD	p value
First period vs. Second period	69.39 101.23	40.10 41.98	0.01	79.58 73.88	39.93 36.72	0.38
First period vs. Third period	69.39 79.45	40.10 35.21	0.39	79.58 81.88	39.93 42.35	0.76
First period vs. Fourth period	69.39 70.40	40.10 35.79	0.91	79.58 83.32	39.93 41.82	0.57
Second period vs. Third period	101.23 79.45	41.98 35.21	0.12	73.88 81.88	36.72 42.35	0.31
Second period vs. Fourth period	101.23 70.40	40.98 35.79	0.01	73.88 83.32	36.72 41.82	0.17
Third period vs. Fourth period	79.45 70.40	35.21 35.79	0.40	81.88 83.32	42.35 41.82	0.85

The comparison was also made between different periods within the year, from which it turned out that a significant difference was observed only in male subjects between the first and second periods, where the value of p=0.01, and between the period of second and fourth, where the value of p=0.01. In female subjects, the comparisons made between different periods did not show a significant difference, and between all comparisons the value of p>0.05.

Discussion

Water-soluble vitamins are essential micronutrients included in the essential metabolic processes necessary for the normal functioning of the human body. People are generally not able to synthesize these micronutrients therefore, the body's supply is mainly based on diets (Tong et al., 2022). A study conducted by Sharma et al. shows that about 78% of the population who had vitamin B12 deficiency were not previously diagnosed. In clinical practice, greater alertness is essential to avoid irreparable damage from late diagnosis (Sharma et al., 2022). Vitamin B12 deficiency is a common condition that may present with non-specific clinical features and in severe cases with neurological or hematological abnormalities (Shipton&Thachil, 2015). Under normal physiological conditions, minimal amounts of vitamins are critical for homeostasis. Several studies have shown that small changes in serum vitamin levels, among other things, can negatively affect human health (Tomasiuk et al., 2022). Our study shows that seasonal variations have an impact on the concentrations of vitamin B12 in the Polog region's population. The highest mean was found in the second period of the year in the male population, but collectively in all age groups and genders, the individuals with vitamin B12 deficiency area small percentage.

Vitamin B12 deficiency is often associated with poor dietary intake, particularly in vegetarians and vegans where a lack of meat consumption creates a risk of vitamin B12 insufficiency*(Watanabe, 2007, Rizzo et al., 2016).*In addition, a significant part of the elderly population suffers from chronic malnutrition and loss of appetite which also been proven in studies carried out byPannérec et al., where it has been shown that both aging and frailty are associated with higher levels of MMA (methyl-malonic acid) indicating a higher prevalence of vitamin B12 deficiency in a study in Singapore*(Pannérec et al., 2017),* whose results correlate with our study where the age group over 61 years have a lower mean compared to the younger age groups.

Similar data was also obtained in the studies of Wong, 2015 and Andrès et al., 2008, which show that the etiology of vitamin B12 deficiency is mainly represented by the malabsorption of food rich in cobalamin and pernicious anemia, and less often by the lack of intake through food (Wong, 2015; Andrès et al., 2008).

Conclusion

Vitamin B12 is present naturally in food products of animal origin, also fortified breakfast cereals are an available source of cobalamin. This means that this vitamin can be sufficiently provided by consuming foods as such. From the results obtained in this study, it appears that the population living in the region of Polog is not at risk of vitamin B12 deficiency. The average values obtained during the study in all periods have satisfactory results within the normal limits. Changes in seasonal concentrations of this vitamin do not endanger the population from vitamin B12 deficiency.

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