ORIGINAL ARTICLE

Evaluation of the Relationship Between Serum Level of Vitamin B12 and Prognostic Indicators of Breast Cancer in Semnan

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ABSTRACT

Introduction: Breast cancer is the most common cancer and the leading cause of cancer death among women in the world. Given the potential relationship between vitamin B12 deficiency and cancer progression, the present study investigated the possible relationship between vitamin B_{12} deficiency and some of the factors affecting the prognosis of breast cancer.

Materials and Methods: This cross-sectional and descriptive-analytical study was performed on 63 patients with breast cancer who met the inclusion criteria. Serum levels of vitamin B_{12} were requested for all patients and the required information was collected in the relevant forms. The relationship between serum vitamin levels and desired factors was investigated by relevant statistical tests.

Results: It was found that only 15.9% of breast cancer patients had normal serum levels of vitamin B12 and others had some degree of deficiency. There was also an inverse relationship between age and serum levels of vitamin B12, but this relationship was not statistically significant (P=0.855). It was also found that the serum level of vitamin B12 is significant with tumor status in terms of PR (P=0.03) and it is notablewith tumor status in terms of ER (P=0.09).

Conclusion: Considering the significant relationship between serum levels of vitamin B12 and PR in breast cancer, further studies with larger sample sizes are suggested to clarify the relationship between these two factors.

Keywords: Breast cancer, Cobalamin, Vitamin B12 deficiency, Estrogen receptor

INTRODUCTION

Breast cancer is the most common cancer in Iran and among women in the world and accounts for 23% of all cancers and 14% of cancer deaths[1, 2]. In Iran, 24.6% of all female cancers are breast cancers and its annual incidence is 27.4 per 100,000 women. Also, the average age of incidence of this cancer is 49.6 years, which is 10 years lower than other countries[3, 4]. Less than 10% of breast cancers are directly related to inherited mutations [3]. Risk factors for breast cancer include old age, family history of breast cancer, early menarche, late menopause, higher first gestational age, nulliparity, and African descent[5, 6].

So far, several factors have been identified as factors affecting the prognosis of breast cancer. Factors such as: axillary lymph node involvement status, tumor size, metastasis to other areas, patient age, degree of histological differentiation of cells (grade), tumor histological subgroup, presence of estrogen and progesterone receptors, expression status of HER_2 proto-oncogene and tumor suppressor genes such as $\mathbf{P_{53}}$[7-9]. It is very important to study the risk factors for change such as pharmaceutical and vitamin supplements[10].

Vitamins with antioxidant properties, maintaining cell integrity and DNA repair, affect the risk of breast cancer[11-13]. Among these vitamins, B vitamins are involved in monocarbon metabolism, which is essential for DNA synthesis[14]. Disruption of the chain of the monocarbon metabolism can interfere with DNA transcription and modification and regulation of gene expression during methylation[15]. This suggests a potential link between B vitamins and the progression of cancers[16]. In fact, imbalances in B vitamins may affect nucleic acid metabolism and participation in carcinogenesis[17]. Vitamin B12 or cobalamin is one of the vitamins in this group, which is an important cofactor for methionine synthase and L-methylmalonyl COA mutase[3]. In fact, vitamin B_{12} catalyzes the transfer of the methyl group from methyl tetrahydrofolate to homocysteine to make methionine and finally S-Adenosyl methionine. Deficiency of this vitamin level can reduce the availability of S-Adenosyl methionine for DNA methylation and thereby affect gene expression[18, 19] and can also cause DNA instability and thus cancer progression[20, 21]. Methionine synthase, which is dependent on vitamin B_{12}, plays an important role in folate metabolism[22]. Severe deficiency of vitamin B_{12} or methionine synthase can cause hypermethioninemia, hyperhomocysteinemia and homocystinuria. This phenomenon leads to the accumulation of deoxyuridylate in DNA, which may cause DNA damage and strand breaks in DNA that are commonly seen in cancers[23, 24].

The normal serum level defined for vitamin B_{12} is 200-900 picograms per ml and by definition, a serum level below 200 pg/ml is considered deficiency of this vitamin[25]. The overall prevalence of vitamin B12 deficiency is unknown. Studies have shown that 39% of US adults are at risk for vitamin B_{12} deficiency[26]. The prevalence of vitamin B_{12} deficiency seems to be higher, especially in developing countries[27-29]. However, the prevalence of vitamin B12 deficiency may increase with increasing gastric bypass surgery, which is used for severe obesity[30]. Serum vitamin B_{12} deficiency is estimated at 46% in South Asia and 27.2% in Iranian women in Iran[28, 31].

Considering the above mentioned points and the relatively high prevalence of vitamin B_{12} deficiency in Iranian women compared to other communities and also a potential link between the deficiency of B vitamins, especially vitamin B_{12}, and the incidence of cancer, and given that a number of past studies have confirmed the link between vitamin B_{12} and cancer, in the current study, serum level of this vitamin and its association with a number of factors that have been considered to determine the prognosis of breast cancer in women with this breast cancer will be measured.

MATERIALS AND METHODS

Study Designer: This descriptive-analytical cross-sectional study was performed **on 63 patients** with breast cancer being admitted to the office or oncology and hematology clinic of Semnan during the two-year period of 2015-2017. By reviewing the records of female patients with breast cancer, after considering the inclusion and exclusion criteria, they were included in the study by simple sampling method if they had the consent.

Inclusion and exclusion criteria: Inclusion criteria: Patients with breast cancer whose disease has just been diagnosed and the diagnosis was confirmed by biopsy and pathology and had the full consent to participate in the study.

Exclusion criteria included patient's lack of consent to continue participation in the study, women with a specific disease that disrupts the vitamin B_{12} cycle (such as pernicious anemia), patients with a history of previous, current cancers or chemotherapy, patients with any malabsorption disease or diabetes, patients with a history of long-term use of drugs such as PPIs or metformin that have side effects such as vitamin B_{12} depletion.

Data collection: Interviews (history taking), examination (patient observation), patient records and blood samples were used to collect relevant data. The data were recorded in the relevant checklist.

Method: After approving the draft in the Research Council of Semnan University of Medical Sciences and receiving the code of ethics from the ethics committee of this university, qualified people entered the study after explaining the objectives and conditions of the study by a medical student. The information about all participating patients, including age, pathological and immunological features, and tumor, was recorded in a checklist previously prepared by the researcher. Then a blood sample was taken from the patients for testing or the patients were introduced to the laboratory for sampling. The patients were advised to bring the test results within a week and this was followed up by the researcher.

Data analysis: For descriptive findings with mean and standard deviation and number and percentage were reported and for analysis, analysis of variance, t-test, Pearson and Spearman correlation coefficient were used. A value less than 5% was considered significant. SPSS software version 21 was used to analyze the data.

Ethical considerations: This study is performed on patients who have the complete consent for taking their history, clinical examination and necessary tests. The relevant checklist is completed with the complete consent of the patients and anonymously, and the information about each patient was kept completely confidential.

Findings: In this study, 63 patients with breast cancer were studied. The mean age of the patients was 59.40±10.3 years. 67.5% of patients were in the age group of 50-69 years. The distribution of patients was not significant in terms of age (P=0.909). In more than half of the patients, the tumor (58.1%) was in T_3. Of the studied patients, 33 patients (52.4%) were histopathologically in G_2 phase. The mean level of vitamin B12 in the studied patients was 282.28±202.5 pg/L (50-1000). Of the patients, 29 (46.0%) had vitamin B_{12} deficiency, 24 (38.1%) had relative deficiency and 10 (15.9%) had normal levels of vitamin B_{12}. Also 57 patients (90.5%) were in the ductal histopathological subgroup and 6 patients (9.5%) were in the lobular histopathological subgroup (Table 1).

Regarding lymph node involvement, findings showed that 33 patients (52.4%) had lymph node involvement and 30 patients (47.6%) did not have lymph node involvement (Graph 1. A) regarding metastasis, 19 patients (30.2%) had distant metastasis and 44 patients (69.8%) did not have distant metastasis (Graph 1. B).

Vitamin B12 levels were not significantly different in patients with different age groups (P=0.201). Frequency distribution of vitamin B12 status in patients according to tumor size showed that patients with different tumor sizes were not significantly different in terms of vitamin B12 levels (P=0.107). Also vitamin B12 status in patients in terms of lymph node involvement showed that patients with different levels of vitamin B12 were not significantly different in terms of lymph node involvement or non-involvement (P=0.434). Patients with different levels of vitamin B12 were not significantly different in the presence or absence of distant metastases (P=0.902). Also, the frequency distribution of the vitamin status in patients in terms of histopathological subgroup and different histopathological stages was not significant (P=0.516 and P=0.781).

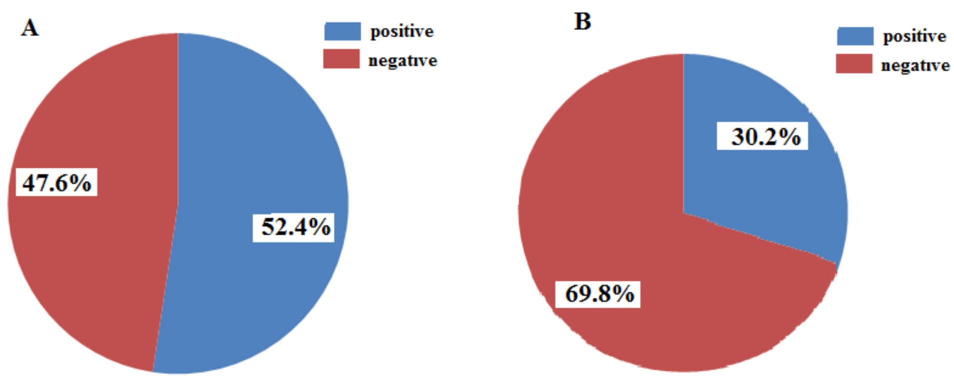
According to Table 3, different levels of vitamin B12 in patients with or without ER hormone receptor were not significantly different (P=0.094). However, the frequency distribution of vitamin B12 status in patients in terms of progesterone receptor (PR) showed that different levels of vitamin B12 in patients with or without PR marker tumor were significantly different (P=0.039).

Also, examination of hormone receptors using t-test showed that the mean level of vitamin B_{12} in people who had negative PR hormone receptors was lower than those who tested positive for these hormone receptors (P=0.35). The difference in vitamin B_{12} levels in other cases was not significantly related to the status of hormone receptors and oncogenes.

According to Table 5, the frequency distribution of vitamin B_{12} status in patients in terms of P_{53}mutation showed that vitamin B12 levels in patients with or without **P_{53}**tumor marker were not significantly different (P=0.291). Also, different levels of this vitamin were not significantly different in patients with or without HER_2 tumor marker (P=0.341).

Table 1: Mean and standard deviation of the main variables of the study in the studied patients

Parameter	Mean	Standard deviation	Number
Age	54/57	11.1	63
	Levels	Number	Percentage
Tumor stage	T_1	11	17.5
	T_2	45	71.4
	T_3	6	9.5
	T_4	1	1.6
Disease stage	G^*_X	17	27.0
	G_1	1	1.6
	G_2	33	52.4
	G_3	12	19.0
Vitamin B_{12} level	Deficiency (less than 200 pg/L)	29	46.0
	Relative deficiency (350-200 pg/L)	24	38.1
	Normal (greater than 350 pg/L)	10	15.9
Subgroup	Ductal	57	90.5
	Lobular	6	9.5
Total		63	100



Graph 1. Frequency of lymphatic involvement and metastasis

Table 2: Frequency distribution of vitamin B_{12} levels in patients according to the studied variables

		Vitamin B12 levels							
Age group		Deficiency		Relative deficiency		Normal		Total	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
	Less than 46	9	69.2	4	30.8	0	0	13	20.5
	46-60	10	34.5	12	41.4	7	24.1	29	41.2
	61 and more	10	47.6	8	38.1	3	14.3	21	33.3
	Total	29	46.0	24	38.1	10	15.9	63	100
Tumor size	T_1	8	72.7	0	0	3	27.3	11	17.5
	T_2	18	40.0	20	44.4	7	15.6	45	71.4
	T_3	3	50.0	3	50.0	0	0	6	9.5
	T_4	0	0	1	100.0	0	0	1	1.6
Lymph node involvement	Yes	16	53.3	9	30.0	5	16.7	30	47.6
	No	13	39.4	15	45.4	5	15.2	33	52.4
Distant metastasis	Yes	21	47.7	16	36.4	7	15.9	44	69.8
	No	8	42.1	8	42.1	3	15.8	19	30.2
Histopathological subtype	Ductal	26	45.6	21	36.8	10	17.6	57	90.5
	Lobular	3	50.0	3	50.0	0	0	6	9.5
Histopathological stage	G_X	8	47.1	7	41.1	2	11.8	17	27.0
	G_1	1	100	0	0	0	0	1	1.6
	G_2	13	39.4	13	39.4	7	21.2	33	52.4
	G_3	7	58.3	4	33.3	1	8.4	12	19.0
	Total	29	46.0	24	38.1	10	15.9	63	100

Table 3. Frequency distribution of vitamin B_{12} levels in patients in terms of hormone receptors

Parameter		Vitamin B_{12} level							
		Deficiency		Relative deficiency		Normal		Total	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
ER hormone receptor	Positive	17	37.8	19	42.2	9	20.0	45	71.4
	Negative	12	66.7	5	27.7	1	5.6	18	28.6
	Total	29	46.0	24	38.1	10	15.9	63	100
PR hormone receptor	Positive	14	35.0	17	42.5	9	22.5	40	63.5
	Negative	15	65.2	7	30.4	1	4.4	23	36.5
	Total	29	46.0	24	38.1	10	15.9	63	100

Table 4. Frequency distribution of hormone receptors and oncogenes in patients

Hormone receptor or oncogene		Number	Percentage	Vitamin B_{12} level		P
				Mean	Standard deviation	
ER	**+**	45	71.4	305.84	226.4	*0.154
	-	18	28.6	225.06	109.4	
PR	**+**	40	63.5	316.65	236.9	*0.035
	-	23	36.5	223.83	10.8	
HER_2	**+**	15	23.8	356.07	280.2	*0.224
	-	48	76.2	259.85	168.7	
27	**+**	9	14.2	239.00	84.5	**0.624
	-	27	42.9	234/67	102.0	
	Unknown		42.9	345.44	279.3	

* Independent T Test

**Kruskal Wallis Test

Table 5. Frequency distribution of vitamin B_{12} status in patients in terms of tumor marker

		Vitamin B_{12} level							
		Deficiency		Relative deficiency		Normal		Total	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Tumor marker P_{53}	Unknown	11	40.8	9	33.3	7	25.9	27	42.9
	Positive	3	33.3	5	55.6	1	11.1	9	14.2
	Negative	15	55.6	10	37.0	2	7.4	27	42.9
	Total	29	46.0	24	38.1	10	15.9	63	100
Tumor marker HER_2	Positive	5	33.3	6	40.0	4	26.7	15	23.8
	Negative	24	50.0	18	37.5	6	12.5	48	76.2
	Total	29	46.0	24	38.1	10	15.9	63	100

DISCUSSION

The results of our study showed that only 15.9% of breast cancer patients had normal serum levels of vitamin B_{12} and others had 38% relative deficiency and 46% actual vitamin B12 deficiency. Mean serum levels of vitamin B_{12} were significantly different in those with a negative progesterone (PR) receptor than in those with positive receptor. This means that in people with PR+, serum levels of vitamin B_{12} were significantly higher. Conversely, in people with PR-, vitamin B_{12} deficiency is greater, and this association was not statistically significant regarding estrogen receptor (ER) but was numerically significant.

Many studies have examined the association between breast cancer risk and vitamin B_{12}. A 2013 meta-analysis by Dr. Zhang et al. found that adequate serum levels of vitamin B_{12} provide clear protection against breast cancer risk[32, 33]. In a prospective study by Martin Lajous et al. on folate and vitamin B_{12} and postmenopausal breast cancer in French women. it was found that there is an inverse relationship between folate and postmenopausal breast cancer, which is stronger in women with high intake of high vitamin B_{12}[34]. According to another consistent study, it was found that vitamins B_6 - B_{12} - methionine and riboflavin do not play an independent and important role in the carcinogenesis of breast cancer [35]. A study by Dr. Vahid et al. in 2018 in Iran found that there is an inverse relationship between breast cancer risk and a number of vitamins, including vitamin B_{12}[36].

As mentioned earlier, these studies have examined the association of overall breast cancer risk with serum levels of vitamin B12, some of which have rejected and some have confirmed the association. In this regard, a consistent study showed that plasma levels of folate and vitamin B12 were not significantly associated with the overall risk of breast cancer and the status of hormone receptors. But there was a correlation between vitamin B12 and breast cancer risk in women with moderate alcohol consumption. B12 levels were also associated with a higher risk of breast cancer in women with lower folate levels. The results of this study on hormone receptors were not consistent with our study that the higher and routine consumption of alcohol in Western societies and its effect on serum levels of vitamin B12 may be one of the reasons for this. It is also important to note that alcohol consumption is a separate risk factor for breast cancer and can be considered as an independent confounding factor [37]. Another study showed that there was no clear relationship between folate and vitamin B12 intake and the polymorphism of related enzymes and breast cancer risk due to the status of hormone receptors[38]. The results of the above two studies are not consistent with the results of the current study, which may be due to the much smaller sample size and the absence of a control group in the current study. Also, the percentage of ER+ and PR+ cases in our study was much higher than people who are negative in terms of these receptors, and this may be considered as a confounding factor.

Another related study found that increased plasma levels of B_6 and B_{12} were associated with a reduced overall risk of breast cancer. It also reduces the risk level of ER+ and PR+ as well as premenopausal and alcoholic individuals. This finding is relatively consistent with our study [39]. In a study by Zhang et al. on dietary intake of folate, B_6, B_{12} and methionine, and breast cancer with respect to the status of hormone receptors, a significant inverse relationship was observed between dietary intake of folate and vitamin B_{12} in a variety of ER and PR statuses[40].A study by Pirouzpanahet al. in 2008 showed the inverse effect of plasma level of vitamin B_{12} on ER hypermethylation status in breast cancer patients [41]. In another study, it was found that high dietary intake of B vitamins was associated with a reduced risk of premenopausal breast cancer and possibly a reduction in ER- and PR-cases [35], which are consistent with the findings of our study. In other words, with increasing plasma levels of vitamin B_{12}, the rate of ER-cancers decreases.

It is important to pay attention to diets containing vitamin B12. The only source of vitamin B12 for humans are foods of animal origin. The recommended dose of this vitamin in adult women through diet is 2.4 micrograms per day. If the intake of this vitamin through the diet is less than 0.1 micrograms per day for a year or more, obvious symptoms of deficiency occur. The most common cause of vitamin B12 deficiency is its malabsorption, and the only other cause is inadequate dietary intake [5].

In the present study, no significant relationship was found for ER+/-. In this regard, in a cohort study conducted in 2013, it was found that there is an inverse relationship between breast cancer and vitamin B12 intake and a positive association with vitamin B12[42]. But no significant association was found for ER+/- and PR+/- types[43]. The reason for this inconsistency with the current study can be the difference in the sample size as well as the difference in the type of study.

In addition to the strengths of this study, as it was done for the first time, this study, like any other study, had a number of limitations. Among the most important ones are the small sample size and measuring the level of vitamin B12 once. Also, due to the wide range of topics and many known and unknown factors affecting the level of vitamin B12 and also the very high risk factors for breast cancer, it was certainly not possible to eliminate all the confounding factors. Defects in patients' records also created limitations in some cases. Also, in many cases, we could not check the exact and sufficient number of lymph nodes to check for involvement or non-involvement, and we only used the information in the records about the positive or negative involvement of lymph nodes. Considering all the mentioned aspects, any definite statement about this relationship needs more and more extensive studies that more accurate results can be obtained by removing the above limitations.

Further studies with larger sample sizes and in a wider statistical population are recommended. Also, conducting prospective and intervention studies can help to eliminate confounding factors. The limit of statistical sample size was the most important limitation of our research. Therefore, a study with a larger sample size is recommended.

CONCLUSION

There was a significant relationship between serum level of vitamin B12 and prognostic factors of breast cancer such as age, tumor size, lymph node involvement or non-involvement, primary tumor metastasis, degree of differentiation, tumor histological subtype, tumor status in terms of positive or negative HER2 and P53 oncogenes. However, this relationship is significant for the hormone progesterone (PR) receptor and notable for the estrogen receptor (ER). According to the above results, further studies with a larger sample size are suggested to clarify the issue.

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