Reduction of Serum Vitamin B12 Levels among the Female Patients with Breast Cancer (Case Study: Sulaimania city- Iraq)

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Abstract

Background and objectives: Breast carcinoma is one of the most common malignant diseases among women worldwide. In Iraq there are noticeable elevation in incidence rates and prevalence of advanced stages of breast cancer. Cobalamin (vitamin B12) is essential micronutrient involved in one carbon metabolism and DNA methylation, which affects cancer. All of these may be change in breast cancer. The present work was designed to estimate and compare serum vitamin B12 among female breast cancer patients (60 ones) and healthy control subjects (60 ones) in Sulaimania city.

Methods: This is a case-control study conducted on sixty cases of newly diagnosed women with breast cancer, the control group include sixty healthy women. Serum vitamin B12 levels were estimated by electrochemiluminescence immunoassay (Elecsys) method. Data was analyzed using the software SPSS (Ver. 22) including frequency and percentage for categorical variables. Pearson chi-square test was used for analysis of all categorical variables.

Results: In this study we found that serum vitamin B12 levels were significantly (p=0.01) lower in breast cancer patients as compared to healthy control subject. There was no association between serum vitamin B12 levels with estrogen, progesterone, and HER2 receptor.

Conclusion: Given the results, it can be concluded that serum vitamin B12 is consistently lower among breast cancer patients. There was no association between serum vitamin B12 levels and hormones receptors status, indicating clinical implications for the interpretation of serum vitamin B12 levels. Therefore, it should be taken into consideration by physicians and cancer specialists.

Keywords: Vitamin B12; Breast Cancer; Sulaimania

Introduction

Breast cancer is the most frequent malignancy in women worldwide with estimated incidence of 252.710 invasive breast cancer and 41.070 deaths expected in 2017 in the United States alone[1]. Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death

after cardiovascular diseases among women, with a cancer-related mortality rate of 23% (2) (3). About 4,115 cases of breast cancer were reported in 2015, accounting for 19.5% of all newly diagnosed malignancies and 34% of the registered female cancers.

Vitamin B12 (cobalamin [Cbl]) is an essential water-soluble vitamin that involved in one-carbon metabolism (4). Vitamin B12 is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism (5). It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin (6), and in the maturation of developing red blood cells in the bone marrow.

Similarly, Vit-B12 is considered as a crucial nutrient which contributes in one-carbon metabolism and cell division. A use of 2 to 5 µg per day, together with efficient absorption, transportation, and transformation, are required for preserving human health (7). Furthermore, total plasma Cbl measurement is highly demanded in clinical approaches for the biochemical assessment of Cbl deficiency (8).

Vitamin B12 is used by the body in two forms, either as methylcobalamin or 5 deoxy-adenosylcobalamin. The enzyme methionine synthase needsmethylcobalamin as a cofactor, which catalyzes the transfer of methyl groups for the conversion of the amino acid homocysteine into methionine, whilemethionine becomes S-adenosyl methionine, which is the universal methyl donor for methylation reaction (9). 5 Deoxyadenosylcobalamin is a cofactor needed by the enzyme that converts l methylmalonyl CoA to succinyl CoA. This conversion is an important step in the extraction of energy from proteins and fats. In addition, succinyl CoA is necessary for the production of hemoglobin which is the substance that carries oxygen in red blood cells (10).

The mechanisms underlying the association of low serum vitamin B12 and breast cancer might be explained by the role of vitamin B12 in DNA methylation. Methionine synthase, a vitamin B12-dependent enzyme, catalyzes the transfer of a methyl group from methyltetrahydrofolate to homocysteine to form methionine, and eventually S-adenosylmethionine, which is the universal methyl donor for methylation reactions (11). Deficient vitamin B12 levels can reduce the availability of S-adenosylmethionine for DNA methylation (11-12) and may thereby influence gene expression (12). Thus, lower concentrations of B12 might result in DNA hypomethylation, which might play a role in carcinogenesis. Through diminished availability of methyltetrahydrofolate, which is involved in reactions generating

thymidylate and purines, lower B12 concentrations might also lead to reduced DNA synthesis and, thus, impaired DNA repair mechanisms (13).

In a similar study among patients with different cancer types, the results indicated a correlation between high Cobalamin (Cbl, vitamin B12) levels and the risk of subsequently diagnosed cancer (14). Likewise, an interaction is seen between plasma folate and vitamin B12 on Esophagus, Stomach, and Liver cancers in a Chinese Population, indicating correlations between plasma folate and vitamin B12 with upper GI cancers (15).

Hence, given the prevalence of breast carcinoma among Iraqi women and while Cobalamin (vitamin B12) is essential micronutrient involved in one carbon metabolism and DNA methylation, which affects cancer, the present aimed at estimation and comparison of serum vitamin B12 among female breast cancer patients (60 ones) and healthy control subjects (60 ones) in Sulaimania city.

Materials and Methods

This is a case-control study conducted from September 2018-February 2019, and the study population include 120 females. Out of these, 60 were of newly diagnosed/untreated histopathologically proven breast cancer

attending Hiwa hematology and oncology hospital in Sulaimani city in Iraq and 60 healthy females. In order to respect ethical issues, an informed consent was obtained from all participants. Breast cancer patients under- radiation or hormonal or chemotherapy, patients receiving therapeutic vitamin B12 supplement, Pregnant or lactating females and those with any associated chronic medical condition were excluded from the study. Also, for the control group members, having the gender female, an age of 25-65 and ability to read and write in Arabic were considered as inclusion criteria and being pregnant/lactating as well as suffering from any kind of cancers were considered as exclusion criteria in this group. Data were collected through an interview using a questionnaire which contains the following variables: age, marital status, marital status, menopausal status, chronic illness, the presence of cancer, treatment, use of vitamins B12 supplements. The date, type of breast cancer, and histopathological grade were reported from the patients' clinical records.

Blood sample were collected from patients and control groups. The quantity of 2.5 ml of the blood were collected in Gel and clot activator tubes were allowed to clot for 30 minute then centrifuged at 4000 rpm for 5

minute to get serum and kept at -20 till the samples were analyzed.

Laboratory test

The Serum Vitamin B12 estimation was done bya competitive test principle electrochemiluminescence immunoassay (Elecsys) method (Cobas e 411, Roche, Germany) by using Vitamin B12 II kite.

Statistical analysis

Data was analyzed using the Statistical Package for Social sciences (SPSS version 22). The results have expressed as frequency and percentage for categorical variables. Pearson chi-square test has been used for analysis of all categorical variables. P value was considered significant if it was less than 0.05.

Results

The study was conducted on 120 samples, 60 newly diagnosed women with breast cancer cases and 60healthy controls group. The details on demographic specification of the both groups are totally presented in Table 1. In addition, Figure 1 compares the members in terms of their age; Figure 2 compares them in terms of their menopausal; Figure 3 does the comparison in marital status; and Figure 4 compares the participants in terms of the Body Mass Index (BMI).
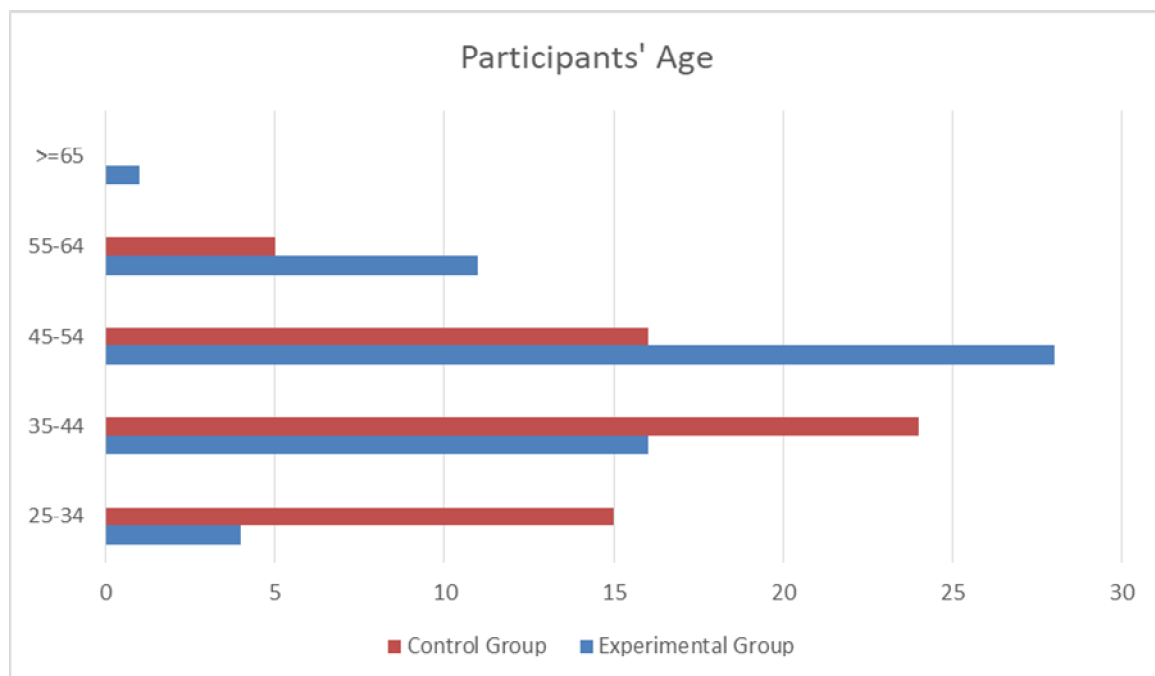

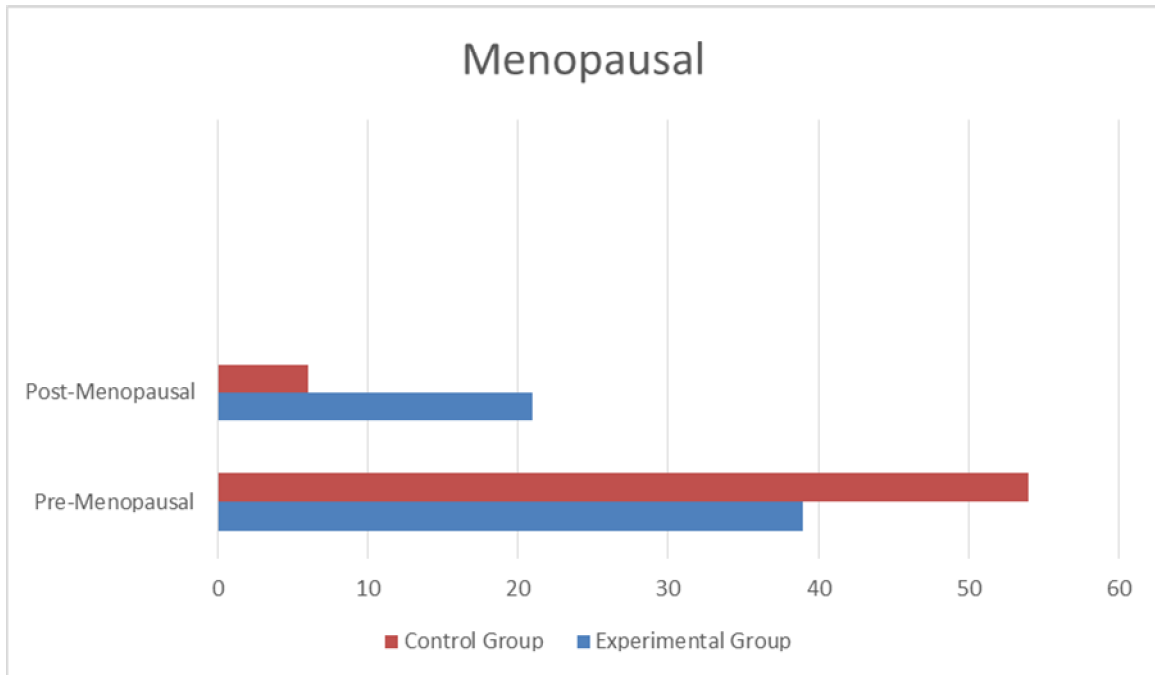**Figure 1.** Comparison of participants' age between experimental and control groups

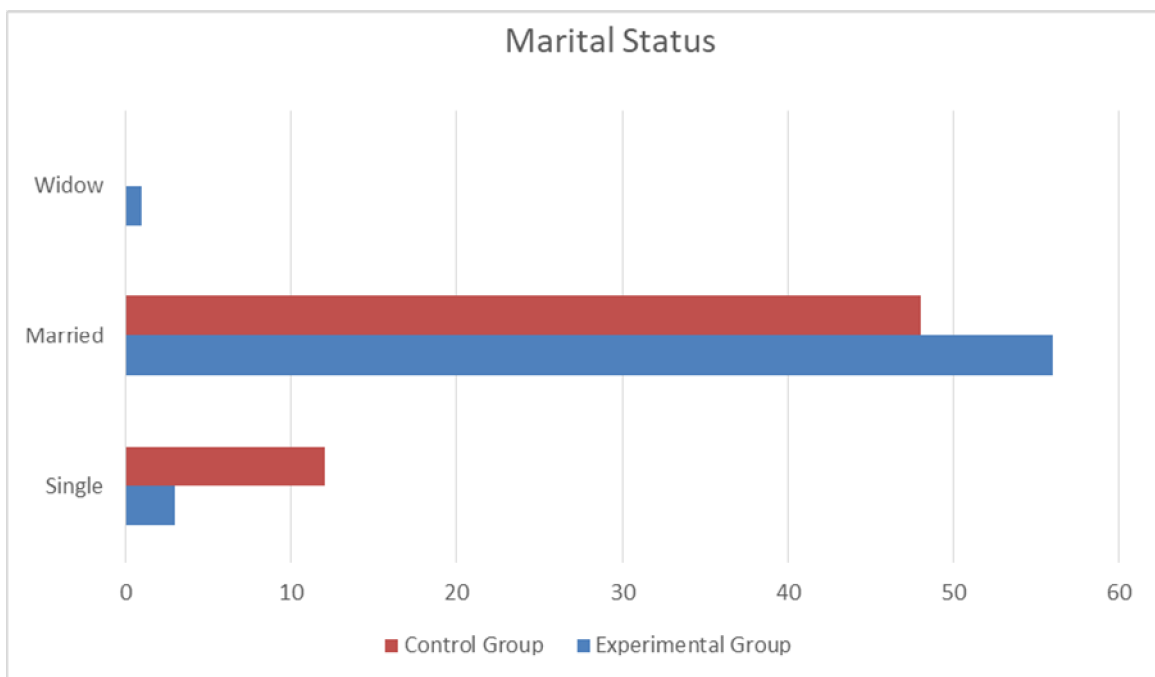**Figure 2.** Comparison of participants' menopausal among experimental and control group members

Figure 3. Comparison of marital status between experimental and control groups' members
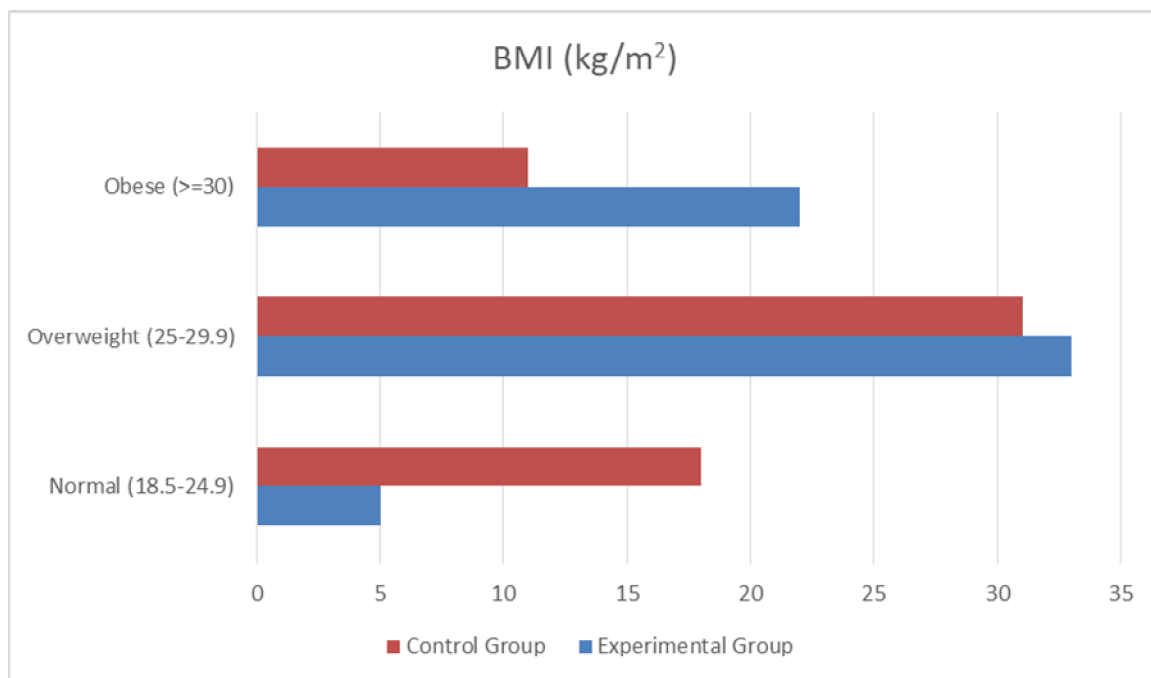
Figure 4. Comparison of Body Mass Index (BMI) between members of the two groups

Table 2 provides frequency and percentage of serum vitamin b12 levels in breast cancer patients and control group. The results obtained reveal that serum vitamin B12 in breast cancer patients significantly lower (p value= 0.01) than those obtained in control group. Table 3 provides the frequency and percentage of serum vitamin B12 in pre- and postmenopausal women in breast cancer patients. The results reveal no significant difference (p-value 0.9) in serum vitamin B12 levels between pre- and post-menopausal women in breast cancer patients. In pre-menopause 30% was Vitamin B12 deficient and 35% was normal, while in post-menopause 17% was Vitamin B12 deficient and 18% was normal.

Table 1: characteristic of breast cancer patients and control group

Data		Breast cancer (n=60)	Control group (n=60)	P value
Age (years)	25 – 34	4(6.67%)	15(25.0%)	0.006
	35 – 44	16(26.67%)	24(40.0%)	
	45 – 54	28(63.6%)	16(26.67%)	
	55 – 64	11(46.67%)	5(8.33%)	
	≥ 65	1(18.33%)	0(0.0%)	
Menopausal	Pre-Menopausal	39(65.0%)	54(90.0%)	0.001
	Post-Menopausal	21(35.0%)	6(10.0%)	
Marital status	Single	3(5.0%)	12(20.0%)	0.025
	Married	56(93.33%)	48(80.0%)	
	Widow	1(1.67%)	0(0.00%)	
BMI (kg/m^2)	Normal (18.5 -24.9)	5(8.33%)	18(30.0%)	0.004
	Overweight (25 - 29.9)	33(55.00%)	31(51.67%)	
	Obese (≥ 30)	22(36.67%)	11(18.33%)	

Table 2: Serum Vitamin B12 levels in Breast Cancer and Control group.

Vitamin B12	Breast cancer n=60	Control group n=60	P value
Low < 211 pg/ml	28 (47%)	15 (25%)	0.01
Normal 211 - 946 pg/ml	32 (53%)	45 (75%)	

Table 3: serum vitamin B12 in pre- and postmenopausal women in breast cancer patients

Variables		Post-Menopause Breast cancer (n=21)	Pre-Menopause Breast cancer (n=39)	P value
Vitamin B12	Low < 211 pg/ml	18(30%)	10(17%)	0.9
	Normal 211 - 946 pg/ml	21 (35%)	11(18%)	

Table 4: serum vitamin B12 and hormones receptor status in breast cancer patients

	Vitamin B12		
Variable	low<211pg/ml	Normal 211-946pg/ml	P value
ER +	20(33%)	24(40%)	0.7
ER-	8(13%)	8(13%)	
PR +	19(32%)	21(35%)	0.8
PR -	9(15%)	11(18%)	
HER2 +	4(7%)	10(17%)	0.1
HER2 -	24(40%)	22(37%)	

Estrogen receptor positive/negative (ER +,-), progesterone receptor positive/ negative(PR +,-), humane epidermal growth factor receptor2 positive/ negative(HER2 +,-).

Table 4 provides frequency and percentage of serum vitamin B12 in hormones receptor status in breast cancer patients. The results show no association between serum levels of vitamin B12 with estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2).

Discussion

In the current study, the serum vitamin B12 level was significantly lower in breast cancer patient as compared to controls group (table 2). We observed that 47% of breast cancer patients and 25% of control group had serum vitamin b12 concentration below 211pg/ml (normal range 211-946 pg/ml). These results were in agreement with two studies who state that low level of serum vitamin B12 increase risk of breast cancer (16)(17).On the other hand, disagree with study(18) who confirmed that vitamin B12 not associated with overall risk of breast cancer, while(19)found positive association between serum concentration of vitamin B12 and risk of breast cancer restricted to women with either high alcohol intake or low folate status only. The mechanisms underlying our observed association onB12 and breast cancer might be explained by the role of B12 as a cosubstrate in the synthesis of methionine, for which a methyl group is transferred from methyltetrahydrofolate to homocysteine. Two

crucial implications derive from this reaction:(a) it is the only means to provide de novo methyl groupsfor many methylation processes that are mediated by S-adenosylmethionine;and (b) it is also the only reaction to regenerate unsubstituted tetrahydrofolate from 5-methyltetrahydrofolate. Thus, lower concentrations of B12 might result in reduced synthesis of de novo methyl groups, leading to DNA hypomethylation, which might play a role in carcinogenesis (13)(20)(21). Through diminished availability of unsubstituted tetrahydrofolate, which is involved in reactions generating thymidylateand purines, lower vitamin B12 concentrations might also lead to reduced DNA synthesis and, thus, impaired DNA repair mechanisms(13).

There was no significant difference (p-value 0.9) in vitamin B12 levels between premenopausal and postmenopausal breast cancer patients (Table 3). This finding is in agreement with other studies(22)(18) who revealed no significant association between biomarkers of vitamin B12 and breast cancer risks in the subgroup analysis by menopausal status.

In the present study as shown in (Table 4) there was no association between serum levels of Vitamin B12 with Estrogen Receptor, Progesterone Receptor, and HER2

Receptor. These results are consistent with the findings from (14)(15) where no association of serum vitamin B12 levels with estrogen receptor, progesterone receptor, and HER2 in breast cancer patients was found.

Conclusion

This study attempted to establish association of serum vitamin B12 in breast cancer patients as compared with healthy individual (control group). Based on study results, it concluded that serum vitamin B12 is consistently lower in breast cancer patients. There was no association between serum vitamin B12 levels and hormones receptors. Despite the fact that our results may have clinical implications for interpreting Cbl levels, further studies are required to assess the possible diagnostic value of Cbl levels.

Of the limitation of present study, one can refer to lack of possibility to generalize the results to other regions of Iraq and even other neighbor countries such as Iran. In addition, this study was dedicated to breast cancer due to limitations in examination, thus, coming studies are proposed to consider other types of cancer, as well.

Declarations

Human subjects

A written informed consent was obtained from all participants.

Conflict of interest

None

Authors' contributions

All authors contributed equally to this work

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