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NUTRITIONAL DEFICIENCY INCREASES RISK FOR BREAST CANCER IN POSTMENOPAUSAL WOMEN: A PILOT STUDY IN DELHI

Oncology

Dr Shilpa Bhardwaj* MD Biochemistry, Rajiv Gandhi Super Speciality Hospital, Tahirpur, Delhi-93 *Corresponding Author

Dr (COL) H.C. Sharma MHA Administration, Rajiv Gandhi Super Speciality Hospital, Tahirpur, Delhi-93

ABSTRACT

Background: Vitamin B12 deficiency is associated with impaired DNA synthesis, repair and methylation. Sufficient intake of vitamin b12 has been hypothesised to lower breast cancer risk. So we conducted this case-control study to assess role of vitamin b12 in breast cancer in postmenopausal women in Delhi, India.
Methods: We included 60 postmenopausal women diagnosed with breast cancer and 60 age matched postmenopausal healthy women as controls. Serum vitamin b12 levels were estimated using chemiluminescence based assay on access 2 (Beckman Coulter systems). Statistical analysis was done using SPSS (IBM, USA).
Results: Serum vitamin b12 levels were found to be significantly lower in breast cancer patients as compared to healthy controls (p value < 0.05). The levels were found to be significantly decreased in patients with ER-/PR- receptor status.
Conclusion: Vitamin B12 may have a role in carcinogenesis and may affect the expression of estrogen/ progesterone receptor in breast cancer.

KEYWORDS

Vitamin B12, Breast cancer, postmenopausal, chemiluminescence, receptor status

INTRODUCTION

Worldwide, breast cancer in women is the most common invasive cancer impacting 2.1 million women each year. In 2018, around 6.2 lac women i.e 15% of all cancer deaths among women were due to breast cancer. [1]. Study conducted by Indian council of medical research showed that for every 2 women newly diagnosed with breast cancer, one woman dies of it in India. [2-3].The relevance of non modifiable risk factors like genes, early age at first menarche, late age at menopause, and late age at first full-term pregnancy in breast cancer has been documented largely but the role of modifiable risk factors is still unexplored.

Vitamin B12 (Vit B12) is a water-soluble vitamin that plays a significant role in DNA synthesis & DNA methylation. Its deficiency is known since historic times in Indian subcontinent owing to vegetarian dietary habits. Even though body stores Vit B12 in sufficient quantities but with increasing age deficiency manifests and goes undetected due to associated folic acid deficiency. Women are more prone to effects of its deficiency due to increased demand during multiple gestations or frequent child births and insufficient intake. Dietary requirement of Vit B12 is minimal as it is the only B vitamin stored abundantly in liver. It is required as a coenzyme for purine and thymidylate synthesis and thus, its deficiency can result in reduced synthesis of these nucleotides leading to defective DNA synthesis & repair which, in turn, might increase mutation rate [4,5]. Moreover, its deficiency simultaneously produces folate trap which further affects DNA synthesis & repair. Vit B12 is also required for synthesis of active methionine which has a role in DNA methylation [6]. Hypomethylated islands of DNA have been suggested to have a role in carcinogenesis [4,7] although specific role in breast cancer in not yet known [8,9]. Several studies have reported an indirect role through effect modification by Vit B12 on the association between folate and breast cancer risk thereby pointing towards possible role of Vit B12 in carcinogenesis [10,11]

So the present study was conducted to find the role of Vit B12in postmenopausal women with breast cancer in Delhi, India. We also correlated the levels of Vit B12 with tissue receptor status [estrogen receptor, progesterone receptor] and Her-2 neu status.

MATERIALS AND METHODS

Study design: A cross-sectional, case-control study was conducted in the department of biochemistry in collaboration with department of surgery of a tertiary care hospital, Delhi, India.

Subjects: 60 postmenopausal women with histologically diagnosed breast cancerformed the case group &age matched 60 postmenopausal apparently healthy women who attended hospital for routine visits for a period of one year and volunteeredformed the control group. Postmenopausal status was determinedat the time of diagnosisbased on age ≥ 45 years and no menses since 365 days to exclude premature menopause. Smoking, drinking and any factors affecting Vit B12 absorption like diarrhoea, drugs, enteropathy were considered as the exclusion criteria. A questionnaire was formed to obtain detailed reproductive history, family history of breast cancer, hormone use, physical activity, and dietary vitamin supplementation. Staging of the tumor was done based on the American Joint Committee on cancer [AJCC].

Methods: Sample was taken after overnight fast under sterile conditions and processed immediately for routine investigations.Vit B12 estimation was done using chemiluminescence based immunoassay on access 2 (Beckman Systems, USA). Laboratory personnel were unaware of case-control status, and each case-control set was analyzed in the same batch using the same reagents. The study was approved by institutions ethic committee. All participants had given informed written consent for the study.

Statistical evaluation: Data analysis was done using Statistical package for Social Science software, version 19(SPSS Inc., Chicago, IL, USA) and Fisher's test was used to confirm the normal distribution of data. Quantitative data was assessed using independent sample t-test and presented as mean values ± Standard Error of Mean (S.E.M). Pearson's correlation analysis was done. $p \leq 0.05$ was considered statistically significant.

RESULTS

The study characteristics of cases and controls have been shown in table 1. Both groups were comparable on the basis of age and menopausal status and no one was on any vitamin supplementation. Vit B12 levels were significantly lower in breast cancer patients as compared to controls.

Table 1: Demographic features and Vit B12 status of study population

PARAMETERS	CASES [postmenopausal women][n =60]	CONTROLS [postmenopausal women][n=60]	p VALUE
Age [years]	54.36 ± 11.45	51 ± 10.76	NS
Family history of breast cancer	60%	None	
History of HRT	5%	None	
Vitamin B12 [pg/ml]	338 ± 96	406 ± 88	0.02 [S]

p value <0.05 is considered as statistically significant

Table 2 highlights the distribution of subjects based on TNM staging and receptor status. ER-/PR-/Her-2 neu + receptor status was more common as compared to ER+/PR+/Her-2 neu – receptor status. T3N0M0 was the most common staging observed.

Table 2: Histological characteristics of breast tumor in cases [n=60]

Variable	no. of patients	%
Age [years]		
45-60	40	66
>60	20	34
TNM staging		
T3N1M0	30	50
T4bN0M0	15	25
T4bN1M0	8	13
T4bN2M0	5	8
T4dN2M0	2	3
Receptor Status		
ER Status		
ER +	15	25
ER -	45	75
PR Status		
PR +	15	25
PR -	45	75
Her-2 neu Status		
Her-2 neu +	45	75
Her-2 neu -	15	25

Table 3 shows the variation in serum Vit B12 levels with receptor status. The difference between mean values of Vit B12 according to ER and PR status were found to be significant whereas insignificant for Her-2 neu status. The mean value of Vit B12 in ER + postmenopausal women was significant at 0.04 level of significance (303 ± 64 pg/ml) as compared to ER- women (230 ± 48 pg/ml). A significant difference was observed in mean Vit B12 levels between PR+ and PR- receptor status (310 ± 73 pg/ml and 205 ± 52 pg/ml respectively) with 0.03 p value. We observed that at cut-off value of 380 pg/ml, relative risk was 2.3 with 95% C.I. of 1.43-3.79 at a p value of <0.001.

Table 3. Vitamin B12 levels correlated with receptor status

Receptor status	no. of subjects	Vit. B12		p value
ER status		Mean	SEM	
ER+	15	303	64	0.04
ER-	45	230	48	
PR status				
PR+	15	310	73	0.03
PR-	45	205	52	
Her-2 neu status				
Her-2 neu +	45	307	55	0.23
Her-2 neu -	15	292	60	

p value <0.05 is considered as statistically significant

Table 4 Depicts Statistical characteristics of Vit B12 [at concentration 380 pg/ml] in Ca Breast patients

Characteristics		95% C.I.
Sensitivity	58%	44.8 - 70.9
Specificity	75%	62.1 - 85.3
positive likelihood ratio	2.3	1.43 - 3.8
negative likelihood ratio	0.56	0.40 - 0.78
PPV	70%	55.4 - 82.1
NPV	64%	51.9 - 75.4

PPV= positive predictive value, NPV= negative predictive value

DISCUSSION

The rising epidemic of breast cancer in India cannot be attributed solely to genes as environmental factors also play a significant role. The quest to identify newer modifiable causes of breast cancer in Indian population despite lower prevalence of risk factors as compared to western population, prompted us to investigate the role of Vit B12 in breast cancer in Indian population.We observed that the Vit B12 levels were significantly lower in postmenopausal women with breast cancer as compared to their apparently healthy controls. A population based case control study showed that Vit B12 intake was associated with lower risk of breast cancer in postmenopausal women [12].The plausible mechanism underlying this association observed in our study can be explained on the basis of role of Vit B12 in the synthesis of an active methyl group donor or active methionine, known as SAM [S-adenosyl Methionine] which plays a crucial role in trans-methylation reactions. Its deficiency may impair DNA synthesis & repair by disrupting one-carbon metabolism and thereby, increases the risk of carcinogenesis [13]. Another study supports our hypothesized role of B12 in one carbon metabolism but suggests a different pathway in carcinogenesis [14]. Several other studies have shown a protective association of Vit B12 with breast cancer [10,15] whereas findings of other studies have been incongruous [9,16].

In the present study, we included 60 postmenopausal women with histological proven breast cancer with the aim to correlate the levels of Vit B12 with receptor status (ER, PR and Her-2 neu). We observed statistically significant levels of Vit B12 in breast cancer postmenopausal women with positive ER and positive PR expression. The mean levels of Vit B12 were higher in women with ER+ and PR+ status as compared to women with ER- and PR- status. Vit B12 accepts methyl group from unusable form of folic acid (methyl-tetrahydrofolate) to form more usable tetrahydrofolate form which is required for de novo thymidylate & purine synthesis and transmethylation reactions. So Vit B12 deficiency may trap folate in unusable form. Studies have suggested that low levels of folate might result in DNA hypomethylation, which may lead to silencing of receptor expression due to aberrant methylation of CpG island promoters for the ER gene [17,18]. Lajous et al suggested in their work that Vit B12 intake may modify the protective effect rendered by folate in breast cancer prevention [12]. We could not find any significant difference between the mean levels of Vit B12 in relation to her-2 neu status. Since, Vit B12 may play a role in the expression of estrogen and progesterone receptors, it may pave a pathway for improving the response to chemotherapy in Indian population where ER + /PR+ cases are comparatively less as compared to western population. A case report revealed that falsely positive levels of CA 153 in cancer patients improved on vitamin B12 supplementation [19].

We conducted this study in a highly specific group of post menopausal women diagnosed with breast cancer with no history of diabetes, smoking, alcohol intake, any other cancer or vitamin B supplementation, thereby, reducing the confounding effect of other modifiable risk factors. But still our study had certain limitations like dietary intake of other B vitamins was not studied so consumption of folate fortified foods could not be ruled out which may influence our findings indirectly as it precipitates Vit B12 deficiency. Moreover, we could not study the genetic variations which have been associated with breast cancer risk [20, 21]. But our results suggest protective role of Vit B12 in breast cancer which is consistent with the findings of other studies. [22]

In conclusion, our findings suggest that Vit B12 deficiency may contribute to increase prevalence of breast cancer especially with ER-/PR- status in post menopausal women in Indian population. Large scale studies may be conducted to establish causal relationship. As it is a modifiable risk factor, simple dietary supplementations may help in preventing the escalation in cancer prevalence and improving prognosis through responsive treatment.

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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