Systemic Chemotherapy Interferes in Homocysteine Metabolism in Breast Cancer Patients

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Background: Hyperhomocysteinemia in breast cancer (BC) patients can be a risk factor for thromboembolic events. This study aimed to evaluate homocysteine and its cofactors (folic acid and vitamin B12) concentrations and platelet count at diagnosis of BC, 3 and 6 months after the beginning of chemotherapy treatment and to correlate them with clinical data. **Methods:** Thirty-five BC patients were included; blood samples were obtained by venipuncture. Plasmatic Hcy and cofactors concentrations were measured by competitive chemiluminescent enzyme immunoassay method. Platelet count was done using an automated analyzer. Sta-

tistical analysis was performed using the software SPSS. **Results:** During chemotherapy, homocysteine ($P = 0.032$) and vitamin B12 ($P < 0.001$) concentrations increased, while folate and platelets decreased ($P < 0.001$). Among the clinical data, the menopausal status showed significant positive correlation ($P = 0.022$) with homocysteine concentration increase. **Conclusions:** Evaluation of homocysteine concentrations during chemotherapy is extremely important because their levels increase during chemotherapy treatment, thus increasing the risk of thromboembolism development. J. Clin. Lab. Anal. 28:157–162, 2014. © 2014 Wiley Periodicals, Inc.

Key words: homocysteine; vitamin B 12; folic acid; breast neoplasms; chemotherapy and thromboembolism

INTRODUCTION

Breast cancer (BC) remains the second most frequent type of cancer in the world and the first among women. According to the National Cancer Institute (INCA) 52,680 new cases of BC were expected in Brazil for the year 2012, with an estimated risk of 52 cases per 100,000 women (1).

Several well-established factors were associated with increased risk of developing BC such as family history, nulliparity, early menarche, advanced age, exposure to ionizing radiation, and personal history of BC (in situ or invasive) (2).

Venous thromboembolism (VTE) is the most common complication in patients with cancer and the second most frequent cause of death in these patients (3, 4). The frequency of venous thrombosis in BC patients is 5–15%

(5, 6) when compared to the healthy population rate of 0.1% (6).

The pathophysiology of cancer-related thrombosis is multifactorial and complex. It can be caused by the formation of procoagulant factors by tumor cells, changes in blood flow and endothelial damage as a result of cancer or chemotherapy. The high rate of venous thrombosis in BC patients may also be explained by hyperhomocysteinemia

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(7,8), an increase of homocysteine (Hcy), a sulfur amino acid obtained from methionine (9,10).

Metabolism of homocysteine involves multiple pathways of synthesis and degradation, with the participation of important cofactors such as vitamin B6, B12, and folate (9–11).

In normal conditions, homocysteine levels are reduced due to its rapid conversion to methionine and cystathionine (9,12). A modification of the enzymes cystathionine synthase and methionine synthase or a transient deficiency of the enzyme cofactors (folate, vitamin B12, and B6) increases homocysteine levels. The modification of hemostatic proteins (N-homocysteinylated or S-homocysteinylated proteins) induced by Hcy or its thiolactone, and links of homocysteine or homocysteine thiolactone to •NO metabolism seems to be the main reason of biotoxicity of homocysteine in cardiovascular diseases (13).

Treatments such as surgery, chemotherapy, and venous catheterization increase the risk of hyperhomocysteinemia development. Patients undergoing surgery have at least twice the risk of deep vein thrombosis and three times the risk of fatal venous embolism than patients without cancer undergoing similar procedures (14,15). Cancer patients receiving chemotherapy account for 13% of all cases of VTE (16). The hormonal manipulation also affects the risk of venous thrombosis and is associated with a lower risk when compared to chemotherapy (17,18). So this study aimed to evaluate circulating homocysteine, folic acid, and vitamin B12 concentrations and platelet count at diagnosis of BC.

PATIENTS AND METHODS

Thirty-five patients diagnosed and under treatment for BC at the Oncology and Thromboembolic Diseases Centre of ABC Medicine Faculty (FMABC) were recruited from February 2008 to February 2010. All patients have written informed consent and the study was approved by the FMABC Ethics Committee (011/2009 protocol). All patients were treated following the chemotherapy standard protocol (cyclophosphamide/metrotoxate and fluoroyil).

Five milliliters of peripheral blood samples were collected by venous puncture (basilic or cephalic veins) and kept in tubes containing EDTA as anticoagulant or in tubes without any anticoagulant (dry tubes). Blood samples were collected at diagnosis and after 3 and 6 months after the beginning of chemotherapy treatment.

Samples were manipulated at the FMABC Clinical Analysis Laboratory. Plasma was obtained after sample centrifugation for 10 min at $1,629 \times g$ at room temperature and stored at -80°C until use (19).

Plasma homocysteine, vitamin B12, and folic acid samples were processed by chemiluminescent enzyme immunoassay method using Immulite 2000® (Siemens).

Homocysteine, vitamin B12, and total folic acid measures were calculated using calibration curves obtained from known concentrations. Reference values for homocysteine are as follows: 5–15 $\mu\text{M/l}$ for normal, 16–30 $\mu\text{M/l}$ for moderate hyperhomocysteinemia, 31–100 $\mu\text{M/l}$ for intermediate hyperhomocysteinemia, >100 $\mu\text{M/l}$ for severe hyperhomocysteinemia, and 200–400 $\mu\text{M/l}$ for homocystinuria. For vitamin B12, reference values are 174–878 pg/ml (128–648 pmol/l), and for folic acid, 3–17 ng/ml (6–39 nmol/l) (19).

Platelet count was performed using flow cytometry analyzer ABX Pentra 120.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 17.0. Quantitative variables were described through mean, standard deviation, and median; qualitative variables were described through absolute and relative frequencies. Student's *t*-test was used in order to compare the mean of two groups. Comparisons among three or more groups were performed using analysis of variance (ANOVA). Variable evolution study was performed using ANOVA with repeated measures. Correlations between two variables were analyzed through Pearson correlation coefficient; when data parametric normality assumption was rejected, Spearman correlation coefficient was used. Considered power test was 95%.

RESULTS

Patients clinical data, such as tumor characteristics and treatment, are summarized in Table 1.

A statistically significant relationship was only observed between the concentration of homocysteine and menopausal status ($P = 0.022$). The analysis showed that the group “no menopause” differs significantly from the menopausal group ($P = 0.018$) but did not differ from the premenopausal group ($P = 0.562$). It was also observed that homocysteine concentrations were higher in the menopausal group. Moreover, the premenopausal group did not differ significantly from the menopausal group ($P = 0.798$).

There was no statistically significant correlation between the remaining clinical data evaluated and the concentrations of homocysteine, vitamin B12, folic acid, and platelets. Figure 1 shows the distribution of the concentrations of homocysteine according to the tumor size at the diagnosis of the patients studied.

Variance analysis with repeated measurements has shown that there was a significant change in vitamin B12 levels ($P < 0.001$) during treatment. Levels at diagnosis were significantly lower than all other time points

TABLE 1. Patients Clinical Data Versus Circulating Homocysteine, Vitamin B12, Folate, and Platelet Concentration at Diagnosis

Clinical data	Vitamin B12 (pg/ml)	Folate (nmol/l)	Homocysteine ($\mu\text{mol/l}$)	Platelet ($\times 10^3/\text{mm}^3$)
Chemotherapy (100%)	282.46	12.47	9.6	329.26
Hormone therapy				
Yes—65.72%	245.64	12.14	10.14	324.91
No—34.28%	313.57	11.23	7.34	293.5
Student's <i>t</i> -test <i>P</i> -value	0.166	0.707	0.061	0.195
Surgery				
Surgery yes—94.28%	264.69	12.65	9.55	331.42
Surgery no—5.72%	383.67	6.3	6.87	195.67
Student's <i>t</i> -test <i>P</i> -value ^a				
Menopausal status				
No menopause—29.41%	227.18	11.38	5.73	285.55
Menopause—50%	257.2	11.09	10.27	246.47
Premenopause—20.59%	259.75	10.64	8.3	342.5
ANOVA <i>P</i> -value	0.791	0.913	0.022 ^b	0.112
Axillary lymph node				
Present—65.62%	259.91	12.17	9.04	319.32
Absent—34.38%	283.92	10.35	8.96	286
Student's <i>t</i> -test <i>P</i> -value	0.921	0.881	0.715	0.897
Nuclear grade				
Grade 1—3.03%	150	12	9.1	244
Grade 2—57.57%	278.95	12.48	9.69	320.71
Grade 3—39.39%	236.79	11.03	8.41	264.79
Student's <i>t</i> -test <i>P</i> -value ^c	0.375	0.158	0.612	0.143
Radiotherapy				
Yes—79.42%	227.57	11.84	9.75	315.75
No—20.58%	406.63	10.85	6.08	288.5
Student's <i>t</i> -test <i>P</i> -value	0.063	0.523	0.202	0.822
Histological grade				
Grade I—3.33%	267	11.3	15.3	358
Grade II—63.33%	226.7	11.63	9.69	312.6
Grade III—16.66%	148.67	12.83	5.6	274.5
Cd1: 3—10%	195.25	8.3	9.35	237
Nottingham grade II—6.66%	342.33	9.97	3.47	252.33
Student's <i>t</i> -test <i>P</i> -value ^d				

^aFor the criterion surgery there are only two cases under “surgery no,” making it impossible the comparison between the groups.

^bStatistically significant *P*-value.

^cIn these comparisons, the parameter “grade I” was not used because the sample size is too small.

^dNo comparisons were made, since groups G1, G2, and Cd1 did not have sufficient number of samples for comparison.

(3 months: $P < 0.001$; 6 months: $P < 0.001$) and levels at the third month were significantly higher than the sixth month ($P = 0.023$) (Fig. 2).

Folic acid concentration at diagnosis was significantly higher than the other ones (3 months: $P < 0.001$; 6 months: $P < 0.001$). No differences were observed between the third and sixth month of treatment ($P = 0.769$) (Fig. 3).

There was a significant change in homocysteine levels ($P = 0.025$) during treatment. Hcy at diagnosis did not significantly differ from the third month ($P = 0.067$), but differed from the sixth month ($P = 0.011$). No differences were observed between the third and sixth month of treatment ($P = 0.286$) (Fig. 4).

There was a significant change in platelet levels ($P < 0.001$) during treatment. Platelet level at diagnosis did not significantly differ from the third month ($P = 0.239$),

but it was higher than the sixth month ($P < 0.001$) (Fig. 5).

A negative significant correlation between homocysteine and folic acid concentrations at diagnosis was observed (Fig. 6).

There was a negative significant correlation between homocysteine and vitamin B12 concentrations at the third month of treatment (Fig. 7).

DISCUSSION

This work aimed to evaluate plasma concentrations of homocysteine, its cofactors vitamin B12 and folate, and plasma concentrations of platelets at diagnosis of BC and during chemotherapy treatment in order to verify correlations between those factors, clinical data and treatment. We verified the increase of Hcy levels when this

Fig. 1. Distribution of the concentrations of homocysteine according to the tumor size at diagnosis of the patients studied.

Fig. 2. Mean and standard deviation values of vitamin B12 concentration, according to the time of evaluation.

Fig. 3. Mean and standard deviation values of folic acid concentration, according to the time of evaluation.

parameter was evaluated during the treatment; we also verified a correlation between Hcy levels and cofactors (B12 vitamin and folate).

Gatt et al. (7) and Chou et al. (8) showed correlation between hyperhomocysteinemia and women with BC. However, those studies considered Hcy concentrations only at the diagnosis of the disease. In the present study, despite the fact homocysteine levels were normal at the diagnosis, we verified the increase of this amino acid during chemotherapy treatment.

Fig. 4. Mean and standard deviation values of homocysteine concentration, according to the time of evaluation.

Fig. 5. Mean and standard deviation values of platelets concentration, according to the time of evaluation.

Fig. 6. Scatter diagram of homocysteine and folic acid concentrations at diagnosis.

The chemotherapeutic agents used in cancer are divided into classes, which are alkylating agents, antimetabolites (among them are the analogues of folic acid—methotrexate), natural products, hormones and their antagonists, and other agents (20). Their most important actions are those that affect DNA synthesis and cell division.

The anti-DNA action of alkylating agents would lead to a reduction of folic acid and vitamin B12 concentrations, which in turn would lead to an increase of homocysteine

Fig. 7. Diagram of dispersion of homocysteine and vitamin B12 concentration at the third month of treatment.

concentration, since they are needed to metabolize this amino acid. In addition, when concentrations of vitamin B12 are inadequate, there is a functional deficiency of other intracellular forms of folic acid (20). Among the folic acid analogs, the best known is methotrexate, an inhibitor of dihydrofolate reductase, which also directly inhibits folate-dependent enzymes in the synthesis of DNA and RNA precursors, and thus also lead to an increase of homocysteine, as described above (20). Furthermore, some neoplastic cells receptors have a high affinity to folate, and since these cells replicate extremely rapid they are extremely dependent on an abundant supply of reduced folate, an additional mechanism which explains the reduction of folic acid in cancer patients (21).

As seen in this study, vitamin B12 and folic acid concentrations decreased with the progression of treatment, and they are inversely related to homocysteine levels. This inverse relationship of plasma concentrations of homocysteine and folic acid was also verified by Gatt et al., 2007 (7).

We noticed that there is a downward trend in plasma concentration of platelets during chemotherapy treatment. The increase of endothelial damage in cancer patients undergoing chemotherapy would be one possible mechanism to explain the reduction of platelets in these patients, since endothelial injury leads to platelet consumption, thereby resulting in increased plasma homocysteine.

When comparing the clinical data of patients with plasma concentrations of homocysteine, folic acid, vitamin B12, and platelets, we found that there was statistically significance only between the concentration of homocysteine and menopausal status. The group “no menopause” was statistically different from the menopausal group, with higher homocysteine concentrations in the postmenopausal group. A study conducted by the National Health and Nutrition Examination Survey (22) found that a higher estrogen status is associated with decreased total serum concentration of homocysteine (tHcy); other studies showed that estrogen

hormone can reduce homocysteine in postmenopausal women (23–25). As expected, our results showed that at diagnosis, Hcy is higher in patients at menopause status (postmenopausal group); however, the Hcy concentrations in these patients increased during chemotherapy treatment. There was no statistically significant correlation between the other clinical data evaluated and the concentrations of homocysteine, vitamin B12, folic acid, and platelets.

According to De Stefano et al. (10), there is a relationship between hyperhomocysteinemia and the occurrence of VTE, but the mechanism that leads to this association is still unknown. Our data suggest that the chemotherapy treatment can lead to hyperhomocysteinemia and may thus increase the risk of thromboembolic event. Thus, homocysteine evaluation during chemotherapy is a very important parameter since its level increases during systemic treatment. Further studies should be conducted to verify the risk of thromboembolism in these patients undergoing chemotherapy.

Finally, the purpose of this study was to verify if the chemotherapy approach could interfere in Hcy metabolism. Despite the lack of patients’ thrombosis data, it is important to clarify that Hcy and its cofactors must be measured in oncology patients during chemotherapy in order to evaluate thromboembolism events.

CONCLUSION

In this present study, we observed a significant increase in homocysteine concentration 6 months after chemotherapy, as well as a significant decrease in vitamin B12, folic acid, and platelets at the third and sixth month after beginning of chemotherapy treatment in women with BC.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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