Is Increase of Homocysteine, Anti-Cardiolipin, Anti-Phospholipid Antibodies associated with Breast Tumors?

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Abstract- Patients with malignancy are at higher risk of thrombotic complications due to the hypercoagulability state. Our objective in this study was to assess the serum concentrations of Homocysteine, anti phospholipid antibodies, and anti cardiolipin in patients with benign and malignant breast tumors and study the effect of chemotherapy on the serum levels of these markers. A case control study was carried out on 100 women with malignant breast tumor and 100 age matched control with benign breast tumors.Serum concentrations of heomocystein, anti cardiolipin antibody (IgG and IgMaCL) and anti-phospholipid antibody (IgG and IgMaPL) were measured in all cases. The malignant group was followed for six months, and serum levels of abovementioned markers were measured again after surgical removal of breast tumor and chemotherapy. Current results showed a significantly higher serum concentration of Homocysteine, IgG and IgMaPL, IgG and IgM aCL in patients with malignant tumor before chemotherapy compared with benign tumor patients. We found a significant decrease in these markers after chemotherapy (*P* Value<0.05).We propose performing these tests (Homocysteine, IgG and IgM aPL, aCL) in patients with breast malignancy and starting prophylactic anti-thrombotic treatment in those with high serum levels of the markers. In addition, since the serum levels of the markers in patients with malignancy reduce after adjuvant therapies, we strongly recommend using adjuvant chemotherapy in these patients.

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Introduction

The thromboembolic disease affects about 15% of cancer patients (1-4). Thrombotic events in malignancies adversely affect the prognosis. According to some studies, the risk of thrombosis in breast cancer is higher than other malignancies (1-4). Various factors including the type of malignancy, stage of disease, diet, chemotherapy, immobility resulting from the malignancy or after surgical procedures, and the use of catheters may contribute to the development of thrombosis (1-4).

Malignancies themselves cause an increased tendency to coagulation and can directly activate coagulation system. Malignancies can indirectly increase the tendency to coagulation by activating mononuclear cells that are responsible for the production of procoagulants (4-6).

Increased serum levels of antiphospholipid antibody (aPL), anticardiolipin antibody (aCL), and Homocysteine are associated with increased incidence of thrombotic events in malignancies (5,7-13). Prophylactic use of anticoagulants such as Enoxaparin and Warfarin could be beneficial to decrease thrombotic events and improve prognosis in these cases (12,15-17). The average levels of these antibodies are higher in patients with solid tumors are compared to those of hematologic and lymphoproliferative malignancies, as were the risk of developing thrombosis (3,17,18).

A decrease in the serum levels of these markers has been documented after appropriate treatments (11-19) as a consequence; these markers can also be used to evaluate patient's response to treatments such as surgery and chemotherapy.

Some studies were conducted on the thrombotic effect of malignancy especially in the patients with solid tumor

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in most parts of the world, but few investigations have been held of thrombotic effect of breast tumors in Iran and our region.

Regarding the high risk of thrombosis in patients with malignancies and the fact that its cause is not yet clearly explained, our objective in this study was to compare the serum concentrations of homocysteine, antiphospholipid antibodies, and anticardiolipin in patients with benign and malignant breast tumors. We also aimed to study the effect of malignancies' treatments on the serum levels of mentioned markers.

Materials and Methods

In the case-control study, 100 women with malignant breast tumors were enrolled as a patient group and 100 age-matched one's benign breast tumor as a control group. The study was carried out at Imam Hospital Complex (TUMS), Tehran, Iran during 2012. Patients with pregnancy, autoimmune disease and antiphospholipid syndrome (APS) or other disorders associated with changes in Homocysteine , anticardiolipin and antiphospholipid antibody were excluded from this study.

Diagnosis of the malignancy or benign pattern of lesions was based on mammography findings as well as needle biopsy pathology report. It was confirmed after the surgical removal of the tumor.

Before surgery, 5-6 milliliters of blood was taken from all case and control patients. Blood samples were taken again six months later from patients with malignancy after surgery and chemotherapy. The clotted blood was centrifuged at 3000 g for 15 minutes and extracted serum was then stored in a -70 centigrade Celsius freezer. All specimens were assessed in terms of homocysteine, anti-cardiolipin antibody (IgG and IgMaCL) and anti-phospholipid antibody (IgG and IgMaPL) following the instruction provided by the manufacturer. Serum levels of homocysteine, anti-cardiolipin antibody (IgG and IgMaCL) and antiphospholipid antibody (IgG and IgMaPL) were measured in all patients. Homocysteine were measured by Diazyme enzymatic assay (Diazym USAkitt) with a limit of detection<1.5 μmol/L. A concentration lower than 15 μmol/L was considered as normal.

aCL antibody was measured quantitatively by indirect solid phase enzyme immunoassay (Orgentec Diagnostika, GmbH, Germany kitt) with the detection limit of 1 U/ml and 0.5 U/ml for IgM and IgG antibodies, respectively. IgG<10 U/ml and IgM<7 U/ml were considered normal.

aPL antibody were measured by quantitative enzyme immunoassay (Orgentec Diagnostika, GmbH, Germany kitt) with the detection limit of 0.5 U/ml. IgG<10 U/ml and IgM<10 U/ml considered as normal.

Serum levels of Homocysteine, aCL, and aPL antibodies were measured again, six months after surgical removal of the breast tumor and chemotherapy in the patient group.

The study was carried out according to the principles of Helsinki. The local ethics review committee of Tehran University of Medical Sciences approved the study protocols.

Statistical analysis was performed by the statistical package for windows (SPSS16, Chicago, Inc). Differences in study biomarkers were compared between patients and control groups, and patients with malignant breast tumor before and after chemotherapy by using T-test, Wilcoxon, and chi-square. *P.* value less than 0.05 were considered statistically significant.

Results

The study population consisted of 100 patients with malignant breast tumor and 100 patients with benign breast tumor as a control group. Mean ages were 49.14 ± 4.3 (within the range of 39 to 55) and 44.6 ± 5.30 (within the range of 36 to 53), respectively in patients and control groups with no significant differences (*P.* Value=0.09).

Table 1 shows mean serum levels of Homocysteine, IgG, and IgM aCL, and IgG and IgM aCL antibodies in patients with malignant breast tumor (before and after therapy) and patients with benign breast tumor with the reference ranges.

Serum Homocysteine levels of all patients with benign breast tumor were in the reference range, 28 (28%) of patients with malignant breast tumor had Homocysteine above normal (>15) before chemotherapy which turned into normal range after chemotherapy. There was a significant difference between mean serum levels of Homocysteine in patients with benign breast tumor and malignant breast tumor patients before treatment (*P.* Value<0.001). Serum Homocysteine was significantly decreased after treatment in patients with malignant breast tumor (*P.* Value =0.001).

A total of 20 (20%) of patients with malignant breast tumor had high IgM aPL level which was declined to normal values after chemotherapy. High IgG aPL were observed in 8 (8%) patients which in 4 of them returned back to the normal range after chemotherapy. However, in all patients with benign breast tumor IgM and IgG

aPL were within normal range.

Mean serum level of IgM and IgG aPL was significantly higher in patients before chemotherapy than in control group (*P.* Value<0.05 and *P.* Value<0.001), this levels significantly declined after chemotherapy (*P.* Value<0.05)

IgM aCL levels in 24(24%) patients with malignant tumor were higher than normal which declined to reference range after chemotherapy. In 12(12%) patients with a malignant breast tumor, IgG aCL levels were above normal range before chemotherapy which in 8(8%) of them decreased to reference values. In the control group, high IgGaCL was seen in only 4(4%) of patients. IgM aCL level was significantly higher in patients with malignant breast tumor compared with control group (*P.* Value<0.001). A significant decreased was seen at this level after chemotherapy (*P.* Value<0.001). IgG aCL level was also significantly higher in patients compared to control group (*P* Value<0.05) with a significant decrease after chemotherapy (*P.* Value<0.05).

Table 1. Mean serum levels of Homocysteine, IgG- IgM aCL and IgG – IgM aCL antibodies in patients with benign and malignant breast tumors

	Benign breast tumor	Malignant breast tumor		Reference Range
		Before treatment	After treatment	
Serum hemocysteine	7.88 µ.mol/L	12.22 µ.mol/L	8.7 µ.mol/L	1.5 – 15 µmol/L
IgMaPL	1.44 U/ml	5.7 U/ml	2.35 U/ml	0.5 – 10 U/ml
IgGaPL	1.35 U/ml	4.94 U/ml	2.01 U/ml	0.5 – 10 U/ml
IgMaCL	1.99 U/ml	5.40 U/ml	3.28 U/ml	1 – 7 U/ml
IgGaCL	2.65 U/ml	5.06 U/ml	2.43 U/ml	0.5 – 10 U/ml

Out of 100 malignant tumors, 96 were invasive ductal carcinoma and 3 were Lobular carcinoma. There was no meaningful association between the pathological category of the malignancy and the level of aPL, aCL antibodies, and Homocysteine.

Discussion

Breast cancer is the second most common cause of death from cancer in women (20), and the burden of this disease is considered to be equal to that of cardiovascular diseases. Despite an increase in the incidence of breast cancer, development of new diagnostic and therapeutic methods has advanced life expectancy of the patients to near that of normal people. Because of attempts made to minimize complications of breast malignancy, new treatments have improved the quality of life of the patients. Thrombosis is a common complication of breast cancer (1,14,21,22).

According to many studies patients with malignancy are at higher risk of thrombotic complications due to the hypercoagulability state. The authors of the current study assessed the serum levels of Homocysteine, aPL antibodies, and aCL and evaluated the changes in the level of these markers in patients with benign tumor and malignant tumor before and after chemotherapy.

Although the mechanism of thrombosis due to hyperhomocysteinemia is still in doubt; the association between homocysteine level and malignancy is well known based on several reports. In a study on patients with chronic myeloproliferative diseases, hyper homocysteinemia was correlated with thrombosis; additionally Homocysteine level depends on the proliferative stage of the disease (11).

In the present study, we found a significantly increased level of Homocysteine in patients with malignant tumor compared to benign breast tumor; which significantly decreased after chemotherapy. This result was in accordance with the results of other studies.

Gatt *et al*. examined the level of Homocysteine in patients with early and metastatic breast cancer and a healthy control group which was 9.43 µmol/l, 11.34 µ mol/l, 7.9 µ mol/l, respectively. In this study, patients with metastatic disease had significantly higher levels of Homocysteine compared with controls but not with patients with early breast cancer. This could explain the higher rate of thrombosis in metastatic patients (13).

aPL is another important marker found to have thrombogenic properties (5,12,14,17,19,23).

The thrombotic event along with aPL antibody could be the first manifestation of malignancy (12).

A review reported by Tincani *et al.,* showed that not only aPL seems to be associated with a higher risk of thromboembolic events in some neoplastic patients but also there is a higher risk of developing malignancy

(especially hematologic) in aPL healthy carriers. It also mentioned the effect of prophylactic use of low molecular weight heparin and consideration of aPL reduction to confirm the remission (17). A study conducted in the series of critically ill cancer patients positive for aPL antibodies by Asherson *et al*., declared that the catastrophic aPL syndrome (CAPS) is associated with higher number of thrombotic events and acute organ dysfunctions (23).

Salluh *et al*., has mentioned aPL antibody as a risk factor for developing thrombosis in cancer patients (5). Gomez *et al.,* conducted a literature-based study on all cases of malignancies having aPL from 1966 to 2003. The main aPL-related manifestations mentioned in this study were thrombocytopenia (25%), cerebrovascular accidents (24%), deep vein thrombosis (19%), and pulmonary embolism (15%), and heart valve lesions (9%). A significant remission in serum aPL antibodies was seen in 35% of patients who recover after proper treatment for malignancy (19).

This finding is in the same line with ours; Current results shows a significantly higher serum level of IgG and IgM aPL in patients with malignant tumor before chemotherapy compared to benign tumor patients. We found a significant decrease in serum levels of IgG and IgM aPL after chemotherapy.

Micsbash W *et al*., conducted a study on 58 cancer patients (39 with solid tumors and 19 patients with haematologic and lymphoproliferative malignancies); in which 46% and 32% of whom developed thrombotic events due to antiphospholipid antibodies; respectively. Despite the higher level of serum anticardiolipin in these patients, no significant correlation between serum aCL and thrombotic events was found (12). Naess *et al.,* found no association between elevated aCL antibody levels and subsequent venous thrombosis on the study recruited in 508 patients with venous thrombosis and 1464 matched controls (24).

It has previously been found that the serum level of these antibodies depends on the stage of the disease and the probability of finding these antibodies is higher in those with advanced or metastatic disease (13). By considering the antibodies as markers of advanced malignancy which can be negative in early stages of the disease; the seronegative of these antibodies in patients with malignant breast tumor in some studies, could be explained. In this study, we recruited 100 patients with advanced malignant breast tumor needed chemotherapy after tumor resection and mastectomy. In addition, genetic and racial differences and the sensitivity of laboratory kits may also influence the antibody levels.

Since in this study aCL and aPL IgM antibodies were found more frequently than IgG antibodies, we propose the hypothesis that these acute reaction antibodies corroborate the association of the antibodies with malignancy. This antibody production might be the result of an immune response against malignant cells especially before starting adjuvant therapies.

Since we studied quantitative serum variables, the effect of genetic and racial variations, drugs, and infections on the final results cannot be overlooked. Using greater samples and multi-center sampling can result in better and more reliable results.

Results of the present study and previous studies suggest the existence of a correlation between these serum markers and an increased probability of thrombosis in malignancies. We propose performing tests for the markers in patients with malignancies and starting prophylactic anti-thrombotic treatment in those with high serum levels of the markers. We also suggest performing screening tests and using the serum levels of the antibodies as indicators of a high chance for future development of malignancies. In addition, since the serum levels of the markers in patients with malignancy reduce after adjuvant therapies, we strongly recommend using adjuvant chemotherapy in these patients.

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