The Correlation Between Homocysteine, Blood Coagulation and the Breast Cancer Risk and Clinicopathological Characters: A Case Control Study

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Research Article

Keywords: breast cancer, homocysteine, coagulation function, risk of onset, clinical staging, molecular classification

Posted Date: March 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1437183/v1

Abstract

Purpose

To investigate the correlation between homocysteine, coagulation function indexes and breast cancer risk and clinicopathological characteristics.

Methods

From January 2018 to December 2018, coagulation function test results and clinicopathological data from 369 female breast cancer patients were collected. 183 women with benign breast disease were selected as the control group. Results: The levels of HCY, Fbg and D-D in the peripheral blood of breast cancer patients were higher than those of the control group ($P < 0.05$); PT, AT-Ⅲ, PC, F-VIII, and α_2-AP were lower ($P < 0.05$). Concentrating on different menstruation status, serum levels of AT-Ⅲ, PC and α_2-AP in menopausal patients were lower than those in the control group ($P < 0.05$); HCY, Fbg and D-D in postmenopausal patients were higher than those in the control group ($P < 0.05$), while the PT, F-VIII and α_2-AP were lower than those of the control group ($P < 0.05$). The levels of HCY, Fbg, AT-Ⅲ, PC, α_2-AP, FDP, and D-D are higher in T_2-T_4 breast cancer patients than in T_{is}-T_1 breast cancer patients, PT and ATPP levels are higher in T_{is}-T_1 breast cancer patients; Fbg, AT-Ⅲ, α_2-AP levels are increased in breast cancer patients with lymph node metastasis. Among premenopausal breast cancer patients, HCY, Fbg, and D-D levels were increased in patients with T_2-T_4 stage ($P < 0.05$); Fbg, AT-Ⅲ, and α_2-AP levels were increased in patients with lymph node metastasis ($P < 0.05$). In postmenopausal patients, the levels of PT (SEC), AT-Ⅲ, PC, α_2-AP, FDP, and D-D in the T_2-T_4 stage are higher than those in the T_{is}-T_1 stage, and the levels of AT-Ⅲ and α_2-AP in the lymph node metastasis increased in the number of patients ($P < 0.05$). The abnormal rate of HCY and PT in the triple-negative patients was higher than that in the Luminal A + B group and the HER-2 overexpression group. The abnormal rate of Fbg in the HER-2 overexpression patients was higher than that in the Luminal A + B group and the triple-negative group ($P < 0.05$).

Conclusions

Homocysteine and abnormal blood coagulation are related to the risk of breast cancer, and tumor size, lymph node metastasis and molecular typing in breast cancer patients.

Introduction

According to global cancer incidence and mortality in 2020, there are approximately 19.3 million new cancer cases and nearly 10 million cancer deaths worldwide [1]. There are about 1.67 million newly diagnosed breast cancer cases every year, and its mortality rate is second to lung cancer [2]. It is the leading cause of cancer-related deaths in women worldwide [3].

Tumor growth and metastasis are related to the development of subclinical hypercoagulability in the host [4]. The abnormal activation of the coagulation system in cancer patients is a common cause of tumor recurrence or metastasis. A unique feature of malignant tumors is the pro-coagulation associated with tumor cells.

These properties lead to the activation of the coagulation cascade, the production of thrombin and fibrin, and the stimulation of platelets. Leukocytes and endothelial cells expose their cell procoagulant properties. Some of these mechanisms can promote the development and progression of tumors. In particular, mp-enriched prethrombotic factors and pro-angiogenic factors play an important role in supporting tumor growth [5] [6]. In general, developing tumors participate in many innate physiological processes simultaneously, including wound repair, immune response, blood coagulation, complement cascade, tissue remodeling, and metabolic homeostasis. Abnormal changes in various indicators of coagulation function are during the coagulation process, and it is important in the biological process of tumor occurrence, development, and metastasis [7]. Many studies have confirmed that homocysteine (HCY), prothrombin time (time) (PT), partially activated thrombin time (ATPP), fibrinogen (Fbg), prothrombin time (TT), Antithrombin III (AT-III), protein C(PC), coagulation factor VIII (F-VIII), α_2-antifibrinolytic activity (α_2-AP), fibrinogen degradation products (FDP), D- Dimer (D-D) and platelets (PLT) can be applied as indicators to indicate the state of blood hypercoagulability, and are closely related to the occurrence, development and metastasis of tumors. HCY is a sulfur-containing, non-protein, and toxic amino acid, which exists in the mutual conversion pathway of methionine and cysteine. HCY is metabolized through two different pathways: remethylating and transsulfur. When these two pathways are abnormal, they will cause abnormal HCY metabolism and increase the level of HCY in the body. The hypomethylation of DNA and impaired remethylating and transsulfur are often related to carcinogenesis [8]. Therefore, elevated HCY levels in patients are often closely related to the occurrence and development of cancer.

PT, ATPP, Fbg, TT, AT-III, PC, F-VIII, α_2-AP, FDP, D-D, PLT are important indicators of the activation of the coagulation/fibrinolysis system, and abnormally elevated levels are often observed in cancer patients or lower, and the blood of these patients is in a hypercoagulable state. Thrombin is a terminal coagulation protein that supports the generation and growth of cancer cells; fibrinogen is a dimeric molecule with many functional properties, including the ability to support cell adhesion through integrins and non-integrins. It is considered a molecular bridge between specific receptors on tumor cells and vascular endothelium, supporting tumor cells to adhere to the endothelium of target organs; tumor cells have vigorous procoagulant activity and can form fibrin around tumor cells. Support the proliferation and metastasis of tumor cells and stabilize the adhesion of tumor cells to the blood vessel wall; Fibrin matrix is also believed to contribute to the formation of interstitial tissues (such as tumor vasculature), providing nutrition and gas exchange for the rapid growth of malignant cells; the formation of tumor cells and platelets mediated by the combination of fibrinogen and integrin microthrombus. Microthrombus can be used as a physical barrier to protect tumor cells from being eliminated by natural killer cells, and enhance the metastatic potential of tumor cells. In breast cancer, fibrinogen has been shown to induce the barrier of endothelial cells. Permeability, and enhance the epithelial to mesenchymal transition of malignant

breast cancer cells through a vecadherin-dependent mechanism [9]. D-Dimer (D-D) is a fibrin degradation product, which is produced by the degradation of the cross-linked fibrin XIIIa by the plasmin produced by plasminogen under the action of the serine protease tissue plasminogen activator [10]. In addition, the platelet itself will produce stromal cell-derived factor-1 (SDF-1) SDF-1, it can also act as a chemotactic agent for cancer cells. Therefore, molecules that are up regulated in the early stages of clot formation and proteins that are activated and aggregated by coagulation as supporting inflammatory cell infiltration may also be involved in tumor development. Tumor cell-induced platelet activation, increased expression of tissue factor in tumor-related blood vessels, up-regulation of circulating monocyte factor phagocytic cells, and endogenous fibrinolysis inhibitors are confirmed to relate to higher invasiveness and metastasis, and poor prognosis [9, 11–14].

In this study we analyze the correlation between homocysteine, coagulation function indexes of breast cancer patients and breast cancer risk, clinicopathological characteristics, and molecular typing.

Method

The clinicopathologic and laboratory examination data of 369 female breast cancer patients admitted to Tianjin Cancer Hospital from January 2018 to December 2018 were collected.

Inclusion criteria: new breast cancer patients; preoperative patients receive neoadjuvant chemotherapy, cases after tumor resection were performed in this hospital; There were complete clinical and pathological data.

In the control group, 183 patients with benign breast lesions treated in our hospital during the same period were selected. Comparison of general data of breast cancer patients and control groups is shown in Table 1.

Table 1
General data of breast cancer patients and control group

factors	BC group (n = 369)	Control group (n = 183)	x^2 value	*P* value
Menstnlation status			0.759	0.416
Premenopausal	196(53.1)	90(49.2)		
Postmenopausal	173(46.9)	93(50.8)		
Reproductive history			0.928	0.350
Yes	357(96.7)	174(95.1)		
No	12(3.3)	9(4.9)		
Smoking			0.141	0.789
Yes	10(2.7)	6(3.3)		
No	359(97.3)	177(96.7)		
alcohol consumption			1.496	0.554
Yes	3(0.8)	0(0)		
No	366(99.2)	83(100)		
History of hypertension			2.229	0.139
Yes	65(17.6)	42(23)		
No	304(82.4)	141(77.0)		
History of heart disease			0.014	1.000
Yes	19(5.1)	9(4.9)		
No	350(94.9)	174(95.1)		
History of diabetes			1.651	0.217
Yes	29(7.9)	9(4.9)		
No	340(92.1)	174(95.1)		
Family History of cancer			0.002	1.000
Yes	82(22.2)	41(22.4)		
No	287(77.8)	142(77.6)		

Laboratory examination: collect 5ml of venous blood in the morning after 12 hours of fasting, Roche Cobas c-701, Sysmex CS5100, Sysmex XN2000 instrument and original reagents to exam serum HCY (enzyme colorimetry), plasma PT (SEC) (coagulation method), PT (INR) (coagulation method), ATPP (coagulation method), Fbg (coagulation method), TT (coagulation method), AT-III (chromogenic substrate method), PC (hair Color substrate method), coagulation factor VIII (coagulation method), α_2-AP (coagulation method), FDP (coagulation method), D-D (enzyme-linked immunofluorescence method), PLT (flow cytometry).

Statistical analysis: SPSS20.0 software was used for statistical analysis. Continuous variables were described by $\bar{x}\pm s$, and *t test* was used to compare the differences between the two groups. D-D were non-normal distribution data, described with Median and compared by Mann-Whitney U test. categorical variables were compared by chi-square test and Fisher's exact test. Logistic regression analysis was used to analyze the risk of breast cancer. $P < 0.05$ indicates that the difference with statistical significances (two-side).

Results

Patients:

A total of 369 breast cancer patients were included in this study, with an average age of 51.39 ± 11.53 years old, of which 173 were premenopausal and 196 were postmenopausal. There were 110 cases at Tis-T1 stage, 259 cases at T2-T4 stage, 306 cases at N0 stage, and 63 cases at N1-N3 stage. The control group included 183 cases with an average age of 50.27 ± 14.15 years, including 93 cases of premenopausal and 90 cases of postmenopausal. The age, birth history, menstruation status, smoking history, and alcohol consumption of the two groups has no significant difference.

Comparison of coagulation function between breast cancer patient group and control group.

The serum levels of HCY, Fbg, and D-D in the breast cancer group were higher than those in the control group, while the levels of PT (SEC), PT (INR), AT-III, PC, F-VIII, and α_2-AP were lower than those of control group, and the differences were statistically significant ($P < 0.05$). The levels of AT-III, PC and α_2-AP in the premenopausal breast cancer group were lower than those in the control group, and the differences were statistically significant ($P < 0.05$). The levels of HCY, Fbg, and D-D in the postmenopausal breast cancer patient group were higher than those in the control group; the levels of PT (SEC), F-VIII, and α_2-AP were lower than those in the control group, and the differences were statistically significant ($P < 0.05$) (Table 2).

Table 2
Comparison of coagulation function between breast cancer patient group and control group

					Perimenopause				Menopause Group		
Project	Case group	control group	t value	*P* value	Case group	control group	t value	*P* value	Case group	control group	t valu
HCY	13.40 ± 5.38	11.54 ± 3.81	4.696	0.000	12.36 ± 4.75	11.43 ± 4.67	1.525	0.128	14.33 ± 5.73	11.65 ± 2.66	5.3
PT(SEC)	10.25 ± 0.52	10.47 ± 0.92	2.996	0.003	10.34 ± 0.54	10.44 ± 0.66	1.412	0.159	10.17 ± 0.49	10.49 ± 1.14	2.5
PT(INR)	0.92 ± 0.72	0.94 ± 0.16	2.277	0.024	0.93 ± 0.68	0.97 ± 0.14	1.801	0.074	0.91 ± 0.073	0.93 ± 0.17	1.3
ATPP	23.67 ± 4.92	23.42 ± 2.70	0.768	0.443	24.67 ± 6.06	24.20 ± 2.94	0.855	0.393	22.78 ± 3.41	22.61 ± 2.15	0.5
Fbg	2.59 ± 0.81	2.46 ± 0.51	2.054	0.040	2.48 ± 1.00	2.37 ± 0.50	0.970	0.333	2.69 ± 0.57	2.55 ± 0.51	2.0
TT	19.63 ± 1.09	19.70 ± 1.05	0.736	0.462	19.58 ± 1.20	19.61 ± 1.19	0.243	0.808	19.67 ± 098	19.79 ± 0.89	0.9
AT-III	94.26 ± 18.26	98.07 ± 12.32	2.551	0.011	92.02 ± 17.52	96.50 ± 12.71	2.175	0.031	96.24 ± 18.70	99.70 ± 11.75	1.6
PC	112.34 ± 21.46	117.38 ± 22.64	2.507	0.012	105.60 ± 24.13	112.47 ± 19.98	2.483	0.014	119.30 ± 15.59	121.71 ± 23.98	1.0
F-VIII	135.38 ± 31.13	144.69 ± 37.52	2.899	0.004	128.74 ± 30.47	132.90 ± 34.62	1.010	0.313	141.24 ± 30.59	156.88 ± 36.68	3.7
α_2-AP	112.84 ± 17.73	122.52 ± 15.49	6.294	0.000	113.71 ± 16.40	122.33 ± 16.83	4.048	0.000	122.07 ± 18.83	122.72 ± 14.06	4.7
FDP	2.70 ± 0.98	2.75 ± 0.66	0.675	0.500	2.62 ± 0.89	2.52 ± 0.56	1.037	0.301	2.77 ± 1.04	2.97 ± 0.68	1.7
D-D*	269.68(62.86 ~ 5757.11)	246.98(82.04 ~ 866.49)	3.012	0.003	242.01(62.86 ~ 4141.9)	214.75(8.04 ~ 866.49)	1.774	0.077	347.53(132.67 ~ 5757.11)	328.28(142.03 ~ 851.00)	2.0
PLT	262.27 ± 63.88	257.07 ± 61.91	0.911	0.363	269.16 ± 64.382	265.37 ± 69.94	0.443	0.658	256.19 ± 62.57	248.49 ± 51.33	1.0

*D-D described with Median and compared by Mann-Whitney U test.

Comparison Of Abnormal Coagulation Rate Between Breast Cancer Patient Group And Control Group

The abnormal rates of HCY, PT (SEC), ATPP, FDP, D-D in the breast cancer patient group were higher than those in the control group; the abnormal rates of F-VIII and α_2-AP were lower than those in the control group, and the differences were statistically significant ($P < 0.05$) (Table 3).

Table 3
Comparison of abnormal coagulation rate between breast cancer patient group and control group

Project	Case group (n = 369)	control group (n = 183)	X^2 value	P value
HCY	118(32.0%)	30(16.4%)	15.142	0.000
PT(SEC)	36(9.8%)	1(0.5%)	16.592	0.000
PT(INR)	15(4.1%)	2(1.1%)	3.620	0.068
ATPP	171(46.3%)	64(35.0%)	6.467	0.013
Fbg	49(13.3%)	22(12.0%)	1.352	0.509
TT	26(7.0%)	17(9.3%)	0.857	0.399
AT-III	14(3.8%)	2(1.1%)	3.171	0.104
PC	4(12.2%)	17(9.3%)	1.036	0.390
F-VIII	106(28.7%)	81(44.3%)	13.181	0.000
α_2-AP	107(29%)	91(49.7%)	22.852	0.000
FDP	11(3.0%)	0(0.0%)	5.566	0.019
D-D	57(15.4%)	13(7.1%)	7.690	0.006
PLT	29(7.9%)	16(8.7%)	0.128	0.724

Logistic regression for coagulation function and the risk of breast cancer.

HCY [OR=0.914 (0.875-0.955)], PT (SEC) [OR=1.581 (1.215-2.0561)], PT (INS) [OR=11.824 (1.767-79.109)], Fbg[OR= 0.708 (0.507-0.990)], AT-III [OR=1.015 (1.003-1.028)], PC [OR=0.990 (0.982-0.998)], F-VIII [OR=1.008 (1.003-1.014)], α_2- AP[OR=1.037 (1.025-1.050), D-D[OR=0.999 (0.998-1.000)] are independent risk factors of breast cancer (Table 4).

Table 4
The correlation between coagulation function indexes and the risk of breast cancer.

Project	Single factor		multi-factor	
	OR(95%)	P value	OR(95%)	*P* value
HCY	0.914(0.875–0.955)	0.000	0.934(0.889–0.982)	0.007
PT(SEC)	1.581(1.215–2.056)	0.001	1.513(1.128–2.030)	0.006
PT(INR)	11.824(1.767–79.109)	0.011	14.084(1.750-113.332)	0.013
ATPP	0.986(0.943–1.031)	0.525		
Fbg	0.708(0.507–0.990)	0.044	0.459(0.290–0.725)	0.001
TT	1.064(0.902–1.254)	0.461		
AT-III	1.015(1.003–1.028)	0.014	1.014(0.999–1.028)	0.063
PC	0.990(0.982–0.998)	0.014	0.978(0.968–0.988)	0.000
F-VIII	1.008(1.003–1.014)	0.002	1.013(1.006–1.020)	0.000
α_2-AP	1.037(1.025–1.050)	0.000	1.048(1.032–1.065)	0.000
FDP	1.062(0.872–1.293)	0.551		
D-D	0.999(0.998-1.000)	0.020	0.998(0.997-1.000)	0.020
PLT	0.999(0.996–1.002)	0.363		

Correlation analysis of coagulation function and clinical TNM staging in breast cancer patients.

The levels of PT (SEC) and ATPP in breast cancer patients are higher in T_{is}-T_1 than in T_2-T_4; Fbg, AT-III, PC, α_2-AP, FDP, and D-D levels are lower in T_{is}-T_1 than in T_2-T_4. The differences were statistically significant (P<0.05) (Table 5). The levels of Fbg, AT-III, and α_2-AP in breast cancer patients were lower in stage N_0 than

in stages N_1-N_3, and the differences were statistically significant (P<0.05) (Table 6).

Table 5
Correlation analysis of coagulation function and clinical T staging in breast cancer patients

Project	T_{is}-T_1	T_2-T_4	*t* value	*P* value
HCY	12.53 ± 4.89	13.69 ± 5.34	1.991	0.047
PT(SEC)	10.53 ± 0.85	10.21 ± 0.54	3.722	0.000
PT(INR)	0.92 ± 0.06	0.91 ± 0.08	0.953	0.341
ATPP	24.48 ± 6.83	23.27 ± 3.61	2.271	0.024
Fbg	2.46 ± 0.48	2.62 ± 0.59	2.794	0.006
TT	19.77 ± 1.22	19.63 ± 1.06	1.151	0.250
AT-III	91.65 ± 15.32	96.09 ± 19.20	2.208	0.028
PC	114.49 ± 17.87	119.15 ± 24.04	2.104	0.036
F-VIII	134.06 ± 30.82	13819 ± 32.49	1.163	0.245
α_2-AP	109.48 ± 19.36	115.42 ± 17.20	2.993	0.003
FDP	2.53 ± 0.71	2.76 ± 1.04	2.550	0.011
D-D*	253.49(62.8 ~ 1736.31)	289.25(84 ~ 575.11)	2.969	0.003
PLT	260.74 ± 61.94	267.50 ± 69.34	0.906	0.366
*D-D described with Median and compared by Mann-Whitney U test.				

Table 6
Correlation analysis of coagulation function index and clinical N staging in breast cancer patients

Project	N_0	N_1-N_3	*t* value	P value
HCY	13.45 ± 5.47	12.83 ± 3.80	0.848	0.397
PT(SEC)	10.32 ± 0.68	10.29 ± 0.58	0.238	0.812
PT(INR)	0.91 ± 0.07	0.92 ± 0.73	0.691	0.490
ATPP	23.59 ± 5.14	23.7 ± 2.82	0.595	0.553
Fbg	2.53 ± 0.55	2.83 ± 0.61	3.951	0.000
TT	19.69 ± 1.10	19.55 ± 1.17	0.900	0.369
AT-III	93.06 ± 18.97	103.60 ± 10.05	4.226	0.000
PC	117.13 ± 23.12	120.72 ± 18.74	1.142	0.254
F-VIII	137.51 ± 33.39	134.71 ± 23.70	0.789	0.432
α_2-AP	112.31 ± 17.48	119.90 ± 19.55	3.050	0.002
FDP	2.67 ± 0.92	2.83 ± 1.15	1.235	0.218
D-D*	263.38(62.86 ~ 5757.11)	331.33(116.23 ~ 1376.65)	0.478	0.633
PLT	266.59 ± 68.51	259.73 ± 61.09	0.730	0.466
*D-D described with Median and compared by Mann-Whitney U test.				

Comparison of coagulation function in clinical TNM staging of breast cancer patients with different menstrual status.

The levels of HCY, Fbg, and D-D in patients with premenopausal breast cancer in T_{is}-T_1 stage were lower than those in T_2-T_4 stages; Fbg, AT-III, and α_2-AP levels in N_0 stage were lower than those in N_1-N_3 stages, and the differences were statistically significant ($P < 0.05$) (Table 7). Postmenopausal breast cancer patients PT (SEC) level in T_{is}-T_1 stage is higher than T_2-T_4 stage; AT-Ⅲ, PC, α_2-AP, FDP, D-D level in T_{is}-T_1 stage is lower than T_2-T_4 stage; The levels of AT-Ⅲ and α_2-AP in breast cancer patients were lower in N_0 stage than in N_1-N_3 stage, and the difference was statistically significant ($P < 0.05$) (Table 8).

Table 7
Comparison of coagulation function in premenopausal breast cancer patients by TNM stage

Project	Tis-T1	T2-T3	t value	P value	N0	N1-N3	t value	*P* value
HCY	11.41 ± 3.99	12.79 ± 4.88	2.030	0.044	12.39 ± 4.79	12.05 ± 3.78	0.331	0.741
PT (SEC)	10.44 ± .50	10.31 ± 0.59	1.430	0.154	10.37 ± 0.55	10.30 ± 0.68	0.428	0.672
PT (INR)	0.93 ± 0.06	0.92 ± 0.07	0.659	0.511	0.92 ± 0.06	0.92 ± 0.08	0.185	0.855
ATPP	25.52 ± 9.00	24.06 ± 3.65	1.553	0.122	24.60 ± 6.32	24.12 ± 2.70	0.371	0.711
Fbg	2.32 ± 0.47	2.51 ± 0.57	2.281	0.024	2.39 ± 0.49	2.84 ± 0.72	3.998	0.000
TT	19.74 ± 1.34	19.53 ± 1.12	1.136	0.257	19.65 ± 1.21	19.24 ± 1.07	1.576	0.117
AT-III	91.53 ± 13.07	93.39 ± 19.73	0.660	0.510	91.29 ± 18.46	101.98 ± 8.80	2.783	0.006
PC	113.40 ± 17.59	114.36 ± 21.02	0.305	0.761	113.98 ± 20.27	113.86 ± 18.08	0.027	0.978
F-VIII	129.83 ± 29.19	131.83 ± 34.63	0.382	0.703	131.70 ± 34.48	129.08 ± 20.13	0.530	0.599
α_2-AP	112.94 ± 20.06	116.37 ± 16.09	1.243	0.216	113.89 ± 17.81	122.89 ± 11.81	2.393	0.018
FDP	2.55 ± 0.75	2.66 ± 0.92	0.792	0.430	2.58 ± 0.75	2.91 ± 1.42	1.122	0.272
D-D*	206.94(62.86 ~ 926.91)	253.28(84.67 ~ 4141.92)	2.210	0.028	235.16(62.16 ~ 4141.92)	331.33(116.23 ~ 1375.85)	1.598	0.112
PLT	272.02 ± 70.44	276.03 ± 72.01	0.335	0.723	278.19 ± 69.95	252.72 ± 79.13	1.104	0.271
*D-D described with Median and compared by Mann-Whitney U test.								

Table 8
Comparison of the level of coagulation function in postmenopausal breast cancer patients by TNM stage

Project	Tis-T1	T2-T3	*t* value	*P* value	N0	N1-N3	t value	*P* value
HCY	13.71 ± 5.46	14.44 ± 5.60	0.845	0.399	14.45 ± 5.89	13.33 ± 3.78	1.104	0.271
PT(SEC)	10.62 ± 1.10	10.13 ± 0.48	3.21	0.000	10.27 ± 0.78	10.29 ± 0.52	0.141	0.888
PT(INR)	0.91 ± 0.05	0.90 ± 0.08	0.515	0.607	0.90 ± 0.07	0.91 ± 0.07	1.286	0.200
ATPP	23.41 ± 3.09	22.61 ± 3.44	1.528	0.128	22.64 ± 3.44	23.71 ± 2.93	1.750	0.082
Fbg	2.61 ± 0.46	2.72 ± 0.59	1.336	0.184	2.66 ± 0.56	2.83 ± 0.53	1.629	0.105
TT	19.80 ± 1.10	19.72 ± 0.99	0.537	0.592	19.73 ± 0.98	19.76 ± 1.20	0.133	0.894
AT-III	91.77 ± 17.46	98.35 ± 18.52	2.312	0.022	94.73 ± 19.34	106.65 ± 10.77	3.013	0.003
PC	115.62 ± 18.25	123.15 ± 25.70	2.020	0.045	120.11 ± 25.22	125.17 ± 18.02	1.154	0.250
F-VIII	138.43 ± 32.09	143.51 ± 29.67	1.073	0.285	143.01 ± 31.46	138.36 ± 25.34	0.841	0.401
α2-AP	105.89 ± 18.09	114.61 ± 18.10	3.088	0.002	110.81 ± 17.08	117.95 ± 23.20	2.145	0.033
FDP	2.51 ± 0.67	2.85 ± 1.13	2.653	0.009	2.75 ± 1.05	2.78 ± 0.96	0.168	0.867
D-D*	325.86(140.06 ~ 1736.31)	328.86(132.67 ~ 5757.11)	2.002	0.047	323.47(132.67 ~ 5757.11)	335.65(167.55 ~ 743.59)	0.485	0.628
PLT	249.07 ± 49.66	260.35 ± 66.42	1.162	0.247	255.63 ± 65.47	264.21 ± 46.55	0.761	0.448
**D-D described with Median and compared by Mann-Whitney U test.								

Comparison of coagulation function and molecular typing in breast cancer patients.

The abnormal rate of HCY in the LuminalA + B group was higher than that of the HER-2 overexpression group and the triple-negative group; the abnormal rate of PT (SEC) and PT (INR) in the triple-negative group was higher than that of the LuminalA + B group. HER-2 overexpression group; the abnormal rate of Fbg in the HER-2 overexpression group was higher than that in the LuminalA + B group and the triple-negative group, and the difference was statistically significant ($P < 0.05$) (Table 9).

The level of HCY, D-D in triple negative type group was higher than that in LuminalA + B group and her-2 over-expression group. The PT(SEC) level in Luminal A + B group was higher than that in her-2 overexpression group and triple negative type group. TT level in her-2 over-expression group was higher than that in

LuminalA + B group and Tri-yin type group, with statistical significance ($P < 0.05$)(Table 10).

Table 9
Comparison of coagulation function abnormal rate and molecular typing in breast cancer patients

Project	LuminalA + B	Her-2 over-expression	Triple negative	χ^2	*P* value
HCY	76(28.1%)	21(38.9%)	21(46.7%)	7.470	0.024
PT(SEC)	18(6.7%)	8(14.8%)	10(22.2%)	12.440	0.002
PT(INR)	7(2.6%)	6(11.1%)	6(13.3%)	13.715	0.001
ATPP	122(45.2%)	30(55.6%)	19(42.2%)	2.296	0.317
Fbg	5(1.9%)	4(7.4%)	3(6.7%)	6.314	0.043
TT	18(6.7%)	5(9.3%)	3(6.7%)	0.473	0.789
AT-III	10(3.7%)	2(3.7%)	2(4.4%)	0.059	0.971
PC	28(10.4%)	8(14.8%)	9(20.0%)	3.746	0.154
F-VIII	78(28.9%)	11(20.4%)	17(37.8%)	3.646	0.162
α_2-AP	76(28.1%)	18(33.3%)	13(28.9%)	0.588	0.745
FDP	10(3.7%)	1(1.9%)	0(0.0%)	2.108	0.348
D-D	38(14.1%)	8(14.8%)	11(24.4%)	3.195	0.202
PLT	23(8.5%)	2(3.7%)	4(8.9%)	1.516	0.469

Table 10
Comparison of coagulation function level and molecular typing in breast cancer patients

Project	LuminalA + B	Her-2 ver-expression	Triple negative	*t* value	*P* value
HCY	13.23 ± 5.19	13.79 ± 4.79	14.94 ± 5.90	4.152	0.042
PT(SEC)	10.29 ± 0.49	10.17 ± 0.54	10.08 ± 0.56	4.009	0.019
PT(INR)	0.92 ± 0.07	0.89 ± 0.08	0.91 ± 0.09	2.612	0.075
ATPP	23.71 ± 5.28	23.66 ± 3.66	23.45 ± 3.99	0.053	0.949
Fbg	2.55 ± 0.58	2.49 ± 0.43	2.72 ± 0.63	2.173	0.115
TT	19.57 ± 1.08	19.98 ± 1.20	19.58 ± 0.95	3.351	0.036
AT-III	93.94 ± 18.67	94.51 ± 18.32	95.91 ± 15.80	0229	0.796
PC	116.21 ± 21.78	119.25 ± 24.93	120.45 ± 27.32	0.912	0.403
F-VIII	134.40 ± 29.82	133.91 ± 24.57	142.98 ± 43.29	1.538	0.216
α2-AP	112.95 ± 18.82	112.61 ± 13.38	112.45 ± 15.73	0.021	0.979
FDP	2.70 ± 1.03	2.61 ± 0.90	2.78 ± 0.70	0.365	0.694
D-D*	262.68(29.53 ~ 4141.92)	259.175(140.06 ~ 1404.57)	353.95(130.04 ~ 5757.11)	5.868	0.003
PLT	263.31 ± 63.12	25.33 ± 72.14	266.76 ± 58.13	0.674	0.510
*D-D described with Median and compared by Mann-Whitney U test.					

Discussion

Breast cancer is one of the major causes of cancer deaths in women worldwide [15]. As the malignancy of tumors increases, the body's hypercoagulable state may be a response to tumor cell invasion and metastasis [16]. Patients with advanced breast cancer have significantly increased coagulation abnormalities and thromboembolic events, which are important factors affecting the quality of life and survival outcomes of patients [17].

It has been reported in the literature that approximately 50% of cancer patients and 94% of metastatic patients exhibit abnormal coagulation/fibrinolysis system indicators [18, 19]. In our study, we observed that the HCY level was abnormally increased in breast cancer patients. When comparing the abnormality rate, we also found that the abnormality rate of HCY in the patient group was higher than that in the control group, compared with Hasan T, Rehman T, etc. The research results [8, 20] are consistent. Wu et al. [21]. showed that in premenopausal and postmenopausal women, high concentrations of HCY mainly play a pathogenic role through the metabolic accumulation of intracellular SAH. SAH is a catechol-O-methyltransferase(COMT)-mediated endogenous and exogenous catechin, which is a strong non-competitive inhibitor of phenol methylation metabolism. The oxidative metabolites of estrogen, including catechol estrogen and 16a-OHE1, are involved in the development of estrogen-induced tumors and human breast cancer in some animal models. COMT methylation is

the inactivation of catechol estrogen. Therefore, when we analyzed the HCY levels of patients on different menstrual status, we found that the HCY levels in the postmenopausal group were significantly higher than those in the control group. Research on HCY and breast cancer risk factors found that HCY level is associated with a higher risk of breast cancer, and the higher the HCY level, the higher the risk of breast cancer. HCY can be used as an independent factor of breast cancer risk. Subsequently, we grouped breast cancer patients with different TNM stages and found that in the premenopausal patient group, the HCY level of T_2-T_3 patients was significantly higher than that of T_{is}-T_1 patients. Thus, the hormone levels in the patients may affect the HCY levels in the body. Thereby affecting the size of the tumor. However, the mechanism needs to be studied in further. In its molecular typing study, it was found that the abnormal rate of HCY in triple-negative breast patients was higher than that of LuminalA + B group and HER-2 overexpression group, which was consistent with the results of Naushad SM et al.[22][23]. In the study of the coagulation/fibrinolysis system indicators, it was found that the levels of PT, AT-III, PC, F-VIII, and α_2-AP were lower in the breast cancer patient group than in the control. The levels of Fbg and D-D in the breast cancer patient group were significantly higher than those in the control group. The levels of AT-III, PC and α_2-AP in the premenopausal group were significantly reduced in breast cancer patients; the levels of F-VIII and α_2-AP in the postmenopausal group showed a decreasing trend in breast cancer patients. The levels of Fbg and D-D showed a significant increase in breast cancer patients in the postmenopausal group. The abnormal rate of PT, ATPP, FDP, and D-D in the breast cancer patient group was significantly higher than that of the control group, and the abnormal rate of F-VIII and α_2-AP in the breast cancer patient group was significantly lower than that of the control group. In the study of the correlation between the coagulation/fibrinolysis system indicators and the risk of breast cancer, we found that PT, Fbg, AT-III, PC, F-VIII, α_2-AP, FDP, D-D, PLT were independent factors related to the risk of breast disease. We analyzed breast cancer patients with different TNM stages and found that in T stage, breast cancer patients with T_{is}-T_1 stage had higher PT and ATPP levels than those with T_2-T_4 stages; The levels of Fbg, AT-III, PC, α_2 -AP, FDP and D-D are higher in patients with stage T_2-T_4 than those with stage T_{is}-T_1, indicating that the increase in coagulation/fibrinolysis index levels may be related to the size of the tumor. In the N stage, the levels of Fbg, AT-III, and α_2-AP are higher in N_1-N_3 stage patients than in N_0 stage patients, which is consistent with the results of Yu et al. [24]., Previous studies [9, 25–27] have shown that shortening of PT and APTT can directly promote thrombosis in patients with malignant tumors, because circulating tumor cells can survive in single cell and can metastasize distantly. Fbg in tumors micrometastasis also plays an important role. It can provide necessary materials and locations for tumor cell micrometastasis, while avoiding tumor cells from being attacked by immune cells; D-D, as the final product of fibrinogen hydrolysis, is used in the differential diagnosis of breast cancer. In our study, it was correlated with clinicopathological characteristics, which are consistent with the results of this study. When analyzing the TNM staging of breast cancer patients in the postmenopausal and premenopause groups, it was found that in the T-stage of the premenopause patients, the Fbg level was higher in the T_2-T_3 stage than in the T_{is}-T_1 stage in the premenopause group, while this did not occur in the postmenopausal group. In the postmenopausal patient group, PT levels are higher in T_{is}-T_1 patients than in T_2-T_3 patients, and AT-III, PC, α_2-AP, and FDP levels are higher in T_2-T_3 patients than T_{is}-T_1 patients. The level of D-D was higher than that of T_{is}-T_1 patients in T_2-T_3 stage patients in the premenopausal group or the postmenopausal group. In the N stage, Fbg levels in the premenopausal group are higher in the N_1-N_3 stage patients than in the N_0 stage patients, but no change was observed in the postmenopausal patients; AT-III and α_2-AP levels are in the premenopausal group and the postmenopausal group N_1-N_3 stage patients are higher than N_0 stage patients. Studies have shown that coagulation/fibrinolysis index levels are irrelevant to the size and metastasis of tumors in breast cancer patients, based on different hormone levels in the patients [28, 29]. The changes in the hormone levels in the patients may cause or exacerbate the abnormality of the coagulation/fibrinolysis system indicators in patients, thereby further inducing and promoting the occurrence, development and metastasis of breast cancer.

The PT in the triple-negative patient group was significantly higher than the Luminal A + B group and the HER-2 overexpression group, while the Fbg in the HER-2 overexpression group was higher than that of the LuminalA + B group and the triple-negative breast Group, consistent with the research results reported in the existing literature [30, 31],indicating that HCY□PT □Fbg□D-D indicators are closely related to the molecular typing of breast cancer, and can better predict the occurrence, development, and metastasis risk and prognosis of breast cancer.

Conclusion

HCY and hypercoagulable state are closely related to the risk of breast cancer. In breast cancer patients, the increase in HCY and changes in hypercoagulable state indicators are closely related to tumor size, lymph node metastasis and molecular typing, but these correlations Affected by the hormone levels in the patient.

Declarations

Ethics approval and consent to participate: This research project was approved by the Ethics Committee of Tianjin Cancer Institute and Hospital. Written consents were obtained from each patient.

Availability of data and material : The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication: Written consents were obtained from each patient to publishing their pathological images as represent Figures.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [zihao Liu],[na Zhang],[zhihong Zhao],[fangxuan Li],[shixia Li] and [xin Ding].The first draft of the manuscript was written by [zihao Liu] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing interests: All authors declare that they have no conflicts of interest.

Acknowledge: None

References

1. Sung H, Ferlay J, Siegel R L, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries[J]. CA: a cancer journal for clinicians,2021,71(3):209–249.
2. Barrios C H, Reinert T, Werutsky G. Global Breast Cancer Research: Moving Forward[J]. American Society of Clinical Oncology educational book,2018,38:441.
3. Torre L A, Siegel R L, Ward E M, et al. Global Cancer Incidence and Mortality Rates and Trends—An Update[J]. Cancer Epidemiology Biomarkers & Prevention,2016,25(1):16–27.
4. Giaccherini C, Marchetti M, Masci G, et al. Thrombotic biomarkers for risk prediction of malignant disease recurrence in patients with early stage breast cancer[J]. Haematologica (Roma),2020,105(6):1704–1711.
5. Xie W Z, Leibl M, Clark M R, et al. Activation of the coagulation system in cancerogenesis and metastasation[J]. Biomedicine & Pharmacotherapy,2005,59(3):70–75.
6. Falanga A, Marchetti M, Vignoli A. Coagulation and cancer: biological and clinical aspects[J]. Journal of Thrombosis and Haemostasis,2013,11(2):223–233.
7. Pitteri S J, Kelly-Spratt K S, Gurley K E, et al. Tumor Microenvironment–Derived Proteins Dominate the Plasma Proteome Response during Breast Cancer Induction and Progression[J]. Cancer Research,2011,71(15):5090–5100.
8. Hasan T, Arora R, Bansal A K, et al. Disturbed homocysteine metabolism is associated with cancer[J]. Experimental & Molecular Medicine,2019,51(2):1–13.
9. Krenn-Pilko S, Langsenlehner U, Stojakovic T, et al. An elevated preoperative plasma fibrinogen level is associated with poor disease-specific and overall survival in breast cancer patients[J]. The Breast,2015,24(5):667–672.
10. Khangarot S S, Gupta N, Goswami B, et al. Correlation of D dimer and factor VIII levels with histopathology in patients with breast carcinoma[J]. Cancer Biomarkers,2011,7(6):305–314.
11. Shu Y J, Weng H, Bao R F, et al. Clinical and prognostic significance of preoperative plasma hyperfibrinogenemia in gallbladder cancer patients following surgical resection: a retrospective and in vitro study[J]. BMC Cancer,2014,14:566.
12. Mego M, Karaba M, Minarik G, et al. Relationship between Circulating Tumor Cells, Blood Coagulation, and Urokinase-Plasminogen-Activator System in Early Breast Cancer Patients[J]. The Breast Journal,2015,21(2):155–160.
13. Walsh M, Moore E E, Moore H, et al. Use of Viscoelastography in Malignancy-Associated Coagulopathy and Thrombosis: A Review[J]. Seminars in Thrombosis and Hemostasis,2019,45(04):354–372.
14. Gu M, Zhai Z, Huang L, et al. Pre-treatment mean platelet volume associates with worse clinicopathologic features and prognosis of patients with invasive breast cancer[J]. Breast Cancer,2016,23(5):752–760.
15. Koizume S. Breast cancer phenotypes regulated by tissue factor-factor VII pathway: Possible therapeutic targets[J]. World Journal of Clinical Oncology,2014,5(5):908.
16. Mi X K, Liu Q R, Zhu L, et al. Mechanism of the high coagulation state of breast cancer tissue factor[J]. Eur Rev Med Pharmacol Sci,2017,21(9):2167–2171.
17. Gerotziafas G T, Taher A, Abdel Razeq H, et al. A Predictive Score for Thrombosis Associated with Breast, Colorectal, Lung, or Ovarian Cancer: The Prospective COMPASS–Cancer-Associated Thrombosis Study[J]. The Oncologist,2017,22(10):1222–1231.
18. Kirwan C C, Descamps T, Castle J. Circulating tumour cells and hypercoagulability: a lethal relationship in metastatic breast cancer[J]. Clinical and Translational Oncology,2020,22(6):870–877.
19. Qi Y, Fu J. Research on the coagulation function changes in non small cell lung cancer patients and analysis of their correlation with metastasis and survival[J]. J BUON,2017,22(2):462–467.
20. Rehman T, Shabbir M A, Inam Ur Raheem M, et al. Cysteine and homocysteine as biomarker of various diseases[J]. Food Science & Nutrition,2020,8(9):4696–4707.
21. Wu X, Zou T, Cao N, et al. Plasma homocysteine levels and genetic polymorphisms in folate metablism are associated with breast cancer risk in chinese women[J]. Hered Cancer Clin Pract,2014,12(1):2.
22. Zhang H, Fredericks T, Xiong G, et al. Membrane associated collagen XIII promotes cancer metastasis and enhances anoikis resistance[J]. Breast Cancer Research,2018,20(1).
23. Naushad S M, Reddy C A, Kumaraswami K, et al. Impact of Hyperhomocysteinemia on Breast Cancer Initiation and Progression: Epigenetic Perspective[J]. Cell Biochemistry and Biophysics,2014,68(2):397–406.
24. Mei Y, Zhao S, Lu X, et al. Clinical and Prognostic Significance of Preoperative Plasma Fibrinogen Levels in Patients with Operable Breast Cancer[J]. PLOS ONE,2016,11(1):e146233.
25. Mi X K, Liu Q R, Zhu L, et al. Mechanism of the high coagulation state of breast cancer tissue factor[J]. Eur Rev Med Pharmacol Sci,2017,21(9):2167–2171.
26. Corsi M P, De Martinis M, Di Leonardo G, et al. [Blood coagulation changes and neoplastic pathology][J]. Recenti Prog Med,2000,91(10):532–537.
27. Lu Y, Zhang L, Zhang Q, et al. The association of D-dimer with clinicopathological features of breast cancer and its usefulness in differential diagnosis: A systematic review and meta-analysis[J]. PLOS ONE,2019,14(9):e221374.

28. Lowe G D, Rumley A, Woodward M, et al. Epidemiology of coagulation factors, inhibitors and activation markers: the Third Glasgow MONICA Survey. I. Illustrative reference ranges by age, sex and hormone use[J]. Br J Haematol,1997,97(4):775–784.
29. Sumino H, Ichikawa S, Sawada Y, et al. Effects of hormone replacement therapy on blood coagulation and fibrinolysis in hypertensive and normotensive postmenopausal women[J]. Thrombosis Research,2005,115(5):359–366.
30. Giaccherini C, Marchetti M, Masci G, et al. Thrombotic biomarkers for risk prediction of malignant disease recurrence in patients with early stage breast cancer[J]. Haematologica,2020,105(6):1704–1711.
31. Wen J, Yang Y, Ye F, et al. The preoperative plasma fibrinogen level is an independent prognostic factor for overall survival of breast cancer patients who underwent surgical treatment[J]. The Breast,2015,24(6):745–750.