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The bicarbonate status of the Chinese primary breast cancer patients with different clinicopathology and molecular subtypes

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Abstract: *This study aimed to investigate the bicarbonate status of Chinese primary breast cancer patients with different clinicopathology and molecular subtypes. A total of 4834 primary breast cancer women and 583 women with breast benign tumors were included from the China Database for Concomitant Disease of Breast Cancer (CDCDBC). Breast cancer patients had significantly lower serum bicarbonate levels compared to women with a benign breast tumor (serum bicarbonate: 24.6±2.50 mmol/L vs 25.2±2.36 mmol/L, P<0.001; the proportion of low bicarbonate status: 25.22% vs 15.69%, P<0.001). Patients with T3/T4-stage had the highest serum bicarbonate levels (T3/T4 vs Tis: Z = -2.944, P < 0.01; T3/T4 vs T1/T2: Z = 4.610, P < 0.001). In the T2 stage, the distribution interval of serum bicarbonate levels in lymph node metastases of ≥ 3, and no lymph node metastases was statistically different (P < 0.01). There are statistical differences in serum bicarbonate values among Luminal A, Luminal B, HER2 overexpression, and Triple-negative breast cancer (TNBC)(Z = 8.330, P = 0.040). The TNBC patients had lower serum bicarbonate levels compared to that in HER2 overexpression-type patients (serum bicarbonate: P<0.01; the proportion of low bicarbonate status: 26.74% vs 21.80%, P<0.05). The ER (+) patients had lower serum bicarbonate levels compared to the ER (-) patients (serum bicarbonate: 24.68±2.52 mmol/L vs 25.03±2.50 mmol/L, P<0.05; the proportion of low serum bicarbonate: 24.02% vs 20.96%, P <0.01), while the PR (+) patients had lower serum bicarbonate levels compared to the PR (-) patients (serum bicarbonate: 24.55±2.52 mmol/L vs 25.02±2.48 mmol/L, P<0.05; the proportion of low serum bicarbonate: 26.48% vs 19.23%, P <0.01) with T1 stages.Breast cancer patients with different clinicopathology and molecular subtypes have different bicarbonate status and those patients with higher T-stages or larger tumor lesions are more acidic.*

Keywords: *Breast cancer, Serum bicarbonate, T- stage, Clinicopathological feature*

1. Introduction

Breast cancer has become the most prevalent malignant tumor and the second leading cause of cancer-related death among women in recent years. One of the important factors for the development and treatment of breast cancer is that the malignant cells release excessive protons, which lower the pH of the extracellular environment of the tumor cells. The extracellular environment pH of malignant tumors is often acidic (ranging from 6.5 to 6.9), while the normal range is 7.2–7.4[1]. Studies have shown that pH regulators are more highly expressed on the plasma membrane of human breast cancer tissues than on normal breast epithelial cells. Moreover, it is reported that the gene expression of pH regulators may vary depending on the molecular subtype of breast cancer[2, 3]. The extracellular pH environment of malignant tumors has been extensively studied, as it is related to various cellular mechanisms, including carbonate dehydrogenase[4], vacuolar ATPase[5], and Na+/H+ exchange[6], which are the main contributors to tumor acidity. Due to the view that acidic pH can greatly enhance the invasion and metastasis of malignant tumor cells, some studies have proposed that neutralizing acidic tumor pH can inhibit tumor cell invasion and slow down tumor metastasis and spread[7]. A recent study has investigated the increased heterogeneity of bicarbonate transfer protein NBDT (Na-driven bicarbonate transporters) expression under hypoxia conditions. It was reported that targeted NBDT may reduce the risk of metastasis in triple-negative breast cancer (TNBC). The metastatic potential provided by the acidic extracellular environment of tumors has also been demonstrated in

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both animals and humans, as evidenced by increased filopodia formation and participation in the expression of invading proteolytic enzymes under acidic culture conditions[8]. Tumor cells exposed to acidic culture for a long time may have increased invasiveness and cathepsin B activity. Carbonic anhydrase (CA)[9-11], Na+/H+ exchange isomer1, SLC9A1 (NHE1), Na^+, HCO_3^-cotransporter NBCn1 (SLC4A7)[12-17] are considered to be the most important factors affecting the acidity of the tumor microenvironment. It has been reported in previous studies that oral bicarbonate can inhibit the metastasis of tumors derived from cell lines of prostate, breast, and colon cancer[18]. However, the relationship between serum bicarbonate levels and tumor size, type, and malignancy degree in breast cancer patients is still unclear. Therefore, this article will investigate the clinicopathological and molecular subtype features of initially diagnosed Chinese breast cancer women with different bicarbonate statu

2. Patients and methods

This retrospective study included 4,834 female patients with initially diagnosed primary breast cancer without distant metastasis from the China Database for Concomitant Disease of Breast Cancer (CDCDBC) and 583 women with breast benign tumors who both undergone serum bicarbonate detection in Chongqing Breast Cancer Center, from November 2012 to July 2020. The exclusion criteria were as follows: age < 18 years old, serum bicarbonate values were absent, metastatic breast cancer, history of other malignant tumors, kidney disease, and other diseases that may affect the level of bicarbonate. Venous blood samples from all participants were collected after fasting for at least 8 hours and analyzed using standard laboratory procedures in the Clinical Laboratory of the First Affiliated Hospital of Chongqing Medical University, which is accredited by the College of American Pathologists (CAP No.7215494). All breast ultrasounds are checked and evaluated by a sonographer at the Health Examination Center. The pathological diagnosis and immunohistochemical results were provided by professional pathologists in the Department of Pathology of Chongqing Medical University.

The Bicarbonate Liquid (CO2-L) method was used for the detection of serum bicarbonate, and the relevant clinical reference interval of serum bicarbonate was 23-29mmol/L. Low levels (less than 23 mmol/L), normal levels (23-29 mmol/L), and high levels of serum bicarbonate (> 29 mmol/L) are classified.

Body Mass Index (BMI) is a person's weight in kilograms (kg) divided by the square of height in meters (m^2)[19]. BMI classification was calculated according to the BMI standard for Asians established by the World Health Organization: underweight (<18.5 kg/m^2), normal (≥ 18.5 kg/m^2, and < 23.0 kg/m^2), overweight (≥23.0 kg/m^2, and < 25.0 kg/m^2) and obesity (≥ 25.0 kg/m^2). According to TNM staging criteria of the eighth edition of the American Joint Board on Cancer Staging Manual[20], T staging: TIS: ductal carcinoma in situ (DCIS); T1: The maximum tumor size is less than or equal to 20 mm; T2: Tumor > 20 mm, but maximum size ≤ 50 mm; T3: Tumor > 50 mm; T4: Tumors of any size that extend directly into the chest wall and/or skin (ulcers or macroscopic nodules); Dermal invasion alone does not qualify for T4. The status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth receptor (HER2) was determined by immunohistochemistry (IHC). If ER/PR is expressed in total tumor cells and staining in ≥ 1% of the nucleus is defined as positive[3]. As for HER2: + negative, ++ uncertain, +++ positive. Cancers with Her-2 2+ should be evaluated additionally by fluorescence in situ hybridization (FISH). In the study, breast cancer was divided into the following five groups: Luminal A (ER and/or PR positive/HER2 negative/low Ki-67), luminal B (ER and/or PR positive/HER2 negative/high Ki-67), HER2 positive (ER and PR negative /HER2 overexpression, ER and/or PR positive/HER2 overexpression/any Ki-67) and triple negative (ER and PR negative /HER2 negative)[21].

3. Statistical analysis

All raw data were entered into Microsoft Excel 2010 for categorization and summary. The mean ± SD or 95% CI of the normally distributed variables and the proportion of categorical variables were used to describe patient characteristics. The Chi-square, unmatched T-test, and Mann-Whitney U test were used to compare the characteristics of the patients. All tests were bilateral, and the obtained data were recorded, statistically tested, and calculated by SPSS 26.0. $P < 0.05$ was considered statistically significant

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4. Main results

1) Serum Bicarbonate in Patients with Malignant Tumor and Benign Breast Tumor:

A total of 4,834 female patients with initially diagnosed breast cancer and 583 female patients with benign breast tumors were enrolled in this study. There was no statistical difference in baseline demographic characteristics (including age, laboratory data of liver function and kidney function, and nutritional status) between the two groups in terms of basic data (TABLE 1). The serum bicarbonate-related electrolytes were lower in the breast cancer group than in the benign group, while there was little difference in the liver and kidney function tests between the two groups. The mean serum bicarbonate level in the breast cancer group (24.6 ± 2.50 mmol/L) was lower than that in the control group (25.2 ± 2.36 mmol/L), which was statistically significant ($P < 0.001$). The rate of less than normal value (23 mmol/L) of serum bicarbonate (25.22%) in the breast cancer group was higher than that in the benign group (15.69%), which was statistically significant ($P < 0.001$). There were statistical differences in the overall distribution of bicarbonate values between benign and malignant patients ($Z = -3.595$, $P < 0.001$), and breast cancer patients had a much lower distribution of bicarbonate values.

Table 1: Baseline data characteristics of Chinese women with breast cancer or breast benign tumor

baseline data	breast cancer (n=4834)	breast benign tumor(n=583)	P-value
Age,y	55.40±11.21	56.86±8.55	.020
Height,cm	156.68±5.25	158.01±5.13	<.0.001
Weight,kg	57.58±8.19	56.89±8.56	.093
BMI,kg/m^2	23.53±3.20	22.93±3.31	.003
Serum bicarbonate, mmol/L	24.69±2.50	25.16±2.36	<.0.001
Na, mmol/L	142.23±2.31	141.53±2.48	.001
K, mmol/L	4.04±0.32	4.07±0.33	.0.07
Ca, mmol/L	2.28±0.10	2.30±0.11	.004
Mg, mmol/L	0.86±0.06	0.87±0.05	.001
P, mmol/L	1.19±0.25	1.23±0.17	.003
Cl, mmol/L	104.93±2.51	105.19±2.37	.019
eGFR, ml/min	106.62±15.38	108.04±15.01	.006
Urea,mmol/L	5.11±1.67	4.92±1.39	.010
Crea,umol/L	60.35±10.867	59.21±9.75	.017
UA,umol/L	274.80±67.62	271.46±66.06	.263
Cys-c,mg/L	0.86±0.29	0.83±0.16	.082
PA,mg/L	228.79±40.35	221.95±40.09	.003
TP,g/L	70.50±5.78	69.75±5.74	.003
Alb,g/L	42.97±4.20	41.80±4.22	<.0.001
GLB,g/L	26.84±3.93	26.64±3.78	.379
A/G	1.67±0.27	1.64±0.30	.085
TBIL,umol/L	10.63±5.44	10.31±4.79	.283
DBIL,umol/L	4.35±2.79	4.07±2.00	.023
IBIL,umol/L	7.76±4.93	7.51±4.91	.469
ALT,U/L	20.13±18.89	21.13±15.08	.219
AST,U/L	20.27±13.89	21.41±13.57	.090
ALP,U/L	68.93±26.50	65.97±25.89	.012
GGT,U/L	23.44±14.55	22.53±12.51	.584
LDH,U/L	220.95±146.56	265.20±152.85	<.0.001
CHE,U/L	8117.20±1729.32	7680.49±1630.33	<.0.001
Hb,g/L	126.86±12.61	125.59±12.16	.024

Data are presented as mean ± SD and percentage. $p < 0.05$ consider significantly different between 2 groups

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2) Serum Bicarbonate of Breast Cancer Patients with Different T Stages:

4834 initially diagnosed breast cancer patients with TNM staging Tis: 205 patients, T1:1796 patients, T2:2054 patients, T3:255 patients, T4:61 patients (TABLE 2/TABLE 3). Multi-group Kruskal-Wallis test ($Z = 23.245$, $P < 0.001$) suggested that the overall distribution interval of serum bicarbonate level in breast cancer patients with different T stages was different, and the serum bicarbonate in patients with higher T stages was much concentrated in the lower interval level, and the proportion of patients with higher T-stage was higher. The T3 group was higher than the Tis group ($Z = -3.161$, $P = 0.002$), the T3 group was higher than the T1 group ($Z = 4.709$, $P < 0.001$), the T3 group was higher than the T2 group ($Z = 4.384$, $P = 0.001$), and the overall distribution of serum bicarbonate value was statistically different. The author considered that the sample size of the T4 group was relatively insufficient due to the small number of samples, so the tumor size of 5 cm was used as the boundary, and the tumor was regrouped into the carcinoma in situ group (Tis group), T12 (T1 + T2) group, and T34 (T3 + T4) group. Compared with the Tis group ($Z = -2.944$, $P < 0.01$) and the T12 group ($Z = 4.610$, $P < 0.001$), the overall distribution of serum bicarbonate value in the T34 group was significantly lower than that in the other two groups. The T34 group had a much higher rate of serum bicarbonate value of less than the normal value of serum bicarbonate. There was no significant difference in the overall distribution of serum bicarbonate between the Tis group and the T12 group ($Z = -0.81$, $P = 0.936$). Compared with the Tis group, the T12 group, and the T34 group, there was a statistical difference in the proportion of serum bicarbonate value of less than the normal value of serum bicarbonate ($P < 0.001$). The T34 group was higher than the Tis group ($P < 0.001$), The T34 group was higher than the T12 group ($P < 0.001$), but there was no statistical difference in serum bicarbonate value between the Tis group and the T12 group. In the T2 stage, the distribution interval of serum bicarbonate levels in lymph node metastases of ≥ 3, and no lymph node metastases was statistically different ($P < 0.01$).

Table 2: Basic situation of serum bicarbonate value in patients with different T stages

T stage	Tis(n=205)	T1(n=1796)	T2(n=2054)	T3(n=255)	T4(n=61)
Mean value	24.76±2.35	24.79±2.49	24.73±2.51	24.08±2.30	24.35±2.64
<23mmol/L	39(19.02%)	407(22.66%)	504(24.53%)	92(36.08%)	19(31.15%)
23mmol/L~25mmol/L	77(37.56%)	551(30.68%)	587(28.58%)	71(27.84%)	18(29.51%)
25mmol/L~27mmol/L	55(26.83%)	495(27.56%)	578(28.14%)	67(26.27%)	13(21.31%)
27mmol/L~29mmol/L	25(12.20%)	265(14.76%)	285(13.88%)	19(7.45%)	10(16.49%)
≥29mmol/L	9(4.39%)	78(4.34%)	100(4.87%)	6(2.35%)	1(1.64%)

Table 3: Statistical analysis of serum bicarbonate values in breast cancer patients with different T stages

Group	Z value	P value	X^2	P value
Tis-T1	0.253	.997	1.405	.136
Tis-T2	-0.074	.998	3.103	.045
Tis-T3	-3.161	.016	16.227	.0001
Tis-T4	-0.878	.997	4.052	.036
T1-T2	0.745	.997	1.867	.092
T1-T3	4.709	.0001	21.834	.0001
T1-T4	1.126	.997	2.403	.084
T2-T3	4.384	.0001	15.778	.0001
T2-T4	0.944	.995	1.440	.147
T3-T4	-1.128	.997	0.525	.285

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Tis-T12	-0.095	.924	2.334	0.072
T12-T34	-4.599	.0001	20.731	.0001
Tis-T34	9.484	.002	15.725	.0001
Overall	23.245	.0001	26.590	.0001

T12 for T1+T2,T34 for T3+T4,Based on Mann-Whitney U Test, Kruskal-Wallis H Test, or chi-square tests.$p < 0.05$ consider significantly different between 2 groups.

3) Serum Bicarbonate of Different Breast Cancer Subtypes and Molecular Types:

Based on the results of pathological examination and immunohistochemistry, breast cancer types were divided into Luminal A, Luminal B, HER2 overexpression, and Triple-negative breast cancer (TNBC), with statistical differences in the overall distribution of serum bicarbonate values ($Z = 8.330$, $P = 0.040$), as seen in TABLE 4. The overall distribution interval of the HER2-overexpressed bicarbonate group was smaller than that of the triple-negative breast cancer group ($Z = -2.860$, $P < 0.004$), and the proportion of serum bicarbonate value of less than the normal in HER2-overexpressed breast cancer group was also higher than that of triple-negative breast cancer ($P = 0.009$).

It was found that the proportion of serum bicarbonate value of less than the normal value of serum bicarbonate in the HER2-overexpressed T3/T4 group was significantly higher than that in Luminal A ($P < 0.001$) and Luminal B ($P = 0.004$) group, respectively. The proportion of serum bicarbonate value of less than the normal value of serum bicarbonate in the TNBC with T3/T4 staging group was significantly higher than that in the Luminal A group ($P < 0.001$) (TABLE 5/TABLE 6). The status of serum bicarbonate of breast cancer patients with different molecular types of the same T stage was analyzed. The mean value of serum bicarbonate in the Luminal A type group was higher than that in TNBC ($P = 0.013$). The mean value of serum bicarbonate in the HER2 overexpression group was higher than that in the TNBC group ($P = 0.022$) in the T1 group. In T3 + T4 breast cancer patients, there were statistical differences in the overall distribution of serum bicarbonate among the four type groups ($P = 0.041$). The overall distribution of serum bicarbonate in the HER2-overexpression group was lower than that in the Luminal B group ($Z = 2.720$, $P = 0.007$).

There was a statistical difference in the overall distribution of serum bicarbonate values among the four type groups of ER and PR($Z = 11.505$, $P = 0.009$), as seen in TABLE 7. The overall distribution interval of serum bicarbonate in the ER (+) PR (+) group was significantly lower than that in the ER (+) PR (-) group ($Z = 2.115$, $P = 0.034$), and the ER (-) PR (-) group ($Z = 2.286$, $P = 0.022$), respectively. The overall distribution of serum bicarbonate in the ER (-) PR (+) group was significantly lower than that in the ER (+) PR (-) group ($Z = 2.225$, $P = 0.026$) and ER (-) PR (-) ($Z = -2.214$, $P = 0.027$).

There was a statistical difference ($P = 0.012$) in the proportion of serum bicarbonate value of less than the normal value of serum bicarbonate in the four types of ER and PR breast cancer population. After pair-to-pair comparison, the proportion of serum bicarbonate value below the normal value of serum bicarbonate in ER (+) PR (+) group is higher than those in ER (+) (PR (-) group ($P = 0.022$) and ER (-) PR (-) group ($P = 0.045$), but lower than that in ER (-) PR (+) group ($P = 0.026$), respectively. The proportion of serum bicarbonate value of less than the normal value of serum bicarbonate in the ER (-) PR (+) group is higher than that in the ER (+) (-) PR group ($P = 0.011$) and ER (-) PR (-) group ($P = 0.016$), respectively.

There was no difference between the ER (+) group and the ER (-) group. The PR (+) group had a much lower bicarbonate value than the PR (-) group ($p = 0.006$). Only in patients with T1 staging: The mean value of serum bicarbonate in ER (+) group (24.68 ± 2.52 mmol/L) was lower than that in ER (-) group (25.03 ± 2.50 mmol/L) ($t = -2.422$, $P = 0.016$), and the overall distribution interval of serum bicarbonate in ER (+) patients was also lower than that in ER (-) patients ($Z = -2.447$, $P = 0.014$). The mean value of serum bicarbonate in PR (+) group (24.55 ± 2.52mmol/L) was lower than that in PR (-) group (25.02 ± 2.48mmol/L)($t = -3.776$, $P < 0.001$), and the overall distribution interval of serum bicarbonate in PR (+) group was much lower than that in PR (-) group ($Z = -3.501$, $P < 0.001$).; At the same time, the proportion of serum bicarbonate value of less than the normal value of serum bicarbonate in patients with PR (+) was lower than that in PR (-) patients ($P < 0.001$). No positive results were found in the remaining T-stage group. No differences were found in the HER 2 (+) and HER 2 (-) group, or the high or low expression of the Ki-67 group, respectively.

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Table 4: The basic distribution of serum bicarbonate values in different molecular types and ER and PR types of breast cancer

Types	<23mmol/L	normal range (23~29mmol/L)	>29mmol/L	Total
Luminal A	237(24.43%)	696(71.75%)	37(3.81%)	970
Luminal B	101(23.05%)	329(75.11%)	8(1.83%)	438
HER2 overexpression	234(26.74%)	616(70.40%)	25(2.86%)	875
Triple negative	189(21.80%)	638(73.59%)	40(4.61%)	867
ER(+) PR(+)	554(26.08%)	1489(70.10%)	81(3.81%)	2124
ER(+) PR(-)	205(22.55%)	662(72.83%)	42(4.62%)	909
ER(-) PR(+)	9(50%)	9(50%)	0(0%)	18
ER(-) PR(-)	317(23.46%)	969(71.72%)	65(4.81%)	1351

Table 5: T stage of different subtypes of breast cancer

T Stage	Tis	T1	T2	T3	T4	Total
Luminal A	46(5.23%)	468(53.18%)	328(37.27%)	33(3.75%)	5(0.57%)	880
Luminal B	7(1.86%)	145(38.46%)	200(53.05%)	19(5.04%)	6(1.59%)	377
HER2 overexpression	50(6.50%)	259(33.68%)	395(51.37%)	53(6.89%)	12(1.56%)	769
triple negative	16(2.06%)	274(35.35%)	427(55.10%)	51(6.58%)	7(0.90%)	775
ER(+)	97(3.61%)	1228(45.72%)	1231(45.83%)	103(3.8%)	27(1.00%)	2686
ER(-)	54(4.41%)	415(33.88%)	655(53.47%)	87(7.10%)	14(1.14%)	1225
PR(+)	69(3.62%)	914(47.95%)	844(44.28%)	66(3.46%)	13(0.68%)	1906
PR(-)	82(4.09%)	728(36.31%)	1043(52.02%)	124(6.18%)	28(1.40%)	2005

Table 6: Pairwise comparison of T stage in different subtypes of breast cancer

	P value	
1-2	.001	
1-3	.001	
1-4	.001	
2-3	.004	
2-4	.555	
3-4	.001	
Overall	.001	
5-6	.001	
7-8	.001	

Remarks: 1 for Luminal A, 2 for Luminal B, 3 for HER2 overexpression, 4 for triple negative,5 for ER(+),6 for ER(-),7 for PR(+), 8 for PR(-) Based on chi-square tests.$P < 0.05$,there was statistical difference.

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Table 7: The distribution and proportion of serum bicarbonate in breast cancer patients with different subtypes and ER/PR types

	Z	P value	X^2	P value
1-2	0.359	.720	0.312	.313
1-3	1.710	.087	1.291	.139
1-4	-1.227	.220	1.783	.100
2-3	1.009	.313	2.084	.084
2-4	-1.330	.183	0.267	.326
3-4	-2.860	.004	5.789	.009
Overall	8.330	.040	6.152	.104
5-6	-2.115	.034	4.229	.022
5-7	1.883	.060	5.270	.026
5-8	-2.286	.022	3.015	.045
6-7	2.225	.026	7.489	.011
6-8	.099	.921	0.254	.326
7-8	-2.214	.027	6.894	.016
Overall	11.505	.009	11.753	.012

Remarks: 1 for Luminal A, 2 for Luminal B, 3 for HER2 overexpression, 4 for triple negative, 5 for ER(+) PR(+), 6 for ER(+) PR(-), 7 for ER(-) PR(+), 8 for ER(-)PR(-),Based on Mann-Whitney U Test, Kruskal-Wallis H Test, or chi-square tests.$P < 0.05$, there was statistical difference.

5. Discussion

We investigated the serum bicarbonate concentration in breast cancer women and found significant variations in serum bicarbonate levels based on tumor size, cancer subtype, immunohistochemistry, and other factors. This is the first study to focus on serum bicarbonate level and its related clinicopathological and molecular subtype features in Chinese breast cancer women since the theory of tumor acidity was proposed.

Previous studies mainly focused on the pH of the tumor, especially the PH status of the tumor microenvironment. A Systemic administration of bicarbonate to buffer extracellular acidity slowed and inhibited the metastasis of breast cancer in a mouse model. Researchers used bicarbonate and dichloroacetate (DCA) to test PH modification therapy in mouse models of metastatic breast cancer[22]. Previous studies have shown that pre-treatment serum bicarbonate can predict the likelihood of recurrence in patients with local non-small cell lung cancer[23]. Low serum bicarbonate levels increase cardiovascular and cancer mortality in patients with type 2 diabetes[24], and in a large cohort of US adults, the evidence that serum bicarbonate concentrations of below normal range are associated with malignancy-related mortality[25] supports the findings of our study.

In our study, we found that by comparing the test data of a large sample size (TABLE 1), the mean level of serum bicarbonate in breast cancer patients was lower than that in benign breast tumor patients, and the biochemical electrolyte indexes related to serum bicarbonate value were lower than those in benign population. The overall range of serum bicarbonate values in breast cancer women is relatively low, and a higher proportion of patients have serum bicarbonate values below the normal range.Such a finding is noteworthy. The main reason for this result may be malignant tumors, which operate at highly acidic extracellular pH conditions with alkaline cytoplasmic pH conditions greater than 7.4 and 6.7-7.1. Reversal of the pH gradient between the cytoplasm and the extracellular environment promotes a malignant phenotype[26, 27]. The large amount of anaerobic glycolysis of cancer cells affects the internal environment around tumor cells and also affects the pH of the internal environment of patients to a certain extent. As a basic test index, it can reflect the progression of tumors in patients to a certain extent. However, the omission of electrolyte examination in the daily test of the physical examination

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group is also one of the main reasons why this factor has not been paid attention to.

According to the above conjecture, we have analyzed the relation between serum bicarbonate concentration and T staging and found (TABLE 2, 3) that serum bicarbonate levels in breast cancer patients may be related to the size of the tumor and its progression to some extent. Regardless of the mean value, distribution range of serum bicarbonate, and the proportion below the normal range of serum bicarbonate, patients with higher T stage tend to be more acidic. Along with some other systemic diseases, the ability of the body to maintain homeostasis in the internal environment is relatively inferior to that of healthy people, and the body is more susceptible to the influence of the tumor-bearing state. The relationship between the clinical T staging and serum bicarbonate levels in a large sample of breast cancer patients has not been analyzed in previous studies.

We systematically analyzed the relationship between the molecular typing and immunohistochemistry of breast cancer: Differences were found in the four common breast cancer types (TABLE 4.5.6). It is worth noting that although the serum bicarbonate level in patients with HER2 overexpression and TNBC was significantly lower than that in patients with Luminal A and Luminal B, it is interesting to note that the serum bicarbonate level in HER2 overexpression was lower than that in TNBC. However, another Chinese study found that luminal A cancer had the best prognosis, HER2 overexpression type was poor, and TNBC had the worst prognosis[28]. Therefore, after sorting out the T stages of different subtypes, it is due to the significantly higher proportion of T3/T4 populations with HER 2 overexpression type and TNBC in our analyzed samples than in the Luminal A and Luminal B groups. So we can postulate that the lower the serum bicarbonate value, the worse the prognosis. In addition, the ratio of T3/T4 of HER2 overexpression type in the samples was higher than that of TNBC, which would result in the lowest bicarbonate. Studies have shown that HER 2-positive tumors tend to have larger volumes than HER 2-negative tumors, often exhibit more lymph node metastases, and may be associated with short-term disease-free survival in patients[29]. The histopathological grading distribution of TNBC is significantly different from that of patients with luminal A, luminal B, and HER2 overexpression. The longest median survival time of advanced TNBC was 12 months, much lower than that of other advanced breast cancer subtypes[30]. It may be that the higher the degree of malignancy of the tumor, the crazy growth of tumor cells and the increase of aggressiveness, and the larger tumor size has a greater impact on the homeostasis of the internal environment, which also confirms the above analysis of T staging and serum bicarbonate concentration.

Finally, different interesting phenomena were found in the analysis of ER, PR, and other indicators: ER (+) PR (+) patients had lower bicarbonate than ER (+) PR (-) and ER (-) PR (-) patients, and PR (+) patients had lower bicarbonate than PR (-) population. We further did correlation analysis of T stages in different ER and PR situations, and found that the possible reason is that the proportion of T3/T4 in ER (-) and PR (-) groups was significantly higher than that in ER (+) ($p<0.001$) and PR (+) ($p<0.001$) groups. The effect of tumor size may directly affect the results of analysis, and the T-stage was still the most important factor affecting tumor acidity. As for the findings mentioned above only in T1 stages, the patients with ER (+) and PR (+) had lower serum bicarbonate concentration than ER (-) and PR (-), considering the possible reason is that when the tumor is small at an early stage, even though it is less malignant than HER 2+ or TNBC, the cancer cells with hormone receptor-positive cells under estrogen stimulation may be more active than TNBC[31], and the early tumor may even be more active than malignant TNBC. When the tumor develops to a certain size, it is more of its subtype that determines and affects its development.

Our study also had some limitations. First, as a cross-sectional study, we cannot determine the natural course and causality of the effects of breast cancer malignancy and serum bicarbonate concentration. However, our study included large sample size and sufficient clinical data, which allowed us to reduce the influence of potential confounding factors on the study. However, the absence of serum bicarbonate data in the physical examination population is a pity for this study due to the omission of electrolyte examination in the Chinese population with healthy examination, and we have not made progress in the efficacy and prognosis of breast cancer patients. All analysis results suggest that tumor acidity is the driving force of tumor invasion and metastasis[32], as previously mentioned. Tumor acidity plays an important role in the occurrence and development of tumor metastatic potential, providing the basis for clinical data research to a certain extent.

6. Conclusion

In this study, it was found that tumor size (T staging), tumor subtype, and molecular type were the

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main factors affecting the changes in serum bicarbonate concentration in breast cancer patients, and larger tumor lesions had a greater impact on the pH of the body's internal environment, which was more acidic. As a common clinical indicator, the long-term detection of serum bicarbonate can evaluate the tumor progression of cancer patients well, and we recommend that serum bicarbonate be included in routine breast cancer screening. Sodium bicarbonate may be more effective when used as an adjunct to other anticancer therapies. As a possible target for clinical anticancer diagnosis and treatment, serum bicarbonate is worthy of expectation, but more theoretical and practical support is still needed.

Availability of data and materials

The datasets analyzed during the current study are not publicly available because all data were recorded in the electronic medical record system of the Quality Control Center of Health Examination in Chongqing. However, they are available from the corresponding author upon reasonable request. The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

The present study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by The Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval number: 2022-K450)

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References

[1] Quade, B. N., Parker, M. D., & Occhipinti, R. (2021). The therapeutic importance of acid-base balance. Biochemical pharmacology, 183,114278.https://doi.org/10.1016/j.bcp.2020.114278

[2] Tavares-Valente, D., Sousa, B., Schmitt, F., Baltazar, F., & Queirós, O. (2021). Disruption of pH Dynamics Suppresses Proliferation and Potentiates Doxorubicin Cytotoxicity in Breast Cancer Cells. Pharmaceutics, 13(2), 242. https://doi.org/10.3390/pharmaceutics13020242

[3] Ko, M., Quiñones-Hinojosa, A., & Rao, R. (2020). Emerging links between endosomal pH and cancer. Cancer metastasis reviews, 39(2), 519–534. https://doi.org/10.1007/s10555-020-09870-1

[4] Swietach, P., Vaughan-Jones, R. D., & Harris, A. L. (2007). Regulation of tumor pH and the role of carbonic anhydrase 9. Cancer metastasis reviews, 26(2), 299–310. https://doi.org/10.1007/s 10555-007-9064-0

[5] Martinez-Zaguilan, R., Lynch, R. M., Martinez, G. M., & Gillies, R. J. (1993). Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells. The American journal of physiology, 265(4 Pt 1), C1015–C1029. https://doi.org/10.1152/ajpcell.1993.265.4.C1015

[6] Bourguignon, L. Y., Singleton, P. A., Diedrich, F., Stern, R., & Gilad, E. (2004). CD44 interaction with Na+-H+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. The Journal of biological chemistry, 279(26), 26991–27007. https://doi.org/10.1074/jbc.M311838200

[7] Carroll, C. P., Bolland, H., Vancauwenberghe, E.et al (2022). Targeting hypoxia regulated sodium driven bicarbonate transporters reduces triple negative breast cancer metastasis. Neoplasia (New York, N.Y.), 25, 41–52. https://doi.org/10.1016/j.neo.2022.01.003

[8] Martin NK, Robey IF, Gaffney EA, Gillies RJ, Gatenby RA, Maini PK(2012). Predicting the safety and efficacy of buffer therapy to raise tumour pHe: an integrative modelling study. British journal of cancer, 106(7), 1280–1287. https://doi.org/10.1038/bjc.2012.58

[9] Becker H. M. (2020). Carbonic anhydrase IX and acid transport in cancer. British journal of cancer, 122(2), 157–167. https://doi.org/10.1038/s41416-019-0642-z

[10] Mboge, M. Y., Mahon, B. P., McKenna, R., & Frost, S. C. (2018). Carbonic Anhydrases: Role in pH Control and Cancer. Metabolites, 8(1), 19. https://doi.org/10.3390/metabo8010019

[11] Supuran C. T. (2017). Carbonic Anhydrase Inhibition and the Management of Hypoxic Tumors.

ISSN 2706-6819 Vol.6, Issue 4: 51-61, DOI: 10.25236/IJFM.2024.060407

Metabolites, 7(3), 48. https://doi.org/10.3390/metabo7030048
[12] Amith, S. R., & Fliegel, L. (2013). Regulation of the Na+/H+ Exchanger (NHE1) in Breast Cancer Metastasis. Cancer research, 73(4), 1259–1264. https://doi.org/10.1158/0008-5472. CAN-12-4031
[13] Lee, S., Axelsen, T. V., Jessen, N., Pedersen, S. F., Vahl, P., & Boedtkjer, E. (2018). Na^+,HCO_3^--cotransporter NBCn1 (Slc4a7) accelerates ErbB2-induced breast cancer development and tumor growth in mice. Oncogene, 37(41), 5569–5584. https://doi.org/10.1038/s41388-018-0353-6
[14] Boedtkjer E. (2019). Na+,HCO3- cotransporter NBCn1 accelerates breast carcinogenesis. Cancer metastasis reviews, 38(1-2), 165–178. https://doi.org/10.1007/s10555-019-09784-7
[15] Paradiso A, Cardone RA, Bellizzi A, Bagorda A, Guerra L, Tommasino M.et al (2004). The Na+-H+ exchanger-1 induces cytoskeletal changes involving reciprocal RhoA and Rac1 signaling, resulting in motility and invasion in MDA-MB-435 cells. Breast cancer research : BCR, 6(6), R616–R628. https://doi.org/10.1186/bcr922
[16] Boedtkjer E, Moreira JM, Mele M, Vahl P, Wielenga VT, Christiansen PM.et al(2013). Contribution of Na+,HCO3(-)-cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). International journal of cancer, 132(6), 1288–1299. https://doi.org/10.1002/ijc.27782
[17] Lauritzen, G., Jensen, M. B., Boedtkjer, E., Dybboe, R., Aalkjaer, C., Nylandsted, J.et al (2010). NBCn1 and NHE1 expression and activity in DeltaNErbB2 receptor-expressing MCF-7 breast cancer cells: contributions to pHi regulation and chemotherapy resistance. Experimental cell research, 316(15), 2538–2553. https://doi.org/10.1016/j.yexcr.2010.06.005
[18] Gorbatenko, A., Olesen, C. W., Boedtkjer, E., & Pedersen, S. F. (2014). Regulation and roles of bicarbonate transporters in cancer. Frontiers in physiology, 5, 130. https://doi.org/10.3389/fphys. 2014.00130
[19] Caballero B. (2019). Humans against Obesity: Who Will Win? Advances in nutrition (Bethesda, Md.), 10(suppl_1), S4–S9. https://doi.org/10.1093/advances/nmy055
[20] Armando E Giuliano 1, James L Connolly 2, Stephen B Edge 3Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual,CA Cancer J Clin. 2017 Jul 8; 67(4):290-303
[21] Goldhirsch, A., Wood, W. C., Coates, A. S., Gelber, R. D., Thürlimann, B., Senn, H. J, et al (2011). Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of oncology: official journal of the European Society for Medical Oncology, 22(8), 1736–1747. https://doi.org/10.1093/annonc/mdr304
[22] Robey, I. F., & Martin, N. K. (2011). Bicarbonate and dichloroacetate: evaluating pH altering therapies in a mouse model for metastatic breast cancer. BMC cancer, 11, 235. https://doi.org/10. 1186/1471-2407-11-235
[23] Sebastian, N., Wu, T., Driscoll, E., Willers, H., Kelly, S., Musunuru, H. B. et al (2019). Pre-treatment serum bicarbonate predicts for primary tumor control after stereotactic body radiation therapy in patients with localized non-small cell lung cancer. Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology, 140, 26–33. https://doi.org/10. 1016/j.radonc.2019.05.014
[24] Li, Y., Gao, R., Zhao, B., & Zhang, Y. (2022). Low Serum Bicarbonate Levels Increase the Risk of All-Cause, Cardiovascular Disease, and Cancer Mortality in Type 2 Diabetes. The Journal of clinical endocrinology and metabolism, 107(11), 3055–3065. https://doi.org/10.1210/clinem/dgac504
[25] Al-Kindi, S. G., Sarode, A., Zullo, M., Rajagopalan, S., Rahman, M., Hostetter, T.et al (2020). Serum Bicarbonate Concentration and Cause-Specific Mortality: The National Health and Nutrition Examination Survey 1999-2010. Mayo Clinic proceedings, 95(1), 113–123. https://doi.org/10.1016/j. mayocp. 2019.05.036
[26] Webb, B. A., Chimenti, M., Jacobson, M. P., & Barber, D. L. (2011). Dysregulated pH: a perfect storm for cancer progression. Nature reviews. Cancer, 11(9), 671–677. https://doi.org/10. 1038/nrc 3110
[27] Ko, M., Quiñones-Hinojosa, A., & Rao, R. (2020). Emerging links between endosomal pH and cancer. Cancer metastasis reviews, 39(2), 519–534. https://doi.org/10.1007/s10555-020-09870-1
[28] Jia, W. J., Jia, H. X., Feng, H. Y., Yang, Y. P., Chen, K., & Su, F. X. (2014). HER2-enriched tumors have the highest risk of local recurrence in Chinese patients treated with breast conservation therapy. Asian Pacific journal of cancer prevention: APJCP, 15(1), 315–320. https://doi.org/10.7314/ apjcp.2014.15.1.315
[29] Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu

ISSN 2706-6819 Vol.6, Issue 4: 51-61, DOI: 10.25236/IJFM.2024.060407

oncogene. Science (New York, N.Y.), 235(4785), 177–182. https://doi.org/10.1126/science.3798106
[30] Dent, R., Trudeau, M., Pritchard, K. I., Hanna, W. M., Kahn, H. K., Sawka, C. A,et al (2007). Triple-negative breast cancer: clinical features and patterns of recurrence. Clinical cancer research: an official journal of the American Association for Cancer Research, 13(15 Pt 1), 4429–4434. https://doi.org/10.1158/1078-0432.CCR-06-3045
[31] Bhardwaj, P., Au, C. C., Benito-Martin, A., Ladumor, H., Oshchepkova, S., Moges, R,et al. (2019). Estrogens and breast cancer: Mechanisms involved in obesity-related development, growth and progression. The Journal of steroid biochemistry and molecular biology, 189, 161–170. https://doi.org/10.1016/j.jsbmb.2019.03.002
[32] Robey, I. F., Baggett, B. K., Kirkpatrick, N. D., Roe, D. J., Dosescu, J.et al. (2009). Bicarbonate increases tumor pH and inhibits spontaneous metastases. Cancer research, 69(6), 2260–2268. https://doi.org/10.1158/0008-5472.CAN-07-5575