Research Article

Analytical Study on Relationship between Cell-Free Plasma DNA Concentration and Clinicopathological Features in Breast Cancer

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Received: 26 December 2019; **Accepted:** 06 January 2020; **Published:** 15 January 2020

Citation: Song-Hak Kim, Un-Rim Sim, Chong-Won Ri , Hui-Myong Pak, Chol-Ho Ri, Yong-Suk Kim, Myong-Nam Kim. Analytical Study on Relationship between Cell-Free Plasma DNA Concentration and Clinicopathological Features in Breast Cancer. Archives of Internal Medicine Research 3 (2020): 026-031.

Abstract

Background: Breast cancer is the most fatal form of cancer for female population and non-invasive method for early detection of human cancers is a promising way to provide more quality health service to the population. Objectives: In this study, we made basic data that can evaluate the progression of breast cancer by explaining the relationship between cell-free plasma DNA concentration and clinicopathological features in breast cancer.

Methods: Cell free DNA levels were measured in plasma of breast cancer, breast benign disease and healthy people.

Results: Cell-free plasma DNA concentration in breast cancer was significantly higher than in healthy individuals and breast benign disease. ($p<0.001$, $p<0.001$, $p=0.002$) Plasma free DNA concentration was significantly higher as the size of tumor was getting bigger, as tumor differentiation was getting lower, and as pTNM stage was getting higher. And also it was higher in a lymph node positive than negative

(p<0.001). Postsurgical plasma free DNA concentration was significantly lower than presurgical concentration. (p<0.001) So we could estimate lymph node metastasis and effect of operation as well as development and progression in breast cancer.

Conclusion: Plasma free DNA concentration is related to progression and metastasis and it can be used as a remarkable biomarker to assess progression of breast cancer, lymph node metastasis and effect of the operation.

Keywords: Breast Cancer; Cell-Free DNA; Plasma Free DNA; Clinicopathological Features

1. Introduction

Many studies have reported that the cell-free DNA level was low in the serum or plasma of healthy people, but it was high in patients with cancer or apparent diseases [1]. Cell-free DNA is released from tumor and normal cells by different mechanisms, including necrosis and apoptosis making it a challenging analyte owing to its high degree of fragmentation [2 ,3 ,4]. Cell-free DNA in cancer patients may be either tumor derived or released from injured host blood or vascular tissues by both apoptotic and necrotic cell death [5, 6]. So circulating cell-free DNA is a important biomarker for early detection of cancer, residual disease, monitoring chemotherapy and other aspects of cancer management [7]. As a result of this, by explaining the relationship between plasma free DNA concentration and clinicopathological features, we attached importance to making basis by which can assess progression of breast cancer.

1.1 Subjects

From September, 2016 to August, 2017, the subjects used in this study were 108 patients with breast cancer who were diagnosed primary breast cancer and received breast cancer surgery at the oncology institution. And there were 36 patients with fibroadenosis, 12 patients of phyllodes tumor and 39 relative healthy volunteers.

2. Methods

We set the clinicopathological features and collected data in accordance with document and consultation, clinicopathography. Plasma free DNA was separated using AxyPrepTM Multi source Genomic DNA Miniprep Kit. Plasma free DNA concentration was measured using Nano-drop for nucleic concentration measurement.

3. Results and Discussion

On the basis of that Plasma free DNA concentration is related to progression and metastasis and it can be used as a remarkable biomarker for treatment effect and prognosis assessment [7, 8, 9, 10, 11, 12] we attached importance to making basis by which can assess progression of breast cancer, lymph node metastasis and effect of operation by explaining the relationship between plasma free DNA concentration and clinicopathological features.

3.1 Plasma free DNA concentration in relative healthy people and patients with breast benign disease and breast cancer

Plasma free DNA concentration was significantly higher in patients with fibroadenosis(117.3 ± 8.5ng•mL^{-1}), phyllodes tumor (224.0 ± 81.4 ng•mL^{-1}), breast cancer (636.9 ± 33.7ng•mL-1) than in realative healthy individuals (53.6 ± 3.9ng•mL^{-1}) (p<0.001). It was significantly higher in phyllodes tumor and breast

cancer than in fibroadenosis (p=0.028, p<0.001) and significantly higher in breast cancer than in phyllodes tumor. (p=0.002) (Table 1). So measurement of plasma free DNA concentration can help us differentiate breast benign disease and breast cancer.

3.2 Relationship between cell-free plasma DNA concentration and clinical features in breast cancer

Table 2 and 3 shows the concentration of plasma free DNA with age, family history of cancer, menstruation related indices, Body Mass Index (BMI) as an obesity index in breast cancer. Correlation coefficient with age was -0.171, so there was somewhat reversed correlation. And the Plasma free DNA concentration was significantly higher in patients aged under 40 (734.5 ± 92.4 ng•mL-1) than aged after 50 (556.1 ± 36.1 ng•mL-1)(p<0.039) (Table 2). There was no significant difference between presence and absence of cancer family history (Table 2). There was no significant difference between presence and absence of menopause, but there was a falling tendency in patients with menopause (Table 2). And then there was no relationship between plasma free DNA concentration and menopausal age (r=-0.038) (Table 3). There was no correlation between plasma free DNA concentration and BMI, the menarche, age in patients with breast cancer. (r=0.036, 0.051) (Table 3) And we examined the relationship between plasma free DNA concentration and related pregnancy, delivery, abortion indices. There was no correlation between plasma free DNA concentration and the numbers of pregnancy, delivery and abortion (r=-0.05, 0.07, 0.062).

3.3 Relationship between cell-free plasma DNA concentration and pathological features in breast cancer

Table 4 shows the concentration of plasma free DNA as tumor size, lymph node metastasis, tumor differentiation, pTNM stage in breast cancer. As shown in table 4, plasma free DNA concentration was significantly higher in more than 2cm tumor (538.3 ± 30.3ng•mL-1), compared to less than 2cm tumor (431.1 ± 34.1ng•mL-1) (p=0.024). So we could determine that the size of tumor affects the concentration of plasma free DNA. And we think that the larger the size of tumor is, the more the proliferative and necrotizing tumor cells are. As shown in table 4, plasma free DNA concentration was significantly higher in group with lymph node positive (629.0 ± 45.3ng•mL-1) than in group with lymph node negative (434.6 ± 22.7ng•mL-1) (p<0.001). So we could determine that these features could be used in explaining the presence of lymph node. And we think the tumor related free DNA is released in metastasized lymph node as well as local tumor and it affects the whole plasma free DNA concentration. As shown in table 4, plasma free DNA concentration was significantly higher in low and undifferentiation (533.9 ± 27.4ng•mL-1) than in high and middle differentiation (435.6 ± 23.9ng•mL-1) (p<0.001). As shown in table 4, plasma free DNA concentration was significantly lower in II and I (494.9 ± 29.2 ng•mL-1, 408.8 ± 34.1ng•mL-1) than in III stage (785.0 ± 25.5ng•mL-1) (p<0.001). Our study suggests that the measurement of plasma free DNA may be used for the assessment of breast cancer development and distant metastasis and so on.

3.4 Presurgical and postsurgical cell-free plasma DNA concentration in breast cancer

As shown in Table 5, postsurgical plasma free DNA concentration(162.7 ± 11.4ng•mL-1) was significantly

lower than the presurgical concentration (496.0 ± 23.4ng•mL-1) ($p<0.001$). So we could decide the effect of operation through the measurement of plasma free DNA concentration. We could conclude that Plasma free DNA concentration may be used for assessment of breast cancer development, progression, distant metastasis as well as the effect of the operation and prognosis and it can be used as a remarkable biomarker in clinical practice.

Division	**No.**	**Plasma free DNA concentration (ng/ml blood)**		***P1***	***P2***	***P3***
		$\overline{X} \pm SE$	**95% CI**			
Realative Healthy People	39	53.6 ± 3.9	45.7~71.6	-		
Fibroadenosis	36	117.3 ± 8.5	99.6~134.9	<0.001	-	
Phyllodes	12	224.0 ± 81.4	114.8~383.2	<0.001	0.028	-
Breast Cancer	108	636.9 ± 33.7	570.1~703.7	<0.001	<0.001	0.002

Table 1: Cell-free plasma DNA concentration in relatively healthy people and breast benign disease and breast cancer.

Features		**No.**	**Plasma free DNA concentration (ng/ml)**		**P1**	**P2**
			$\overline{X} \pm SE$	**95% CI**		
Age	≦39	12	734.5 ± 92.4	531.0~937.9	-	
	40~49	50	627.1 ± 36.5	553.7~700.4	0.226	-
	50>	46	556.1 ± 36.1	483.3~628.7	0.039	0.172
P1; vs patients under 39, *P2;* vs patients aged from 40 to 49						
Family history of cancer	+	18	561.9 ± 52.3	451.6~672.2	0.409	
	-	90	618.9 ± 28.6	562.1~675.8		
P; vs presence of family history						
Presence of menopause	+	48	554.8 ± 35.4	483.5~626.1	0.058	
	-	60	652.0 ± 35.1	581.8~722.1		
P; vs presence						

Table 2: Relationship between cell-free plasma DNA concentration and clinical features (1).

Division	$\overline{X} \pm SE$ **(ng/ml)**	**95% CI**	**Correlation coefficient**
BMI (kg/m^2) (n=108)	21.9 ± 0.1	21.6~22.2	0.036
Plasma free DNA concentration	636.9 ± 33.7	570.0~703.7	
menarche age (n=108)	15.4 ± 0.2	14.9~15.9	0.051

Plasma free DNA concentration	636.9 ± 33.7	570.0~703.7	
menopausal age (n=48)	48.6 ± 0.5	47.7~49.5	-0.038
Plasma free DNA concentration	554.8 ± 35.4	483.5~626.1	

Table 3: Relationship between cell-free plasma DNA concentration and clinical features(2).

Variable	**No.**	**Plasma free DNAconcentration (ng/ml)**		P_1	P_2
		$\overline{X} \pm SE$	**95% CI**		
size					
<2.0	30	431.1 ± 34.1	361.4~500.7	-	
≥2.0	46	538.3 ± 30.3	477.1~599.5	0.024	
Lymph node metastasis					
negative	52	434.6 ± 22.7	388.9~480.2	-	
positive	24	629.0 ± 45.3	535.2~722.8	<0.001	
Differentiation					
High, middle differntiation	22	435.6 ± 23.9	385.9~485.2	-	
Low, undifferntiation	54	533.9 ± 27.4	478.9~588.8	<0.001	
pTNM staging					
I	26	408.8 ± 34.1	338.6~478.9	-	
II	42	494.9 ± 29.2	435.9~553.8	0.065	-
III	8	785.0 ± 25.5	724.7~845.3	<0.001	<0.001
P_1;vs I stage, P_2;vs II stage					

Table 4: Relationship between cell-free plasma DNA concentration and pathological features.

Division	**Plasma free DNA concentration (ng/ml)**		***P***
	$\overline{X} \pm SE$	**95% CI**	
Presurgical	496.0 ± 23.4	449.3~542.6	-
postsurgical	162.7 ± 11.4	139.9~185.4	<0.001

Table 5: Presurgical and postsurgical cell-free plasma DNA concentration (n=76).

4. Conclusion

In summary, we analyzed cell-free plasma DNA concentration and examined the relationship between plasma free DNA concentration and clinicopathological features in breast cancer First, Cell-free plasma DNA concentration in breast cancer was respectively higher by 1188%, 543%, 284% than in healthy individuals, Fibroadenosis and Phyllodes tumor. (p<0.001, p<0.001,

p=0.002) Second, Plasma free DNA concentration was respectively 24%, 23%, 59% higher as the size of the tumor was getting bigger, as tumor differentiation was getting lower, and as pTNM stage was getting higher. And also it was 45% higher, in lymph node positive than negative. (p<0.001) Third, Postsurgical plasma free DNA concentration was significantly lower by 33% than presurgical concentration. (p<0.001) These findings suggest that plasma free DNA concentration may be a useful factor in determining progression of breast cancer, lymph node metastasis and effect of operation. Furthermore, it may be used in devising strategy of breast cancer treatment in the future.

Funding

None

Conflict of Interest

The authors declare that they have no conflict of interest.

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