The Plasma DNA Concentration as a Potential Breast Cancer Screening Marker

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Abstract Increased levels of plasma DNA have frequently been noticed in the blood plasma of cancer patients. The possibility of using plasma DNA level as the indicator of tumor stage in breast cancer was investigated in plasma samples obtained from 100 breast cancer patients and 100 healthy women who were included as controls. Circulatory plasma free DNA was extracted from plasma samples and quantified by fluorometer. The median concentration of plasma DNA in the plasma samples from breast cancer patients classified by TNM staging system as stage I, II, III, IV and breast surgical patients were 0.5, 235, 422, 1,280 and 0.5 ng/ml, respectively. The level of plasma DNA in the stage II- IV group was significantly higher than those in the surgical group with breast cancer and control group (P value < 0.001). The plasma DNA concentration in stage II, III and IV of breast cancer were higher when compared with healthy group. These tumor size, TNM stage and metastasis were significantly correlated with plasma DNA. The cut point of 120 ng/ml was early screening and treatment follow up breast cancer.

Keywords Breast cancer · Stage · Plasma DNA ·
Cut point · Screening

Introduction

Breast cancer is the most common malignant disease in woman and the second leading cause of cancer death in Thailand [1]. Early diagnosis of breast cancer and accurate identification of hematologic metastasis can improve the success of treatment [2–4]. For a definitive diagnosis fine needle aspiration, mammography, ultrasonography and a tumor biopsy is required [5]. Therefore, a marker that can screen the risk of breast cancer by noninvasive and simple method would be useful for all people who are at risk for breast cancer. DNA damage is very frequent and appears to be a fundamental problem of life [6, 7]. The incidence of DNA damage increased during unfavorable conditions such as inflammation [8], cancer [9–11]. Most DNA damages are recovered by DNA repair enzyme, but these repair process are not 100 % efficient [12]. Therefore, nuclear free DNA in the plasma of cancer patients was introduced as a tool for detection and surveillance of cancer [13]. Previously, the amount of circulating DNA was detected by real time quantitative polymerase chain reaction (PCR).

The aim of this study was to determine the concentration of plasma DNA in patients with stage of breast cancer and surgical breast cancer patients. This study presents an easy method of quantification of plasma DNA by fluorometry technique that is sensitive enough to detect small amounts of DNA. Stage of breast cancer was TNM classification of the American Joint Committee on Cancer staging system. The cancer stage and plasma DNA levels in the cohort of breast cancer were compared with those in normal controls. The cut point for screening breast cancer and correlations between the factors that affected plasma DNA in breast cancer patients were revealed.

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Materials and Methods

Samples

In total, 250 peripheral blood samples were analyzed in this study. These samples were from 100 breast cancer patients with and without breast surgery at the Breast Cancer clinic Buddhachinaraj Hospital in Phitsanulok Thailand. The remaining 100 samples from healthy women were used as controls. All diagnoses were biopsy confirmed by pathology. All blood samples were collected by sterile EDTA tubes and started DNA extraction within 2 h [4].

The study was approved by the institutional review board of Naresuan University and Buddhachinaraj Hospital. Written informed consent was obtained in all cases.

DNA Extraction

The blood samples were centrifuged at 800 g for 10 min. The plasma was immediately separated from blood cells and centrifuged again at 1,600 g for 10 min [11]. Cell free DNA was extracted from 240 µl plasma sample using NucleoSpin® Plasma XS (MACHEREY–NAGEL GmbH & Co. KG, Germany) according to the manufacturer's protocol. The DNA preparations were eluted by 20 µl elution buffer. DNA extractions were measured directly.

Quantitative Analysis of Plasma DNA Sample

A total of 5 µl of DNA extraction was mixed with 195 µl Quant-iT™ working solution (1:200 Quant-iT™ reagent in Quant-iT™ buffer). The total solution of 200 µl was mixed for 3 s, incubated for 2 min at room temperature and the concentration of plasma was measured by Qubit™ fluorometer using excitation and emission filter at 502 and 523 nm, respectively. The concentrations of plasma DNA were proportional to the fluorescence signal and expressed as ng/ml.

Statistical Analysis

Data were analyzed with the Mann–Whitney *U* test for comparing the median of the level of plasma DNA in healthy controls, patient groups categorized by TNM stage and surgical breast cancer patients. Spearman rank correlation test and Eta value were performed to indicate the relationship between the level of plasma DNA and each factors. The statistical significance is *P* value < 0.05.

Results

The patients and healthy controls were recruited in the study between June and August 2011. Table 1 shows that

Table 1 Comparison of plasma DNA concentration of the healthy and breast cancer patients

Sample	<i>n</i>	plasma DNA Median (ng/ml)	Range (min–max)	<i>P</i> value ^a
Healthy controls	100	0.5	0.5–148.0	
Patient	100			
Stage I	3	0.5	0.5–218	0.552
Stage II	12	235	100–1,900	<0.001
Stage III	17	422	150–1,500	<0.001
Stage IV	3	1,280	1,010–1,980	<0.001
Surgery breast	65	0.5	0.5–376	0.001
Total	200	0.5	0.5–1,980	

^a Compare with healthy controls by Mann–Whitney *U* test

Table 2 The relationship of plasma DNA concentration with factors of breast cancer patients

Patient	Correlation Eta/r	<i>P</i> value
Age (years)	0.184	0.291
Location of tumor	0.241	0.164
Tumor spread to axillary	0.117	0.504
Tumor size (cm)	0.403	0.016*
TNM stage	0.474	0.046*
Tumor metastasis	0.404	0.011*

* *P*-value of < 0.05 was considered to be a significant correlation

the median concentration of plasma DNA and variation, minimum and maximum, in the samples of healthy, stage I, stage II, stage III, stage IV and breast surgical patients were 0.5, 0.5, 235, 422, 1,280 and 0.5 ng/ml, respectively. The plasma DNA concentrations were significantly higher in patients with stage II–IV and breast surgical group compared with healthy controls (*P* value < 0.001). Except stage I, the concentration of plasma DNA was not significantly different from healthy controls (*P* value = 0.552). Furthermore, the higher stage of breast cancer trend increased plasma DNA concentrations.

The Table 2 shows the relationship between plasma DNA concentrations and variance factors, age, the location of breast tumor (left and right), tumor spread to axillary (No or Yes), tumor size, TNM stage, and tumor metastasis (No or Yes). There were the tumor size (0.40), TNM stage (0.47), and tumor metastasis (0.40) that were significantly correlated with plasma DNA (*P* value < 0.05). Whereas an age, the location of breast tumor and tumor spread to axillary were not significantly correlated with plasma DNA (*P* value > 0.05).

The cut point values of plasma DNA for screening breast cancer were shown in Table 3. The ROC curve analyzes cut points for breast cancer and represents sensitivity, specificity and area under curve (AUC) that measure

Table 3 The cut-off points of plasma DNA for screening breast cancer

Cut-off point of plasma DNA (ng/mL) ^a	Sensitivity (%)	Specificity (%)
90	96.5	94.3
100	97.0	92.7
120	100.0	88.6

^a By ROC curve and AUC was 0.956

the discrimination accuracy. High sensitivity means strong discrimination power of breast cancer patients and high specificity means strong discrimination power of healthy women. Therefore, the proper cut point of plasma DNA was 120 ng/ml because the 100 % sensitivity implied all breast cancer patients were included for screening with this method.

Discussion

The elevated plasma DNA in breast cancer could result from apoptosis [14] and necrosis [15] of tumor tissue. The plasma DNA concentration should be a marker for breast cancer screening that was easily accessible and noninvasive [16]. In addition, high sensitivity and specificity of the plasma DNA with fluorometric method provide a diagnostic advantage in screening. The concentration of plasma DNA correlated with the tumor size, TNM stage, and tumor metastasis. These results are in accordance with previous experiments that tumor size and extent of spread are most important factors in predicting the prognosis in this disease [2].

Elevated levels of plasma DNA were found in Stage II, III and IV. While surgery breast cancer patients had low the plasma DNA concentration (0.5 ng/ml). Out 2 of 65 surgery patients had high concentration (140 and 376 ng/ml), and both patients had undergone operation less than 3 months before blood samples were collected. Therefore, the plasma DNA may retain in plasma that may use for monitoring the success of surgical patients.

We established the proper plasma DNA cut point for breast cancer screening was 120 ng/ml. The sensitivity was 100 % and the specificity was 89 %. This test was easy to perform, low cost and did not need high technology instruments.

Conclusions

Using commercial kit extraction and a portable fluorometer, the level of plasma DNA in patients with breast cancer can be quickly and easily quantified. The plasma DNA

concentration in stage II, III and IV of breast cancer were higher when compared with healthy individuals and patients with occult tumor or monitoring after surgery [4, 17]. Blood sampling is easier and less painful than bone marrow aspiration or other diagnostic procedures. Therefore, it would be very important to develop large scale studies with breast cancer patients and healthy controls that would clarify early breast cancer and recurrent monitoring.

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