

# Plasma DNA as a potential biomarker for breast cancer detection

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## ABSTRACT

Breast cancer is a major malignancy among Indonesian women. It is often diagnosed in the later stages of cancer, which leads to poor prognosis and survival of the patients. This study investigated plasma DNA concentration as a potential biomarker for breast cancer. The benefit of using this detection is the cost-effectiveness and the samples can be collected from patients using non-invasive methods. Plasma samples were obtained from healthy controls (n = 18) and cancer patients (n = 22). Each sample was split into two equal portions for DNA isolation using two different methods for the NaI method and a commercially available kit (Qiagen/ QA) method. The DNA concentration was determined by using a GeneQuant spectrophotometer (Pharmacia). The t-test was used for statistical analysis, which was performed using the SPSS 17.0 software. Compared to the commercial method, extraction using NaI yielded higher DNA concentration, both from samples of healthy controls and cancer patients (p = 0,008 and p = 0.000, respectively). Furthermore, regardless of the isolation method used, the plasma DNA concentration was higher in healthy controls than in cancer cases (p = 0,032 and p = 0.005, for NaI and QA methods, respectively). In conclusion, isolation methods significantly affect DNA concentrations. The plasma DNA concentration of healthy controls is significantly higher than those of the cancer cases, suggesting that plasma DNA concentration might be a potential biomarker for breast cancer detection with less invasive sampling method than tissue biopsies.

## ABSTRAK

Kanker payudara adalah keganasan utama pada wanita di Indonesia. Penyakit ini seringkali terdiagnosis pada stadium lanjut sehingga mengakibatkan rendahnya keberhasilan pengobatan dan ketahanan hidup pasien yang buruk. Studi ini meneliti potensi DNA plasma sebagai penanda biologis untuk mendeteksi kanker payudara. Penggunaan DNA plasma sebagai penanda biologis dianggap menguntungkan mengingat analisisnya yang ekonomis dan menggunakan pendekatan yang tidak invasif. Sampel plasma darah diperoleh dari individu sehat (n = 18) dan pasien kanker payudara (n = 22) setelah mendapat persetujuan dengan menandatangani informed-consent. Setiap sampel dipisahkan menjadi 2 bagian dengan volume yang sama untuk diekstraksi menggunakan 2 metode yang berbeda yaitu metode Na-I dan kit ekstraksi komersial (Qiagen/QA). Konsentrasi DNA plasma diukur dengan spektrofotometer (Genequant, Pharmacia). Analisis statistik t-tes digunakan untuk melihat perbedaan kedua metode tersebut. Metode ekstraksi Na-I menunjukkan konsentrasi DNA yang lebih tinggi dibandingkan kit komersial, baik pada individu sehat (p = 0.008)

maupun pasien kanker payudara ( $p=0.000$ ). Sejalan dengan hasil di atas, konsentrasi DNA plasma pada individu sehat lebih tinggi daripada penderita kanker payudara menggunakan 2 metode yang berbeda (Nal  $p=0.032$  dan kit komersial  $p=0.005$ ). Dapat disimpulkan bahwa metode ekstraksi DNA menentukan konsentrasi DNA yang diperoleh. Terdapat perbedaan bermakna antara konsentrasi DNA individu sehat dan penderita kanker payudara. Hal ini semakin memperkuat potensi DNA plasma sebagai penanda biologis untuk deteksi kanker payudara yang kurang invasif dibandingkan biopsi jaringan.

**Key words** : plasma DNA – biomarker – detection - breast cancer

## INTRODUCTION

Breast cancer is a major malignancy among women in Indonesia and rest of the world. The incidence of breast cancer is increasing in developing countries, and the majority of the cases are diagnosed at an advanced stage, leading to poor therapeutic outcomes.<sup>1</sup> Early and accurate diagnosis of breast cancer can increase therapeutic efficacy.<sup>2</sup> Therefore, early detection of circulating analytes may help clinicians in providing optimal therapy. Plasma DNA can be detected in the blood circulation. Several studies have reported that plasma DNA of cancer patients present genetic and epigenetic changes that are specific to the type of cancer, indicating that this DNA is tumor-derived. Although the precise source of this DNA is still being investigated, it is expected to come from a combination of apoptosis, necrosis, and release by the tumor cells.<sup>3</sup> Quantitative changes in plasma DNA has been observed in several cancers, including the prostate cancer,<sup>4,5</sup> lung cancer,<sup>6</sup> pancreatic cancer,<sup>7</sup> colon cancer,<sup>8,9</sup> ovarian cancer,<sup>10</sup> and breast cancer.<sup>11</sup> High plasma DNA concentration has been correlated with tumor metastasis,<sup>12</sup> response to therapy, and relapse.<sup>13</sup> This suggests that the plasma DNA has the potential to be a tumor-specific biomarker for diagnosis, prognosis, and early detection of cancer.

Methods of plasma DNA isolation range from using a simple manual method to

using the different kits produced by various manufacturers that offer convenience and more efficient time. However, these methods differ in their efficiency. Therefore, in this study we analyzed the performance of a simple manual method of extraction and compared it effectiveness, efficiency, and cost-effectiveness to that of a commercially available method in the isolation and quantification of plasma DNA. This study is important to find a possible alternative biomarker for early detection of cancer diagnosis, prognosis, and therapy monitoring compared to the invasive methods, such as tissue biopsy. Furthermore, the findings of this study can be used to identify the difference of plasma DNA concentration between healthy controls and cancer cases.

## MATERIALS AND METHODS

### Study participants

Healthy participants were recruited from the local Red Cross chapter. The inclusion criteria were as follows: female aged 25 to 35 years, healthy (no history of chronic diseases, such as diabetes mellitus type 2 and/or cardiovascular disease), and a willing to participate in the study by signing the informed consent. The cancer cases were comprised of breast cancer patients admitted to the Dr. Sardjito General Hospital, Yogyakarta between the years 2009 and 2010. The Ethical

Clearance, number KE/FK/625/EC, issued by the Health and Medical Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta), stated that this research meets all ethical requirements. Written informed consent was obtained from all participants included in this study.

### **Plasma DNA Isolation**

Ten mL of blood samples were withdrawn from study participants by using a vacutainer with EDTA. Plasma samples were isolated by centrifuging the vacutainer at 1200 g for 10 minutes. Each sample collected from the controls and patients were divided into two equal portions for extracting DNA using two different methods, namely the NaI method and the extraction method using a commercially available kit (QiaAmp DNA mini kit, cat #51104). The NaI method, which was developed by Ishizawa *et al.* in 1991,<sup>14</sup> involved solubilization of proteins with a high concentration of NaI (chaotropic reagent) and N-lauryl-sarcosinate, followed by the precipitation of the nucleic acids with isopropanol.

### **Plasma DNA Quantification**

The plasma DNA isolated was quantified by using a Gene Quantspectrophotometer (Pharmacia), and the residual samples were stored at 4°C.

### **Statistical Analysis**

Data analysis was performed by using the SPSS 17.0 software. The Student's t-test was used to find the significant differences

between the plasma DNA concentrations obtained using the two isolation methods and to find the significant differences between the plasma DNA concentrations of the controls and the cancer patients. For identifying the significant differences between the plasma DNA concentrations from two isolation methods, a t-test with equal variance was used, whereas a t-test with unequal variance was used for identifying the significant differences between the plasma DNA concentrations of controls and patients.

## **RESULTS**

The previous reports on the source and mechanisms of the sourcing of plasma DNA into the circulation is often contradictory. Nevertheless, it is thought that originated from different sources, including the apoptotic and necrotic cells.<sup>3</sup> It has been suggested that all living cells actively release DNA into the circulation.<sup>15</sup> There are many DNA extraction kits available currently that offer high efficiency fast and easy procedures. However, they are costly. Therefore, we compared the performance of the standardized protocol provided by the commercially available extraction kit from Qiagen to that of the manual protocol developed by Ishikawa.

The result of this study showed that the NaI method is more efficient and cost-effective than the commercial kit. The NaI method yielded higher plasma DNA concentrations. Furthermore, the results showed that regardless of the isolation method, the plasma DNA concentrations in healthy controls were significantly higher than that in the cancer cases (TABLE 1 and FIGURE 1).

TABLE 1. Comparison of plasma DNA concentrations (ng/mL)

Sample	Salting out (NaI)	Commercially available kit (QA)	p
Healthy controls	69.69 ± 31.83	34.04 ± 15.82	0.008*
Cancer cases	40.64 ± 19.14	14.20 ± 4.22	0.000*
p	0.032 <sup>#</sup>	0.005 <sup>#</sup>	

\*Statistical analysis was performed using Student's t-test with equal variance

<sup>#</sup>Statistical analysis was performed using Student's t-test with unequal variance

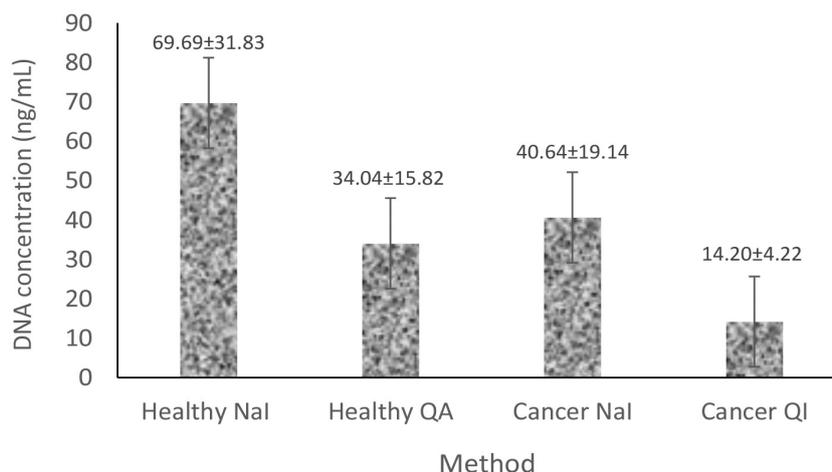


FIGURE 1. Illustration of plasma DNA concentrations

## DISCUSSION

As a highly malignant disease that causes increased mortality rate among women in Indonesia, breast cancer is still a challenge due to the poor diagnosis and prognosis of the disease that could hinder therapy. The routine diagnosis and prognosis of breast cancer typically depends on solid tissue as the source of analysis. Tissue can be harvested only using an invasive surgical methods. It is not always possible to surgically harvest patient's tissue for monitoring the disease and outcome of the therapy. Therefore, an analyte that circulates in the body can be used for non-invasive or minimally invasive diagnostic, prognostic, and monitoring purposes. Plasma DNA is an ideal candidate for these purposes.

Analysis of plasma DNA is a challenging method due to the high degree of DNA fragmentation and its low concentration in the circulation. An earlier study on the source of circulating plasma DNA revealed that although the concentration of DNA in the serum can be 2 to 24 times higher than that in the plasma, plasma is a better source than serum for the analysis of circulating DNA.<sup>16</sup> The higher abundance of DNA in the serum was attributed to the contamination from cells that occur during the clotting process. Therefore, many laboratories recommended the use of plasma as a source of circulating DNA.

Although the ranges of plasma DNA concentrations in healthy individuals and cancer patients remain unknown, this study showed that regardless of the isolation

methods, there is a significant difference between the plasma DNA concentrations of controls and cancer patients. Further studies are needed to fully understand whether the plasma DNA concentrations reflected DNA in the tissues or the clinical manifestations. The non-invasive nature of sample collection and the efficient and cost-effective of its isolation method enable the use of plasma DNA concentration as a potential biomarker for breast cancer. This biomarker can be used for early diagnosis, prognosis, and therapy monitoring purposes.

Majority of the earlier studies found that DNA concentration in the plasma of cancer patients is higher compared to healthy controls. This finding contradictive with our results. The differences in the time elapsed between the sample collection and the start of the isolation procedure likely the reason for this contradiction. The plasma from patients' blood samples were isolated immediately after receiving the samples, while the samples from healthy controls were isolated 2 to 4 weeks later. Therefore, the higher abundance of plasma DNA in healthy controls was likely due to the release of fragmented DNA from lysed cell during this elapsed time.

Selection of an isolation method that ensures the extraction of a sufficient amount of high-quality DNA is critical, and pre-analytical factors of blood sampling and processing can strongly affect DNA yield.<sup>17</sup>

## **CONCLUSION**

The present study shows that isolation methods significantly affected the DNA concentrations. Furthermore, there is a significant difference between the plasma DNA concentrations of healthy controls and of cancer cases, suggesting its potential utility as a biomarker for breast cancer that the

sampling method is less invasive than tissue biopsies.

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