ORIGINAL ARTICLE

# Effect of Chemotherapy on Gingival Crevicular Alkaline Phosphatase Level in Patients with Breast Cancer

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## ABSTRACT

**Introduction:** Chemotherapy is one of the modalities for treating breast cancer. Chemotherapy is conducted by administering cytostatic drugs to intervene in the genetic and cellular division of cancer cells. *In vitro* studies show that exposing osteoblasts to chemotherapeutic agents results in impaired function and activity as indicated by decreased alkaline phosphatase (ALP) activity. Gingival crevicular fluid (GCF) is considered a biomarker of the oral cavity and can be used to detect cellular events in periodontal pockets. The aim of this study was to determine the effect of chemotherapy on ALP activity in gingival crevicular fluid. **Methods:** Patients diagnosed with breast cancer at the "Tulip" Integrated Cancer Installation of Dr. Sardjito General Hospital who were scheduled to undergo chemotherapy were recruited as research subjects. Gingival crevicular fluid was taken from the maxillary anterior teeth before the subjects underwent chemotherapy and 7 days after chemotherapy. The ALP assay of gingival crevicular fluid was conducted with an ALP Kit (DiaSys) and absorbance was determined using a UV-Vis spectrophotometer at a wavelength of 405 nm. Data were analyzed by the paired t-test. **Results:** The results showed significant differences ($p < 0.05$) before and after chemotherapy. **Conclusion:** Chemotherapy could affect gingival crevicular ALP activity in patients with breast cancer at the "Tulip" Integrated Cancer Installation of Dr. Sardjito General Hospital.

**Keywords:** Chemotherapy, Breast cancer, Gingival crevicular fluid, Alkaline phosphatase

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## INTRODUCTION

Breast cancer (BC) is a major cause of death for women in developed and developing countries. BC ranks first in cancers with the highest number of cases and deaths in Indonesia (1). BC is a very heterogeneous type of carcinoma. Five types of BC based on molecular subtypes include: BC with human epidermal growth factor 2 positive (HER 2) +, estrogen or progesterone hormone receptor positive BC, BC with positive hormone receptors and positive HER 2, and triple negative BC (2). Standard treatments for BC therapy include surgical treatment, radiotherapy, hormone therapy, and chemotherapy. Conventional chemotherapy produces a heavy toxic burden on the body (3). The high mitotic rate of tissues, such as bone marrow, the gastrointestinal tract, hair follicles, and the oral mucosa are affected by chemotherapeutic agents (4). Side effects in the oral cavity include mucositis, hyposalivation, xerostomia, dysphagia, pharyngitis, discomfort, and changes in taste (3).

Many chemotherapeutic agents are myelosuppressive; thus, facilitating the prompt establishment of opportunistic microorganisms in ulcerated and nosocomial tissues and increased formation of plaque. Prevalent complications in patients receiving intensive chemotherapy protocols include nervous system toxicity (65%) and mucosal inflammation (21%), and these are often associated with neutropenia and candidiasis. Gingivitis and periodontitis due to bacterial contagions also cause moderate complications, and more than 25 % of all infections have been recorded in patients with acute lymphocytic leukemia (5). In vitro studies show that chemotherapeutic agents have an adverse effect on bone metabolism (6), and also show that there are significant effects of chemotherapeutic agents using clinically relevant concentration to type 1 collagen synthesis, alkaline phosphatase (ALP) levels, and mineralization of primary osteoblast-like cells.

Gingival crevicular fluid (GCF) has potential diagnostic value, as tissue breakdown products can be detected in GCF (7). As a serum exudate, GCF contains components of serum and various host derived products. GCF ALP is chosen as a biomarker to investigate chemotherapeutic effect on periodontal tissue because it is sensitive to bone remodeling, inflammation and periodontal regeneration. Study on the effect of amino-bisphosphonate therapy on

gingival crevicular fluid alkaline phosphatase activity in cancer patients confirmed that GCF ALP could be considered as a biomarker of bone turnover rate rather than serum analysis (8). Changes in serum and bone ALP enzymes have long been used as markers of bone metabolism in some diseases. Increased levels of ALP accompany bone formation. ALP is considered a reliable marker of osteoblast activity and an indicator of bone formation activity (9, 10).

The response of osteoblasts after exposure to chemotherapeutic agents in vivo is thought to be determined by assessing the ALP level of GCF. This study determined the effect of chemotherapy on ALP levels in GCF. The hypothesis that the authors propose is that chemotherapy decreases the levels of osteoblastic ALP in GCF in patients with BC at the "Tulip" Integrated Cancer Installation of Dr. Sardjito General Hospital.

## MATERIALS AND METHODS

This study was analytic observational study with pre and post design. Ethical approval of this study was from the Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (reference number: KE/FK/596/EC/2018).

### Subject Selection

Inclusion criteria for this study were female patients with BC in age range 30-65 years old who were scheduled to undergo chemotherapy, able to open their mouth, have at least four teeth and have not receive periodontal therapy in the last six months. The exclusion criteria were patients with smoking habit, alcohol consumption habit and those who unable to open their mouth. Subjects who met the inclusion criteria and were willing to participate in the study were asked to complete the informed consent form and the patient identification form as a statement of the patient's willingness to participate in the study. Other systemic conditions rather than breast cancer and the amount of medication were not considered in inclusion and exclusion criteria. The limitation of this study was the lack of consideration about patient comorbidity and other systemic condition.

### Sampling Technique

GCF samples were taken from two sites in the mouth with the deepest periodontal pockets using sterile paper points number 30. Each paper points was left 1 mm in a pocket for about 30 sec. Then, the paper point was placed in a 1.5 mL microcentrifuge (Eppendorf) tube containing 0.9% physiological solution (350 µL) and centrifuged with a Microcentaur (UK) machine for 5 min at 2,000 rpm to completely elute the GCF component. Then, the paper point was taken and the supernatant was stored at -20°C until all samples were collected. The GCF ALP assay was conducted with the ALP test using *Alkaline Phosphatase FS DGKC* (DiaSys, Germany) in the Biochemistry Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. The baseline GCF sample was collected before the patient underwent chemotherapy at the "Tulip" Integrated Cancer Installation. The second sample was taken after chemotherapy.

### Data Analysis

The ALP levels were recorded before and after chemotherapy and measured by the ALP test. The results were read with a UV-Vis spectrophotometer (U-1800, Hitachi, Japan) at a wavelength of 405 nm. The data obtained in the observations were quantitative scale-ratio data. The data were tested for normality. Normally distributed data were subjected to a parametric analysis (paired t-test). The Shapiro–Wilk test and paired t-tests were performed with SPSS version 22 software (SPSS Inc., Chicago, IL, USA). A p-value $< 0.05$ was considered significant.

## RESULTS

The data are the results of GCF ALP level measurements in patients with BC undergoing chemotherapy at the "Tulip" Integrated Cancer Installation of Dr. Sardjito General Hospital in Yogyakarta. Data taken before patients underwent chemotherapy were compared with data taken after chemotherapy. ALP levels are expressed in units of U/L. Ten research subjects completed the entire research series. Two set data of ALP measurements were obtained after study completion. The age range of the study subjects was 37–58 years old. The chemotherapeutic agents administered to the research subjects were not the same.

The chemotherapeutic agents were chosen by the oncologist who was treating the subject. All chemotherapeutic agents administered to the patients were part of a combination chemotherapy treatment, with a combination of at least two chemotherapeutic agents (Table I). The most widely used chemotherapeutic agents were cyclophosphamide and doxorubicin. There were three combinations of two chemotherapeutic agents, namely docetere-cyclophosphamide, docetaxel-carboplatin, and doxorubicin-cyclophosphamide. The three combinations of three chemotherapy agents, such as taxotere-carboplatin-herceptin, cyclophosphamide-doxorubicin-5- fluorouracil (5-FU), and 5-FU, epirubicin, and cyclophosphamide. The chemotherapeutic agents were administered intravenously to patients. ALP was

**TABLE I: Various Combination of Chemotheraphy agents received by patients**

| No | Combination of Chemotheraphy agents |
|---|---|
| 1. | Docetere-Cyclophosphamide |
| 2. | Taxotere-Carboplatin-Herceptin |
| 3. | Cyclophosphamide-Doxorubicin-5FU |
| 4. | Docetaxel-Carboplatin |
| 5. | 5-FU, epirubicin, cyclophosphamide |
| 6. | Doxorubicin-Cyclophosphamide |

measured in the GCF taken from the subjects using a spectrophotometer with a wavelength of 405 nm. Then, the results were used to calculate the ALP levels (Table II).

ALP levels increased in the ten subjects after chemotherapy. Data on ALP levels that were obtained were then tested for normality. The two-tailed paired t-test results showed a value of p = 0.002, indicating that the difference between the before and after ALP levels was significant.

**TABLE II: Changes in Mean level of Alkaline Phosphatase (ALP) GCF before and after Chemotherapy**

| | Before Chemotherapy | After Chemotherapy | Changes | Sig |
|---|---|---|---|---|
| **mean ±SD** | 409.37±13.98 | 428.07±16.88 | 4.5% (increase) | 0.002 |

## DISCUSSION

The results showed an increase in mean GCF ALP level after chemotherapy. The average ALP level after chemotherapy increased by 4.5% from 409.37 to 428.07, as shown in Table I. The increase in ALP activity was contrary to the hypothesis of this study. Previous in vitro studies have shown that ALP osteoblast activity decreases after exposure to a single chemotherapeutic agent at therapeutic doses (6, 11, 12). Chemotherapeutic agents cause changes in osteoblast gene expression in periodontal tissues, delay maturation of preosteoblasts to adult osteoblasts, and impair ALP mRNA transcription, so these agents were predicted to cause a decrease in GCF ALP level.

The increased ALP activity observed in this study was possibly due to several factors. Some adverse reactions from chemotherapeutic agents are thought to affect GCF ALP activity through an indirect mechanism. Adverse effects of drugs are dangerous reactions that arise as a result of use of a drug. They also represent the danger that may arise if administration of the drug is continued. Options include: prevention, specific treatment, dose change, or cessation of drug administration (13).

The chemotherapeutic agents received by the study subjects were doxorubicin, cyclophosphamide, docetaxel, and 5-FU, which have adverse effects in the form of neutropenia, leukopenia, decreased immune response, increased risk of infection, and mucositis/stomatitis (14). A periodontal infection, as a detrimental effect of chemotherapeutic agents, can trigger neutrophils to release ALP. ALP is stored in specific secretory granules and vesicles and is generally released during migration to the infection site (15). ALP neutrophil activity increases when a bacterial infection occurs (16). Bacterial infected periodontal tissue is very likely to cause an increase in ALP according to studies suggesting that an increase in GCF ALP indicates active periodontal tissue damage (17). The changes in periodontal tissue of cancer patients who are receiving chemotherapy include gingivitis, gingival bleeding, and periodontal infection (18).

Changes in ALP levels can also occur due to the side effects of chemotherapeutic agents. ALP levels can be very high in patients taking certain drugs. The chemotherapeutic agents administered to the subjects came from different drug classes (Table II) (16). Carboplatin has been reported to increase serum ALP level by 24% (19). Similarly, docetaxel increases serum ALP levels by 4 to 7%. The side effects of carboplatin and docetaxel may have contributed to the increase in GCF ALP although there are still arguments regarding the association between GCF ALP and serum ALP (20, 21).

Factors thought to contribute to the increase in GCF ALP are comorbidities of systemic diseases. A number of systemic conditions are known to cause an increase in serum ALP. High ALP levels can occur due to a bile duct obstruction. Abnormally high osteoblast activity while forming bone as in Paget's disease also results in increased ALP (21). Conditions, such as hyperparathyroidism, vitamin D deficiency, damage to liver cells, celiac disease, rheumatoid arthritis, healing of bone fractures, osteomalacia, and metastatic neoplasms also increase ALP (16, 22).

Local factors or conditions of periodontal disease that already exist can affect the increase in GCF ALP. ALP activity is significantly higher in areas that experience periodontitis compared to healthy areas or areas with newly developed gingivitis (15). There was no plan to intervene in the status of the periodontal tissue in the subjects of this study. Generally, the longer a patient has periodontal disease the more severe the disease (23). Without an intervention from professionals and a change in the patient's behavior, existing periodontal disease is progressive; thus, ALP activity increased at the last measurement point.

The GCF sampling period was also thought to contribute to an increase in GCF ALP. In vitro studies of the expression of osteoblast genes during osteoblast differentiation indicate that ALP osteoblast activity is not observed during the first week after induction. After the first week, ALP osteoblast activity begins to increase slowly and osteoblasts begin to differentiate during the third week after induction (24). Based on these studies, suspected ALP osteoblast activity in GCF will appear to decrease after 3 weeks of chemotherapy. The post-chemotherapy data were obtained 7 days after the subjects underwent chemotherapy. It is possible that ALP taken on day 7 after chemotherapy was neutrophil ALP. ALP exists as several types of isoenzymes or

isoforms that are classified based on the specificity of the expressed tissue (20, 22).

ALP in the GCF consists of several types of ALP isoenzymes derived from phosphatidylinositol ALP derivatives and ALP from periodontopathogenic bacteria. ALP is released by neutrophils through the mechanism of bacterial challenge and the host response mediated by neutrophils in the GCF. On the other hand, ALP activity also increases when bone formation increases. This contradictory ALP behavior is evidence that there are four types of ALP in humans, namely tissue-nonspecific ALP, intestinal ALP, placental ALP, and placental-like ALP (19). This study investigated total ALP levels in GCF. The limitations of this study in investigating specific types of ALP isoenzymes that may experience an increase in GCF caused the associated ALP source to be unknown. It is probable that ALP measured in GCF in this study was not ALP from osteoblasts but total ALP consisting of ALP from neutrophils, ALP from fibroblasts, ALP from osteoblasts, and ALP from periodontopathogenic bacteria. Eighty percent of ALP contributed to GCF originates from polymorphonuclear cells (25).

## CONCLUSION

This study concluded that chemotherapy increases ALP levels in GCF of BC patients at the Tulip Integrated Cancer Installation, Dr. Sardjito General Hospital.

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