ORIGINAL ARTICLE

Detection of Radiation-Induced DNA Damage in Breast Cancer Patients by Using Gamma H2AX Biomarker: A Possible Correlation with Their Body Mass Index

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ABSTRACT

Radiotherapy is one of the most important options for treating breast cancer in humans. The development of biomarkers to monitor radiosensitivity is scarce. The aim of this study is to investigate the γH2AX levels in the human blood samples 0.5 h after radiotherapy compared to the levels before radiotherapy in breast cancer patients in relation to their respective body mass index (BMI). Blood plasma samples were collected from a total of 20 breast cancer patients before and after radiotherapy to measure γH2AX levels with an antibody against γH2AX based on enzyme-linked immunosorbent assay technique. The median BMI of the patients was 30 kg/m^2. γH2AX was differentially expressed in breast cancer patients before radiotherapy. γH2AX levels significantly increased in 14 patients after radiotherapy ($P = 0.006$), whereas γH2AX levels decreased in three patients after radiotherapy, and three patients were excluded. There was no correlation between γH2AX values after radiotherapy and BMI ($P = 0.5$, $r = 0.1$). Our results suggest that γH2AX can be used by ELISA technique to measure γH2AX in the blood plasma of breast cancer patients undergoing radiotherapy and can be considered a biomarker of radiosensitivity.

Key words: Body mass index, DNA damage, H2AX, radiotherapy

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Introduction

Breast cancer is considered the second most common cancer; among women, breast cancer is the leading cause of death worldwide.[1] Ionizing radiation is increasingly used in therapy and diagnosis; radiotherapy is used to treat 50%–60% of cancers[2] and is the main treatment option for all stages of breast cancer.[3] However, biological markers to measure radiosensitivity are important to monitor individual response to radiotherapy and evaluate its benefit. Several assays, including double-strand break (DSB) repair, chromosomal aberrations, and radiation-induced apoptosis in *ex vivo*-irradiated blood lymphocytes, have been described as predictors of radiosensitivity.[4] Biomarkers of radiosensitivity may also be useful in normal tissue toxicity. In addition, several factors are known to enhance tumor response to radiation exposure, including total dose, fractionation, tumor potential doubling time, hypoxia, and intrinsic radiosensitivity.[5] DNA DSBs are one of the most important types of DNA damage. Ionizing radiation causes DNA damage,[6] and DNA DSBs are in turn responsible for the activation of three phosphatidylinositol 3-kinase-like kinases: ataxia telangiectasia-mutated (ATM), ataxia telangiectasia and Rad3-related, and DNA-dependent protein kinase (DNA-PK),

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which catalyze the phosphorylation of H2AX at serine 139 in the C-terminus named γH2AX.[7] The phosphorylated H2AX plays a functional and structural role in DNA damage recognition and repair. Therefore, γH2AX may serve as a sensitive marker for detecting DSBs, which in turn may indicate genomic instability and potentially contribute to cancer development and progression.[8] In breast cancer, γH2AX has been associated with triple-negative breast cancer,[9] breast cancer gene (BRCA) 1, and p53 mutations.[10] Meanwhile, γH2AX is a sensitive indicator of DNA DSBs and is considered an important regulator of DNA damage and repair.[11] The commonly used techniques to measure γH2AX level are immunostaining (analyzed by fluorescence microscopy), western blot, flow cytometry, and enzyme-linked immunosorbent assay (ELISA).[12] The aim of this study is to measure the γH2AX concentration in the blood plasma of breast cancer patients before and after radiotherapy 0.5 h after sample collection by ELISA and then to investigate a possible relationship between γH2AX concentration and body mass index (BMI).

Materials and Methods

Study population

A total of 20 breast cancer patients who received chemotherapy and/or surgery before being subjected to radiotherapy were included. Patients were randomly selected to participate in this study according to whether they had undergone radiotherapy. Blood samples were collected before and after radiotherapy. All patients were informed of their concerns through individual written form. In this regard, the study was approved by the Ministry of Health in Khartoum, Sudan.

Sample preparation and analysis

Venous blood samples were collected in EDTA from breast cancer patients before and after radiotherapy. Samples were centrifuged at 2500 rpm to separate plasma for analysis. ELISA was used to quantitatively measure γH2AX (Human Gamma H2AX ELISA Kit; Cat. No. SG-14818, Sino Geneclon Biotech Co., Ltd). The γH2AX antibody was used based on the principle of ELISA technique. Then, the optical density at 450 nm was determined, and the concentrations were estimated using the standard curve for quantification of γH2AX.

Body mass index

BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2) and classified as categories of normal weight (BMI <25), overweight (≥25), and obese (≥30) breast cancer patients.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 8 software (GraphPad Software Inc., San Diego, California, USA). Correlation coefficient was used for the association between γH2AX levels and BMI. Paired *t*-test was used to determine the significance between groups. Significance was considered at the level of $P < 0.05$.

Results

A total of 20 breast cancer patients with a median age of 44 years were considered for the present study. The median BMI was 30 kg/m^2; therefore, mainly obese patients participated in the present study. Patients were treated with an absorbed dose of 40.5 Gy at 2.7 Gy/fraction. The γH2AX values for all patients before and after radiotherapy are explained in Table 1. Of 20 patients, three were excluded because of very low γH2AX concentrations. In 14 patients, γH2AX levels were increased by 0.5 h after radiotherapy ($P = 0.006$), as shown in Figure 1. Meanwhile, no correlation was found between BMI and γH2AX levels ($P = 0.5$, $r = 0.1$), as shown in Figure 2.

Discussion

γH2AX is a biomarker for detecting DNA double-strand damage. In this study, the level of γH2AX was measured in the blood samples from breast cancer patients who had undergone radiotherapy. Our results showed that γH2AX is expressed in breast cancer patients. In this regard, we found that another study confirmed this finding.[8] In these findings, increased γH2AX level seems to be associated with high BMI in this study because most of the patients showed high BMI; however, several studies found that obesity is associated with breast cancer.[13-15] In another study, an increase in γH2AX levels was observed in breast cancer with a BMI of <25.[16] Obesity is a condition characterized by an increase in the mass of body adipose tissue and is associated with disturbances in lipid and glucose metabolism, chronic inflammation, and oxidative stress.[17] Obesity is associated with DNA damage accumulation,[18] and low body weight has been associated with a reduction in the extent of DNA damage.[19] In addition, obesity can induce DNA damage and inhibit DNA repair mechanisms. The accumulation of DNA damage leading to the activation of various proteins can induce adipocyte differentiation, inflammation, and cell metabolism disorders.[20] A relationship between BMI and DNA damage has been established.[13] γH2AX levels increased in obese patients after 0.5 h of radiotherapy, indicating a strong DNA damage response leading to increased γH2AX levels after radiotherapy. After irradiation, histone H2AX is rapidly phosphorylated by ATM and/or DNA-PK at or near DNA DSB to form γH2AX.[21] Several studies reported that γH2AX is a good biomarker for

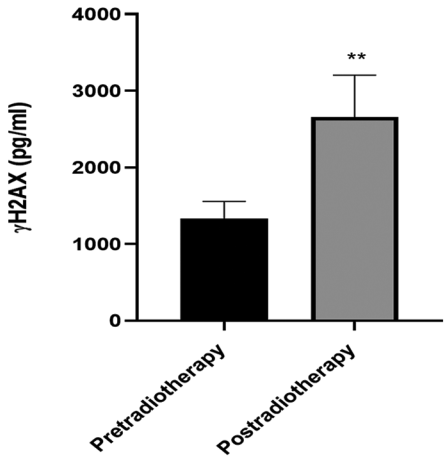

Figure 1: Mean levels of breast cancer patients showed increased γH2AX levels postradiotherapy ($P = 0.006$)

Table 1: Individual γH2AX levels expressed in a study population of breast cancer patients with normal weight, overweight, and obese

Serial number of patients	BMI (kg/m²)	Plasma γH2AX preradiotherapy (pg/ml)	Plasma γH2AX postradiotherapy (pg/ml)	Status of γH2AX at 0.5 h
1	30	597	1692	+
2	26	1126	1710	+
3	31	1656	2356	+
4	30	2454	2975	+
5	28	516	1225	+
6	32	1925	2212	+
7	28	1025	1396	+
8	30	1396	1512	+
9	20	1037	3935	+
10	27	2030	220	-
11	21	1185	1378	+
12	34	1288	2894	+
13	34	929	4509	+
14	37	9045	4005	-
15	26	4050	3915	-
16	29	148	705	+
17	31	3379	8700	+

+: Increase and –: Decrease, BMI: Body mass index

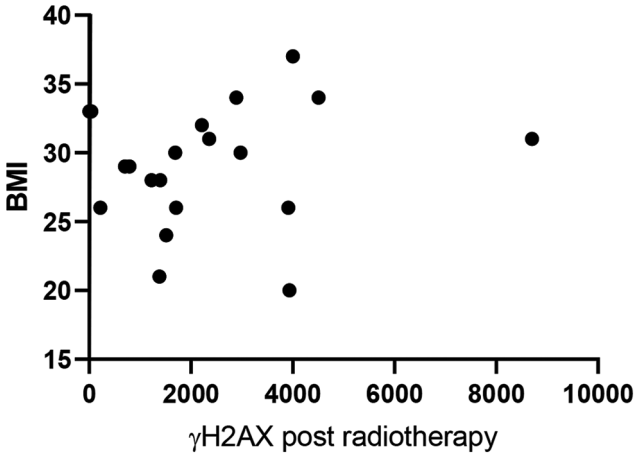

Figure 2: Correlation between body mass index and γH2AX ($P = 0.5$, $r = 0.1$)

radiosensitivity of human tumor cells.[22-24] Phosphorylation of γH2AX is induced by DNA DSBs. Since tumor cells usually exhibit deficiencies in DNA damage response, it has been suggested that constitutive expression of histone γH2AX may indicate DNA damage response dysfunction and genomic instability.[25] Several studies have found that radiosensitivity increases with genetic defects in DNA repair[26] and that the DNA damage marker γH2AX can be used to assess the radiation exposure of biological samples.[27] Thus, γH2AX can be used as a sensitive biomarker for DNA damage caused by ionizing radiation[28] and can be used as a therapeutic marker against cancer.[29]

Our data are limited by sample size, and further studies are recommended to support our findings. γH2AX can be measured in the bloodstream, and there are several techniques to measure its concentration, including ELISA. The development of new biomarkers to monitor radiosensitivity could be useful to optimize treatments and improve patient care. The initial effect of ionizing radiation or its early detection, e.g., after 0.5 h, is important for studying the DNA damage response, and this could be revealed by γH2AX. However, the changes in γH2AX concentrations after radiotherapy in breast cancer patients seem to enhance this histone variant by ionizing radiation.

Conclusion

Individual γH2AX values in breast cancer patients with normal and high BMI increased significantly after radiotherapy. The decrease in γH2AX levels in some patients might indicate a low DNA damage response. On the other hand, no correlation was found between the increase in γH2AX and BMI in our data. The results show that γH2AX can be measured in the blood plasma of breast cancer patients and can be considered as a biomarker for radiosensitivity.

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Conflicts of interest

There are no conflicts of interest.

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