Status of *H2AX* and *TP53* (Non- phosphorylation) for DNA Damage in Cancer Patients on Radiotherapy-A Case-Control Study

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ABSTRACT

Introduction: *Radiotherapy is widely used in the treatment of cancer patients, and some genes affect the response to radiotherapy. In addition, phosphorylation is a hallmark of DNA damage response. In this study, we aim to investigate the status of H2AX and the non-phosphorylation forms of tumor suppressor p53 (TP53) before and after radiotherapy to reveal their roles in DNA damage response and radio-sensitivity.* ***Materials and Methods:*** *ELISA technique was used to determine the H2AX concentration and TP53 expression status in plasma of 29 cancer patients and 29 healthy individuals of the same age and sex as a control group. Patients were treated with radiotherapy, while the control group was not irradiated. H2AX and TP53 were measured before the start of radiotherapy on the first day of treatment and then 30 minutes after radiotherapy on the last day.* ***Results:*** *Both H2AX and TP53 had higher levels in cancer patients before radiotherapy than in healthy subjects. In 21 patients, H2AX levels were significantly decreased after radiotherapy compared with levels before radiotherapy (p=0.002), and TP53 levels were significantly increased after radiotherapy compared with levels before radiotherapy (p=0.0008). In eight patients (n=8), H2AX levels were increased after radiotherapy and TP53 expression was decreased after radiotherapy.* ***Conclusion:*** *The results show that both H2AX and TP53 can be induced by DNA damage. The decrease of H2AX level after radiotherapy refers to the conversion of this histone protein to the phosphorylated form (gamma-H2AX) after radiotherapy, so H2AX can be used as an indicator of DNA damage response and radiation sensitivity by the phosphorylation form (gamma-H2AX) in early exposure. While TP53 is activated by radiotherapy because its expression is increased after radiotherapy in cancer patients, indicating that TP53 is not yet phosphorylated, TP53 can also be used as a marker to indicate DNA damage response in early radiation exposure.*

Keywords: *H2AX*, Radiotherapy, *TP53*, DNA damage

INTRODUCTION

A radiotherapy is a primary option for the treatment of cancer patients. It is used either alone or in combination with surgery or chemotherapy to inhibit tumour development [1]. During radiotherapy, some tumour cells may develop radioresistance, which affects the response to radiotherapy. However, radioresistance is related to epigenetics, which affects cell regulation [2]. DNA double-strand breaks induced by ionizing radiation can trigger a series of cellular

DNA damage responses such as cell cycle arrest and DNA repair [3]. *H2AX* is one of several genes encoding the histone *H2A* family. In humans and other eukaryotes, DNA is wrapped around histone groups in chromatin consisting of core histones *H2A, H2B, H3*, and *H4* [4]. *H2AX* is phosphorylated at serine 139 and then forms gamma-*H2AX*, in response to DNA Double-Strand Breaks (DSBs) after ionizing radiation exposure [5]. *H2AX* increases with time after irradiation and reaches a maximum (20-30) min after irradiation [6]. The human *H2AX* gene (*H2AFX*) is mapped to chromosome 11 at position 11q23, in a region that frequently has mutations in a large number of human cancers [7]. In squamous cell carcinomas of the head and neck, amplification occurs in chromosomal region 11q13 followed by loss of distal 11q, the region containing *H2AFX* [8]. The increased chromosomal instability in these cells is associated with the loss of 11q and *H2AX*, which contributes to progression, resistance, and tumour development in this cancer subtype [9]. In contrast, the tumour suppressor P53 is key to various stress signals in cellular responses such as DNA damage, reactive oxygen species, apoptosis, and senescence [7]. Tumour suppressor P53 is an important regulator of cellular response to radiation [10]. TP53 is a multifunctional transcription factor; upon radiation exposure, the level of P53 protein increases in cells due to activation of the DNA damage response [11]. Activation of p53-mediated signalling pathways facilitates DNA repair and initiates the intrinsic pathway of apoptosis. Therefore, P53 plays an important role in controlling cell activity after irradiation [12]. Therefore, TP53 expression in cancer cells after radiotherapy could be associated with the efficacy of radiotherapy [13]. Therefore, understanding the TP53 status in the response of a cancer cell to irradiation could help to solve the problem of radioresistance and improve the outcomes of radiotherapy.

Total dose fractionation is a powerful technique used in modern radiotherapy [14]. It involves dividing the total dose to the patient into small doses that are administered separately and then recover over hours (approx. 12-24 hours), which helps both cancer cells and normal cells respond to radiation [15]. However, the recovery of the previous dose infractions requires hours, so the levels of *H2AX* and *TP53* after radiotherapy usually indicate DNA damage 30 minutes after the last dose. In the present study, *H2AX* and *TP53* status were determined 30 minutes after radiotherapy to understand their roles in DNA damage in radiotherapy, which could serve as biomarkers for radiation sensitivity and therapeutic response monitoring to improve the outcomes of radiotherapy in cancer patients.

MATERIALS AND METHODS

Study Population

A total of 29 cancer patients undergoing radiotherapy participated in this study after obtaining ethical approval from the competent authority. Blood samples were collected before and 30 minutes after the last session of radiotherapy total dose fractions. Twenty-nine healthy individuals who were not exposed to radiation voluntarily participated in this study as a control group and matched the patients in age and sex. The participating patients were treated at the National Cancer Institute Clinic, the second centre for cancer treatment in Sudan.

Included Criteria of Cancer Patients

Patients undergo radiotherapy as a second line of treatment either after chemotherapy or surgery. Patients were selected according to two cancer types: breast, head, and neck cancer, as breast cancer is classified as one of the most common cancers in Sudanese women with increasing incidence, while head and neck cancer are the most commonly diagnosed cancers in men, women, and children <15 years [16].

External Beam Radiotherapy

Cancer patients treated with Cobalt 60. A conventional simulator planning system was used to adjust the radiation dose as needed to ensure that the beam was accurately focused on the tumour with minimal exposure to normal tissue. All patients were treated at early detection with an α/β ratio of 10.

Collection and Preparation of Blood Samples

Blood samples (3 ml) were collected from cancer patients and healthy donors matched for age and sex. Blood samples were collected in EDTA vacutainer tubes and then separated by centrifugation at 2500 rpm for 10 min at room temperature to collect plasma and then stored at -20°C until analysis.

Reagents

Human *H2AX* Enzyme-Linked Immunosorbent Assay (ELISA) kits from Lifespan Bioscience, Inc. Company were used to measure *H2AX* concentrations in plasma of patients and control, and the kits were developed for research purposes only. The assay is based on the sandwich ELISA principle. *H2AX* was detected by using specific monoclonal and polyclonal antibodies against *H2AX* in human plasma samples. The assay was performed in two runs, then the mean value was determined calorimetrically at 450 nm and the match was established (Pg/ml).

The TP53 antibody used against non-phosphorylation of TP53 was from CUSABIO (CSB-PA003677). The methodology was based on the protocol corresponding to the principle of the ELISA technique. Subsequently, the optical density was determined at 450 nm.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 8 software (Graph Pad Software Inc., San Diego, California, USA). The student's t-test (paired and unpaired) was used to determine the significant differences in *H2AX* values between groups. Significance was considered at the $p < 0.05$ level.

RESULT

29 cancer patients participated in this study, 20 breast cancer patients, and 9 patients with head and neck cancer, including oesophagus, lip, mandible, nasopharynx, and thyroid. The patients were 6 male and 22 female. The age range of breast cancer patients was 30 to 65 years. For head and neck cancer patients, the age range was 12-77 years. The absorbed dose for breast cancer was 40.5 Gy (2.7 Gy/day). For head and neck cancer, the dose ranged from 20 Gy-66 Gy (2 Gy/day). Regarding *H2AX* levels in cancer patients compared to the control group, *H2AH* levels were higher in cancer patients than in the healthy group (Figure 1). Whereas *H2AX* significantly decreased in cancer patients after radiotherapy compared with the values before radiotherapy (p=0.002) (Figure 2). Of the total patients, 21 patients showed a decrease in *H2AX* levels after radiotherapy at 30 minutes of total dose fractions. In 8 patients, *H2AX* levels were increased after radiotherapy and *TP53* levels decreased after radiotherapy; four of the eight patients were head and neck tumours. *TP53* non-phosphorus level significantly increased in cancer patients compared with the control group (p=0.006) (Figure 3). *TP53* also increased significantly in 21 patients after radiotherapy compared with levels before radiotherapy (p=0.0008) (Figure 4).
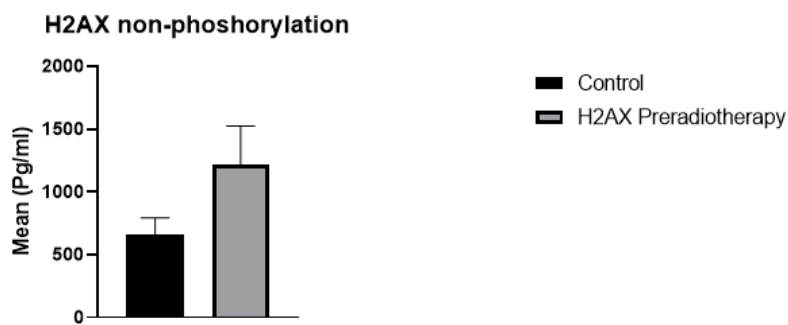

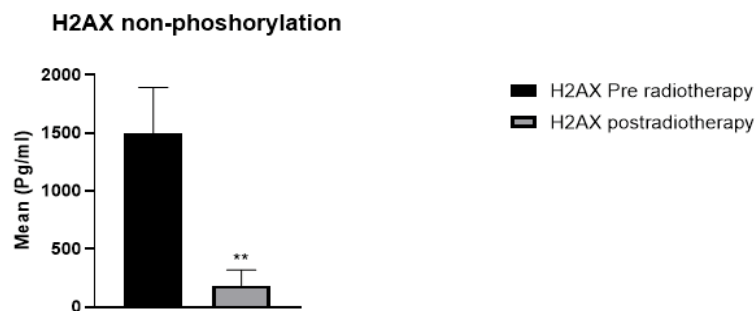**Figure 1 Explain the *H2AX* concentrations in control and cancer patients undergoing radiotherapy**

Figure 2 *H2AX* concentration in cancer patients (n=21) before and after radiotherapy shows significant difference (p=0.002)
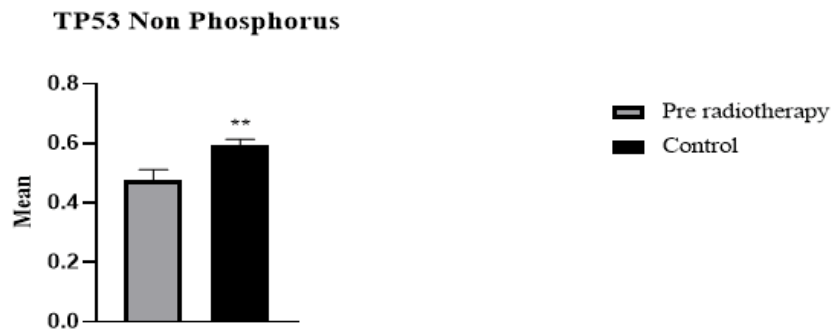
TP53 Non Phosphorus

Figure 3 Explain *TP53* expression in control and cancer patients after radiotherapy (p=0.006)
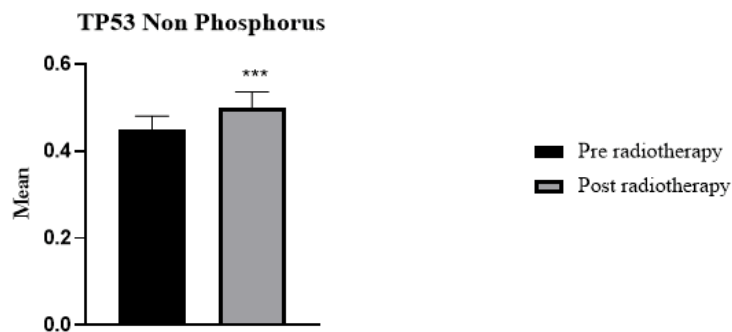
TP53 Non Phosphorus

Figure 4 *TP53* in cancer patients (n=21) before and after radiotherapy difference (p=0.0008)

DISCUSSION

Biomarkers to monitor the efficacy of radiotherapy in cancer patients are essential for radiation oncology. *H2AX* has become an important tool for monitoring DSBs in translational cancer research. Previous studies have used various techniques to assess the response to DSBs using *H2AX* [17]. Researchers in radiation biology are focusing their attention on radiation biodosimetry, emphasizing the potential of the assay to determine the radiosensitivity of patients [18]. Moreover, in the present study, the total amount of *H2AX* and TP53 status were investigated based on the ELISA principle. For total *H2AX, H2AX* levels decreased after radiotherapy compared with levels before radiotherapy, suggesting that the decay of *H2AX* after radiotherapy is due to the DNA damage response caused by radiotherapy, but also that *H2AX* levels decrease or fall because *H2AX* is phosphorylated in the form of γ-*H2AX* after irradiation. Several studies on radiation-induced γ-*H2AX* reported that *H2AX* foci were observed on fibroblasts and lymphocytes after acute external irradiation [19]. In another in vitro study, the decay of γ-*H2AX* foci was found to correlate with the repair of lethal damage, which is part of the radiation damage that can be affected after irradiation in prostate cancer cell lines [20]. Several studies reported that *H2AX* is increased in cancer patients compared with normal patients, confirming our findings [21]. As expected, *H2AX* levels could show the effects of DNA damage after radiotherapy, indicating radio-sensitivity after total dose fractionation. In this study, some patients (n=8) showed increased *H2AX* levels after radiotherapy, explaining that *H2AX*, which is not phosphorylated, could be activated by radiation. Moreover, activation of the non-phosphorylated form in these patients seems to be responsible for a low DNA damage response after 30 minutes, because phosphorylation is a hallmark of the DNA damage response. However, the Biologically Effective Dose (BED) in particular is useful to quantify treatment expectations, such that patients treated with a high α/β ratio (e.g., 10 Gy) may indicate radiation resistance and increased morbidity [22].

As for TP53 expression status, the present study showed that radiotherapy activated *TP53* (non-phosphorus) and its expression increased after radiation exposure in cancer patients. Several studies confirmed that *TP53* is induced by radiation [23]. *TP53* is an important tumour suppressor gene that is expressed at low levels in cells [24]. However, when cells are stressed by toxic agents such as radiation, the expression of P53 increases. This mechanism plays an important role in maintaining the genomic stability of the cell and inhibiting carcinogenesis [25]. These findings explain the role of *H2AX* and *TP53* in DNA damage response after early radiotherapy such as 30 minutes. These

results show that *H2AX* and *TP53* are in a non-phosphorylated form that is activated by radiation. This explains that *TP53* can be used for DNA damage response after radiotherapy. However, *H2AX* can be used to detect DNA damage response as early as 30 min after radiotherapy, and *TP53* activation is strongly induced by DNA damage and drives the response to radiation. The present results demonstrate the potential effects of *H2AX* and *TP53* on DNA damage response after radiotherapy. Furthermore, understanding the responses of cancer cells after radiotherapy will potentially lead to improved therapeutic efficacy. The importance of radio-sensitivity in radiation oncology is critical to monitor drug response and increase the accuracy of the absorbed dose delivered to the patient. In addition, the development of biomarkers for radio sensitivity is needed.

CONCLUSION

The decreased *H2AX* levels after radiotherapy refer to the conversion of *H2AX* to the phosphorated form (gamma *H2AX*) after radiotherapy. Thus, *H2AX* can be used to detect DNA damage after radiotherapy, and the phosphorylated form gamma *H2AX* can be used to monitor radiation sensitivity in cancer patients. On the other hand, the increased expression of *TP53* (without phosphorylation) after radiotherapy suggests that this protein is activated by radiation and induced by DNA damage, which explains its role in DNA damage response, so *TP53* can be used as a marker of DNA damage response in early radiotherapy.

DECLARATIONS

Ethical Approval and Consent to Participate

The proposal was approved by the Ethics Committee of the Research Centre of the Federal Ministry of Health. All patients were informed of their concerns by a written individualized form, except for one child <15 years of age who was consented to by his or her parents.

Consent for Publication

Consent was obtained for publication.

Competing Interests

The authors declared no conflict of interest.

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Author Contributions

Alkhansa designed the study, performed the statistical analysis, wrote the article, and received funding. Ammar ME. Hassan contributed to sample preparation, interoperation results, and review of the paper. Amna Yousif and Raheel Mussa performed the patient consent forms and sample collection. Omer M. Abdalla, Amna Ali, and Nuha M. Hassan contributed to the analysis of the samples. Fawzia Elbashir and Ahmed Omer were involved in the selection criteria for the patients in the clinic and radiotherapy department.

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