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# Phosphorylated P53 (TP53) in Cancer Patients Undergoing Radiotherapy

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## Abstract

***Introduction:*** *Cancer is a common disease worldwide, and radiotherapy is an important option for cancer treatment. P53 tumour suppressor has a role in apoptosis and cancer treatment response. P53 is phosphorylated in response to ionizing radiation by kinases of the ataxia telangiectasia mutation family (ATM). The aim of this study was to detect the DND damage response in cancer patients before and after radiation therapy through measurements the expression levels of phosphorylated P53 on T18.*

***Material and methods:*** *Total of 28 cancer patients on radiotherapy were participate in this study to collect blood samples pre and post radiotherapy compared to 28 healthy people matched in age and sex as control group. P53 antibody used against Phospho-p53 (T18) was obtained from CUSABIO using enzyme linked immunosorbent assay (ELISA).*

***Results:*** *21 of patients were breast cancer, and 7 of patients were Head and Neck. 6 male and 22 female. Median of age was 44 years old. Median of body mass index (BMI) for breast cancer patients was 30 while BMI for head and neck was 23. The absorbed dose for breast cancer was 40.5Gy. While the doses for Head and neck cancers were between 20Gy- 66Gy. Phosphorylated P53 expression increased significantly (P= <0.0001) in the patients preradiotherapy compared to the control group. While no significant difference observed between preradiotherapy and postradiotherapy groups (P=0.7). Individually, 19 patients showed increased in phosphorylated P53 expression postradiotherapy, while, nine patients were showed low P53 postradiotherapy, 8 of them diagnosed with breast cancer and 1 diagnosed with Oesophagus.*

***Conclusion:*** *phosphorylated on T18 can be consider a predictive marker for cancer. Phosphorylated P53 can be indict the DNA damage and response through its activation and proapoptotic effects. Protein expression such as P53 can be use as biomarker to demonstrate individual radiation sensitivity in cancer patients.*

**Keywords :** Radiotherapy, P53, BMI, ELISA

## Introduction

The tumour suppressor p53 (TP53) or P53 is the major cellular response to various stress signals such as oncogene activation, DNA damage (Hafner et al., 2019), hypoxia and reactive oxygen species (ROS) (Bykov et al., 2018). The tumour suppressor protein p53 functions as an important barrier against cancer development and progression (Hassin & Oren, 2023). Upon its activation, p53 triggers numerous cellular responses, including cell cycle arrest, genetic integrity and apoptosis (cell death) (Levine, 2020). In normal cells, the level of p53 protein is low. DNA damage and other stress signals can trigger the increase of p53 proteins, which have three main functions includes growth arrest, DNA repair and apoptosis (Cotter, 2009). Therefore, radiotherapy is a major strategy approaches for cancer patients' treatment (Chen et al., 2017), it is applied in 45–60% of all cancer patients (Borras et al., 2016). In meanwhile, internal radiosensitivity is individually in radiation oncology depends on the genetic background and anticancer treatment plan for each patient (Deycmar et al., 2020). However, certain cells can develop radioresistance, which counteracts the effects of radiotherapeutic efficacy (Poon et al., 2021). The development of radioresistance is related to the genetic and epigenetic of cells regulation (Wu et al., 2020). Protein expression is one of markers can indict ionizing radiation (IR) response such as P53 (Fei et al., 2003). Thus, IR causes DNA double-strand breaks (DSBs) that activate DNA damage checkpoints to trigger cell death; TP53 has been identified as a major checkpoint protein that lead to the diversity of responses to IR (Lee et al., 2013) through multiple cellular pathways includes ataxia-telangiectasia mutated (ATM) (Ditch & Paull, 2012). The aim of study to demonstrate the expression levels of Phosphorylated

P53 in cancer patients on treatment and its role in DNA damage and repair for drug response compared to health subjects.

## Material and Methods

### Study Population

Total of 28 cancer patients on radiotherapy as second line of cancer treatment by cobalt 60, were participate in this study in this study after obtaining ethical clearance from the relevant authority to collect blood samples pre and post radiotherapy treatment (30 minutes after the last fraction dose of radiotherapy total dose). All, the patients informed concern individually. Total of 28 healthy people matched in age and sex have been participated voluntary as control group.

### Phosphorylated P53 assay

P53 antibodies measured in plasma prepared from the blood samples collected from the study population. P53 antibody used against Phospho-TP53 (T18) Antibody (CUSABIO, CSB-PA000603) by enzyme-linked immunoassay (ELISA) according to the protocol manufacture.

### Statistical analysis

Statistical analysis was performed with Graph pad Prism 8 software (Graph Pad Software Inc., San Diego, California, USA). Student's t-test was used to determine the significance between the study groups. Significance was considered at $p<0.05$.

## Results

21 of patients were breast cancer, and 7 of patients were Head and Neck. 5 male and 23 female. Median of age was 44 years old. Median of body mass index (BMI) for breast cancer patients was 30 while BMI for head and neck was 23. The absorbed dose for breast cancer was 40.5Gy. While the doses for Head and neck cancers were between 20Gy- 66Gy.

TP53 expression increased significantly (P=<0.0001) compared to the control (figure 1). While TP53 in preradiotherapy and postradiotherapy didn't showed significant difference (P=0.7). Although, P53 phosphorylated increased in the patients postradiotherapy except in 9 patients, 8 of them diagnosed with breast cancer and 1 patient diagnosed with esophagus as in table 1.

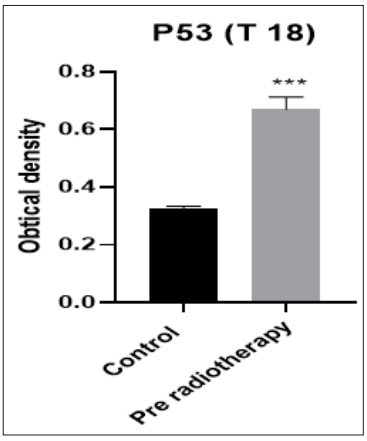


**Figure1:** Phosphorylated P53 in control and cancer patients

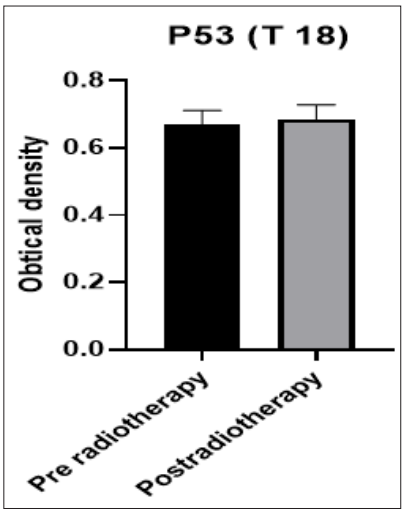


**Figure 2:** P53 Phosphorylated P53 in cancer patients pre and postradiotherapy

| Patients Number | Sex | Diagnosis | BMI | Pre Radiotherapy (O.D) | Postradiotherapy (O.D) |
|---|---|---|---|---|---|
| 1 | Male | Mandible | 24 | 0.659 | 0.696 |
| 2 | Female | Breast | 30 | 0.578 | 0.618 |
| 3 | Female | Breast | 26 | 0.738 | 0.746 |
| 4 | Female | Breast | 31 | **0.678** | **0.655** |
| 5 | Female | Breast | 30 | 0.555 | 0.695 |
| 6 | Female | Breast | 24 | 0.724 | 0.82 |
| 7 | Female | Breast | 28 | **0.585** | **0.511** |
| 8 | Female | Breast | 32 | 0.64 | 0.649 |
| 9 | Female | Breast | 34 | 0.675 | 0.798 |
| 10 | Male | Oesophagus | 25 | **1.056** | **1.021** |
| 11 | Female | Breast | 33 | 0.694 | 0.809 |
| 12 | Female | Breast | 30 | **0.751** | **0.698** |

| | | | | | |
|---|---|---|---|---|---|
| 13 | Female | Oesophagus | 23 | 0.831 | 0.836 |
| 14 | Female | Breast | 20 | **0.86** | **0.821** |
| 15 | Female | Breast | 26 | 0.948 | 0.949 |
| 16 | Female | Breast | 21 | 0.796 | 0.841 |
| 17 | Female | Breast | 34 | **0.691** | **0.623** |
| 18 | Male | Lip | 20 | 1.013 | **0.98** |
| 19 | Female | Breast | 34 | 0.878 | **0.90** |
| 20 | Male | Nasopharynx | 24 | 0.664 | 0.984 |
| 21 | Female | Breast | 33 | **0.825** | **0.715** |
| 22 | Female | Breast | 37 | 0.701 | 0.678 |
| 23 | Female | Breast | 26 | **0.801** | **0.724** |
| 24 | Female | Breast | 28 | **0.309** | **0.296** |
| 25 | Male | Nasopharynx | 12 | 0.256 | 0.276 |
| 26 | Female | Oesophagus | 22 | 0.241 | 0.275 |
| 27 | Male | Thyroid | 19 | 0.278 | 0.3 |
| 28 | Female | Breast | 31 | 0.333 | 0.294 |

**Table 1:** Explain the patients characterization and P53 phosphorylated status in the patients

## Discussion

The findings showed high expression of P53 in cancer patients pre radiotherapy compared to healthy subjects which confirm that p53 can be considers as a predicative marker for cancer therapy response (Riley et al., 2008) due to its a major role to block cell cycle progression and/or to induce apoptosis, in response to cellular stresses such as DNA damage (Hanahan & Weinberg, 2000). Genetic instability is one of the most striking features of malignant tumours, however, P53 plays an important role for detecting DNA damage and repairing the genome. When p53 responds to DNA damage, it triggers either cell cycle arrest or apoptosis (Okazaki, 2022). Radiotherapy destroys cancer cells via DNA damage; DNA damage is causing increase in p53 expression via ATM kinase activation (Cui et al., 2022). In this study, most of patients were reported increased in p53 expression which refer to DNA damage response. While some of patients were showed decreed expression levels postradiotherapy refer to DNA repair. Thus, the P53 was demonstrate at 30 minutes after radiotherapy for demonstrate DNA damage, and the patients who reported low p53 expression appears to low radiosensitivity because cells still in DNA repair because apoptosis may be occurred during several hours after radiotherapy (Sia et al., 2020); sometimes, cells immediately initiate apoptosis when DNA damage repair fails (Sanders et al., 2020), it showed the effect of the previous radiation dose. On the other hand, the treatment of patients usually with strategies such as chemotherapy and/or radiotherapy (Richards et al., 2020). Previous studies confirmed our findings and suggested that DNA damage and apoptosis are important in radiation exposure and can be used as biomarkers for ionizing radiation damage response (Deycmar et al., 2022; Nikitaki et al., 2016). P53 signaling pathway plays critical role in DNA damage, DNA repair and apoptosis (Santivasi & Xia, 2014). Therefore, Repairable DNA damage is normally transient, whereas P53 activation causes cells to initiate an apoptotic pathway (Ragunathan et al., 2020). Another study reported that P53 can be use as biodosimitry marker to demonstrate the radiosensitivity in early exposure within 24 h after IR exposure (Li et al., 2022). P53 is an important tumor suppressor gene that is expressed at low levels in cells, when cells are exposed to stress-induced stimulation, the expression of p53 increases, preventing genome destruction, which assist in maintaining cell stability and preventing cancer development (Kong et al., 2021). However, in radiotherapy of tumors, the status of p53 expression in cancer cells is associated with the efficacy of radiotherapy. Therefore, it is importance to understand how the expression of p53 affects the cellular response to radiation in order to solve the problem of radioresistance and improve the radiotherapy outcome (Fei et al., 2003; Gudkov & Komarova, 2003). However, decrease of phosphorylated P53 postradiotherapy have been seen slightly in cancer patients with high BMI. However, obesity is an important risk factor for breast cancer regardless of menopausal status (Kolb et al., 2022; McKenzie et al., 2015; Alkhansa et al., 2015). Several studies showed that obesity associated radiresistance (Sabol et al., 2020; Zhao et al., 2021; McCall et al., 2018), which highlighted to improve radiotherapy and increase mortality of obese women with breast cancer. So that, biomarkers development for monitoring radiosensitivity in cancer patients is necessary to improver radiotherapy response. In addition, our previous studies have shown that nuclear proteins such as P53 and γH2AX are stimulated by radiation and can be measured as plasma proteins in blood serum (Mahmoud et al., 2022). This is strongly recommended for cancer patients because the samples can be easily collected from patients, the test can be performed quickly and inexpensively in the clinical laboratory, and the techniques for measuring protein levels in patients' blood samples are accurate. Thus, P53 is one of the

most important proteins associated with apoptosis and plays a key role in DNA damage and repair. It can be measured using a variety of techniques, including ELISA.

## Conclusion

Measurement of P53 protein expression was potentially useful in cancer therapy response. phosphorylated P53 can be consider a predictive marker for cancer. Phosphorylated P53 (T18) induced by ionizing radiation that refer to DNA damage and repair, so that it may be useful for indicator of internal radiosensitivity for cancer patients individually.

## Competing Interests

The authors declared no conflict of interest.


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