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ORIGINAL ARTICLE

Radiation-induced effects on genes regulating DNA repair and cell cycle in breast cancer patients

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Abstract

Background: Background: Ionizing radiation is a major DNA damaging agent. One of the most common sources of exposure is through medical diagnostics or treatments such as cancer radiotherapy.

Aim: This study aimed to assess the radiation-induced changes in the expression of DNA repair and cell cycle regulation genes (POLH, PCNA, DDB2, and XPC) in the blood of breast cancer patients and to evaluate their potential as predictive biomarkers for treatment responses.

Methods: In this study, the levels of circulating PCNA, POLH, XPC, and DDB2 were evaluated in 51 females: 31 cancer patients and 20 healthy volunteers as a control group. The genes were extracted from whole blood samples and cDNA was synthesized; qRT-PCR was used to assay the expression pattern of these genes.

Results: Circulating DDB2 showed a significant difference in the relative expression among the three studied groups. Also, there was a significant difference in the relative expression of circulating DDB2 in the pre-radiotherapy and post-radiotherapy groups relative to the control group. Also, relative to the control group, the circulating levels of the four genes were higher in the pre-radiotherapy group to about double that in the post-radiotherapy group. The four genes combination was capable to discriminate the pre-radiotherapy group from the post-radiotherapy group significantly.

Conclusion: The relative expression of these four genes as radiation-responsive genes that are involved in cell cycle regulation and DNA repair could be changed due to radiotherapy. Also, their circulating levels in breast cancer patients could be promising predictive biomarkers for radiotherapy responses.

Keywords: Breast Cancer; radiation-responsive genes; radiotherapy

INTRODUCTION

The major cause of tumour recurrence and relapse are radio- and chemotherapy-resistant cancer cells. While some tumour cells are resistant intrinsically, others that are initially sensitive acquire resistance as a result of radio- or chemotherapy [1, 2].

Resistance to radio- and chemotherapy continues to be a major and serious obstacle in oncology affecting the majority of patients. According to

the current understanding of cancer resistance, the presence of cancer stem cells (CSC) contributes to de novo radio- or chemoresistance, whereas epigenetic alterations in the dysregulation of tumour suppressor genes or oncogenes lead to acquired chemoresistance. Recent research shows that CSC, radio- and chemotherapy-resistant cancer cells share common characteristics. It is anticipated that improved therapeutic strategies and targeting optimization of these cells will be mastered by a better understanding of oncogenic networks among these types of cells [1, 3].

Both internal and external genotoxic stressors, including UV light, reactive oxygen species (ROS), ionizing radiation (IR), chemicals, chemotherapy, and radiotherapy, can result in single strand breaks (SSB) or double strand breaks (DSB). Cells can eliminate the different types of DNA damage through the five repair pathways: homologous recombination (HR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), DNA mismatch repair (MMR), and base excision repair (BER). A DNA lesion activates the DNA damage response (DDR), a multifunctional signalling process that contributes to cell destination, cell cycle checkpoints regulation, and DNA damage repair [4,5,6,7]. The DDR is comprised of multiple pathways, each of which is involved in a lot of cross-talk

with other signalling systems and within the netw ork.

Chemoresistance and the activation of DDR path ways have been connected to the stemness of cancer cells in recent studies [1, 8].

After receiving chemotherapy or radiation therapy, a particular threshold of DNA damage determines the fate of the cells. DDR triggers p53 signalling and apoptosis when more significant DNA damage takes place. Similar to normal stem cells, chemo-/radioresistant and CSC populations are quiescent or slow-proliferating cells [9,10]. Both resistant cancer cells and CSC may share common characteristics as biomarkers. In turn, chemoresistant triple-negative breast cancers (TNBC), revealed an elevated expression of CSC-associated genes in their biopsies. The aforementioned similarities also can be viewed as the results of closely activated pathways. For instance, Wnt/β-catenin pathway is active in both resistant cells and CSC [1, 11, 12, and 13].

The DNA repair machinery and cell cycle checkpoints are stimulated in response to IR-induced DNA damage. BER is the predominant repair pathway, which eliminates damaged bases and DNA SSBs by DNA polymerase, and ligation of DNA ends to fill in gaps. The major pathway for the repair of bulky DNA damages that result in DNA helical distortion is NER. NER proteins, such as Xeroderma pigmentosum, complementation group G (XPG), and Xeroderma pigmentosum, complementation group C (XPC) are also engaged in the repair of oxidative damage through stimulation of BER, demonstrating cross-talk between these two repair pathways [14,6]. This research focuses on XPC, damaged-DNA binding protein 2 (DDB2), polymerase eta (POLH), and Proliferating cell nuclear antigen (PCNA) as DNA repair and cell cycle regulation genes in addition to their transcriptional responsiveness to radiation [14,15,16,17,18].

Currently, animal models and in vitro research are the main sources of our knowledge and understanding of the underlying radiobiological processes. There are few in vivo data despite the use of a variety of cell types to study the kinetics of the transcriptional response to IR. For practical or ethical reasons, data points in humans were rarely designed to collect samples at numerous short time points following the first RT fraction. To bridge these gaps in knowledge, we designed this study to investigate gene expression responses after radiotherapy fractions in breast cancer patients. To assess their predictive added value, a panel of four circulating genes responsive to ionizing radiation was chosen. But even so, one of our limitations was the need to collect samples at numerous short time points after the first fraction of RT.

SUBJECTS AND METHODS

Subjects

The present study was conducted on 51 females: 31 cancer patients and 20 healthy volunteers as a control group. The control group was with matched demographics; free from any smoking data or malignancy and not suffering from any chronic health problem. They freely volunteered to participate in the study and informed written consents were collected from them before inclusion.

Patients were selected from those admitted to the oncology departments, at Alexandria university hospitals, Egypt. The purpose of the eligibility requirements was to make sure that participants weren't affected by known factors that could change the levels of the analytes under study. The study was implemented following the ethical declaration and approved by the Ethical Committee of the Medical Research Institute (MRI), Alexandria University (IOROH: lORG 0008812). According to the instructions of the

Ethics Committee, signed consents were received from all individuals involved in the study. Available Clinical data including medical history and routine laboratory investigations were collected from all patients. Because this study was conducted during Covid-19 peaks and the search in a paper medical record is limited, not all the clinical data were collected, and it is considered a limitation in this study. For all post-radiotherapy patients, a blood sample was collected from them after total exposure of 40 Grey.

Collection of samples

In this cross-sectional study, a 5 ml blood sample was collected from each individual according to the instructions of ethics committee, signed consents were received from all individuals involved in the study, and blood was used for total RNA isolation for the assessment of gene expression.

Gene expression of POLH, PCNA, DDB2, and XPC

500 µL of blood was used for total RNA extraction using QIAamp RNA Blood mini kit (cat. no. 51104) according to the manufacturer's instructions. Nanodrop analysis was used to determine the concentration and integrity of the extracted RNA. The reverse transcription of the extracted RNA was performed using Thermo scientific Revertaid™ first Strand cDNA synthesis kit according to the manufacturer's instructions. The genes expression was quantified in the cDNA by Rotor-Gene Q qPCR (Qiagen, USA) using TaqMan™ Gene Expression Assay (FAM) - (cat.no. PN 4371134). Quantitative PCR amplification conditions were adjusted as an initial denaturation at 95°C for 5 minutes and then 45 cycles of PCR for amplification as follows: Denaturation at 94 °C for 20 sec, annealing at 55 °C for 20 sec and extension at 70 °C for 15 s. The relative expression of POLH, PCNA, DDB2, and XPC genes were quantified relative to the expression of the reference gene (GADPH) in the same sample by calculating and normalizing the threshold cycles (Ct) values of target genes to that of GADPH The relative change in mRNA expression in samples was estimated using the $2^{-\Delta\Delta Ct}$ method. The fold change in gene expression relative to a reference gene by $2^{-\Delta Ct}$ statistical analysis of the data was calculated.

Statistical analysis of the data

Data were expressed as mean ± standard deviation (SD) or median and Inter quartile range (IQR). The used tests were Mann Whitney test, Kruskal Wallis test, Post Hoc Test, Receiver operating characteristic curve (ROC), Sensitivity, Specificity, Positive Predictive value (PPV), and Negative Predictive value (NPV). A value of $p < 0.05$ was considered statistically significant. A Spearman coefficient was calculated to evaluate the correlation between relevant parameters. Statistical analyses were conducted using the statistical software package SPSS version 20 (SPSS Inc., Chicago, USA).

RESULTS

The age description and the received radiation doses of the studied groups are illustrated in table (1).

Figure (1) revealed the relative expression of the four circulating genes relative to the control group to assess the transcriptional changes after RT fractions (40 Gy) in breast cancer patients in this cross-sectional study. Relative to the control group, the circulating levels of POLH, PCNA, DDB2, and XPC were higher in breast cancer patients before radiotherapy (2.3397 ± 1.2736, 4.1285 ± 1.9003, 7.8767 ± 4.0575, and 3.5249 ± 2.0339 folds respectively) as illustrated in table (2). Also, the results represented in table (2) shows the circulating levels of POLH and XPC in the post-radiotherapy group (1.4349 ± 0.4417, and 1.3244 ± 0.3661 folds respectively) were slightly higher than that in the control group, but PCNA circulating level in the post-radiotherapy group was lower than that in the control group (0.7542 ± 0.2379 folds).

Circulating DDB2 showed a significant difference in the relative expression among the three studied groups (p= 0.030*). Also, there was a significant difference in the relative expression of circulating DDB2 between the control group and the pre-and post-radiotherapy groups (p1=0.017*, p2=0.037*) respectively. On the other hand, DDB2 relative expression was 7.8767 ± 4.0575 and 3.8720 ± 1.7570 in the pre-and post-radiotherapy groups respectively as illustrated in figure (1) and table (2).

Relative to the pre-radiotherapy group, the relative expression of the circulating POLH, PCNA, DDB2, and XPC were lower in post-radiotherapy group (0.17875 ± 0.0550, 0.07278 ± 0.0230, 0.02235 ± 0.0101, 0.14038 ± 0.0388) respectively as illustrated in table (3) and figure (2).

Relative to the control group, In the Pre-Radiotherapy group, XPC relative expression showed a positive and significant correlation with POLH, PCNA, and DDB2 (p= <0.001*, <0.001*, and 0.001* respectively). Also, DDB2 relative expression showed positive and significant correlation with POLH, and PCNA (p= <0.001* and <0.001* respectively). And finally, PCNA

relative expression showed a positive and significant correlation with POLH (p= <0.001*). Moreover, Relative to the control group, In the post-radiotherapy group, XPC relative expression showed a positive and significant correlation with POLH, PCNA, and DDB2 (p= <0.002*, 0.034*, and 0.003* respectively). Also, DDB2 relative expression showed a positive and significant correlation with POLH and PCNA (p= <0.001* and 0.013* respectively), and finally, PCNA relative expression showed a positive and significant correlation with POLH (p= <0.007*) as explained in table (4).

Table (5) and figure (3) show that the four genes combination measured by qRT-PCR was capable to discriminate the pre-radiotherapy group from the post-radiotherapy group significantly (p= 0.018*) with a sensitivity of 87.50% and specificity of about 60%.

Table (1): Comparison between the three studied groups according to demographic data and total radiotherapy dose.

	Control	Pre-Radiotherapy	Post- Radiotherapy
	(n = 20)	(n = 15)	(n = 16)
Age (years)			
≤60	20 (100%)	13 (86.6%)	13 (81.25%)
>60	0 (0%)	2 (13.3%)	3 (18.75%)
Mean ± SD.	39.15 ± 9.46	49.31 ± 12.42	51.0 ± 11.95
Median (Min. – Max.)	37.5 (25 – 60)	50 (25 – 68)	50 (27 – 70)
Radiation doses (Gy)			
Total doses	0	0	40 Gy

Table (2): Comparison between the three studied groups according to different genes relative to control.

	Control	Pre- Radiotherapy	Post-Radiotherapy	H	p
POLH	**(n = 20)**	**(n = 15)**	**(n = 16)**		
Mean ± SE.	1.0 ± 0.48	2.3397 ± 1.2736	1.4349 ± 0.4417	2.185	0.335
Median	0.1135	0.2861	0.6097		
(Min. – Max.)	(0.0007 – 8.5417)	(0.0012 – 18.8243)	(0.0024 – 5.2946)		
PCNA	**(n = 20)**	**(n = 15)**	**(n = 14)**		
Mean ± SE.	1.0 ± 0.4183	4.1285 ± 1.9003	0.7542 ± 0.2379	0.345	0.842
Median	0.1387	0.2159	0.4934		
(Min. – Max.)	(0.0008 – 7.7744)	(0.0003 – 23.2430)	(0.0003 – 2.6735)		
DDB2	**(n = 20)**	**(n = 15)**	**(n = 16)**		
Mean ± SE.	1.0 ± 0.6344	7.8767 ± 4.0575	3.8720 ± 1.7570	7.041*	0.030*
Median	0.1325	1.5194	1.3916		
(Min. – Max.)	(0.0009 – 12.8481)	(0.0022 – 59.0342)	(0.0004 – 28.9097)		
Sig. bet. grps.	p_1=0.017*,p_2=0.037*,p_3=0.745				
XPC	**(n = 20)**	**(n = 15)**	**(n = 16)**		
Mean ± SE.	1.0 ± 0.5645	3.5249 ± 2.0339	1.3244 ± 0.3661	1.71	0.425
Median	0.1112	0.856	0.7932		
(Min. – Max.)	(0.0011 – 11.2026)	(0.0007 – 30.8192)	(0.0003 – 5.0482)		

SE: **Standard error** H: H for **Kruskal Wallis test,** pairwise comparison between each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the three studied groups
p_1: p value for comparing between **Control** and **Pre- Radiotherapy**
p_2: p value for comparing between **Control** and **Post- Radiotherapy**
p_3: p value for comparing between **Pre- Radiotherapy** and **Post- Radiotherapy**
*: Statistically significant at $p \leq 0.05$
Note: There were two missed readings of PCNA in the post-radiotherapy group.

Table (3): Comparison between the two studied groups according to different genes (relative to Pre Radiotherapy).

	Pre- Radiotherapy	**Post- Radiotherapy**	**U**	**p**
POLH Min. – Max. Mean ± SE. Median (IQR)	**(n = 15)** 0.00015 – 11.62822 1.0 ± 0.7239 0.05847 (0.0030 – 0.4180)	**(n = 16)** 0.00029 – 0.65955 0.17875 ± 0.0550 0.07595 (0.0160 – 0.2423)	124.0	0.897
PCNA Min. – Max. Mean ± SE. Median (IQR)	**(n = 15)** 0.00003 – 10.02401 1.0 ± 0.6256 0.03555 (0.0028 – 0.7234)	**(n = 14)** 0.00003 – 0.25799 0.07278 ± 0.0230 0.04762 (0.0007 – 0.1272)	93.500	0.448
DDB2 Min. – Max. Mean ± SE. Median (IQR)	**(n = 15)** 0.00001 – 15.31786 1.0 ± 0.9548 0.00975 (0.0020 – 0.0700)	**(n = 16)** 0.000002 – 0.16691 0.02235 ± 0.0101 0.00803 (0.0022 – 0.0256)	114.0	0.616
XPC Min. – Max. Mean ± SE. Median (IQR)	**(n = 15)** 0.00008 – 10.39547 1.0 ± 0.6580 0.09137 (0.0009 – 0.4636)	**(n = 16)** 0.00003 – 0.53510 0.14038 ± 0.0388 0.08408 (0.0163 – 0.2496)	121.50	0.606

SE: **Standard error** IQR: **Inter quartile range**

U: Mann Whitney test
p: p value for comparing between the two studied groups
Note: There were two missed readings of PCNA in the post-radiotherapy group.

Table (4): Correlation between the different genes relative to control.

			PCNA	**DDB2**	**XPC**
Pre-Radiotherapy	**POLH**	r_s	0.882	0.855	0.843
		p	<0.001*	<0.001*	<0.001*
	PCNA	r_s		0.868	0.900
		p		<0.001*	<0.001*
	DDB2	r_s			0.798
		p			0.001*
Post-Radiotherapy	**POLH**	r_s	0.688	0.904	0.706
		p	0.007*	<0.001*	0.002*
	PCNA	r_s		0.644	0.569
		p		0.013*	0.034*
	DDB2	r_s			0.694
		p			0.003*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Table (5): Agreement (sensitivity, specificity) for circulating POLH, PCNA, DDB2, XPC and combination of the four genes measured by qRT-PCR to discriminate Pre- Radiotherapy from Post- Radiotherapy groups. (Relative to Pre- Radiotherapy)

	AUC	p	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
POLH	0.516	0.880	0.308 – 0.723	≤0.12759	68.75	43.75	55.0	58.3
PCNA	0.583	0.442	0.371 – 0.794	>0.01918	56.25	42.86	52.9	46.2
DDB2	0.555	0.598	0.351 – 0.759	>0.00877	50.0	50.0	50.0	50.0
XPC	0.553	0.601	0.351 – 0.756	>0.04117	56.25	47.06	50.0	53.3
Combination of the four genes.	0.754	0.018*	0.581 – 0.928		87.50	57.14	70.0	80.0

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value

Figure (1): The graphical representation of the changes in the circulating level of the four genes among the three studied groups.

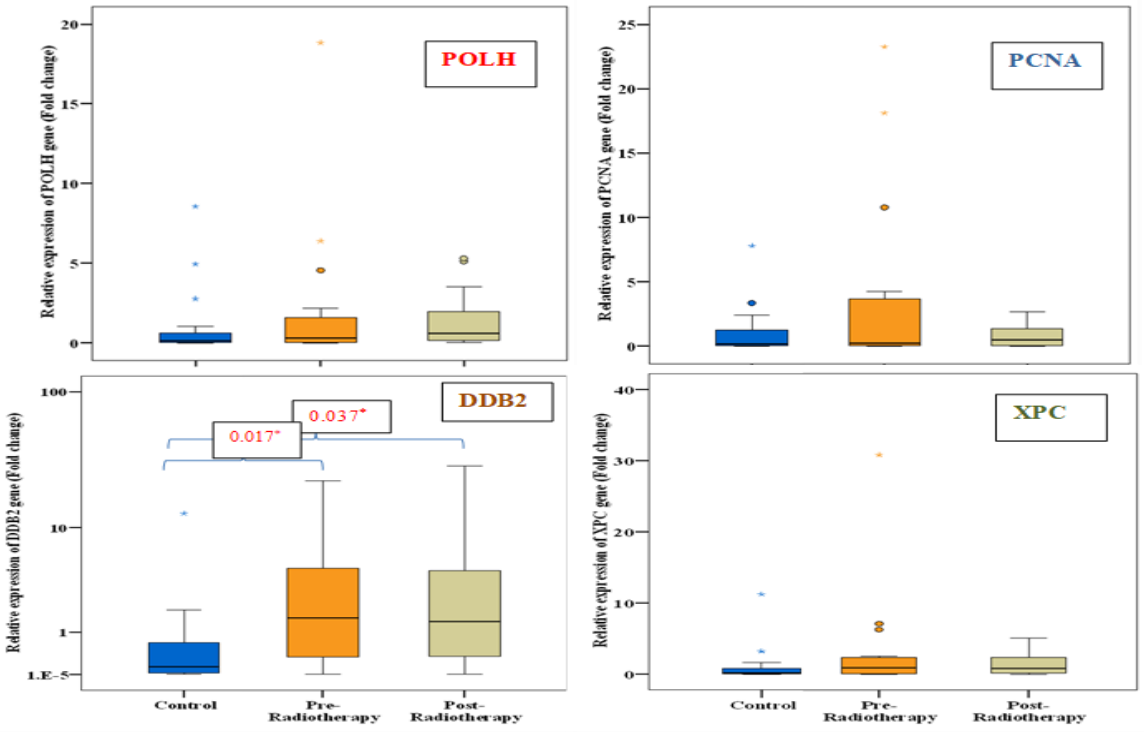


Figure (2): The changes in the circulating level of the four genes relative to Pre- Radiotherapy group.

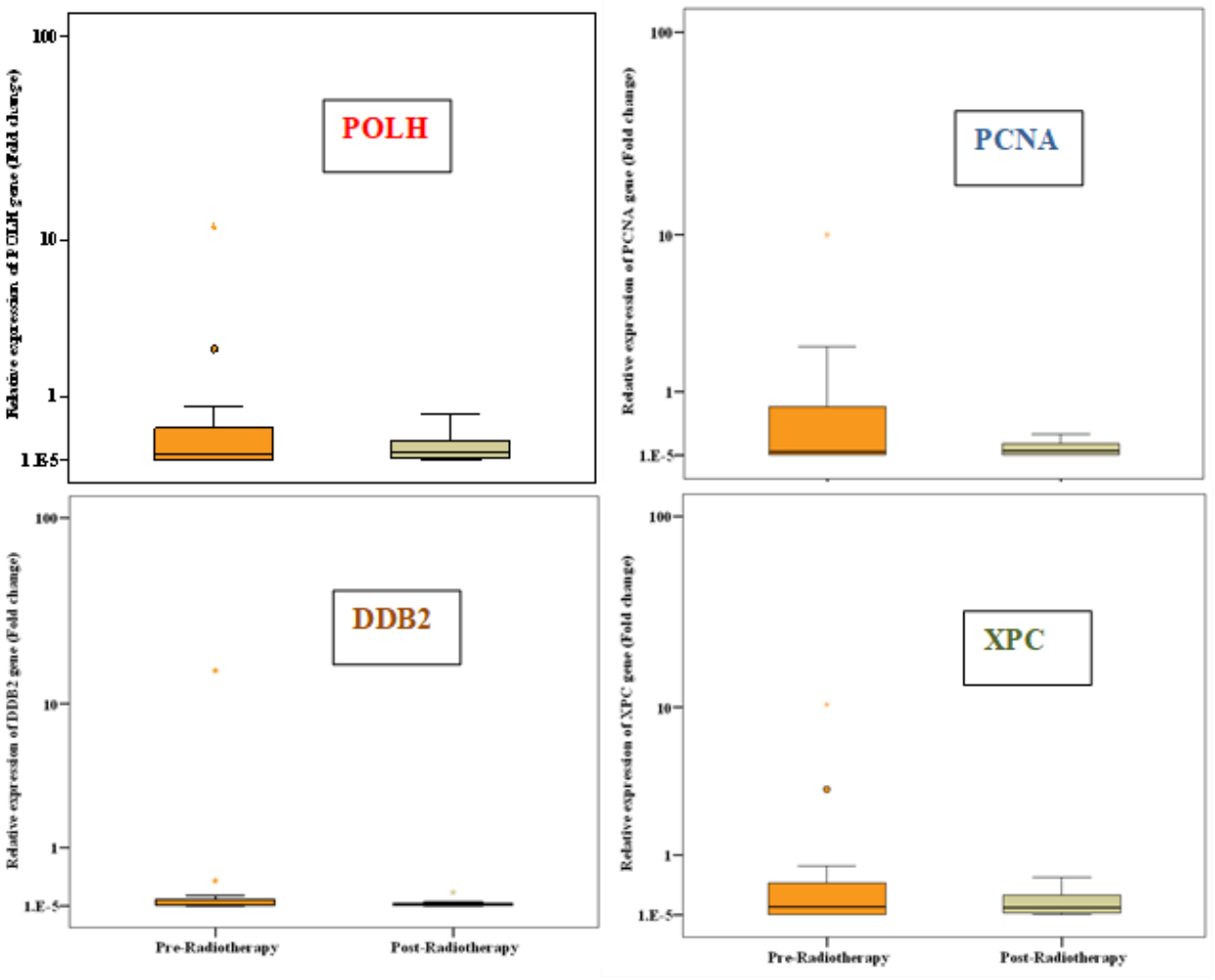
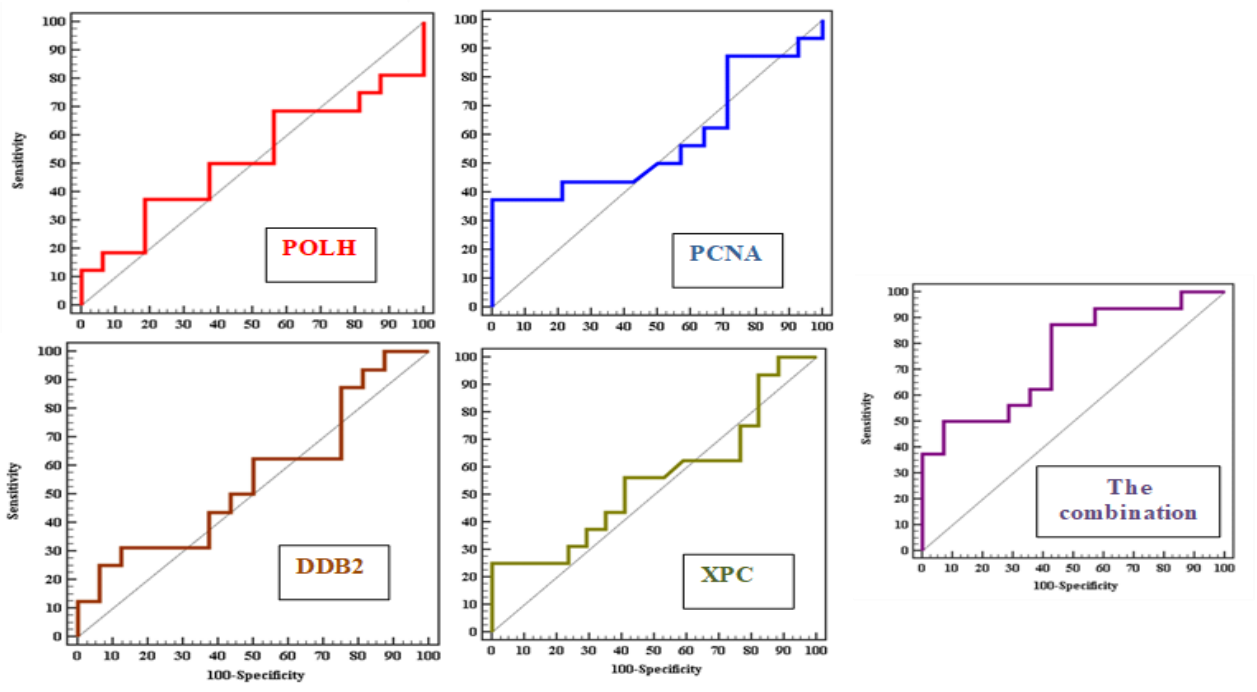


Figure (3): The ROC curves of circulating POLH, PCNA, DDB2, XPC and combination of the four genes measured by qRT-PCR to discriminate Pre- Radiotherapy from Post- Radiotherapy groups. (Relative to Pre-Radiotherapy).



DISCUSSION

A DNA lesion activates the DNA damage response (DDR), a multifunctional signalling process that contributes to cell destination, cell cycle checkpoints regulation, and DNA damage repair [19,4,5,6,7].

The DDR is comprised of multiple pathways, each of which is involved in a lot of cross-talk with other signalling systems and within the netw ork. Activation of DDR pathways can be responsible for the radio-/chemoresistant cancer cells [1, 8]. This study aims to assess the radiation-induced changes in the expression of DNA repair and cell cycle regulatory genes (POLH, PCNA, DDB2, and XPC) in the blood of breast cancer patients and to evaluate their potential use as predictive biomarkers for treatment responses.

Forrester and colleagues used exon arrays, for all known DNA repair gene exons, to assess the effect of IR on the expression of DNA repair genes in cells derived from lymphoblastoid cell lines (LCLs) and primary fibroblasts. They discovered that 21 and 16 DNA repair genes were modulated in LCLs and primary fibroblasts, respectively when applying a p-value cut-off of 0.05 to compare sham-irradiated to those irradiated with 10 Gy at 4 hours post-IR. POLH, DDB2, PCNA, XPC, and RRM2B were the top five genes in lymphoblastoid cells. They validated their results for many of these genes using qRT-PCR. The results of the qRT-PCR analysis were consistent with the exon array-derived data [20]. Forrester and ours used qRT-PCR to assess XPC, POLH, DDB2, and PCNA because these genes have previously been identified as responsive to DNA damaging agents including IR [15,16,17,18]. But the experimental designs were completely different from the perspectives of subjects, time course, and doses. These differences could explain the differences in results.

In numerous cell lines, Rashi-Elkeles' meta-analysis found a core of 374 genes that respond to IR, pointing to a large group of genes that together regulate a significant volume of biological activity. Additionally, they observed that the apoptotic pathway genes were markedly overrepresented in this core (using a combination between microarrays and computational analysis). Our findings do not agree with their meta-analysis, which found that the XPC, DDB2, and PCNA genes were upregulated in response to ionizing radiation. This disagreement could be due to the subject type, radiation dose and dose rate because they used cell lines and selected genes were assayed after exposure to IR (5 Gy) and the corresponding mock-irradiated controls (4h, without IR) [21,22].

Additionally, Rashi-Elkeles and colleagues concluded that analyses of gene expression profiles naturally draw functional conclusions based on the protein products of the identified transcripts (for example, the balance between p53's life and death decisions). The relationship between the kinetics of gene expression and the related proteins should be investigated using the current proteomic techniques [22]. We agree with their conclusion and the protein results will be published within less than a year to deepen multi-layered understanding of the DNA damage response especially DNA damage repair (DDR) proteins have become a promising approach in precision cancer therapy [23].

In the global genome NER, the damage is detected by the UV-DDB (DDB1 and DDB2) and XPC-RAD23B complexes [24,25,26]. DDB1 takes part in NER through DDB2 DNA-binding and cullin 4A ubiquitin ligase activity. The DDB1-CUL4-ROC1 complex ubiquitylates XPC, which may improve its ability to bind DNA and encourage NER [27,28]. Firstly, The DDB complex recognizes the CPD lesions and recruits XPC [24,29,26], whereas XPC can independently recognize 6-4PP lesions. Cullin 4A-mediated proteolysis of DDB2 protein at DNA damage sites regulates lesion recognition by XPC. The recruitment of XPA, XPG, and TFIIH components by XPC facilitates the opening of the DNA helix surrounding the damage site to form a bubble [30].

Ray and colleagues found that immunofluorescence data demonstrated that DDB2 and XPC facilitate ATR and ATM recruitment to the damage sites and have an impact on their functional activation. They came to the conclusion that DDB2 and XPC have a novel role in maintaining a vital cross-talk with checkpoint proteins and thereby coordinating subsequent repair and checkpoint activation [31]. These results were consistent with this study results of correlations.

High levels of DDB2 protein and mRNA have been observed in breast cancer models, according to the findings of Gilson and colleagues, suggesting the oncogenic function of DDB2 in mammary cancer cell growth. The dual activity of DDB2 on cancer cell proliferation was explained by its in vitro antiproliferative effects in ovarian and prostate malignancies [32,25]. These results were consistent with ours because our selected genes were of higher relative expression in breast

cancer patients (XPC, DDB2, POLH, and PCNA levels are 3.5249 ± 2.0339, 7.8767 ± 4.0575, 2.3397 ± 1.2736, and 4.1285 ± 1.9003 respectively). These results were also in line with the findings of Zilal Kattan and colleagues, who demonstrated for the first time that DDB2 can play a role as an oncogene and may become a promising predictive marker in breast cancer [25].

Deficiencies of many genes in DNA repair pathways have been identified in humans, characterized, and could result in radiosensitivity [20]. On the other hand, exposure to ionizing radiation modulates the expression of numerous genes. Identification of specific genes may allow the determination of pathways important in radiation responses [33]. Nevertheless, the radioresistance of cancer cells remains a significant limitation for RT applications. To improve the outcomes of RT, efforts are continuously ongoing to develop radiosensitizers and find sensitizing targets. IR-induced DNA lesions can cause a variety of cellular DNA damage responses (DDRs), including those helping cells recover from radiation injuries, such as cell cycle arrest, DNA repair, and activation of DNA damage sensing and early transduction pathways. These protective DDRs undoubtedly confer tumour radioresistance. A potential method for overcoming tumour radioresistance is targeting DDR signalling pathways [34].

Rui-Xue Huang and Ping-Kun Zhou concluded the predictive value of the radiation-responsive genes [34,35]. This study showed much lower relative expression in the post-radiotherapy group (relative to pre-radiotherapy group, XPC, DDB2, POLH, and PCNA levels are 0.14038 ± 0.0388, 0.02235 ± 0.0101, 0.17875 ± 0.0550, and 0.07278 ± 0.0230 respectively). This study results, as well as Rui-Xue Huang's conclusion, and Lourdes Cruz-Garcia's findings led us to recommend these genes assessment at different time points to predict the treatment responses [34,35]. This recommended study may reveal inter-individual variability and would provide individual biological dosimetry information.

While this study successfully reached the conclusion, there were some limitations. Because this study was conducted during Covid-19 peaks and the search in paper medical records was limited, the first issue was a lack of patient's clinical data. Another limitation was the need to collect samples at multiple short time points after the first fraction of RT.

Conclusion

According to this present study, we can conclude the followings:

- Genes involved in cell cycle and DNA repair, such as POLH, PCNA, DDB2, and XPC, were altered due to radiotherapy.
- The relative expression of circulating POLH, PCNA, DDB2, and XPC in breast cancer patients could be promising predictive biomarkers for treatment responses.
- These radiation-responsive genes could have an oncogenic role in mammary Breast cancer patients.
- Assessment and targeting of the circulating POLH, PCNA, DDB2, and XPC in breast cancer patients could be an attractive strategy for overcoming tumor radioresistance.

Recommendations:

- Assessing the four genes at different time points during radiotherapy.
- Validating their predictive values in a larger number of breast cancer patients.

Availability of data and materials

The datasets and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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