Chromosomal Aberrations and Gene Expression Study in Breast Cancer Patients Undergoing Radiotherapy

AbdulSahib K. Ali¹, Wiaam A. Al-Amili², Rafid A. Abdul-Kareem², Amel J. Muttar¹, Shatha K. K.¹, Adil H.E¹

¹Ministry of Science and Technology / Central Laboratories Directorate.
²University of Baghdad / Institute of Genetic Engineering / Iraq.

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Abstract: The present study aims to use the chromosomal aberrations and gene expression analysis as biomarkers for detection of the effects of ionizing radiation exposure in breast cancer patients (BC) undergoing radiotherapy about 20-30 Gy locally gamma cells, which may effect DNA of cancer patients. This study was carried out on thirty, Iraqi women patients with breast cancer patients during radiotherapy treatment at Al-Amel National Hospital for cancer Management in Baghdad during time 2-13 years, non-smokers and non-alcoholic, aged (30 - 59 year), with stage (grade) I - III, as well as thirty apparently healthy individuals females collected randomly from population living Baghdad, aged ranged (30 - 59 year) which are non-smokers non-alcoholic as control group. Using two molecular genetic end-points parameters were studied to determine genotoxic effects of radiotherapy in peripheral blood lymphocytes of some Iraqi breast cancer patients and compared with control groups. Investigations were carried out by using the chromosomal aberrations (CA) and gene expression were performed on peripheral blood lymphocytes for breast cancer patients and control groups. The present study showed significant increase (p<0.01) in the unstable chromosomal aberration types (CA) fragment, ring and dicentric chromosomes for the breast cancer patients during radiotherapy as compared with the control group. Also, This study including twenty, Iraqi women patients with breast cancer after radiotherapy treatment about 20-30 Gy locally exposure to gamma rays, aged (35 - 55 years), as well as twenty female blood samples, aged (35 - 55 years) which are non-smokers or alcoholic as control group. Total RNA was isolated from blood for BC patients and control groups. The RNA concentration was determined spectrophotometrically by measuring their absorbance that depend on the ratio A_{260}/A_{280} of the wavelength, which leads to the determination of RNA purity, which ranged from 1.79-2.1 in two groups. Complementary DNA was used in amplification of genes used in the present study, three types of specialized primer genes were selected for the genes CDKN1A, BRCA1, and BRCA2 which have a relation with ionizing radiation in addition to the primers for internal control (β-actin) genes. Gene expression analysis revealed statistically significant (ΔΔCt comparative Ct method) transcriptional changes in two genes CDKN1A and BRCA2 up-regulated while BRCA1 gene down-regulated. In conclusion, the results indicated that there is a possibility of using the changes at the level of CA as useful biomarkers for the detection of the effect of radiotherapy in peripheral blood lymphocytes for BC cancer patients. several genes involved in cell cycle regulation and DNA repair were found to be significantly induced by radiation treatment.

Keywords: Radiotherapy, Breast Cancer, CA, Gene Expression.

Corresponding author: (Email: sahib1966@yahoo.com).

Introduction:

Breast cancer (BC) is diagnosed worldwide in approximately one million women annually and radiation and chemical therapy is an integral part of treatment. Radiotherapy is a toxic cellular treatment that destroys rapidly dividing cells such as cancer cells. Many factors have risk for breast cancer like environment factor or genetic factor like BRCA 1, BRCA 2, CHEK 2 and...
Radiotherapy is generally accepted and an important therapeutic modality, particularly in patients with advanced stage non-small cell cancer. However, individual patients may show quite different patterns of response to radiotherapy. This individual variation reflects the underlying mechanisms controlling response to radiation damage, which is determined by multiple cellular events that are controlled by a large pool of genes and their interactions (1). Radiotherapy or chemotherapy reduces the risk of distant metastases by approximately one-third; however, 70–80% of patients receiving this treatment would have survived without it (2). Hereditary breast and ovarian cancer due to mutations in genes BRCA1 and BRCA2 is the most common cause of hereditary forms of both breast and ovarian cancer and occurs in all ethnic and racial populations. This early damage may lead to chromosomal aberrations (CA) and thus to increased risk of mutagenesis and carcinogenesis (3). Cytogenetic studies on individuals occupationally exposed to radiation have been carried out extensively. High frequency of chromosomal aberration (CA) such as dicentrics, rings and acentrics in the peripheral blood lymphocytes of personnel handling diagnostic X-ray machines has been reported by Kasuba et al., (4) and Gadhia et al., (5).

Among the various cytogenetic parameters employed, the most reliable biological indicators of ionizing radiation exposure are CAs in peripheral blood lymphocytes (6,7). Radiation is an effective anti-cancer therapy but leads to severe late radiation toxicity in 5%–10% of patients. Assuming that genetic susceptibility impacts this risk, we hypothesized that the cellular response of normal tissue to X-rays could discriminate patients with and without late radiation toxicity. Radiotherapy is one of the most effective treatments for cancer. The success of radiotherapy depends on its ability to kill cancer cells while sparing normal tissue (8).

Toxicity risk is affected by radiation dose and volume as well as age and condition of the patient. Clinical trials have demonstrated that escalation of the radiation dose increases local tumor control (9,10) but may also increase the risk of late complications (5). Frequencies of chromosomal aberrations in ex vivo irradiated peripheral blood lymphocytes are generally increased in patients displaying normal tissue toxicity after radiotherapy. However, the correlation is too weak to allow pretreatment identification of such patients (11). The cellular and molecular heterogeneity of breast tumors and the large number of genes potentially involved in controlling cell growth, death, and differentiation emphasize the importance of studying multiple genetic alterations in concert. Systematic investigation of expression patterns of thousands of genes in tumors using cDNA microarrays, and their correlation to specific features of phenotypic variation, might provide the basis for an improved taxonomy of cancer (10,12).

The aim of the present study measuring the genetic damage for two genetic end-points was studied in some Iraqi breast cancer patients during radiotherapy using the CA and gene expression profiles in some Iraqi patients with breast cancer treated radiotherapy. Also, the purpose of this
study was to investigate the molecular basis underlying response to radiotherapy in breast cancer.

**Materials and Methods:**

**Subjects and blood sample collection:**

During the period January 2018 till January 2019, this study was carried out on thirty Iraqi women patients with breast cancer after radiotherapy treatment, non-smokers and non-alcoholic, aged (30-59 year), with stage (grade) I to III, breast cancer patients treated at Al-Amel National Hospital for cancer Management in Baghdad during time 2-13 years, as well as thirty apparently healthy individuals females collected randomly from population living Baghdad, aged (30 - 59 year) which are non-smokers, non-alcoholic as control group, all of them (100 %) were females. Five ml of human peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

**Assay procedure:**

The dicentric chromosome aberration was performed according to the description by IAEA (13).

**Blood sampling and lymphocyte cultures:**

Samples of 4 ml whole blood were obtained using heparinized tubes. The cultures was added 0.5 ml of heparinized blood to 4.5 ml RPMI 1640 medium (sigma), supplemented with 20% fetal calf serum (sigma), and 0.1 mg/ml streptomycin and penicillin (Sigma-Aldrich). Phytohemagglutinin (PHA) at a concentration of 10 μl / ml was used to stimulate lymphocyte proliferation. Blood cultures were incubated at 37°C for 56 hours for CA. Colchicine was added to a final concentration of 0.5 μg /ml before 2 h the end of the CA incubation.

**Treatment with hypotonic solution and fixation:**

After 56 h of incubation, the CA cultures were harvested by centrifugation, suspended in hypotonic solution (0.075 mol/L KCl), incubated for 20 minutes at 37°C and fixed in three changes of methanol: acetic acid (3:1). Cell suspensions were dropped on wet, cold slides and dried. Then the slides were routinely stained with Giemsa for 20 min. (13).

**Microscopic examination:**

The coded slides were scored and analyzed at magnification of 1000 X. A total of 200 cells / individual were screened in the breast cancer group and in the control group, and different types of aberrations were classified.

**Gene Expression study on breast cancer patients undergoing radiotherapy:**

This study was carried out on twenty Iraqi women patients with breast cancer after radiotherapy treatment locally about 20-30 Gy gamma rays, non-smokers and non-alcoholic, aged (35-55 year), with stage (grade II only), breast cancer patients treated at Al-Amel National Hospital for cancer Management in Baghdad, as well as
twenty apparently healthy individuals females collected randomly from population living Baghdad, aged (35 - 55 year) which are non-smokers non-alcoholic as control group, and all of them (100 %) were females. Five ml of human peripheral blood from all select subjects were collected and placed into sterile lithium heparin tube. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

**Total RNA extraction:**

Fresh human bloods were used for genomic RNA isolation directly after collection, genomic RNA was extracted using the Trizol method (Invitrogen, USA) according to the manufacturer’s instructions (14). The RNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 \( A_{260} \) and 280 \( A_{280} \) by Nano spectrophotometer. A total of 2 μg RNA was used for reverse transcription (RT) with the *Trans Script* First-Strand cDNA Synthesis Super Mix according to the manufacturer’s instructions (Promega, USA). All primers were designed by the program primer and synthesized (Table 1). The total volume of PCR reactions was 25 μL containing 2.5 μL 10×Taq buffer (Promega), 1.25 U Taq DNA polymerase (Promega), 5mM dNTPs (Promega), 50 pmol of each primer and 100ng of cDNA, 1.25 U Taq DNA polymerase (Promega), 5mM dNTPs (Promega), 50 pmol of each primer and 100 ng of template DNA. PCR reactions were performed on the Master cycler gradient. PCR conditions were: 94°C, 5min; 38cycles of 30 s at 94°C, 30 s at each Tm as appropriate and many seconds as appropriate (60 s/kb) at 72°C; and 72°C for 10 min. The PCR product was analyzed by 1.5% agarose gel electrophoresis and one band was obtained. The QRT-PCR amplification conditions were: 95°C, 3min ; 95°C, 30 sec, 60, 65 °C as appropriate, 30 sec and 72°C, 15 sec for 35 cycles. Melt curves were obtained by increasing the temperature from 56°C to 95°C at 0.5°C/sec for 10 sec, then cooling at 25°C for 30 sec. Each primer set amplified a single product as indicated by a single peak present for each gene during melting curve analyses.

**Table (1): Primers sequence and molecular weight used for QPCR validation and additional expression profiling.**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Primer sequence (5′–3′)</th>
<th>Target size (bp)</th>
<th>Tm (°C)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN1A</td>
<td>Forward: 5′-GACAGCAGAGGAAGACCATGT-3′ Reverse 5′-GGCGTTTGGAGTGGTAGAAATC-3′</td>
<td>185</td>
<td>65</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Forward: 5′-TGCTTGAAGTCTCCCTTG-3′ Reverse 5′-CTTCCATTGAAGGCTCTG-3′</td>
<td>267</td>
<td>57</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Forward: 5′-ACCCTTTTACGGTCTAATGG-3′ Reverse: 5′-TGCGCTGCTTTACTGCAAG-3′</td>
<td>268</td>
<td>60</td>
</tr>
<tr>
<td><em>β</em>-actin</td>
<td>Forward: 5′-GAT GAG ATT GGC ATG GCT TT-3′ Reverse: 5′-ATT GTG AAC TTT GGG GGA TG-3′</td>
<td>100</td>
<td>60</td>
</tr>
</tbody>
</table>

*β*-actin was used as loading internal control.

**Data Analysis and Statistics:**

The data of these studies were compiled into the computerized data file and frequency, distribution and Statistical description (mean, SE) were divided using SPSS Statistical software. We used statistical analysis of variance
(ANOVA) test and least significantly difference (LDS) test by probability of less than 0.01 (p<0.01) according to (15).

The relative quantitative gene expression level was evaluated using the ∆∆Ct comparative Ct method. The ∆Ct values were calculated by subtracting the RPL32 Ct value for each sample from the target Ct value of that sample. Fold inductions were calculated using the formula 2^ (ΔΔCt), ∆Ct=cycle of threshold, ∆Ct=Ct (housekeeping gene) - Ct (target gene), ∆∆Ct=ΔCt (treated) − ΔCt (control). The data thus generated can be analyzed by computer software to calculate relative gene expression in samples. A one sample T-test was used to statistically analyze the difference of the derived expression ratios of irradiated versus non-irradiated samples (16).

Results and Discussion:

Cytogenetic study on breast cancer patients undergoing radiotherapy:

Characteristics and the results of multiple regression analysis including age, gender, smoking status, chromosomal aberration in breast cancer patients undergoing radiotherapy and control groups are summarized in (Table 2). These various types of average As observed in control were fragments (0.00575 ± 0.00076 Frg. /cell), rings (0.00025 ± 0.000251 rings /cell) and dicentrics (0.00175 ± 0.000559). While, the CAs in breast cancer patients undergoing radiotherapy showed a more damage values in fragments (0.00925 ± 0.0011 Frg. /cell), rings (0.00075 ± 0.000412 ring /cell) and dicentrics (0.00375 ± 0.000873 Dicentric /cell). The showed a significant (p<0.01) variation between the average CAs frequency of control and breast cancer patients treatment radiotherapy values (Table 2).The increase frequencies of chromosomal aberration in treated breast cancer patients indicate the cumulative effect of radiotherapy (17,18). It is known that the probability of carcinogenesis is greater in populations exposed to radiation, since ionizing radiation can raise the frequency of CA and spontaneous mutations. Cytogenetic monitoring of treated breast cancer patients has been carried out by means of analyzing the frequencies of chromosomal aberration (19,20), comparing the results with those of a control group. A biologically significant result was obtained a greater frequency of acentric chromosomes in breast cancer patients undergoing radiotherapy, when compared with control individuals (21). The present observations support the findings of other investigators and agree with many cytogenetic studies carried out in breast cancer patients undergoing radiotherapy (20-22). (Table 3) shows the results of influence of three grades on the Level of DNA damage in breast cancer patients after treatment. A borderline association between age and CA parameter frequency was observed in the Iraqi breast cancer patients undergoing radiotherapy group. The statistical analyses of CA parameters in radiotherapy received breast cancer patients after treatment with grade (Table 3). The current study demonstrated that radiotherapy is accompanied by significant. Increased levels of DNA damage in peripheral blood leukocyte of BC patients according to grade. The CA type fragment and dicentric were increased significantly (p < 0.01) according to grade (Table 3).
Table (2): Characterization of the samples (Healthy individuals women control and breast cancer patients women groups undergoing radiotherapy) for Chromosomal aberration study

<table>
<thead>
<tr>
<th>Sample Characteristics</th>
<th>Subject Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy individuals Women group</td>
</tr>
<tr>
<td></td>
<td>Breast Cancer Patients women group</td>
</tr>
<tr>
<td>Number of individuals</td>
<td>30</td>
</tr>
<tr>
<td>Age (years) (Range)</td>
<td>30-59</td>
</tr>
<tr>
<td>Sex (n (%))</td>
<td>Female 30 (100 %)</td>
</tr>
<tr>
<td></td>
<td>Male 0 (0.00 %)</td>
</tr>
<tr>
<td>Smoking status (n (%))</td>
<td>Non-Smoker 30 (100 %)</td>
</tr>
<tr>
<td></td>
<td>Smoker 0 (0.00 %)</td>
</tr>
<tr>
<td>Alcoholic drinking</td>
<td>Non-alcohol 20 (100 %)</td>
</tr>
<tr>
<td>Status (n (%))</td>
<td>Alcohol 0 (0.00 %)</td>
</tr>
<tr>
<td>Chromosomal aberration</td>
<td>No of Metaphase 6000</td>
</tr>
<tr>
<td></td>
<td>Fragment / cell (Mean ± SE)</td>
</tr>
<tr>
<td></td>
<td>0.00575 ± 0.00076 *</td>
</tr>
<tr>
<td></td>
<td>0.00925 ± 0.0011 *</td>
</tr>
<tr>
<td></td>
<td>Ring / cell (Mean ± SE)</td>
</tr>
<tr>
<td></td>
<td>0.00025 ± 0.000251 *</td>
</tr>
<tr>
<td></td>
<td>0.00075 ± 0.000412 *</td>
</tr>
<tr>
<td></td>
<td>Dicentric / cell (Mean ± SE)</td>
</tr>
<tr>
<td></td>
<td>0.00175 ± 0.000559 *</td>
</tr>
<tr>
<td></td>
<td>0.00375 ± 0.000873 *</td>
</tr>
</tbody>
</table>

* Similar latter in a row mean there is no significant difference (p < 0.01).

Table (3): Influence of grade on the CA (Mean ± SE) in breast cancer patients undergoing radiotherapy.

<table>
<thead>
<tr>
<th>Grade Types</th>
<th>CA (Mean ± SE)</th>
<th>Frag./cells (Mean ± SE)</th>
<th>Ring /cells (Mean ± SE)</th>
<th>Dic./cells (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>0.0072 ± 0.0011</td>
<td>0.00072 ± 0.00069</td>
<td>0.0038 ± 0.0015</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>0.0110 ± 0.0025</td>
<td>0.00070 ± 0.00071</td>
<td>0.0042 ± 0.00174</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>0.0103 ± 0.0019</td>
<td>0.00082 ± 0.00076</td>
<td>0.0034 ± 0.0017</td>
<td></td>
</tr>
</tbody>
</table>

Similar letter in a column mean there is no significant difference (p < 0.01)

- Critical Range CA
- (0.01 Frag ,grad ) = 0.0030, 0.0035, 0.0039
- (0.01 rings , grad )= 0.00081, 0.00083, 0.00086
- (0.01 Dic, grad)= 0.00182, 0.00186 , 0.00189

Gene Expression study on breast cancer patients undergoing radiotherapy:

Total RNA was extracted from the Peripheral blood from each patients treated with radiotherapy and control groups. The RNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 (A260) and 280 (A280) by Nano spectrophotometer. The concentration of total RNA ranged from 47 to 199 ng/μl with a Mean ± SE of 123.2 ± 7.85 ng/μl in patients treated with radiotherapy. For control group it ranged from 52 to 197 ng/μl with a Mean ± SE of 112.2 ± 7.70 ng/μl. The yield of RNA from peripheral blood leukocytes acutely depends on the physiological state of the human, reflecting the dynamic shift in circulating white blood cell fraction with subsequent wide-ranging variability on RNA constituents and their yields (23-25). In the present study, the RNA has been isolated from whole blood with a purity ratio ranging from 1.75 to 2.1 for both groups BC treated with radiotherapy and control. If there is contamination with protein or phenol, this ratio will be significantly
less than the values given above, and accurate quantitation of the amount of RNA will not be possible. Relative quantitative gene expression levels for three CDKN1A, BRCA1 and, BRCA2 genes in peripheral lymphocytes for patients treated with radiotherapy regarding to their occupation using ∆Ct method in the present study are shown in (Table 3). These genes showed significant differences between patients treated with radiotherapy and control groups (p < 0.01). The genes CDKN1A, BRCA1 and, BRCA2 were analyzed by RT PCR, to invalidate that these genes generally are induced by irradiation. The average Ct value of CDKN1A gene in the present study is shown in (Table 3). The range of Ct value for CDKN1A gene in the BC Patients treated with radiotherapy group was 22.10 -25.05 with average Ct value for CDKN1A gene a mean ± SE (22.10-25.05).In the control group it ranged from 21.78-22.18 with Ct value for β-actin a mean ± SE (21.135 ± 0.154).

Table (4): Relative quantitative gene expression levels for six genes in peripheral lymphocytes for BC Patients treated with radiotherapy and control regarding to their occupation using ∆Ct method.

<table>
<thead>
<tr>
<th>Gene</th>
<th>BC Patients treated with radiotherapy group(n=20)</th>
<th>Control group (n=20)</th>
<th>LSD value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE of Ct value</td>
<td>Range</td>
<td>Mean ± SE of Ct value</td>
<td>Range</td>
</tr>
<tr>
<td>CDKN1A</td>
<td>23.618±0.272</td>
<td>22.10-25.05</td>
<td>21.135 ± 0.154</td>
<td>21.78-22.18</td>
</tr>
<tr>
<td>BRCA1</td>
<td>25.633± 0.795</td>
<td>25.12-28.73</td>
<td>25.055 ± 1.455</td>
<td>21.51-25.19</td>
</tr>
<tr>
<td>BRCA2</td>
<td>27.135±0.916</td>
<td>25.45-29.41</td>
<td>27.028 ± 0.476</td>
<td>25.88-28.21</td>
</tr>
</tbody>
</table>

As shown in Figure (1) the expression of the CDKN1A gene was found to be up-regulated in peripheral lymphocytes for BC Patients treated with radiotherapy, compared with control group. The up-regulation of numerous cell cycle genes suggests that ionizing radiation has a major effect on cell proliferation, in BC Patients treated with radiotherapy. However, the up-regulation CDKN1A may indicate that the preferential target is the G1/S phase checkpoint and not the G2/M phase checkpoint (26). The expression of CDKN1A is controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli (45).

As shown in Figure (1) the expression of the CDKN1A gene was found to be up-regulated in peripheral lymphocytes for BC Patients treated with radiotherapy, compared with control group. The up-regulation of numerous cell cycle genes suggests that ionizing radiation has a major effect on cell proliferation, in BC Patients treated with radiotherapy. However, the up-regulation CDKN1A may indicate that the preferential target is the G1/S phase checkpoint and not the G2/M phase checkpoint (26). The expression of CDKN1A is controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli (45).

Figure (1): QRT-PCR graphs showing the relative fold expression levels for CDKN1A gene in peripheral lymphocytes for BC patients treated with radiotherapy and control groups.
Altered expression of a few genes playing specific roles in DNA repair/cell cycle control such as CDKN1A, DDB2, XPC, GADD45A and PCNA have been reported earlier (27). Our findings suggest that DNA repair gene expression may be helpful to identify biodosimeters of exposure to radiation.

The average Ct value of BRCA1 gene used in the present study is shown in Table (3). The range of Ct value for BRCA1 gene in the BC Patients treated with radiotherapy group was 25.12-28.73 with average Ct value for BRCA1 gene a mean ± SE 25.63± 0.795.In the control group it ranged from 21.51-25.19 with Ct value for β-actin a mean ± SE (25.055 ± 1.455). As shown in (Figure 2), gene expression levels displayed by BRCA1 gene was down-regulated in peripheral lymphocytes for BC Patients treated with radiotherapy group compared with control group. It has been shown that BRCA1 mRNA levels are down-regulated in BC patients treated with radiotherapy as compared with the control. Since a very high proportion of BRCA1-associated hereditary breast cancers have p53 abnormality. Recently, we have been able to demonstrate that BRCA1 down-regulation and p53 abnormality work synergistically to induce chromosomal instability in sporadic breast cancers (28,29).

The BRCA1 gene combines with DNA damage sensors, other tumor suppressors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (30). As a consequence of this defect in homologous recombination, tumors that arise in BRCA1 carriers are likely to be more sensitive to ionizing radiation exposure .The BRCA1 protein associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. Thus, this protein plays a role in transcription, DNA repair of double-strand DNA breaks (31), ubiquitination, transcriptional regulation as well as other functions (32).

The average Ct value of BRCA2 gene used in the present study is shown in (Table 3). The range of Ct value for BRCA2 gene in the BC patients treated with radiotherapy group was 25.45-29.41 with average Ct value for BRCA2 gene a mean ± SE( 27.135± 0.916) .In the control group it ranged from 25.88-28.21 with Ct value for β-actin a mean
± SE (27.028 ± 0.476). A significant difference was found in between these groups regarding the mean Ct value of β-actin, \( (p=0.012: \ p<0.05) \) with an LSD value of (0.31). BRCA2 gene was found significantly up-regulated by real time PCR analyses. As shown in (Figure 3), gene expression levels displayed by BRCA2 gene was up-regulated in peripheral lymphocytes for BC Patients treated with radiotherapy group compared with control group.

Although BRCA1 mRNA expression is reported to be down-regulated in BC Patients treated with radiotherapy as compared with the control, BRCA2 mRNA expression is reported to be up-regulated in a significant proportion of BC patients treated with radiotherapy. BRCA2 is also a typical tumor suppressor gene, and loss of a wild allele has been reported in almost all breast tumors arising in BRCA2 germ line mutation carriers (33). Other up-regulated genes in BC patients treated with radiotherapy was CDKN1A whose increased expression levels were also confirmed by the real time PCR analysis. As BRCA1 and BRCA2 are involved in the DNA double strand breaks repair (34) which can be caused by exposed to low levels of ionizing radiation, it has been hypothesised that carriers of BRCA1 and BRCA2 mutations might have increased radiosensitivity. We hypothesized that BRCA carriers could have increased radiosensitivity because of impaired DNA repair mechanisms. We observed increased risks of breast cancer among BRCA1 and BRCA2 mutation carriers at dose levels considerably lower than those at which increases have been found in other exposed to radiation. A pooled analysis of eight cohorts exposed to radiation estimated a relative risk of about 2.0 at a dose of 1 Gy, assuming an age at exposure of 25 years (35). BRCA1 and BRCA2 are normally expressed in the cells of breast and other tissues, where they help repair damaged DNA, or destroy cells if DNA cannot be repaired. They are involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double-strand breaks (31,36). If BRCA1 or BRCA2 itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer (37).

![BRCA2 gene expression](image)

**Figure (3):** QRT-PCR graphs showing the relative fold expression levels for BRCA2 gene in peripheral lymphocytes for BC Patients treated with radiotherapy and control groups.
Our present observation that BRCA2 mRNA levels were significantly higher than BRCA1 mRNA expression levels seems to be consistent with this report.

Two gene up-regulated genes in BC patients treated with radiotherapy were CDKN1A and BRCA2 whose increased expression levels were also confirmed by the real time PCR analysis. The results of the current study are compatible other studies that increased BRCA2 gene expression of radiation-responsive genes in chronically exposed \( (25,38) \). Several genes involved in cell cycle regulation and DNA repair were found to be significantly induced by radiation treatment. Mutations were found in the TP53 gene in 39% of the tumours and the gene expression profiles observed seemed to be influenced by the TP53 mutation status \( (39) \). Among these 12 genes, we found no overlap between sham and irradiated samples for 8 biomarkers \( (\text{BBC3, FDXR, CDKN1A, GADD45a, PCNA, XPC, DDB2 and POLH}) \), and found only slight overlaps for the other 4 biomarkers \( (\text{XRCC1, BAX, CCNG1, LIG1 and RAD51}) \) \( (40,41,42) \).

**Conclusions:**

In the light of the findings of the present study the increase frequencies of CA in breast cancer patients undergoing radiotherapy indicate the effect of radiotherapy in inducing some genetic damage. CA could be used as non-invasive and inexpensive biomarkers with predictive value for the estimation of the high increased breast cancer risk assessment in medical surveillance programs. The results indicate the cumulative effect of anticancer agent in inducing some genetic damage in DNA. The presence of a significant reduction \( (p <0.01) \) in the amount of gene expression of the BRCA gene in BC patients treated with radiotherapy. The existence of significant elevation \( (p <0.01) \) in the amount of genes expression of the, CDKN1A, and BRCA2 genes in BC patients treated with radiotherapy. It was found that this gene having up-regulation level in the blood of BC Patients treated with radiotherapy group comparison with the control group. The investigation of genes expression such as CDKN1A, BRCA1 and BRCA2 after exposed to ionizing radiation could serve as a potential molecular marker for such biodosimetry.

**References:**


