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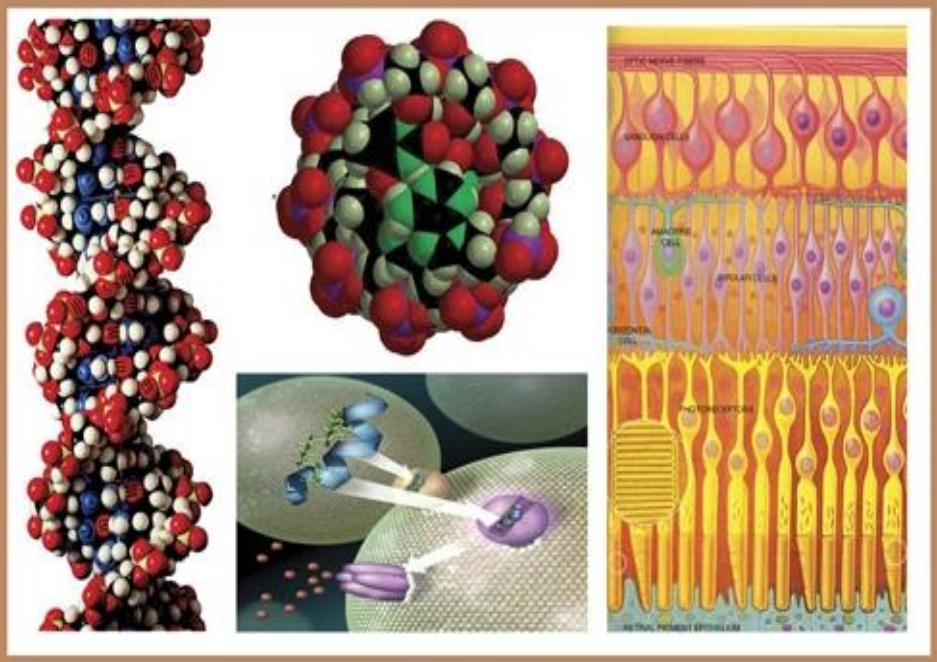

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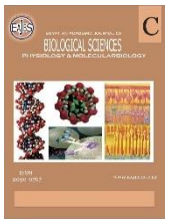

The Effect of Ionizing Radiation on Tumor Suppressor Gene (TP53) In Cancer Patients Who Are Receiving Radiotherapy

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ABSTRACT

Ionizing radiation has an effect on health and genetics and its recently used as therapy for different types of cancer. The study aimed to investigate the effect of therapeutic radiation on the tumor suppressor gene, TP53Three regions of TP53 were investigated, exon-7, intron-7 and exon-8. A total of 77 cancer patients who had radiation therapy were examined in this study for genetic analysis with 80 healthy volunteers (control). Genetic testing was carried out before and after radiation treatment. Three ml of blood sample was taken from each patient for the purpose of DNA extraction. In *blood cell DNA,* TP53 was PCR amplified and sequenced to check any mutation which may occur after radiation. The result demonstrated short-term radiation there was no significant mutation in TP53 gene after radiation therapy. However, there was a novel mutation indicated in the area of the study, in intron-7 region of TP53 gene, C14166T. This mutation was for the first time observed in the study area and in the world. The mutation exists in both health control (26.6%) and cancer patients (73.3%). The female gender experienced a higher rate (60%) of this mutation than male at the rate and it's more common in breast cancer patients, 33.3%. It is observed in patients older than 40 at 66.6%. Short-term radiation therapy may not cause a serious gene mutation in patients undergoing radiotherapy. A novel mutation in TP53 exists in the study area which is more common in cancer patients. This novel mutation is higher in females and in old ages. This single mutation might be useful in the future to be used as a diagnostic marker and this needs more study to investigate.

INTRODUCTION

Radiation has an effect on genes and causes different types of mutation in DNA. Ionizing radiation leads to a break in DNA strands which requires DNA checkpoint of the cell to repair the change. If there are abnormalities in DNA repair mechanism, it leads to producing a cancer cell. One of the crucial repair mechanisms and checkpoints in the cell is TP53 (Tumor suppressor gene 53) (Levine, 1997, Bozkurt *et al.,* 2003, Iyer and Lehnert, 2000).It is clarified that 50% of those people who have cancer have a mutation in tumor suppressor gene, P53 such as lung, colon, oral, and breast cancers (Iyer and Lehnert, 2000) (Lowe *et al.,* 1994). However, there are few studies on the sensitivity and response of p53 to radiation and the underlying mechanisms are not clear until now.

The sensitivity of TP53 was examined in many studies exposed to ionizing radiation ranging from zero to 9 Gyr Gamma ray. It found that TP53 is sensitive to ionizing radiation and its losses its ability to repair cells properly and TP53 containing mutations is more sensitive to radiation (Paulovich *et al.,* 1997).

The effect of radiation on the human body is based on the radiation quantity exposure and the sensitivity of the person who is exposed to radiation. The biomarkers are involved in cell processes such as DNA, repair, apoptosis, and cell cycle control, and can be used for susceptibility testing (Matsui *et al.,* 2001, Geva-Zatorsky *et al.,* 2006). P53 is one of the effective genes in the cell mainly related to cell division control especially cancerous cell control and it is known as a cell protector against radiation. Stress signals in the cell clearly change the behavior of TP53 expression level which indicates the powerful effect on cell cycle control (Efeyan and Serrano, 2007) (Vogelstein et al., 2000). Therefore, checking the sensitivity of TP53 to radiation or any carcinogenic material is very necessary.

The current investigation was undertaken to study the effect of radiation on developing mutation in a tumor suppressor gene, TP53 which is a very sensitive gene for radiation and the most important gene for preventing cells to become cancer cells. This study was performed on those people who have cancer treatment by ionizing radiation. The DNA sequence of TP53 was analyzed after radiation in different periods of time to find whether radiation treatment causes certain mutations of the gene. In addition, an attempt was made to find a single nucleotide polymorphism in P53 gene in the study area among the Kurdish population.

MATERIALS AND METHODS

Study Design and Sample Collection:

The study includes blood sample collection from 77 different types of cancer patients who visited the hospital (Zhianawa radiotherapy Hospital center in Sulaimani City/Iraq) for radiation treatment including Prostate, chest, breast, lung, bone cancer, and skin cancer. In addition, blood samples from eighty volunteers were taken to be used as control samples (Healthy and normal people). All of the patients were treated with 3D CRT (Three-Dimensional Conformal Radiation Therapy) as part of Planning radiotherapy Patients were treated with Total Dose in Centigray (cGy), using (machine model: Elekta synergy) between 4/7/2021 and 1/9/2021. The patients were aged between 10 to 89 years.

Three ml of peripheral blood was taken from 77 cancer patients and 80 controls. The blood was taken from patients before and after radiation treatment between (4/7/2021 to 1/9/2021). The blood samples were collected and kept cold until arrived at the laboratory for DNA Extraction.

DNA Extraction and Sequencing:

DNA extraction was performed according to the manufacturer protocol using EasyPureTM Genomic DNA Kit (Trnas Gen Biotech Co., Beijing, China). The TP53 gene was amplified by PCR using a pair of primers:

F: GCTTGCCACAGGTCTCCCC
R: GCTTCTTGTCCTGCTTGCTT

The amplified gene is composed of 700bp which compromises exon-7, intron-7 and exon-8 genomic regions. All the amplified and purified DNA were sequenced via Sanger sequencing (CHU de Québec-Université Laval, Québec City, Canada). Finally, ClustalW multi-alignment algorism was used to align the sequences and the neighbor-joining (NJ) method (http://www.phylogeny.fr) was used to build phylogenetic analysis (Dereeper *et al.,* 2010).

Sequence Analysis:

The analysis included 80 control nucleotide sequences, retrieved from the plasma of 80 people who had no diagnosed pathologies and from the plasma of 77 nucleotide sequences retrieved from people with different types of cancer diagnosed. For people in care, a sequence before and after

radiological treatment was obtained. All sequences containing the specific p53 genomic regions from exon-7 to exon-8 (thus, including intron-7).

The cellular tumor antigen p53 reference consensus and numeration of nucleotide positions used was published in GenBank database (accession number: X54156).

Multiple sequence alignments of exon-7, intron-7 and exon-8 genomic regions were performed by using ClustalX and manually edited with the BioEdit software, as previously described.

Finally, to analyze mutations, the frequency of all mutations in p53 exon-7 and exon-8 amino acid positions and in the p53 nucleotide position in intron-7 were calculated (Dimonte *et al.,* 2013, Dimonte, 2017).

RESULTS

Prevalence Of P53 Exon-7, Intron-7 And Exon-8 Mutations:

The study includes blood sample collection from 77 for different types of cancer patients who visited the hospital (Zhianawa radiotherapy Hospital center in Sulaimani City/Iraq) for radiation treatment including Prostate, chest, breast, lung, bone cancer, skin cancer (Table 1a and 1b).

Table 1a: The distribution of the samples, patients and controls.

Control		
Male	Female	Median age
38 (47.5%)	42 (52.2%)	30.2

Patients			
Male	Female	Median age	
		Male	Female
32 (41.6%)	45 (58.4%)	52.5	45.3

Table 1b: The distribution of the types of cancer in cancer patients who received radiotherapy.

Cancer types		
	Male	Female
Adenoid cystic cancer	1 (3.1%)	0
Bone metasis	3 (9.4%)	3 (6.7%)
Bone metastasis	4 (12.5%)	2 (4.4%)
Brain (whole brain)	1 (3.1%)	0
Breast cancer	1 (3.1%)	33 (73.3%)
Fibromatosis	0	1 (2.2%)
Gastric cancer	1 (3.1%)	0
Gastrointestinal Stromal Tumor	1 (3.1%)	0
Left cervical and axilla	0	1 (2.2%)
Lift parieto -temporal scalp	1 (3.1%)	0
Liver cancer	1 (3.1%)	0
Lung cancer	2 (6.2%)	1 (2.2%)
Lung carcinoma	1 (3.1%)	0
Metastasic thyroid cancer	1 (3.1%)	0
Mid rectal cancer	1 (3.1%)	0
Naso pharyngeal	1 (3.1%)	0
Nodular sclerosis Hodgkin lymphoma	1 (3.1%)	0
Oro phorynyx	0	1 (2.2%)
Pancreas cancer	0	1 (2.2%)
Prostate	8 (25.0%)	0
Right femoral soft tissue cancer	1 (3.1%)	0
Sarcoma of left chest woll	0	1 (2.2%)
Skin cancer	2 (6.2%)	0

Analyses of blood plasma sequences of p53 exon-7, intron-7 and exon-8 domains detected a conserved variability between pre-post radiological treatment, thus *identifying* patterns of stability (Table 2).

Similarly, in sporadic cases of mutations, these are preserved in the same patients before and after radiological treatment (Table 2).

Table 2: p53 mutations detected in peripheral blood samples

Aminoacid mutation exon-7			Nucleotide mutation intron-7			Aminoacid mutation exon-8		
Control	Pre-Therapy	Post-Therapy	Control	Pre-Therapy	Post-Therapy	Control	Pre-Therapy	Post-Therapy
R261G (1.8%)	T230P (2.4%) T231P (2.4%) I232S (2.4%)	T230P (2.4%) T231P (2.4%) I232S (2.4%)	C14166T (8.9%) T14187G (3.6%)	C14166T (8.9%) T14187G (2.4%)	C14166T (8.9%) T14187G (2.4%)	S269I (1.8%) E287D (1.8%) K292R (1.8%) P301R (1.8%)	Nil	Nil

Mutations on Exon-7:

In the p53 exon-7 the aminoacidic mutation T230P, T231P and I232S, were observed with very low variability, 2.4% (2 same patients), in both before and after radiological treatment, respectively. The mutation at position 232 was observed in the colorectal tissue of a patient with colorectal carcinoma: in fact, Ito T. *et al*. discovered the mutation I232F (Ito *et al.,* 2003).

Mutations on Intron-7*:*

Differently, the nucleotidic mutation C14166T in the intron-7 was observed with a moderate variability of 8.9%, albeit always in the same 11 patients before and after radiological treatment, respectively (table 2a and 2b). Moreover, the nucleotidic mutation T14187G in the intron-7 has a lower variability but is also present in the control and cancer patients: 3.6% and 2.4%, respectively in the control and cancer patients, respectively (Table 2a and 2b).

Frequency Of Intron-7 Mutations in Different Cancer Patients*:*

Type 2 Mutation (T14187G) was found in both control 40% (of healthy cases) and cancer patients 60% (Table 3). 80% of the mutations were observed in females and only 20% were in males. This type of mutation was also found previously in the USA (Figure 1 and 2).

The other type of mutation was Type1 Mutation (C14166T) and this novel mutation was observed only in the studies area (Figure 1 and 2). The latter mutation was more frequent, especially among cancer patients. It was observed in control (healthy) at 26.6%, but it is more common among cancer patients at the rate of 73.3% (Table 3). It exists in both genders but it is higher in females at 60%. It distributed relatively and evenly among different types of cancer but its slightly higher among breast cancer patients, 33.3%. The age of cancer patients who bear a novel mutation is mostly above 40 years old, 66.6% and its less observed in age groups 10-41 at 33.3% (Table 3).

Table 3: Distribution of P53 mutations according to ages and types of cancer.

TYP1 Mutation				TYP2 Mutation			
	Gender	Age	Type of cancer		Gender	Age	Type of cancer
C19	Female	33		A53	Female	47	Left side breast cancer
C59	Female	31		A64	Female	15	
A6	Male	67	Bone metastasis	67	Male	10	Brain (whole brain)
6R	Male	67	Bone metastasis	60	Female	54	Pancreas cancer
A60	Female	54	Pancreas cancer	54	Female	53	Breast cancer
80	Female	71		A60	Female	54	Pancreas cancer
				A59	Male	78	Prostate
				C11	Female	20	
				6R			
				A59R	Male	78	Prostate
				64R	Female	15	
				16	Male	81	Skin cancer
				24	Male	76	Prostate
				54	Female	53	Breast Cancer
				A16	Male	81	Skin cancer
				A6	Male	67	Bone metastasis
				67	Male	10	Brain (whole brain)
				60	Female	54	Pancreas cancer
				69R	Female	42	Breast cancer
				CA64	Male	22	
				C28	Female	26	
				C59	Female	31	

Mutations on Exon-8*:*

Finally, in the p53 exon-8 only in the control dataset, were observed the following aminoacidic mutations S269I, E287D, K292R and P301R, but in a single sample (1.7%) (Table 2).

The residue K292 is targeted by MDM2 (E3-ligase for p53): this mutant demonstrated the most significant decrease in activity though it retained the ability to bind DNA. Authors of this observation hypothesized that the loss of transcriptional activity seen in the K292R-mutant supports the idea that post-translational modification at this site might be involved in regulating p53 transcriptional activator function (Landré *et al.,* 2017)

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3           CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
Pt-80       CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
Reference   CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
Pt-A41      CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
C10         CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
Pt-41       CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
A31         CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCATCTTGGCCTGCTGGGCTCC
A6          CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
Pt-A59      CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
A16         CACTGGAAGACTCCAGGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCC
            ************************************* . ********** ** **

3           AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTTCCATACTACTAC
Pt-80       AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTGCCATACTACTAC
Reference   AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTTCCATACTACTAC
Pt-A41      AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTTCCATACTACTAC
C10         AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTTCCATACTACTAC
Pt-41       AGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGATTTCTTCCATACTACTAC
A31         AGCCTCTGCTTGCCTCTGACCCAGGAGCCCACCTCTTACCGATTTCTTCCATACTACTAC
A6          AGCCTCTGCTTGCCTCTGACCCCTGGGTCCACCTCTTACCGATTTCTGCCATACTACTAC
Pt-A59      AGCCTCTGCTTGCCTCTGACCCCTGGGTCCACCTCTTACCGATTTCTTCCATACTACTAC
A16         AGCCTCTGCTTGCCTCTGACCCCTGGGTCCACCTCTTACCGATTTCTTCCATACTACTAC
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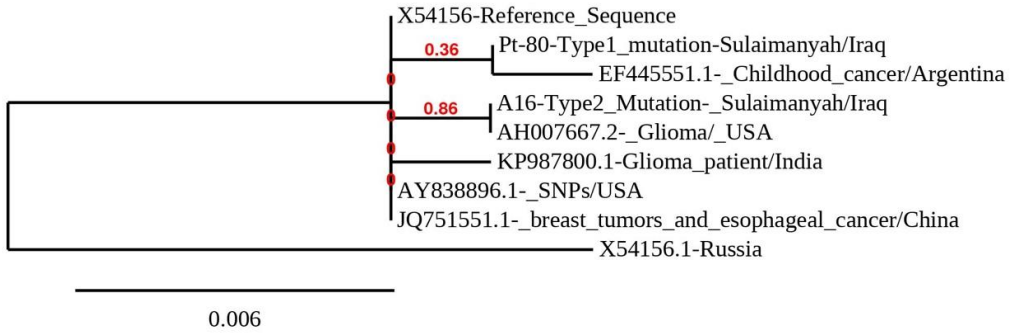
Multi-sequence alignment

Fig. 1: Multi-sequence alignment of p53 mutation in Kurdistan region with global sequences.



Fig, 2: Phylogenetic tree of p53 gene sequences in Kurdistan region in comparison to global sequences.

DISCUSSION

In this study, we demonstrated the impact of therapeutic radiation on the tumor suppressor gene, p53 in cancer patients who have different types of cancer (Table 1b). The patients included both males and females of different ages who visited Zhean radiotherapy Hospital center in Sulaimani City for radiation treatment of cancer. The rate of the participated male was 41.6% with a median age of 52.5 and the female rate was 58.4% with a median age of 45.3 (Table 1a). The rate of female cancer patients was higher because of the high number of breast cancer incidences among women 73.3% (Table 1b). The effect of radiation was checked through point mutations in the gene by sequencing after radiation. The radiation duration was different according to the number of doses and length of time ranging from one-day treatment to two-month treatment. The tumour suppressor gene was chosen because of its crucial role in the cell and because it's one of the most sensitive genes in the cell for radiation. For that purpose, the DNA was extracted from peripheral blood cells for genetic work. The P53 gene sequencing was carried out two

times: one time was before radiation and the second time was after the last dose of radiation to see if there any mutation is generated.

Our results indicate that: there is no mutation observed after radiation treatment (Table 1). It indicates that the radiation for treatment may have less effect on genetic mutation. Maybe one of the reasons is the power of radiation. Because the radiation is not very intense and uses for treatment in lower power of Gyr. The second reason could be related to the duration of radiation. For radiation therapy, patients were exposed to low doses in short times (one to two doses) according to the least requirements of radiation. This is consistent with another study. In which its found that there is no association between radio-sensitivity and TP53 mutations (Koch *et al.,* 1996). In addition, the radiation is local. The therapy was directed only to the local places where cancer developed and other parts of the body were usually protected and this is confirmed again in previous studies (Koch et al., 1996).

The interesting result was finding single nucleotide polymorphism (SNP) among local residences of the city. In this study, three sensitive regions of P53 gene were investigated. Exon-7, Intron-7, and Exon-8 because most of the life-changing mutations were observed in the previous studies. Some of the mutations were missense mutations and caused changes in the amino acids, however, this mutation was rare and appeared only in single cases and it was not repeated among many patients. Two different single nucleotide mutations were observed in intron-7 and this is common among cancer patients and also exists among control people (healthy) (Table 2).

Type 2 mutation (T14187G) was discovered in both control (40%) and cancer patients (60%), but its mostly observed in females, 80%. This mutation was found in only five people. So it concludes that this mutation exists in Sulaimani/Iraq. But it's not new and it was previously observed in USA in Glioma cancer. The present of mutation in TP53 is problematic because its found previously that radiotherapy cure will fail when there is mutation in TP53 (Alsner *et al.,* 2001).

The other mutation, Type1 Mutation (C14166T), is common in the studies area and this mutation is a novel mutation because it is for the first time to be noticed globally (Fig. 2). The mutation exists in both healthy and cancer patients but it is much higher in cancer patients, 73.3% (Table 3). therefore, it may have a role in developing cancer. The mutation is more frequent in females (60%) and among all mutant cases, the rate is obviously higher in breast cancer at 33.3%. Therefore, healthy people who carry this mutation may experience cancer at any stage of life if they don't stay in healthy life especially breast cancer. The age of cancer patients who bear a novel mutation is mostly above 40 years old, 66.6% and its less observed in age groups 10-41 at 33.3% (Table 3). This may be related to age and it may cause cancer in older people. In addition, this novel mutation is found at high rates among cancer patients, therefore it might be the reason for developing cancer in carrier people and this mutation maybe become a biomarker to find for the diagnosis of cancer, especially in the early stages of cancer.

Conclusion

The role of radiation in radiotherapy was studied carefully in cancer patients to create mutations in the human gene. For this purpose, P53 gene was used in this study because it is very sensitive to mutation and radiation and because of its crucial role in the cell in cell cycle control. The present study concluded that short-term radiation used for the treatment of cancer patients is less likely to cause mutation in P53 gene. The area of study contains two common mutations in P53 gene which exist in both healthy and cancer patients. One of the mutations is a novel mutation that exists only in the studied area. The novel mutation is more common among cancer patients and is higher in the female gender. This novel mutation is higher in breast cancer patients and in old ages. This mutation might be

useful in the future to be used as a biomarker and this needs more study to investigate.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

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