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SOX4 as Prognostic Significance for Individual's Exposure to LDIR

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Abstract

X-ray impacts on the systems of human body, some the used techniques recognize molecular properties at the exposure of X-ray .The detection of *SOX*4 (SRY-related HMG-box gene4) the expression via QRT- PCR consider essential technique in the current work. The X-ray genotoxicity can observe in low dose such as 1cGy, depending on the Center of Radiation Protection, there workers exposed to weak dose from γ-rays about (2- 10) cGy. The groups involved the (exposure group) twenty, also the workers in Radiographerthat existing close to the source (X-ray) , (control group) consist of five volunteers from male at various age, they didn't subject to any radiations whether x-ray or radiotherapy. Whole blood collects of all persons. The finding appear that the expression of *SOX* increased in individuals exposed for more than 1cGy , and decreasing in control group . The little doses of X-rays lead to genotoxicity then increased the expression set of genes that responsible of repairing such as *XRCC1*, *XRCC2*,*XRCC3* and other genes like *FOXM1* , the *SOX* gene is one of these genes was Stimulates by oxidative stress of X-ray . SOX4, as one of the SRY-associated HMG-box (SOX) which considers from the family of transcription factor and it has been proved to be participated in the tumorigenesis for many malignancy in human.These study depending on Previous treatise it indicated a correlation between expression in *FOXM1*and *SOX*4

Keywords: SOX 4 ; transcription factor ; Biomarker ;X-ray ; Diagnosis; Therapy; LDIR

1.Introductions:

Classifications of Radiation into two: non-ionizing and ionizing (IR), (NIR). The bimolecular effects by ionizing more than non-ionizing as result for the ionizing ability for stimulating of the atom ionization .the main sources of ionizing radiations are radioisotopes or called(radio nuclides) that be unstable, the particles emit from these radioisotopes with high energy for displacing electrons in atom, where the chain reaction becomes easy the happen[1]. The UV rays ofeffects that consider from NIR on the biological molecular is studiedrecently ,the ionizing radiations employed the most widely in the research centers or the therapies are sources of man-made as elements Co-60, or Cs-137 and the Europium 152[2,3].One of imaging techniques is Radiography that used either (X or γ) rays, [1] in order to display the internal body parts . Radiography can be applied in medical radiography as "diagnostic" or as "therapeutic" also used in industrial radiography. Airport security considers one of similar techniques of radiography or that called is body scanners, where it utilizes backscatter of the X-ray generally. So as to produce an image in the ordinary radiography the X-ray generator generates a ray of X-rays shed towards the object. The objects absorb a specific amount of these rays or any other radiations based on the density and composition of the objects [4]. The purpose of this study is bring to focused the interactions between biological molecules with ionizing radiations and the utilizing radioisotopes in the industry, medicine and science that needs to be administered in way for keeping the people and environment safe for long time ,in the environment may occur ionizing radiations artificially and naturally , the effects of both types on healthy human may be itself. The first case recorded in 1902 for cancers that induced via the radiations [5] in 1917 and until 1926 registered other cases involved the

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painters who deal with the element of radium **[**6**]** as results to some the factors of environmental and the stress like physical factors (**γ**-ray) and chemical reagent, the mechanisms of cell activated to repair of DNA ,also base expression repair (BER) and single strand break repair (SSBR) , besides there other mechanisms like death of cells (apoptosis), cell cycle control for all the parts of body that impacted with ionizing radiations however the response rely on a dose and a period of the exposure. as well known that the tissue , like the blood impacts with ionizing radiations and give rise to the damage of DNA , in mammalian have the cells sundry types of systems of repair DNA where it becomes major role for correcting diverse types from the DNA damage , also The Ionizing radiations cause DNA damage either immediately or un immediately and leads usually to base DNA of damage which it can repair via "base excision repair "(BER) [7]. The gene of SOX encodes the conserved group of the transcription regulatory factors involving of "414-amino- acid polypeptides" for a extremely conserved in the box of high mobility group (HMG**)** [8,9]. The box encodes about 79-amino-acid in the domain DNA-binding , together with two arms L-shaped which it can be bended to ATTGTT or linked the DNA sequence cop in the minor channel via recognize the sequence 5′-(A/T)(A/T)CAA(A/T)-3′, leading to extending of the minor channel releasing of the helix of DNA and then the bending of DNA [10] it can consider as the suppressors of tumor or one of the carcinogenesis promoters. The genes of SOX were proved to be interested the expansion of the different cancers, involving breast [11], lung [12], hepatocellular [13], and gastrointestinal cancers [14]. as result of the high mortality and morbidity of the gynecological cancers (GCs) every year ,SOX genes have the roles of in these GCs, involving cervical (CC) , or ovarian (OC),), also endometrial cancer (EC)[15].the Studies are investigated the relation between odd expression of the genes of SOX and the GCs development , it found that some the genes of SOX that have high expression level expects to make as the regulators of oncogenic that enhancing the GCs progression while as it has the opposite effects at the low expression level i.e. As the suppressors the *SOX*genes of expression level may be as biomarkers related with features the clinical to patients and GCs, involving the histopathologic grade ,also the International Federation of the Gynecology and the Obstetrics (FIGO) stage, and disease-free survival (DFS), in addition to the response of treatment [16- 19]and can considered as biomarkers for exposure to IR. The expression of SOX4 is upraised in board diversity of tumors, involving colorectal cancer, the leukemi, also lung and breast cancer, indicating a major role in the evaluation for these the malignancies. In most of cancers, the developmental factor can be deregulated expression that linked with increased the proliferation of cancer cell, it can inhibition apoptosis by cell survival and the progression tumor during the epithelial induction to the transition of mesenchymal and the metastasis [20]

1-1 The domain structure of SOX4 and function

SOX4 considers from transcription factors, which be from the highly conserved group C SOX (SOXC) that contain of two members extra : SOX11 , SOX12.[21] the members of SOXC are genes of the single exon , collected on the fundamental of the sequence high degree similarity in the domain of HMG existed in the N-terminal half for the protein, furthermore ,can distinguish from other transcription factors of SOX via "their conserved C-terminal transactivation domain (TAD)"

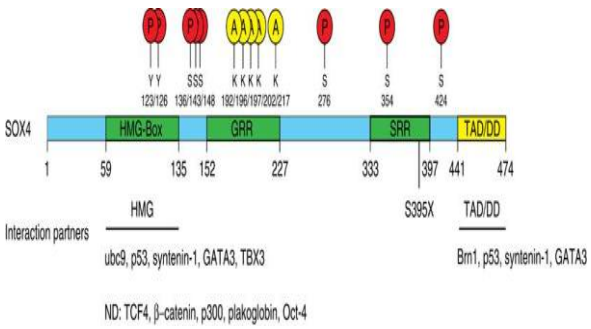

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Figure1. SOX4 structure

.In The research is recognized for the association between genes encode regulatory transcription factors (SOX4) and low dose of IR LDIR

2. Procedure

2.1. **Sampling:**

The blood samples obtain of 20 male individual's workers as Radiography examiners and takes 5 of the healthy donors with no history of radiotherapy, about their ages 30- 65 years old without the medicine consumption or the alcohol. All the blood samples were pulling out in the tubes. To study the genes expression, 250 µl bloods was transformed straight for tube that containing on the Trizol reagent in order to protect RNA from the analysis. for each tube contains the Whole blood were processing directly , also isolation of RNA then inverse into cDNA, the extraction of RNA was as stated by the kits recommendation .The kits were permit an rate yield of above 20 µg of overall mRNA. Whole RNA was reversely transcribed to complementary DNA (cDNA) utilizing ***AccuPower*** **RT Premix** Kit. Whole RNA was reverse transcribed to complementary DNA (cDNA) utilizing AccuPower RT Premix Kit.The proceedings is achieved of 50 µl as the reaction volume according to the industrialist 's ,The volume of total RNA to be copy reversely is (20µl). also added A volume of 20 µl from the total RNA into a tube contains AccuPower RT Premix plus pipetted upward and downward for two times ,then mixed inside the tube is inbrief centrifuged to spin - down the contents and for removing any the bubbles of air .The tube was placed on ice, for performing cDNA synthesis reaction and experimental protocol as following :

42 C , 60 min. (cDNA synthesis)

94 C , 5 min.(RTase in activation)

Table 1 :**experimental protocol**

	Reaction volume	20**µ**l reaction	50**µ**lreaction
Template RNA	Total RNA	0.5-1.0 **µ**g	1.0-2.0**µ**g
	Poly(A) RNA	0.05-0.1**µ**g	0.1-0.2**µ**g
Primer	OLIGO Dt_{18}	0.5**µ**g(100pmole)	1.0**µ**g(200pmole)
	Sequence specific	10-30 pmole	

2.3. The analysis mRNA expression of SOX4 by (QRT-PCR):

The primer sequence of SOX4 was 5- AGGATTCAAACGCAACTCAAAT-3 Forward and 5-AAAGAAATACGAGGATGGAGCA-3 as Reverse, the primers sequences of the genes have been prepared according to[15] where made by Alpha DNA Ltd (Canada) with GAPDH (House –Keeping gene) as shown in the table 2 ,the final volume of PCR reaction was 20 µl, 10µl from SYBR green , 1µl forward primer ,1µl revers primer ,2µl c DNA and completes to 20 µl with the free nucleus water .

Table 2: Primers used in the study.

SOX4	
Forword	AGGATTCAAACGCAACTCAAAT
Reverse	AAAGAAATACGAGGATGGAGCA
GAPDH (House –Keeping gene) 100pb	
Forword	GAAATCCCATCACCATCTTCCAGG
Reverse	GAGCCCCAGCCTTCTCCATG

The amplified of samples by 40 cycles for 5 min at 95 ºC, 95 ºC for 30 sec. also annealing at 62ºC for 60 sec , then the Ct value of analysis [22 رافد] for SOX4 in order to determine the times of folding expression for each group ,the levels of expression SOX4 are normalized to housekeeping gene expression Table 3

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Table 3: Thermal profile of SOX4 genes expression.

Step	Temperature	Duration	Cycles
Enzyme activation	95 ºC	5min	Hold
Denature	95 ºC	30sec	40
Anneal/extend	60 ºC	60 sec	
Dissociation	1 min /95ºC-30 sec /55ºC30 sec/95ºC		

3-Results and Discussion

3-1 Normalization of Ct (cycle threshold) Values SOX4

In currently research, QPCR gives the report analyzed about the expression mRNA of ***SOX4***comparing with the expression of healthy group, exposed individuals group. The gene expression calculations of fold can be changed when using the relative quantification [23]. It bases on the Ct values normalization which is calculating, "where the ΔCtdefined that the difference between the mean Ct values of replication of ***SOX 4*** **cDNA** amplification of each single case and that of the ***GAPDH***. **Table (4)** shows the mean of ΔCt(normalization Ct values) of each study group. ΔCt means in exposed group, healthy control group were **(4.88)**,and **(7.61)** respectively. The difference of significant that can be noticed among the study groups (p=0.001).Results of $2^{-\Delta Ct}$ revealed significantly higher for the group of workers exposed than the group of control (p= 0.0001). To calculate the gene expression folds in relation to the housekeeping genes the result of $2^{-\Delta Ct}$ of each group was measured in relation to that of healthy control group. The results are shown in **Table (4).**The gene expression folds in exposed workers were higher than the group of Healthy control in **6.64** times, as shown in **Table (5).** These findings appear a significantly increase in the gene expression of ***SOX4*** in exposed groups and proved role of *SOX4* as radio resistance. In calculation, the relative expression of SOX4 gene in all study groups the $2^{-\Delta\Delta Ct}$ results was applied. A calibrator was used and it was one of the samples of the controls with high expression of SOX4

Table (4) Fold of sox expression. Depending on $2^{-\Delta Ct}$Method

Groups	Means Ct of SOX_4	Means Ct of *GAPDH*	ΔCt (Means Ct of SOX_4 - Means Ct of *GAPDH*)	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Exposure	26.10	21.22	4.88	0.0339	0.0339/ 0.0051	6.64
Control	28.78	21.17	7.61	0.0051	0.0051/0.0051	1

As shown in Table (5) this the significant difference among these groups regarding for the mean $2^{-\Delta\Delta Ct}$, (p = 0.0001)

This result agrees with my last study about irradiation suppressed on XRCC1 also we confirm that the irradiation of induced SOX-4 can mediate to DNA repair. The results give the evidence about the effects of irradiation anti-tumor in the latest years, studies of gene expression on large-scale have applied to discover the novel genes that related with cancer, where can be utilized as curative targets or prognostication markers,

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there studies identify the elevated expression for SOX4 in almost all possible the prime human cancers, involving lung, the breast, he brain, the prostate, the colorectal, the bladder, the pancreatic and also the ovarian cancer, denoted the centric role in development for various tumors**[24]**

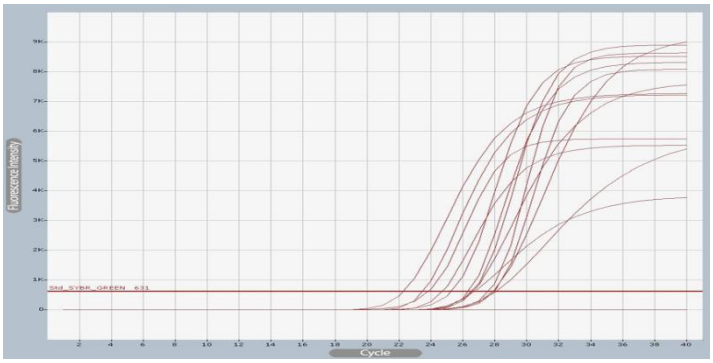

Figure (2) plots of the SOX4 amplification via QPCR. The samples involved the group exposure of individuals'. The range of Ct values at 22.27 to 28.10 ,where the photograph took of QPCR machine directly.

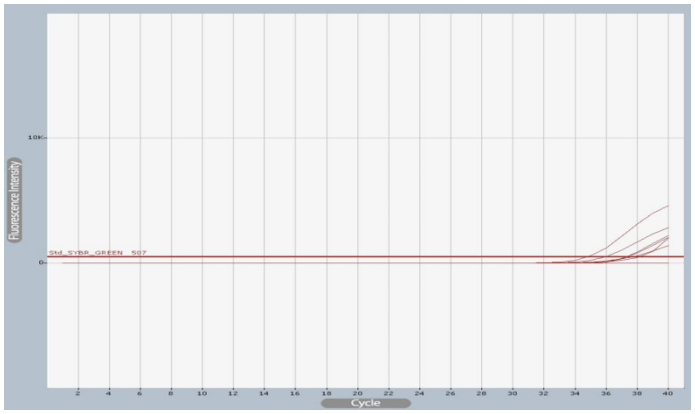

Figure (3)plots of the SOX4 amplification via QPCR.

The samples involved the control group. The range of Ct values at 28.28 to 35.02 , where the photograph took of QPCR machine directly.

Also the results indicate that mentioned mRNA can suggest as specific markers or sensitive in whole blood at the oxidative stress in exposed individuals, the findings are consistent as well with those of [25] .Whose results indicate that knockdown efficiently the genes of SOX4 sensitize the cells of colon tumor for the radiation in vivo or in vitro as a molecule over expressed in primary colon tumor cells, these results coincide with those reported by [26-**28]** who revealed that the mRNA expression of SOX4 in whole blood has clinical value be a significant to the diagnosis of instability genetic and the cancer, with respect to [28]who provided a little portion of harm induced by mean of IR cannot repair in the derived cells from persons with xerodermapigmentosum , this leads to suggestions that IR create `some large damages that cannot be separated through the base excision, As an Alternative , a portion of no the large base damage (8-hydroxyguanine, thymine glycol) may remove via the NER pathway, particularly in the cells long-lived like neurons that afford a large deal of the oxidative damage spontaneously [29-**30**],involved in repaired of DNA double strand breaks by HRR [31] Results the gene expression Sox4 in the cells of colon cancer and also the tissues of **CRC**. In analyses plot , generally, the cell lines of colon cancer will express the Sox2 protein at levels when compare to the positive control of MCF7 and**[25].**Expression of SOX4 gene in exposure individuals and this in turn was the highest from the control group. It is important in reflecting the original mRNAs present in the samples. It is evident from these results that the worker group is associated with the highest copy number of mRNAs reflecting its higher expression.

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Table (5) Fold of SOX expression. Depending on 2-ΔΔCtMethod.

Groups	Means Ct of *sox*	Means Ct of *GAPDH*	ΔCt (Means Ct of*sox* - Means Ct of *GAPDH*)	$2^{-\Delta Ct}$	ΔΔCt	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Exposure	26.10	21.22	4.88	8.98	4.01	16.11	16.11/2.58	6.24
Control	28.78	21.17	7.61	8.98	-1.37	2.58	2.58/2.58	1

On the other hand, exposure to LDIR causes different Ct values for the normal groups .The results show difference of Ct values between the exposed workers and control groups and important evidence that SOX4 gene expression

increases in exposure groups so it is possible to use SOX4 gene as biomarkers for the detection about DNA damage early, SOX family (SOX2–SOX4) that has an essential role in regulating to repair the damages in DNA that induced by the Ionizing radiations .These results agreed with [32]

These possibilities may explain why over expression of SOX4, and that there was no expression of SOX4 mRNA in the peripheral blood of healthy people for that reason, the valuation expression of mRNA of SOX4 in whole blood had remarkable clinical value to diagnosis and the prognosis of ionizing radiation low dose exposed .Each quantificative PCR reactions run in the duplicate of any sample. In each run, samples from worker, and healthy were run in addition to non-template and non- primer controls. This was important to make the statistical calculation of each group and in order to specify the calibrator. Plots of each run were recorded including the plots of amplification and the curves of dissociation, Figures (2) and Figure (3) table show the plots amplification and the curves of dissociation for SOX4.

Conclusion

In this research, it has conclusion that the exposed persons to low doses from LDIR noted that occurrence slightly symptoms cannot notice through outside symptoms as in high doses, therefor it has studied sets changes in the gene expression such as XRCC1,XRCC2,FOXM1 and in this work, it has been studiedSOX4 in order to know the effects LDIR on this gene .

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