

X-Ray Repair Cross Complementing 4 (XRCC4) Gene Expressions as Biomarkers for Detection of Ionizing Radiation Exposure

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Summary

The present study aims at using the gene expression as biomarkers in the identification of the biological effects of low and high doses of ionizing radiation (X-ray) in white mice *Mus musculus* Balb/C, ages 4-6 weeks, weight 30-40 grams. Seventy-two white mice (36 males and 36 females) were divided into two groups; their whole body was exposed to 5 cGy (rad) and 100 cGy (rad) of X-ray radiation at a dose rate of 200 cGy/min, in addition to the control group. Total RNA was successfully isolated using Trizol method from blood and liver samples of mice after 6, 48 hours and 10 days of exposure to radiation as well as of the control group. The RNA concentration was determined spectrophotometrically by measuring their absorbance using nucleic acid and protein analyzer that dependent on the ratio A_{260}/A_{280} of the wavelength which lead to the determination of RNA purity, it ranged from 1.79-2.1 in all mice groups. RNA integrity and quality were confirmed by agarose gel electrophoresis. Three bands such as 28s, 18s and 5s appeared in a visible manner. This study involved the reverse transcription (RT) of the RNA for the manufacture of complementary DNA (cDNA) using the polymerase chain reaction (PCR) for investigation on above-mentioned groups of animals. Complementary DNA was used in amplification of genes used in the present study, one type of specialized primers were selected for the gene as X-Ray repair cross complementing group 4 (XRCC4), which have a relation with ionizing radiation in addition to the primers for internal control (β -actin) gene. The optimal conditions for PCR were determined using a dye (SYBR[®]Green 1). This should be done before using the device quantitative real time-PCR (QRT-PCR) in experiments. The products of replicated specialized primers for the genes concerned and the cDNA for the studied samples were electrophoretically separated in agarose gels. The banding profiles were visualized by ethidium bromide staining, as the molecular weight was 183 bp nitrogen-base pair for XRCC4 gene. The changes in the gene expression for the genes concerned were determined by measuring the quantitative levels of expression in the blood and liver samples of the group of mice after 6, 48 hours and 10 days of exposure to X-ray, in addition to the control group using the device QRT-PCR. The presence of significant reduction ($p < 0.05$) in the amount of gene expression for the XRCC4 gene in samples of blood and liver from mice exposed to both doses of 5 cGy and 100 cGy after 6, 48 hours and 10 days of exposure to radiation. This gene was down-regulation after 6 hours in the blood samples of mice exposed to these doses compared to the control group. In contrast, there were to these finding, significant increases ($p < 0.05$) in the amounts of gene expression of this gene in the liver tissues of mice exposed to both doses after 6 and 48 hours of exposure to radiation. This gene also showed up-regulation after 48 hours in the liver tissue of mice exposed to 5 cGy doses, while the up-regulation was appeared after 6 hours of exposure to 100 cGy dose of radiation. In conclusion, the results indicated that there is a possibility of using the changes in the level of XRCC4 gene expression as useful biomarkers for the detection of organism's exposure to ionizing radiation.

استخدام التعبير الجيني لـ XRCC4 كمؤشر بيولوجي في كشف التعرض للأشعة المؤينة

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الخلاصة

تضمنت الدراسة الحالية استخدام التعبير الجيني لـ XRCC4 كدليل بيولوجي في الكشف عن تأثير الجرعة المنخفضة والعالية من الأشعة المؤينة (الأشعة السينية) في الفئران البيض *Mus musculus* من سلالة Balb/c، بعمر 4-6 أسابيع، ووزن 30-40 غم. قسم اثنا وسبعون فأراً أبيضاً (36 ذكر و 36 أنثى) إلى مجموعتين بعد تشيع كامل الجسم بالجرع 5 و 100 سنتي غراي (راد) من الأشعة السينية بمعدل جرعة 200 سنتي غراي / دقيقة إضافة إلى مجموعة السيطرة. عزل الرنا (RNA) بنجاح من جميع عينات الدم والكبد لكامل الفئران بعد مرور 6، 48 ساعة و 10 أيام من التعرض للأشعة المذكورة إضافة إلى مجموعة السيطرة باستخدام طريقة تريزول (Trizol method)، وقيست كمية الرنا الكلية total RNA (µg/ µl) بوساطة جهاز تحليل الحامض النووي والبروتين (Nucleic acid and protein analyzer) واعتماداً على قيمتي الطول الموجي A_{280} / A_{260} حددت نقاوة الرنا إذ تراوحت بين 1.79-2.1، وتم التأكد من نوعية وسلامة الرنا RNA من التحلل بوساطة الترحيل الكهربائي على هلام الاكروز من خلال وجود ثلاث حزم 5S, 18S, 28S بشكل واضح. وشملت الدراسة الاستنساخ العكسي (Reverse Transcription (RT) للحامض النووي الرنا لتصنيع الحامض النووي الدنا المتمم cDNA باستخدام اختبار تفاعل السلسلي البوليمرازي (Polymerase Chain Reactions (PCR) لكامل الدراسة المذكورة آنفاً. استخدم الدنا المتمم في تضخيم الجين المستخدم في الدراسة الحالية إذ تم اختيار البادئة (Primers) المتخصصة للجين XRCC4 والتي له علاقة بالأشعة المؤينة إضافة إلى بادئة السيطرة الداخلية (β-actin) internal control، ويلعب هذا الجين دوراً مهماً في تنظيم الدورة الخلوية وعملية إصلاح الدنا، لذا فإن دراسته سوف تساهم في إمكانية استخدامه كدليل بيولوجي في الكشف عن التعرض أو التلوث بالإشعاع وبالتالي قد تساهم في فهم بعض الآليات غير المعروفة التي ربما تحدث خلال عملية تكوين السرطان بسبب الإشعاع. حددت الظروف المثلى لتفاعل السلسلي البوليمرازي (PCR) باستخدام الصبغة المتفلورة السايبر الاخضر 1 (SYBR® Green 1) قبل إجراء التجارب باستخدام جهاز تفاعل السلسلي البوليمرازي الفوري الكمي (QRT-PCR) (Quantitative real time-PCR) وحلتل نواتج التضاعف للبادئة المتخصصة للجين المستخدم مع حامض الدنا المتمم للعينات المدروسة بوساطة الترحيل الكهربائي ولوحظ الحزم بعد صبغ الهلام بصبغة بروميد الايثيديوم (Ethidium Bromide)، وكان الوزن الجزيئي 183bp زوج من القواعد النايتروجينية للجين المدروس. درس التغيير في التعبير الجيني geneExpression للجين XRCC4 المستخدم وذلك بقياس المستوى الكمي لذلك التعبير لنماذج الدم والكبد لكامل الفئران بعد مرور 6، 48 ساعة و 10 أيام من التعرض للأشعة السينية المذكورة آنفاً إضافة إلى مجموعة السيطرة و باستخدام جهاز تفاعل السلسلي البوليمرازي الفوري الكمي QRT-PCR. بينت نتائج الدراسة وجود انخفاض معنوي ($p < 0.05$) في كمية التعبير الجيني لـ XRCC4 في عينات دم الفئران المعرضة للجرعتين 5 و 100 سنتي كري وبعد مرور 6، 48 ساعة و 10 أيام من التعرض للإشعاع. كما وجد بان لهذا الجين مستوى تنظيمي واطئ down-regulation بعد مرور 6 ساعات في عينات دم الفئران المعرضة لهاتين الجرعتين مقارنة بمجموعة السيطرة. وعلى عكس ذلك لوحظ زيادة معنوية ($p < 0.05$) في كمية التعبير الجيني لذلك الجين في نسيج الكبد للفئران المعرضة للجرعتين المذكورتين بعد 6 و 48 ساعة فقط من التعرض للإشعاع. كما اظهر هذا الجين مستوى تنظيمي عالي up-regulation بعد مرور 48 ساعة في نسيج الكبد للفئران المعرضة لجرعة 5 سنتي كري، بينما ظهر المستوى التنظيمي العالي up-regulation بعد 6 ساعات من التعرض للجرعة الاشعاعية 100 سنتي كري. نستدل من نتائج الدراسة إلى إمكانية استخدام التغير في مستوى التعبير الجيني لـ XRCC4 مؤشراً بيولوجياً مفيداً يمكن استعماله في الكشف عن تعرض الكائنات الحية إلى الأشعة المؤينة.

Introduction

Exposure to ionizing radiation (IR) produces several forms of cellular DNA damage, including single-strand breaks and double-strand breaks (Kastan and Kuerbitz, 1993; NCRP, 2001). Thus X-rays can cause DNA and protein damage which may result in organelle failure, blocks cell division, or cause cell death (Morgan and Turner, 1973; Gutteridge and Cross, 1992). Cell cycle checkpoint responses to IR-induced DNA damage employ a complex network of gene products that cooperate to delay progression through the interphase compartments of the cell cycle and enhance repair of damaged DNA (Pajalunga *et al.*, 2008). When DNA damage is irreparable, checkpoints also inactivate clonogenic survival by permanent cell cycle arrest or apoptosis. The process of producing a biologically functional molecule of either RNA or protein is called [gene expression](#) (Amaral *et al.*, 2008), several steps in the gene expression process may be modulated, including the [transcription](#) step and [translation](#) step and the [post-translational modification](#) of a protein. Gene regulation gives the [cell](#) control over structure and function, and is the basis for [cellular differentiation](#), [morphogenesis](#), the versatility and adaptability of any [organism](#) (Jen and Cheung, 2003). Analysis of the X-ray repair cross complementing group 4 (XRCC4) genes has played an important part in understanding mammalian DNA repair processes, especially those involved in double-strand break repair. Double-stranded breaks can be generated from clusters of oxidized bases induced by high doses of low- linear energy transfer (LET) radiation. High-LET radiation can also generate clusters of base damage, or can produce both SSB and DSB directly independently without base damage (Ward, 1988). The ionizing radiation exposure results in up-regulation of XRCC4 and XRCC6 which have provides the cell with a means of assuring either proper DNA repair or appropriate response to DNA damage (Kevin *et al.*, 2000). Among them, mutants in XRCC4-7 groups exhibit an extremely high sensitivity to ionizing radiation and show severe defects in DSB and V (D) J recombination (Jackson and Jeggo, 1995; Riballo *et al.*, 2004). The gene expression changes in the radiation biomarker targets CDKN1A, BAX, GADD45A, XRCC4 and DDB2 genes over several days across a broad dose range in both *in vivo* and *ex vivo* irradiated human peripheral blood lymphocytes and measured using a quantitative reverse transcriptase polymerase chain reaction assay in whole blood model (Grace *et al.*, 2009; Al-zayadi, 2009). The aims of the present

study to assess the effect of ionizing radiation on the expression of XRCC4 gene and using the gene expression to the identification of possible candidate a biomarker for whole body radiation exposure .

Materials and Methods

Experimental Animals

Seventy-two males and females mice (*Mus musculus*) were used in the present study, weighting 30-40 gm, ages 4-6 weeks. They were purchased from Lab house of College of Animal Science and Veterinary Medicine, Huazhong Agriculture University, China. Mice were housed in an environmentally room temperature with food and water.

Irradiated Animals

The mice were divided into 2 groups contain 48 mice (24 males and 24 females) and control contains 24 mice (12 males and 12 females):

Group (A): included 24 mice (12 males and 12 females), it was exposed to low dose of X-ray 5 cGy.

Group (B): included 24 mice (12 males and 12 females), it was exposed to high dose of X-ray 100 cGy.

Group (C): included 24 mice were used a controls without radiation.

Group (A) was divided into 3 groups:

Group (A1): included 8 (4 males and 4 females) mice, the blood and liver were collected after 6 hr of post-irradiation with control group 8 mice (4 males and 4 females).

Group (A2): included 8 (4 males and 4 females) mice, the blood and liver were collected after 48 hr of post-irradiation with control group 8 mice (4 males and 4 females).

Group (A3): included 8 (4 males and 4 females) mice, the blood and liver were collected after 10 days of post-irradiation with control group 8 mice (4 males and 4 females).

Group (B) was divided into 3 groups:

Group (B1): included 8 (4 males and 4 females) mice, the blood and liver were collected after 6 hr of post-irradiation with control group 8 mice (4 males and 4 females).

Group (B2): included 8 (4 males and 4 females) mice, the blood and liver were collected after 48hr of post-irradiation with control group 8 mice (4 males and 4 females).

Group (B3): included 8 (4 males and 4 females) mice, the blood and liver were collected after 10 days of post-

irradiation with control group 8 mice (4 males and 4 females). The whole body of mice was irradiated by X-ray (6 kV, 15.5 mA) in Hubei Province Cancer Hospital (Wuhan, China), with an X-ray machine type primus (seminus Co. Ltd., Germany). The dose rate was 200 cGy / min.

Gene expression step methods

Fresh mice blood were used for genomic RNA isolation directly after collection, liver from each mice was snap frozen in liquid nitrogen after collection and stored at -80°C until RNA extraction by manufacturers instructions. Genomic RNA was extracted using the RNA isolation kit (TakaRa) or Trizol method (Sambrook & Russel, 2001). RNA integrity and concentration were evaluated by agarose gel electrophoresis and DU 640 Nucleic Acid and Protein Analyzer (BACKMAN, U.S.A). A total of 2 µg RNA was used for reverse transcription (RT) with the TransScript First-Strand cDNA Synthesis SuperMix according to the manufacturer's instructions (Beijing TransGen Biotech Co., Ltd., China). All primers were designed by the program Primer and synthesized by the commercial company (Invitrogen). The total volume of PCR reactions was containing 2.5 µL 10×Taq buffer (TaKaRa), 1.25 U Taq DNA polymerase (TaKaRa), 5mM dNTPs (TaKaRa), 50 pmol of each primer and 100ng of template DNA, 1.25 U Taq DNA polymerase (TaKaRa), 5mM dNTPs (TaKaRa), 50 pmol of each primer and 100ng of template DNA. PCR reactions were performed on the Mastercycler gradient (eppendorf). PCR conditions were: 94°C, 5min; 38cycles of 30 s at 94°C, 30 s at each T_m as appropriate (Table 1), 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.

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

Gene Symbol	Primer sequence (5'-3')	Target size (bp)	T _m (°C) ^a
XRCC4	Forward: TGGAAGCACCGATGAAG Reverse: TCCTGTAGCGGCAACTC	183	55
Housekeeping gene (β-actin) MAcb*	Forward: CAGCCTTCCTCTTGGGTAT Reverse: TGGCATAGAGGTCTTTACGG	100	60

* β-actin was used as loading internal control.

QPCR was performed on the IQTM5 Real Time PCR Detection System (Bio-Rad) using SYBR[®] Green Realtime PCR Master Mix (TOYOBO CO., LTD, Japan) as the readout. The QRT-PCR amplification conditions were: 95°C, 3min ; 95°C, 30 sec, 55-60°C as appropriate, 30 sec and 72°C, 15 sec for 40 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.

Data Analysis and Statistics

The relative quantitative gene expression level was evaluated using the $\Delta\Delta C_t$ comparative C_t method. The ΔC_t values were calculated by subtracting the RPL32 C_t value for each sample from the target C_t value of that sample. Fold inductions were calculated using the formula $2^{\Delta\Delta C_t}$, $\Delta C_t = C_t$ (target gene) - C_t (housekeeping gene), $\Delta\Delta C_t = \Delta C_t$ (treated) - ΔC_t (control). Quality of the PCR product was monitored using post-PCR melt curve analysis. 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 (Vandesompele, *et al.*, 2002).

Results

The rang amount of total RNA isolated in this study were (0.18-1.41), (0.12-0.72) and (0.21-1.40) µg/ µl from 1 - 1.5 mL whole mice blood at 6 hr, 48 hr and 10 days after exposure to 5 cGy irradiation , respectively, in comparison with control (0.28-1.50) µg/ µl. Whereas for rang amount of total RNA isolated (0.26-1.32), (0.22-1.14) and (0.13-1.47) µg/ µl from 1-1.5 mL whole mice blood at 6 hr, 48 hr and 10 days after exposure to 100 cGy irradiation, respectively, in comparison with control (0.29 -1.52) µg/ µl . The amounts of total RNA isolated in the present study were ranged between (4.15-12.86), (4.05-11.56) and (4.45-10.43) µg per 100 mg mice liver at 6 hr, 48 hr and 10 days, respectively after exposure to 5 cGy irradiation when compared with control (4.82-12.66) µg per 100 mg mice liver. Whereas the amounts of RNA were (3.77-13.05), (4.60-9.15) and (4.90-11.75) µg per 100 mg mice liver at 6 hr, 48 hr and 10 days, respectively after exposure to 100 cGy irradiation when compared with control (4.52- 13.46) µg . Figure 1 illustrates the electrophoretic experiment of the present

study in which the integrity of the RNA is evident on 1% agarose gel electrophoresis where 28s, 18s and 5s bands are clearly visible, no extra fragments have been observed, and there have been no signs of genomic DNA contamination. As a result, it was judged that most samples were indeed composed of intact RNA and appeared to be comparable in quality.

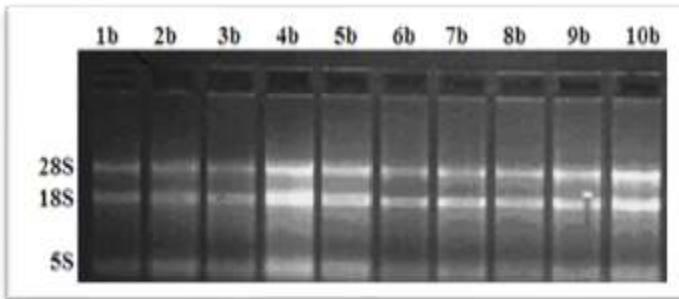


Figure (1): Evolution of RNA integrity by 1% agarose gel electrophoresis in 1xTBE using 6V/cm for 30 -45 min. the lanes 1b- 8b represent partial samples

RNA was isolated and reverse transcribed into cDNA by using an anchored oligodT primer and subsequently amplified by PCR. The PCR products were analyzed on 1.5% agarose gel electrophoresis to detect the absence and presence of band patterns. The amplified cDNA with β -actin gene was 100 bp in length of all mice blood and liver samples in this study. These results have also shown that there was a single pattern DNA band was clearly visible in each samples, which indicate the DNA and mRNA were undegraded and no primer-dimer formation. In all successful PCR reactions, the β -actin product 100 bp molecular weight was observed, this considered as a mandatory sign of successful RT reaction upon gel electrophoresis, and its band was located in 100 bp ladder DNA marker that composed of 2000 bp (Fig.2).

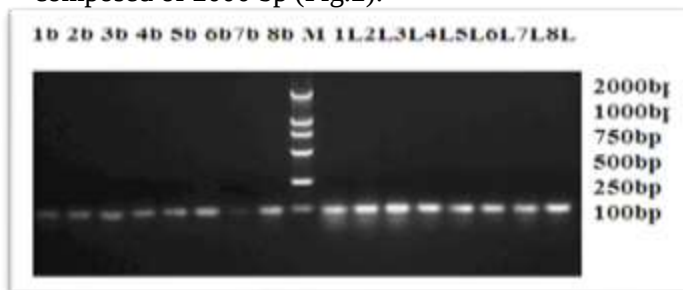


Figure (2): RT- PCR detection of Housekeeping (β -actin) genes, the lanes 1b- 8b represent partial blood samples, the lanes 1L- 8L represent partial liver samples, M: marker DNA ladder 2000 bp. Agarose 1.5% in 1xTBE using 6V/cm for 30 min

A pre-experiment for QRT-PCR, in the present study, showed that the amplified cDNA with β -actin primer gene was 100 bp in length of all mice blood and liver samples (Fig. 3). The presence of XRCC4 primer gene was identified the molecular weight was 183 bp. These results also showed that there was a single DNA band was clearly visible in each sample, which indicate no primer-dimer formation (Fig.3).

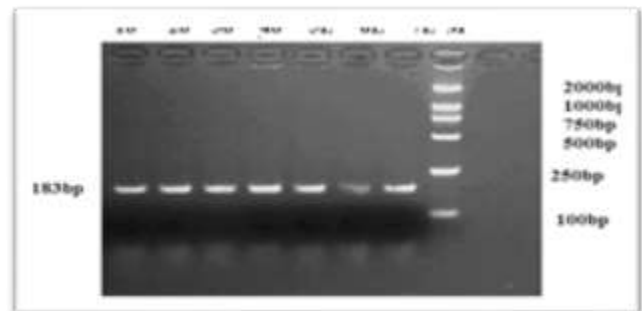


Figure (3): RT-PCR XRCC4 gene detection by agarose gel electrophoresis before QRT-PCR. the lanes 1b-7L represent partial samples: marker DNA ladder 2000 bp. Agarose 1.5% in 1xTBE using 6V/cm for 30 min

Also, as shown in figure 4, the Melt peak chart of these genes in the mice blood and liver after 5 cGy and 100 cGy of whole body x-ray irradiation. 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.

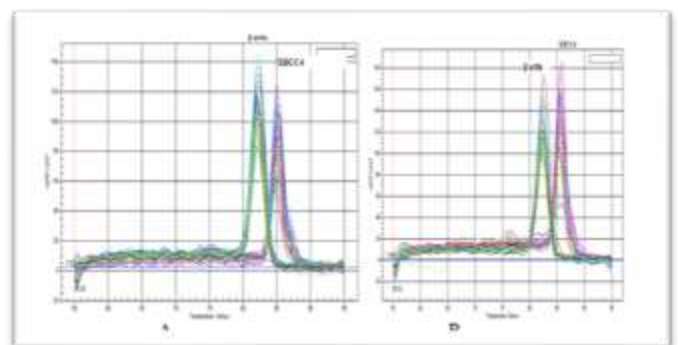


Figure (4): Melt peak of XRCC4 expression in mice at 0 hr, 6 hr, 48 hr and 10 days after 5 cGy (A) and 100 cGy (B) of whole body X-ray irradiation. Expression levels of β -actin are used as the internal control

In this study, table 2 showed the results of fold expression levels for XRCC4 gene in the blood and

liver of mice which was measured at 6, 48 hr and 10 days post-irradiation 5 cGy of whole body X-ray irradiation. A significant decrease ($p < 0.05$) was observed after 6 hr, 48 hr and 10 days of radiation (2.32-, 1.00- and 1.12- fold decrease, respectively) in mRNA expression of whole body irradiation, compared with normal nonirradiated controls (37.53- fold). The analysis of expression by real-time PCR in the mice blood also was observed a significant decrease ($p < 0.05$) in fold expression of the XRCC4 gene at 6 hr, 48 hr and 10 days after 100 cGy whole body irradiation (1.53-, 1.00-, and 2.35- fold decrease, respectively) compared with normal non-irradiated controls 8.57-fold (Table 2). The present study also measured the fold expression of XRCC4 gene in the liver of mice after exposure to 5 cGy and 100 cGy of whole body X-ray irradiation. The QRT-PCR analysis showed that the fold expression level of these genes in the liver of mice after exposure to 5 cGy with no significant difference ($p > 0.05$) after 6 hr (1.87-fold) but increased significantly ($p < 0.05$) after 48 hr (4.15-fold) in mRNA expression of whole body irradiation, compared with 10 days and normal non-irradiated controls (1.00- and 1.54- fold, respectively). The quantitative of XRCC4 gene expression level level of this gene in the liver of mice after exposure to 100 cGy which was highly expressed after 6 hr. A significant increase ($p < 0.05$) was observed after 6 hr, 48 hr of radiation (3.03-, 3.40-fold increase, respectively) in mRNA expression of whole body irradiation, compared with 10 days and normal non-irradiated controls (1.00-, 1.07- fold, respectively) (Table 2).

Table (2): Fold expression levels for XRCC4 gene in the blood and liver of mice after 5 cGy and 100 cGy of whole body X-ray irradiation

Samples	Exposure times for X-Rays	Low dose 5 cGy				High dose 100 cGy			
		fold expression	fold expression SD	Mean Ct	Ct SD	fold expression	fold expression SD	Mean Ct	Ct SD
Blood	0 hr	37.53 a	45.08	24.12	0.82	8.57 A	28.35	25.21	1.80
	6 hr	2.32 *a	3.28	22.38	0.50	1.53 *A	1.06	23.12	0.66
	48 hr	1.00 *a	0.70	23.41	0.62	1.00 *A	0.59	25.58	0.38
	10 days	1.12 *a	1.27	22.41	0.80	2.35 *A	0.74	23.69	0.34
Liver	0 hr	1.54 b	1.80	20.40	1.10	1.07 B	1.22	20.15	1.11
	6 hr	1.87 b	1.83	20.50	0.62	3.03 *B	8.40	23.36	2.59
	48 hr	4.15 *b	3.40	18.34	1.08	3.40 *B	8.55	20.74	0.79
	10 days	1.00 NS	0.80	18.69	0.70	1.00 NS	0.53	19.38	0.47

*a (XRCC4) significant diff. ($P < 0.05$) blood 5 cGy Compared with the control (a);

*A ((XRCC4) significant diff. ($P < 0.05$) blood 100 cGy, Compared with the control (A).

*b ((XRCC4) significant diff. ($P < 0.05$) liver 5 cGy, Compared with the control (b);

*B ((XRCC4) significant diff. ($P < 0.05$) liver 100 cGy, Compared with the control (B). NS=Non significant.

Ct=Cycle threshold, SD=Standard error.

As shown in Figure (5A) the expression of XRCC4 gene which was down-regulated at 6 hr, 48 hr and 10 days after 5 cGy in blood of irradiated mice, compared with normal non irradiated controls. Also, as shown in Figure, this gene whose expression was down-regulated in the blood at 6 hr after 100 cGy whole body radiation. whereas the expression of XRCC4 gene which was unchanged at 6 hr but up-regulated at 48 hr after 5 cGy whole body radiation exposure in mice liver (Figure 5 B). Also, as shown in this figure the expression of XRCC4 gene was found to be up-regulated at 6 hr and 48 hr after 100 cGy whole body radiation exposures in mice liver, but not significantly altered after 10 days, compared with normal non-irradiated controls.

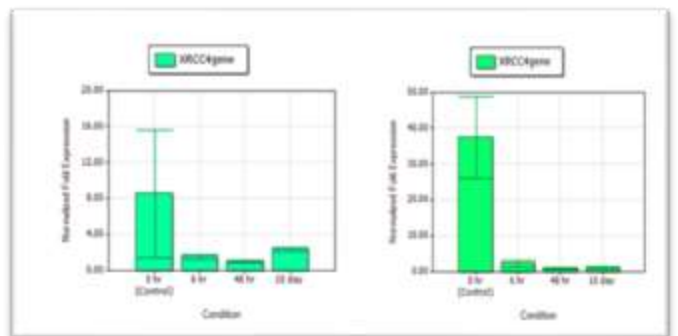


Figure (5 A): QRT-PCR graphs showing the relative fold expression levels for XRCC4 gene in the blood of mice after 5 cGy (right) and 100 cGy (left) of whole body X-ray irradiation

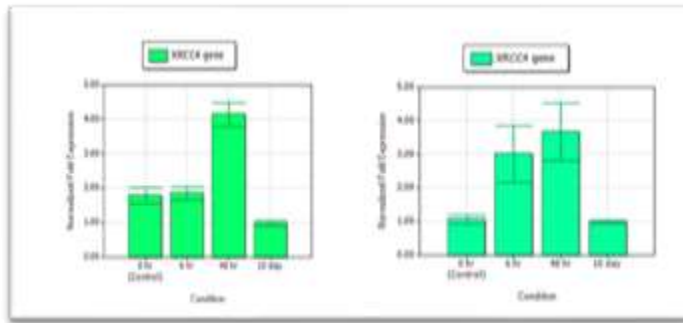


Figure (5 B): QRT-PCR graphs showing the relative fold expression levels for XRCC4 gene in the liver of mice after 5 cGy (right) and 100 cGy (left) of whole body X-ray irradiation

Discussion

The yield of RNA from peripheral blood leukocytes acutely depends on the physiological state of the mice, reflecting the dynamic shift in circulating white blood cell fraction with subsequent wide-ranging variability on RNA constituents and their yields (Alizadeh *et al.*, 1998 and Otto *et al.*, 1998). Although peripheral blood is a readily accessible tissue source, whole blood contains extremely high concentrations of serum proteins and derivatives of hemoglobin that can interfere with efficient RNA extraction, and subsequent amplification extraction (Alkane, 1996 and Daniel, 1997). The amount of RNA available for isolation varies with cell types; blood and liver cells are metabolically active and produce relatively large amounts of RNA per gram of tissue (Brisco *et al.*, 1997). In this study, the RNA has been isolated from mice blood and liver with a purity ratio ranging from 1.8 to 2.1 in all mice groups. However, in some sample the purity ranged below 1.8 between 1.78 -1.79. Pure preparations of RNA have OD₂₆₀ / OD₂₈₀ values of 1.8 to 2.1. 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 (Sambrook *et al.*, 1989). In this study, the RNA samples in all studied groups exhibited banding patterns characteristic of RNA isolated from mice blood and liver, no apparent degradation of RNA was observed indicating that RNA resulting from present study was relatively free of RNases. Further, the band integrity and RNA concentration were found to be different according to the amount of yielded genomic RNA and its purity which depended on the amount of RNA in the samples. Total RNA isolated from mice should produce 2 bright 28s and 18s rRNA and the ratio

of the intensities of 28S to 18S rRNA bands approximately twice as intense as the 5s rRNA band. The integrity of RNA is a major concern for gene expression studies and traditionally has been evaluated using the 28s to 18s rRNA ratio. The results of the current study are similar to those reported by Yan *et al.*, (2002) in which the 28s to 18s rRNA 2:1 ratios therefore the (28s:18s) is a good indication of RNA integrity. A pre-experiment for QRT-PCR, in the present study, showed that the amplified cDNA with β -actin primer gene was 100 bp in length of all mice samples. The presence of XRCC4 primer gene was identified the molecular weight was 183 bp. These results also showed that there was a single DNA band was clearly visible in each sample, which indicate no primer-dimer formation. Table (3-6) show's no significant difference in fold expression levels in 6 hr, 48 hr and 10 days post -irradiation. Non-homologous end joining is the major DNA double-strand break repair pathway in mammalian cells and this pathway predominates over the homologous recombination (HR) pathway and therefore may account for the low level of HR events that occur in irradiated mice (Andreassen *et al.*, 2006 and Bertolini *et al.*, 2007). The XRCC4 gene was described to play a role in the sensitivity of mammalian cell towards ionizing irradiation. Cells with a mutation of this gene present with decreased single-strand break repair and reduced recombination repair, showed increased double-strand breaks repair (Hacker and Zdzienicka, 2004). The regulation of the gene activities associated with this recombination pathway has been still largely unknown (Vyacheslav, *et al.*, 2006). The findings of the present study are in agreement with those reported previously by Dimitry *et al.* (2008) who have showed evidence suggests that genetic alterations in XRCC4 and ligase IV may promote genomic instability and radiosensitivity. This result confirms that the decrease in XRCC4 is due to the inhibition of the fusion gene and these genes were identified through their ability to correct DNA damage hypersensitivity in cell by several different repair pathways including base-excision repair and non-homologous end joining repair (Thacker and Zdzienicka, 2003). The results of the current study are compatible with those mentioned by Bertolini *et al.* (2007) in which the significant decrease in the expression of a linear XRCC4 and ku70 genes in mammalian cells, while it is different from the result found by other studies which referred that the ionizing radiation exposure results in up-regulation of XRCC4 and XRCC6 in order to provide the cell with a means

of assuring either proper DNA repair or appropriate response to DNA damage (Kevin *et al.*, 2000).

Conclusions

The use of quantitative Real-time QRT-PCR in the study of gene expression changes as a biomarker offers rapidity and sensitivity to be applied for the detection of exposure to ionizing radiation and using the XRCC4 gene expression to the identification of possible candidate a biomarker for whole body radiation exposure. The XRCC4 gene expression has showed down-regulation after 6 hours in the blood samples of mice exposed to both doses 5 cGy and 100 cGy. In contrast, this gene has showed up-regulation after 48 hours in the liver tissue of mice exposed to dose 5 cGy, while the up-regulation has been appeared after 6 hours of exposure to 100 cGy dose of radiation. using the gene expression to the identification of possible candidate a biomarker for whole body radiation exposure .

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