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Research Article

Molecular Study of AHR Gene Polymorphisms among Iraqi Women Staff Working in Radiotherapy Units

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ABSTRACT

Radiation may impact the DNA directly, causing its ionization or cause an indirect action in this case, the radiation interacts with non-critical target atoms or molecules, usually water which results in the production of free radicals. These free radicals can then attack critical targets such as the DNA. Radiation is considered carcinogenic. in this study were the evaluation the Ahr gene polymorphisms among Iraqi staff working in radiotherapy units. This case control study include two groups : fifteen healthy women as control group and fifteen radiologist. Ahr polymorphism has been done at two loci G1721A and G1768A using alleles specific PCR. Nucleotide polymorphism variants and different genotypes of *Ahr* gene have important roles in the development of cancer. This was mainly seen in Ahr polymorphism in GG and GT variants of G1721A, GG and GT variants of G1768A. High risk of radiation damage was noticed in the GG1768 genotype where (OR=3.25(0.5185-20.3704),p=0.2082)and (RR=2.49) also GT1721 genotype It can reflect radiation risks where (OR=1.62(0.2303-11.464)and (RR=1.5). All these results imply that SNPs studies must be conducted on cancer patients and radiation staffs as routine investigation to watch for and prevent DNA damage as much as possible.

Keywords: Ahr gene, polymorphism, Iraqi women, radiotherapy units.

INTRODUCTION

Physics, radiation is defined as electromagnetic waves or as moving subatomic particles, especially high energy particles which cause ionization. Radiation is energy in the form of waves of particles. Radiation whether natural or used for treatment is harmless, the damage starts at the cellular level when radiation is absorbed in a cell so that it targets cell components the most important of which is the DNA causing cell death, mutation, and carcinogenesis[1]. Radiation may impact the DNA directly, causing its ionization[2] or cause an indirect action in this case, the radiation interacts with non-critical target atoms or molecules, usually water which results in the production of free radicals. These free radicals can then attack critical targets such as the DNA[3]. Radiation is considered carcinogenic. The *aryl hydrocarbon receptor (Ahr)* is a member of the basic helix-loop-helix / PER-AHR nuclear translocator (ARNT)-SIM superfamily of nuclear receptors[4]. It regulates a wide range of developmental and toxicological processes including cell proliferation and xenobiotic metabolism[5-7]. In addition, it is regarded to play a contributory role in cancer[8,9]. The human AHR gene is highly polymorfic and most important genetic changes are in exon 10 of *AhR gene* there are two polymorphic loci G1721A and G1768A this results in the production of functionally altered prteins[10].

PCR Procedure:

(PCR) contained 1.5µl genomic DNA, 5µl taq PCR pre Mix, 10 picomols/µl of Primer Forward,10 picomols/µl

This study aim to

Evaluate the genotype distribution of Ahr gene among radiologists in an attempt to explore the effect of radiation on their polymorphisms.

MATERIALS AND METHODS

Determination of AHR gene

AHR genotyping at the loci of G1721A and G1768A.

AHR genotyping was performed by allele specific PCR (AS-PCR). two primers were separating used as shown in the primer sequences table (1-1). Primers were designed according to the cDNA sequence of exon 10 of AHR gene .two primers were used for detecting G1768A polymorphism. The sequence of these primers were revealed in table (1-3)[11].

Preparation of primers

The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).

Primers selection

The specific primer of AHR gene

The specific primer G1721A of Primer Reverse and 16.5 µl Distilled water And thus becomes the final volume 25µl As shown in the table (1-4). Thermal cycling conditions for the G1721, AHR gene and G1768 AHR gene. The touch down PCR procedure

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Table (1-1): (1) Sequence of primers used in the Step one for G1721A (335bp) (GG).

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- ACTCTCTCAATCCTAGTTCC- 3'	50.5	45	335
Reverse	5'- TTTCATTCTGCATGTGTC- 3’	47.7	38.9	base pair

(2) sequence of primers used in the Step Two for G1721A (335bp)(GT).

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- ACTCTCTCAATCCTAGTTCC- 3'	50.5	45	335
Reverse	5'- TTTCATTCTGCATGTGTT- 3’	46	35.2	base pair

(B) The specific primer G1768A of gene:

Table (1-2): (1) sequence of primers used in the Step one for G1768A (383bp)(GT

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- ACTCTCTCAATCCTAGTTCC- 3'	50.5	45	383
Reverse	5'- GTCAATGTCTCTGAAGTCAAT-3’	50.1	38.1	base pair

(2) Sequence of primers used in the Step two for G1768A (383bp)(GG):

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- ACTCTCTCAATCCTAGTTCC- 3'	50.5	45	383
Reverse	5'- GTCAATGTCTCTGAAGTCAAC- 3’	50.1	38.1	base pair

Table (1-3): Reagents of PCR mixture for detection Ahr (G1721A), Ahr (G1768A) Mutation gene polymorphism.

Material	Volume
i-Taq DNA Polymerase	5U/ µl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Table (1-4): Mixture of working solution.

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl
Reverse primer	10 picomols/µl
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table (1-5): PCR program that was applied in the thermo cycler devices.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	40 cycle
2-	Denaturation -2	95°C	45 sec	
3-	Annealing	°C58	45 sec	
4-	Extension-1	72°C	50 sec	
5-	Extension -2	72°C	10 min.	

was chosen for AHR genotyping. The program initial denaturation 1 step for 3 minutes at95°C, followed by 1 cycles and {denaturation 2 step for 45 seconds at 95°C, Annealing step for 45 seconds at 58°C, extension 1 step for 50 seconds at 72 °C} followed by 40 cycle. The final extension 2 step was performed at 72°C for 10 minutes. The essential components of polymerase chain reaction were adopted As shown in the table (1-5) The products of PCR from amplification of G1721A and G1768A were then electrophoresed on 2% agarose at 5volt/ cm^2.1X TBE buffer for 1:30 hours. N:DNA ladder (100). The presence of bands of 333bp was indicative of the G1721A genotypes, 385 bp was indicative of the G1768A genotypes.

RESULTS AND DISCUSSION

AHR polymorphism

G1721A

The study analyzed the distribution AHR G1721A and G1768A polymorphism in study groups. The study groups included, radiologist and control. The distribution of genotype and allele frequencies among investigated groups compared with control for the G1721A is shown in table (1-6).Were G allele is a major one in the studied population. this allele is more common in radiologists (0.93,0.9) than in control with significant different when compared radiologist with control (P=0.002). The main genotype is GG in the investigated group with signification association when compare radiologists to control (12 vs13 p= 0.001). No significant different in relation GT genotypes any investigated groups. The odd ratio that appeared for the G1721A of radiologist, explain that GG genotypes of radiologist reduces the association with breast cancer whiles, GT increases with this association (OR=0.61 and 1.62) respectively. The difference is significant P=0.06262 as shown in the table (1-7).

The relative risk RR of radiologists is 0.93, this suggests that this genotype decreases the probility of contracting breast cancer. The GT genotype in radiologists increases the probility of contracting breast cancer (RR 1.5). As shown in the table (1-8).

It seems that that the search for such a polymorphism is a sensitive test for searching for DNA damage by radiation specially in heterozygote group GT in radiologists group. However, it is only specific for breast cancer patients and radiologists group whereby both GT and GG genotype were associated with significant difference. These

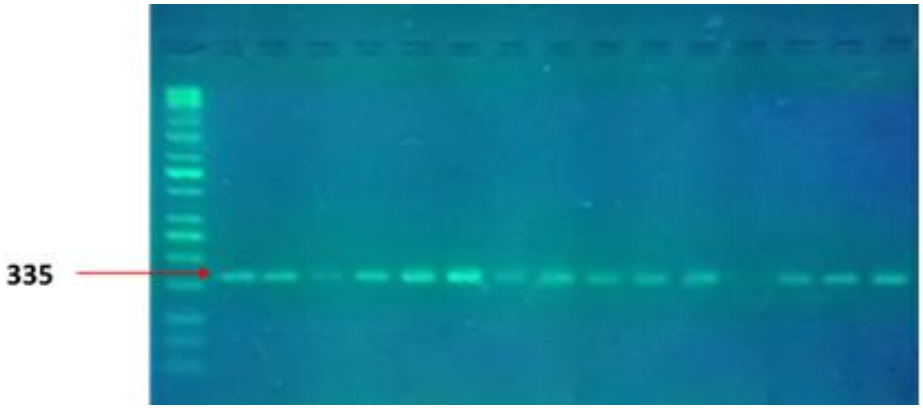

Figure 1: PCR products of G1721A genotypes the band size 35bp.

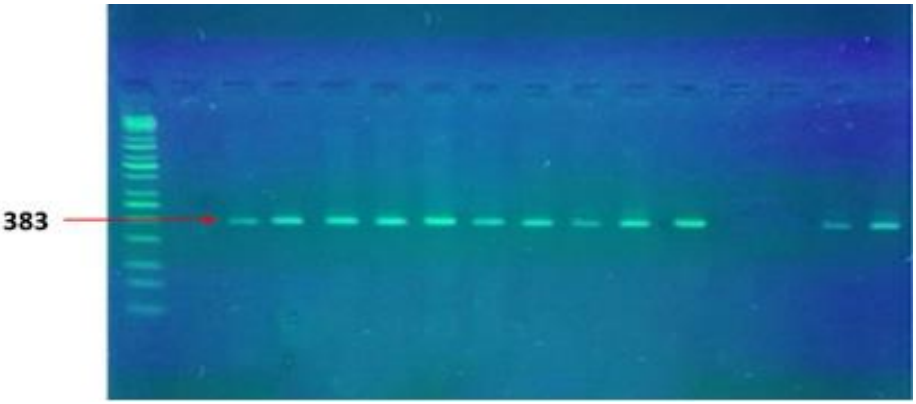

Figure 2: PCR products of G1768A genotypes, the band size 383bp.

Table (1-6): Distribution of genotypes and allele frequency at G1721A locus in investigated groups.

Parameter Groups	Genotype No.(%)			Allele frequency No. (%)	
	GG	GT	TT	G	T
Control	13 (86.67)	2 (13.33)	0(0.00)	14(0.93)	1(0.07)
Radiologist	12(73.33)	3(26.67)	0(0.00)	13(0.9)	2 (0.1)
Chi-square calculated	14.942	1.500	0.00	12.911	0.750
P-value	0.001	0.472	0.00	0.002	0.687
Significant	significant	Non-Sig.	Non-Sig.	significant	Non-Sig.

Table (1-7): Odd ratio of radiologists and control by AHR genotype at G1721A

AHR polymorphism at G1721A	Frequency %		P-value	OR(95%CI)
Genotypes	Control	radiologist		
GG	86.66(13)	80(12)	0.6262	0.61(0.0872-4.3414)
GT	13.33(2)	20(3)	0.6262	1.62(0.2303-11.464)
TT	0	0	-	-

Table (1-8): Relative risk (RR) of radiologists by AHR genotype G1721A.

Groups	Genotype%			Allele frequency	
	GG	GT	TT	G	T
Control	86.7	13.3	0	0.93	0.07
radiologists	80	20	0	0.9	0.1
RR (CI 95%)	0.93(0.588-1.217)	1.5(0.24-9.32)	0		
P-value	0.3692	0.3692	0		

polymorphisms do not reflect radio toxicity either since they were associated with lower frequencies.in other words, they were associated with lower risk of radiation effect. These results are in agreement with Sangrajrang; 2009 who found the lys allele of the AhR Arg^{554}lys polymorphism was associated with increased risk of breast cancer in Thai women (OR=1.34 (95%CI)). On the other hand these results were not agreement with[12], this

Table (1-9): Distribution of genotype and allele frequency in study groups at AHR G1768A locus.

Groups	Number	Genotype			Allele frequency	
		GG	GT	TT	G	T
Control	15	2(13.33)	13(86.67)	0	0.56	0.44
Workers in x ray units	15	5(33.4)	10(66.6)	0	0.66*	0.34

Table (1-10): Relative risk of Radiologist group by AHR genotypes at G1768A locus.

Groups	Genotype%			Allele frequency	
	GG	GT	TT	G	T
Control	13.33	86.67	0	0.56	0.44
Radiologist	33.4	66.6	0	0.66	0.34
RR (CI 95%)	2.49(0.4291-9.3213)	0.076(0.5880-1.2177)	0		
P-value	0.3692	0.3692			

Table (1-11): Odd radio of radiologists and control by AHR genotype at G1768A.

AHR polymorphism at G1768A	Frequency %		P-value	OR(95%CI)
Genotypes	Control	radiologists		
GG	13.33(2)	33.33(5)	0.2082	3.25(0.5185 -20.3704)
GT	86.66(13)	66.66(10)	0.2082	0.31(0.0491-1.9286)
TT	0	0	-	-

study showed no associated between AhR Arg^{554}lys polymorphism and breast cancer.

G1768A

Table (1-9) reveals genotype distribution at AHR G1768A.G allele is more common in radiologists individuals 0.66 than control groups (P> 0.05). The individuals with GT genotypes are more frequent among other genotypes in the investigated group with significant difference when comparing radiologists group to control (P=0.01). There is no significance association when compared G allele in radiologist with control group P=0.687 whereas this association is significance in ration to T allele (P=0.002) As shown in the table (1-9). (1-10) displays the relative risk of investigated population by AHR genotypes at G1768A locus. in radiologists, the GG genotype increases the probility of contracting breast cancer (RR 2.49).

The genotype GG in radiologists appears to be in association with contracting breast cancer (OR= 3.25) whereas the GT genotype reduce this association (OR=0.31). the difference is significant P= 0.2082 as showed in table (1-11).

Genotyping analysis results of AhR –G1721A of radiologist Showed that there was a risk of breast cancer with heteromutant GT1721 (OR=1.62 (0.2303-11.464), P=0.6262) and (RR=1.5). While homomutant GG1768 is more dangerous to the radiologists where (OR=3.25(0.5185 -20.3704), p=0.2082) and (RR=2.49). Unlike the polymorphism G1721A, polymorphism G1768A Where the latter reflects the risk of radiation. For this reason it would work as a sensitive test for screening DNA damage in radiation exposure.

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