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XRCC1 codon 194 polymorphism in Iraqi population

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Abstract: XRCC1 is one of the DNA repair genes, which play an important role in maintains DNA stability via DNA repair by base excision repair and single strand break, As its acts as a scaffold to other repairing proteins. In this study the polymorphism at the codon 194 had been studied in (202) of Iraqi population, distributed in two groups (106) Arab and (96) Kurdish. The alleles for the 194 codon, were investigates by PCR-RFLP techniques. It's found that (94) represented 88.6% Arab individuals carrying the CC dominant homozygous genotype, while (12) individuals represented 11.4% who carry the CT heterozygous genotype, and no one had the recessive TT genotype. In Kurdish group (94) represented 97.9% individuals had the CC dominant homozygous genotype, while only (2) represented 2.1% had the CT heterozygous genotype, also no one had the recessive TT genotype. The allele frequency for the Arab individuals was 0.9434 for the C which encoding for Arginin and 0.0566 for the T allele which encoding for Tryptophan. In Kurdish individuals the frequency of C allele was 0.99 while the frequency of the T allele was 0.01. These results may indicates that the C allele is the most common allele in Iraqi population in both Arab and Kurdish, and there is a slightly high allele frequency were observed in Kurdish may due intermarriage leading to reduce the recessive allele in the population.

Keywords: *XRCC1* gene, Polymorphism, Iraqi population.

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Introduction:

Mutations and DNA damage considered as the main cause of carcinogenesis (1). Removing of DNA damages is under tightly control of several mechanisms leading to maintain the genome integrity and stability by repairing DNA damages. These mechanisms include Base excision, nucleotide excision, mismatch repair, single and double strand breaks repair (2). X-Ray repair Cross Complementing group1, abbreviated as XRCC1, is one of the early responsive proteins to DNA base Damages and single strand breaks, which may caused by exposure to X-ray, ionizing radiation or toxic chemicals (3,4,5). XRCC1 acts as scaffold to DNA polymerase β and ligase III during repair processes taking place (6). At least, there are eight polymorphic variants of XRCC1, only three of them have functional activities(7). *XRCC1* composed of 17 exons expanding over 32kb on chromosome 19q13.2.Its encodes for 633 amino acid with70KD (5, 7).

The codon 194 located on the 6th exon with nonsynonymous substitutions (C>T) changing the encoded amino acid from Arginin to Tryptophan. It's considered as a potential polymorphism associated with cancer risk (8). Codon 194 polymorphism status varies in ethic and geographic distanced populations (9) , there for this study aimed to detect allele frequency of each of wild and variant alleles at codon 194 in two

major components of Iraqi population, Arab and Kurdish.

Sampling:

Peripheral blood samples were collected from 202 Iraqi individuals. The samples distributed into 106 non relative Arab origins from Baghdad governorate and 96 non relative Kurdish origins from Daiyala/ khanaqin, and Sulaymaniyah/ Kalar cities. Blood samples were stored at -20 °C till DNA extraction.

DNA Extraction:

Genomic DNA was extracted according to Promega/ Relia Prep^{TM} Blood gDNA miniprep System instructions. Extracted DNA concentration were measured by nanospectrophotometer(Maxuell, USA), then stored at 20 °C till it used.

Polymerases chain reaction and Restriction Fragment Length Polymorphism:

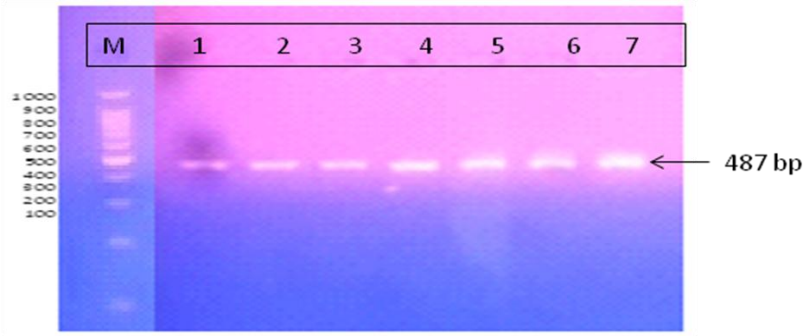
Optimization was done for annealing temperature of the primers in the polymerase chain reaction. Primers sequence for XRCC1 codon 194 were; F: 5'CCCGTCCCAGGTA 3' and the R: 5'AGCCCCAAGACCCTTTCACT 3' to amplify region of 487 bp (5). The reaction were performed in final volume 25µl by mixing 12.5 µl of Green Master Mix (Promega, USA), 1µl of each forward and reverse primers and 2µl of extracted gDNA, then volume were completed by distilled water. Reaction conditions were: one cycle at 95 °C for 7 minutes for initial denaturation, 35 cycles of denaturation for 1 minute, annealing at 60 °C for 30 seconds then extension for 1 minute at 72 °C, then final extension at 72°C for 4 minutes then cooling for 4 minutes. Restriction fragment length polymorphism detection was done by incubating 10µl of PCR products with 0.25µl of *MSP1* for 30 minutes at 37 °C for all samples, and then products were electrophoresed in 3% Agarose gel for 75 minutes at 80 Volts. Bands were visualized and documentation by UV transilluminator (Optima/ Japan).

Statistical analysis:

Statistical analysis was used to analysis the Hardy-Weinberg equation, Chi-square and allele frequency according to SSPS program.

Results:

The Codon 194 is located within the 487 bp amplified region in exon 6 of the XRCC1 gene. Figure (1) shows amplified PCR products. Figure (2&3) shows Fragment length restriction polymorphism products were obtained by mixing PCR products with 0.25µl of *Msp1*. The Arginin' s wild type allele were represented by 3 bands with 292 bp, 20 bp and 174 bp, while Tryptophan's polymorphic allele were represented in 2 bands 313pb because of the loss of restriction site in the band 313. The band 174 bp were appeared in all reaction as an internal control to successful restriction by *Msp1*.



Figure(1): PCR Amplified region of exon6, XRCC1 gene with 487bp band, on 2% Agarose gel, at 80volt,75 miuuets, M: 100bp DNA ladder, 1-7 were samples of PCR products.

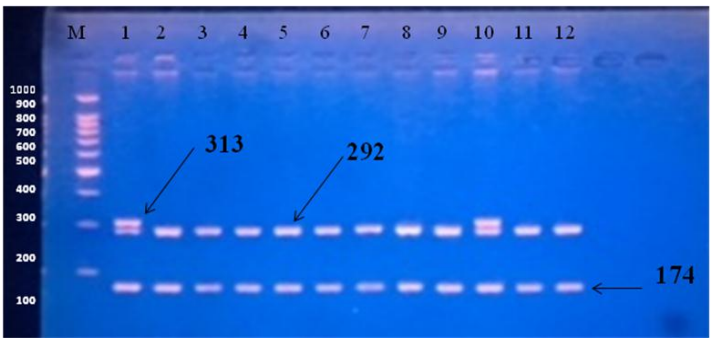


Figure (2): Restriction patterns of XRCC1 exon 6 by *MspI* for Arab individuals (on 3%Agarose gel for 75 minutes at 80Volt), showing homozygous Arginin wild type alleles in the samples (2-9 and 11,12) showing 3 bands 292 bp , 174 bp and 20 bp which it doesn't appear because it's too small but can be detected as the 292 bp band appears. Heterozygous samples were 1 and 10, showing the 313 band for Tryptophan allele as well as the Arginin 292 bp, 20 bp and 174 bp. The band 174 bp appears in all samples as internal control for successful restriction by *MspI*.

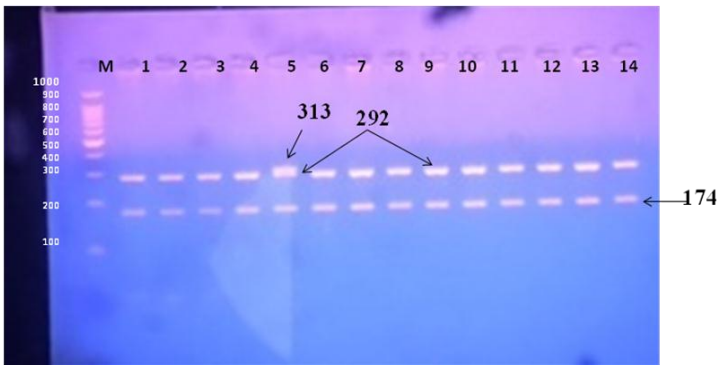


Figure (3): Restriction patterns of XRCC1 exon 6 by *MspI* for Kurdish individuals (on 3%Agarose gel for 75 minutes at 80Volt), showing homozygous Arginin wild type alleles in the samples (1-14 except 5) showing 3 bands 292 bp , 174 bp and 20 bp which it doesn't appear because it's too small but can be detected as the 292 bp band appears. Heterozygous sample was 5, showing the 313 band for Tryptophan allele as well as the Arginin 292 bp, 20 bp and 174 bp. The band 174 bp appears in all samples as internal control for successful restriction by *MspI*.

Table (1): Genotype distribution for Arab and Kurdish individuals.

Genotypes / Groups	Arg/Arg	Arg/Try	Try/Try	Total	Chi Square
Arab	94 88.6%	12 11.4%	0 0%	106	X^2= 0.38
Kurdish	94 97.9%	2 2.1%	0 0%	96	X^2= 0.01

Table (2): Codon 194 Alleles frequency for Arab and Kurdish individuals.

Alleles / Groups	Arg C	Try T	Arg/Try CT
Arab	0.9443	0.0566	0.106
Kurdish	0.99	0.01	0.0198

Discussion:

Polymorphism in DNA repair genes is attractive subject to be investigated for their direct relationship to maintain genomic integrity and stability. XRCC1 gene plays a crucial role in early response to DNA damage caused by radiation or accumulation of chemical toxic substances (5). It involve in base excision and single strand break repairing mechanisms. Its efficiency in DNA damage repair may be modulated by allelic polymorphism. Polymorphism at codone 194 may present in two allelic forms: the wild allele with Arginin which encoded by the sequence CGG, where the recognition site of *Msp* is represented, or the variant allele with tryptophan which encodes by the sequence TGG, leading to distinct polymorphic patterns on Agarose gel.

Codon 194 is found in the evolutionary, well conserved region of XRCC1. Tryptophan with aromatic non polar structure causes a conformational change protein resulting in lowing XRCC1 efficiency of repair the DNA damaged region. Accumulation of unrepaired damages is associated with different types of genetic induced diseases and cancers. The association is still controversial among different populations and ethnic groups. In Caucasian population as Norwegian, English, Trukish (4) and polish populations as well as the Wight American , mexcican, Brazilian (5) and the Asian like Indian, Chimes and twain populations(6,7) the Arginin had been found the dominant allele. But in the same time Tryptophan allele was found increased the risk of chronic myeloid leukemia(8,9), Head and Nick (10), bladder(5),prostate(11), colorectal(12), gastric(12) and breast cancers, with interesting founding that tryptophan allele may become a protective allele after menopause(14). No association had been found between Arg194Try and osteosaecoma in chines (15). In Kurdish population, Jalali *et.al* and Ahmadi *et.al*, found that Tryptophan encoding alleles may be a risk factor for breast cancer in Iranian Kurdish ethnicity(16,17). In our study to detect

XRCC1 codon 194 polymorphism in two ethnic groups in Iraq, Arab and Kurdish we found that, the Arg allele is the dominant allele in 0.94 Arab and 0.99 of Kurdish individuals with slight higher percentage of the Arg/Arg, genotype in Kurdish 0.97.9 than arab 88.6%. Arabic population, may be related to intermarriages which lead to homogeneity in Kurdish population rather than the Arabic population. This slightly high allele frequency may give the Kurdish population protection against genetically caused disease and cancers.

In Al Mutairi et. al, study on breast cancer in Arab Saudi population, they found that the allele frequencies for the healthy controls were 0.96, 0.4 and 0.0 for Arg/arg, Arg/Try and Try/Try respectively (18).These results are slightly higher than the frequencies for Arab Iraqi population but in both the Arginin allels is the dominant. In our study, the genotypes frequency for the Kurdish population were 0.99,0.01 and 0.019 respectively. While in Jalili et. al. study(16) on Iranian Kurdish population they found that allele frequencies for Iranian Kurdish healthy women were 0.45,0.40 and 0.15 for Arg/Arg, Arg/Try and Try/Try respectively, comparing with breast cancer patients which was much higher and associated of tryptophan allele with breast censer higher risk (16). There is a notable verity in allele frequency for Arab in Iraq and sudia Arabia, but in both Also the allele frequency is higher in Iraqi Kurdish population than Iranian Kurdish population in Jalilii et. al. study(16). This variation may be due to several reasons like the study sampl size and sub-ethnic origins, intermarries.

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