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Molecular detection of a polymorphism in the p53 codon 72 gene and its association with prostate cancer

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***Abstract*---**The role of the current study in detecting polymorphisms in the gene *P53codon72*, a gene that has morphological polymorphism and has an important role in the incidence of diseases, especially cancers such as prostate cancer, samples for the study were collected from 50 patients with prostate cancer from the auditors of the Imam Hussein Center for Cancer and Diseases Blood in Al-Hussein, peace be upon him, Teaching Hospital - Holy Karbala Governorate - Iraq After diagnosis by a specialist surgeon and 50 phenotypically healthy individuals as a control group, the (DNA) was extracted from the blood of both healthy and all patients study groups, and molecular detection of polymorphism of nucleotides was performed in Genes of the study using PCR-RFLP Molecular detection results in *P53codon72* The results showed that there were highly significant differences between the genotype (Pro-Pro) and between the infected and healthy groups as well as the genotype (Arg-Arg), while there was no significant relationship between the genotype (Arg-Pro) and between the infected and healthy groups. The results also showed highly significant differences between the three genotypes (Arg/ Arg, Aro/Pro, Pro/Pro) and between the two groups sick and healthy.

***Keywords*---**molecular detection, polymorphism, gene, prostate cancer.

Introduction

The prostate gland is the largest accessory gland in the male reproductive system since a healthy human prostate is usually slightly larger than a walnut (Benabdelkrim and Berredjem, 2018). Prostate cancer is one of the most common types of cancer that affects men (Lopez *et al.*, 2021), as the world records 1,276,106 new cases of prostate cancer annually and 358,989 deaths due to it (Jawad, Kamal and Awad, 2020). Prostate cancer ranks second after lung cancer As a cause of death in men in the United States and the European Union, and the sixth leading cause of all cancer deaths in men worldwide (Van *et al.*, 2021) prostate cancer increases in people over 50 years of age (Jawad, Rasool and Awad, 2020; Louis *et al.*, 2021). The incidence of prostate cancer has increased significantly in developing countries, including Iraq, according to data published by the World Health Organization (WHO) in recent years (Taha and Ali, 2020), where the Central Bureau of Statistics in the Iraqi Ministry of Planning recorded its last statistic in the year For the year 2017, (853) prostate cancer cases, i.e. (6.82%) among the types of cancer that affect males, at a rate of (4.55) per (100,000) people, and thus ranked fifth among the ten most prevalent sites of cancer among males in the year 2017. In Iraq, as shown in Table (2-1), and in 2018, the number of cases increased to (1023) new cases of prostate cancer, at a rate of (7.52%) of the total number of male cancer types, at a rate of (5.31).) for every (100,000) people and thus ranked fourth among the ten most prevalent sites of cancer among males in Iraq, and this indicates a significant increase in the number of prostate cancer cases throughout Iraq(Jawad and Awad, 2020).

Genes play a major role in coordinating and regulating the cell cycle, during which the cell increases in size and then undergoes division. In the regulation of the cell cycle (Boo,2021) , the cancerous tumor is in response to damage to the DNA, causing dysregulation in the cell cycle, which is an early stage of the development of cancer (Jawad *et al.*, 2018). Tumor suppressor genes have an important role in the formation of cancers when exposed to mutations, so they lose their function in tumor suppression, as they work naturally to regularize the normal cell life cycle and stop DNA damage(Huang and Zhou, 2021).

Apoptosis or programmed cell death genes play an important role in preserving the cell when a defect or error occurs during DNA replication in the process of cell growth, through special enzymes that repair DNA damage, which treat errors to allow the cell to continue dividing, But sometimes the repair process is impossible because of the accumulation of defects in the DNA, and thus an irreparable genetic defect will be formed, then the cell will initiate programmed death (Apoptosis) and programmed death is a genetically controlled process that directs the cell to die, which helps the organism to get rid of cells carrying accumulated genetic errors Then it prevented the formation of cancerous cells (Gems, 2021). Among the important tumor suppressor genes with polymorphism associated with the risk of prostate cancer (*P53 codons 72* polymorphism), this gene has a polymorphism located at codon 72 and associated with the encoding of the two alleles (proline and arginine), which have a significant relationship with the increase in the risk of infection. For a number of cancers such as bladder cancer, lung cancer, breast cancer, uterine cancer and prostate cancer, more than one single nucleotide polymorphism (SNPs) were found to be associated with a

number of cancers, including prostate cancer (Kaya *et al.*, 2021). The *P53 codons 72* gene is located on chromosome (17q 13.1), and it encodes a protein containing 393 amino acids *P53 codons 72* has been described as the guardian of the genome(Cannarella *et al.*, 2021).

The *P53Codon72* gene has three genotypes:

(Arg -Arg) Homozygous
(Pro-Pro) Homozygous
(Pro-Arg) heterozygous (Tiwari and Fleshner, 2021).

Materials and working methods

Patient and control samples

The study samples were collected for patients with prostate cancer from the auditors of the Imam Hussein (peace be upon him) Center for Oncology and Hematology at Imam Hussein (peace be upon him) Teaching Hospital in the Holy Karbala Governorate / Iraq. It included 50 patients after prostate cancer was diagnosed clinically by a specialist surgeon, in addition to collecting 50 healthy volunteers from the disease (control group) for the period from 25August to 30November, 2021.

Blood collection and DNA isolation

4 ml of venous blood was withdrawn from the patients and control group and placed in anticoagulant tubes (Ethylene diamine tetra acetic acid) EDTA and the sample was agitated to prevent clotting, and DNA was extracted from the blood according to the instructions of the extraction kit supplied by Geneaid company (korea) Samples containing DNA under -20°C until the day of the molecular examination.

Genotyping analysis and amplification *p53codon72* gene

P53Codon72 polymorphism analysis was performed by PCR and PCR-RFLP The primers for one-step amplification were

F: 5′-GCT CTT TTC ACC CAT CTA CAG-3′ and
R: 5′-TGA AGT CTC ATG GAA GCCAGC-3′,

were Perform PCR amplification with an initial denaturation step at 95 °C for 5 min, then amplify for 35 cycles of plate denaturation at 95 °C for 30 sec, annealing at 58 °C for 30 sec, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min.

The results of the molecular diagnosis of the study samples (patients and healthy people) using PCR technique for the *P53 codons 72* gene are shown in Figure (1) the PCR reaction products after being electrophoresed on agarose gel at a concentration of 2% at 60 V for 50 minutes, where column M represents the volume guide. (bp) size (100-2000) and columns (1,2,3,4,5,6,7,8) represent the

DNA bundles of *P53 codons 72* gene (bp279), and column M represents the guide size (bp size 100-2000) and this agrees with The results of the study (Doosti and Dehkordi, 2011), which showed p53 codon 72 gene bundles with a size of (bp279), in agreement with the results of the current study.

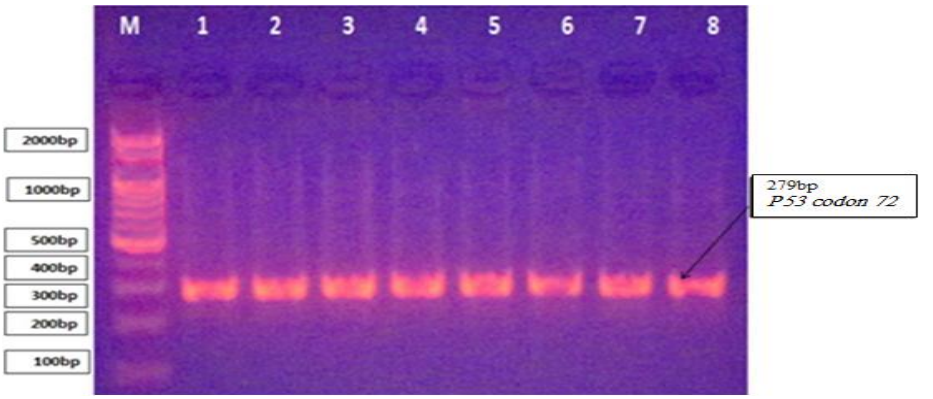

Figure (1) Results of the polymerase chain reaction (PCR) for *P53 codon 72* gene after being electrophoresed on agarose gel at a concentration of (2%) at 60 V for 50 minutes

Molecular detection of the polymorphism gene *P53 codon 72*

The PCR products for the *P53 codon 72* polymorphism gene were cut by using the BstUI restriction enzyme by adding µl5 of the polymorphism gene P53 codon 72 amplification product in a small tube and then adding µl1 of the BstUI trimer enzyme with (1X) µl5 of 10XNEBuffer to make the volume of the mixture Total 50 µl After completing the volume by adding deionized water, the mixture was incubated at 37°C for 12 hours.

In the study, one of the modifications of the polymerase chain reaction (PCR) was used to study the polymorphisms of the *P53 codons 72* gene, which is the technique of restriction fragment length polymorphisms, which is known as RFLP. Figure (2) shows the genotypes of the polymorphism of the *P53 codons 72* gene, where The PCR reaction was cut using the cutting enzyme BstUI and electrophoresis was carried out on a 2% agarose gel for 50 minutes at a voltage of 60 V.

Columns (1,2,3,4) indicate the homozygous arginine (arginine) genotype (160, 119 bp), while bars (5,6,7) represent the Heterozygotes Pro-Arg genotype (279, 160, 119 bp) , (8) column indicates the homozygous (pro) proline size genotype package (279 bp) and column M represents the bp size guide (100–2000 bp). The result of the current study agreed with the study (Doosti and Dehkordi, 2011) in terms of the appearance of the genotypes of the *P53 codons 72* gene with the same study package sizes using RFLP technology.

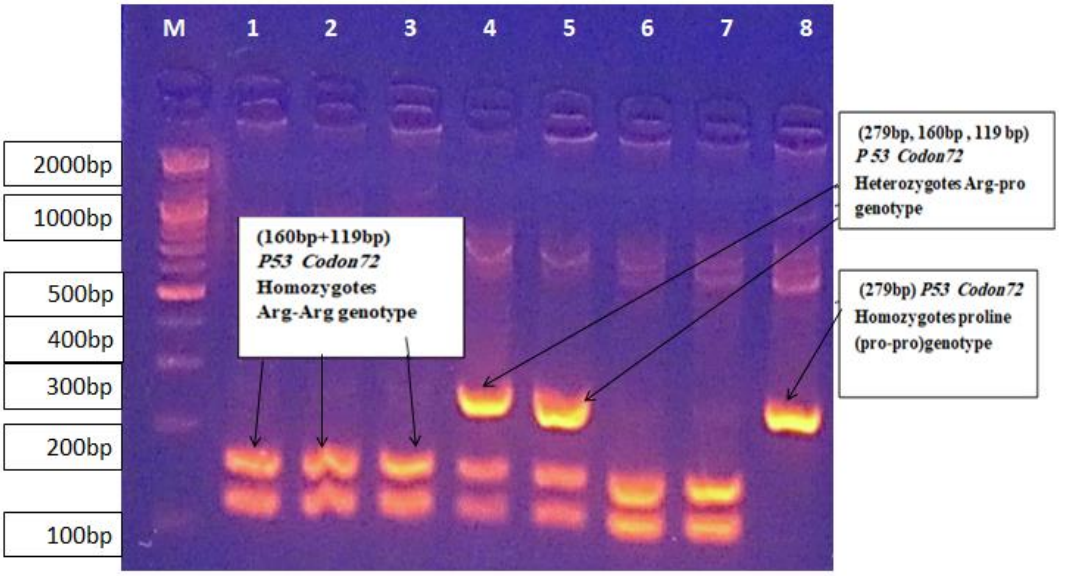

Figure (2) Electrophoresis of samples for *P53 codons 72 gene* using *BstUI* cutting enzyme on agarose gel at a concentration (2%) at 60 V for 50 minutes

Statistical Analysis

The Statistical Analysis Program (SPSS) Special Packages of Social Since V.22 was used to analyze the results of the study, and the level of significance $P \leq 0.01$ and $P \leq 0.05$ was adopted to find out the statistical differences for the study samples.

Results and Discussion

Molecular diagnosis of the polymorphism in *p53Codon 72* gene using RFLP technique

The results of the study of the disease using RFLP technology, shown in Table (1), showed that 20 (40%) represented the Pro-Proline genotype (Homozygotes proline) in the group of patients, while the percentage for the same genotype was 36 (72.0%) of the healthy group, In the genotype Arg-Arg (Homozygote arginine), 20 (40%) appeared in patients and 8 (16%) in healthy subjects, and 10 (20%) were recorded in prostate cancer patients carrying the genotype Pro-Arg (Heterozygotes proline-arginine) As for the healthy subjects, the percentage was 6 (12%).

The results of the statistical analysis recorded a statistical significance with a high significant relationship between the genotype (Homozygotes proline) and the groups of healthy people and patients with prostate cancer. The statistical analysis also showed a significant relationship between the genotype (Homozygote arginine) and between patients and healthy subjects, unlike the genotype (Heterozygotes proline-arginine), for which the statistical results did not record a significant relationship between it and the two groups of patients and healthy subjects, while the results recorded a highly statistically significant relationship between the genotypes. The three genes (Pro-Pro, Arg-Arg, Pro-Arg) for the codon

72 p53 gene and between the two groups of patients with prostate cancer and those without it.

The variation between individuals in the metabolism of toxic and carcinogenic substances, as well as the regularity of the cell cycle and the ability to suppress tumors, is due to the polymorphism in their genes and the emergence of active and inactive genotypes, which may cause or prevent several diseases, the most important of which is cancer (Skandalaki, and Theocharis, 2021), the Codon 72 p53 gene, for example, has polymorphisms, and it is one of the most important tumor suppressor genes. These polymorphisms have shown a lot of association with dangerous diseases such as various cancers (Donehower *et al.*, 2019; Jawad, Mohammed and Jeddoaa, 2019). The p53 gene has polymorphism and contains coding and non-coding regions. Most of these polymorphisms contain single nucleotide polymorphisms (SNPs), and these polymorphisms are located on code 47 and code 72, and this code is functionally important because it is associated with coding for the arginine and proline alleles, which are associated with the occurrence of many types of cancer, including prostate cancer (Mostaid *et al.*, 2021), my study (Doosti and Dehkordi, 2011; Mittal *et al.*, 2011) indicated a highly significant association of genotypes (Homozygotes proline & Homozygote arginine) for the polymorphism of the *p53Codon 72* gene with prostate cancer, while no significant relationship of the (Heterozygotes proline-arginine) genotype with the disease has been proven(Huang *et al.*, 2004; Rogler *et al.*, 2011) which did not prove a significant association between any of the genotypes of the Codon 72 p53 gene with prostate cancer, and justifies the reason for the emergence and disappearance of genotypes and its association or not with prostate cancer in the world to the variation of gene expression according to Environment and Ethnicity (Hong *et al.*, 2019).

Table No.(1):Distribution of samples (patients - healthy) by genotype of the *P53Codon 72* gene polymorphism

Genotype	The number and the ratio	sample		p.v
		Patients	Healthy	
Pro- Pro	number	20	36	0.03
	ratio	40.0%	72.0%	
Arg-Arg	number	20	8	0.02
	ratio	40.0%	16.0%	
Pro-Arg	number	10	6	0.32
	ratio	20.0%	12.0%	
Significance between *p53codon 72* genotypes and groups of patients and healthy subjects				0.004

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