How to Cite:
Abdulla, A. J., & Jawad, Z. N. (2022). Molecular detection of GSTM1 and GSTT1 gene polymorphisms and their association with prostate cancer risk. *International Journal of Health Sciences*, *6*(S3), 6656–6663. https://doi.org/10.53730/ijhs.v6nS3.7482

Molecular detection of GSTM1 and GSTT1 gene polymorphisms and their association with prostate cancer risk

Ali Jabbar Abdulla
Department of Biology, College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq
*Corresponding author email: ali.jabbar@s.uokerbala.edu.iq

Zainab Nizar Jawad
Department of Biology, College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq
Email: zainab.n@uokerbala.edu.iq

***Abstract*---**The role of the current study in the detection of polymorphisms appears in genes *GSTT1& GSTM1* polymorphisms ,They are genes that have polymorphisms and have an important role in the occurrence of diseases, especially cancers such as prostate cancer, and Samples for the study were collected from 50 patients with prostate cancer (from the patients of the Imam Al-Hussein Center - peace be upon him - for cancerous tumors and blood diseases at Al-Hussein Teaching Hospital, peace be upon him - Holy Karbala Governorate – Iraq) After diagnosis by the oncological surgeon and 50 samples collected from phenotypically healthy individuals as a control group the (DNA) was extracted from the blood of both patients group and healthy group. Molecular detection of the nucleotide polymorphism in the genes under our study was carried out using Multiplex- PCR .the results of molecular detection of the *GSTT1* gene showed a highly significant correlation between its genotypes and between prostate cancer patients and healthy individuals, while the results did not show significant differences between the genotypes of the *GSTM1* gene and between prostate cancer patients and people whom don't have prostate cancer, as well as the results did not show significant differences between the genotypes of the two genes *GSTM1, GSTT1* combined. With patients with prostate cancer and healthy people.

***Keywords*---**molecular detection, polymorphisms, association, prostate cancer risk.

Introduction

Cancer is a serious challenge and a major cause of death in the world, which affects any part of the body, as it appears in the form of abnormal growth of cells in the body due to a defect in the natural cellular systems that control cell division and reproduction, Which leads to it continuing to grow outside the control of the body and the formation of abnormal cells, which produces masses of tissue known as cancerous tumors. Prostate cancer is one of the types of cancers specialized in the infection of the prostate gland in males, and it is one of the most prevalent types among them, especially among those over the age fifty of them (Jawad *et al.*, 2018; Kohestani, 2021). Prostate cancer usually does not cause any symptoms in its initial stages, and its symptoms are similar to those of benign prostatic hyperplasia. These symptoms include frequent urination, especially during the night, difficulty urinating, and failure to maintain a constant flow of urine

The appearance of bloody urine with pain in its advanced stages, in addition to sexual problems that accompany it, such as erectile dysfunction or loss of Malignant tumor sexual abilities(Galvin, Garland and Wibowo, 2021). It consider the sixth type of cancer causing death globally and the second in theUnited States of America(Jawad, Kamal and Awad, 2020; Jemal *et al.*, 2021). In Iraq, specifically in 2018, it ranked fourth among the ten common types of cancer, as it recorded (1023) new cases of prostate cancer, with a rate of (7.52%) of the total number of male cancer types, and at a rate of (5.31) per (100,000) people (Iraqi Ministry of Planning 2019). Clinical and epidemiological data indicate that the occurrence of prostate cancer has several interconnected causes [source]. Smoking, dietary habits, lifestyle factors, and environmental factors, as well as geographic/ethnic factors and genetics may be primary causes of PCa. And the modification in the genes of food metabolism that causes cancer may play a crucial role in the occurrence of PCa because these genes produce enzymes that have important functions such as detoxification (Glutathione-*S*-transferees) Gene group encoding enzymes.

Which remove toxins, so it is necessary to protect cells from oxidation and damage, in addition to the metabolism of carcinogenic compounds such as polycyclic aromatic hydrocarbons resulting from cigarette smoking or resulting from burning meat and diesel vehicle exhaust products (Rögner *etal* 2021)Glutathione is naturally produced in the body by the liver, and it can be obtained through food. It is found in dairy products, cereals and bread in a small percentage, but fruits and vegetables contain an average percentage, while fresh meat contains a high percentage of glutathione in addition to its presence in many nutritional supplements(Jawad and Kamal, 2020). GSTs are a multigene family has a secondary gene families including Alpha, Mu, Omega, Pi, Sigma and Theta (source) (Medjani *et al.*, 2020) Two of those genes, GSTM1 and GSTT1, have been studied extensively because their genetic polymorphism accompanies increased susceptibility to various diseases such as cardiovascular and respiratory diseases, in addition to several types of cancer such as ovarian, colon, breast, lung and prostate cancer (Khan *et al.*, 2014)The GSTM1 gene belongs to the sub-family Mu, which includes five related genes on the short arm of the first chromosome in humans. It is located on chromosome 1q13.3 and consists of 8

exons and four allelic variants (A, B, C and - or null) and the form GSTM1-null or (GSTM1-) is the most common polymorphism that encodes a detoxifying glutathione enzyme other than the *GSTM1* Wild or (*GSTM1*$^{+}$) genotype that encodes the inactive glutathione detoxifying enzyme (Hasan, 2018),As for the *GSTT1* gene, it belongs to the secondary family Theta and is located on the long arm of the human chromosome 22 at the site (22q11.2). It has the function of removing toxins and carcinogens, causing an increased risk of developing prostate cancer The aim of this study was to evaluate the effect of glutathione S-transferase (*GSTM1, GSTT1*) on PCa risk (Drozdz *et al.*, 2020).

Procedure
Study Samples

The study samples were collected from patients with prostate cancer who are attend to the Imam Hussein (PBUH) Center for Oncology and Hematology at Imam Hussein Teaching Hospital in the Holy Karbala Governorate - Iraq. It included 50 patients after prostate cancer was diagnosed clinically by the specialized surgeon, in addition to collecting samples from 50 healthy volunteers from the disease (control group).

Molecular analysis of genetic polymorphisms of the *GSTM1* and *GSTT1* genes
Blood collection and DNA isolation

4ml of venous blood was drawn from healthy and patient individuals and placed in anti-coagulant tubes (Ethylene diamine tetra acetic acid) EDTA. The sample was agitated to prevent clotting, and then it was transferred in a cooler box to the postgraduate laboratory in the Biology Department, Faculty of Education Pure Science University of Karbala so we can do the molecular examinations for them, the DNA was extracted from the blood according to the instructions of the extraction kit supplied by (korea) Geneaid Company. The genomic DNA samples were stored at -20°C until the day of molecular detection.

Genotyping *GSTM1* and *GSTT1*

GSTM1 and *GSTT1* gene deletions were analyzed simultaneously by multiplex PCR. β-globulin was used as an internal control, confirming successful PCR amplification to confirm that *GSTM1*-null and *GSTT1*-null were due to deletion of *GST* alleles and not due to PCR failure. For detection of *GSTM1* deletion, the primers used were as in Table (1)

Table 1
Primers used for molecular detection of mutations and single nucleotide polymorphisms (SNPs) in the study genes

Name of gene	Sequence of Primers*	Product Size(bp)	Reference
GSTM1	F :5′-GAACTCCCTGAAAAGCTAAAGC-3′ R :5′-GTTGGGCTCAAATATACGGTGG-3′	230	Medjani *etal.*, (2020)
GSTT1	F (5′-TTC CTTACTGGTCCTCACATCTC-3′) R: (5′-TCACCGGATCATGGCCACCA-3′	480	

β-globulin	F 5′-ACA CAA CTG TGT TCA CTA GC–3′ R 5′-CAA CTT CAT CCA CGT TCA CC-3′	110	

The PCR conditions were: 5 min of initial denaturation at 95 °C, followed by of 37 cycles of denaturation at 94 °C for30sec., annealing at 60 °C for 1 min and extension at 72 °C for 1 min with a final extension at 72 °C for 5 min. The *GSTM1* fragment was 230 bp, the *GSTT1* fragment was 480 bp, and the *β-globulin* fragment was 110 bp in size. Multiplex- PCR technology is one of the PCR modifications, as it is characterized by its speed in detecting deletions and gene amplification. Samples were diagnosed in healthy and molecularly healthy prostate cancer patients using Multiplex-PCR technology.

Figure (1) shows the electrophoresis for detection of the *GSTM1* and *GSTT1* polymorphisms on 2% agarose gel at 60 V. for 50 min, where column M represents the volume guide(100-2000 bp), and a gene (β-globulin) was added to the interaction as an internal control. If the genes (*GSTM1* & *GSTT1*) are deleted while the (*β-globulin*) gene remains this indicates the deletion of the alleles of the genes ($GSTM1^-$, $GSTT1^-$).The bands in the two columns (1,2) indicate the presence of the two genes *GSTT1* and *β-globulin* with a size of 230pb and 110pb, respectively, which means that the genotype of those columns is ($GSTT1^+$- $GSTM1^-$), while the bands in columns (3,4) indicate the appearance of the genes (*GSTM1*, *GSTT1* and *β-globulin*) with a size of (480 pb, 230pb, and 110pb) , respectively, and this indicates the genotype *(*$GSTT1^+$ - $GSTM1^+$), while column (5) indicates the presence of the *GSTM1* gene and the *β-globulin* gene with a size of (480 pb and 110pb), respectively, indicating that The genotype ($GSTM1^+$-$GSTT1^-$) and the columns (6,7,8) refer to the genotype ($GSTM1^-$, $GSTT1^-$), where only one bundle of size 110pb appeared in it, which refers to the *β-globulin* gene, and the column M represents the volume guide size (100-2000 bp).

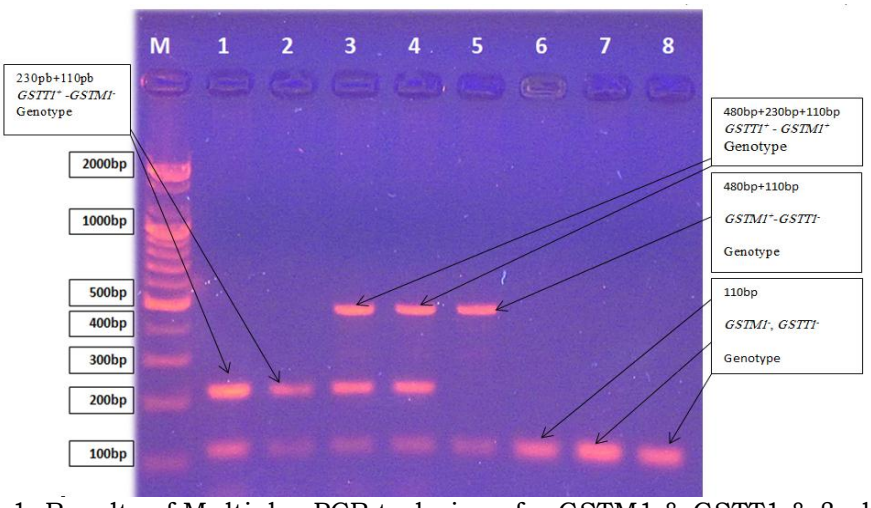

Figure 1. Results of Multiplex PCR technique for GSTM1 & GSTT1 & β-globulin genes after being electrophoresed on agarose gel at a concentration of (2%) at 60 V for 50 minutes

Statistical Analysis

The statistical analysis program (SPSS) packages were used since version 22 to analyze the results of the study, and the significance level $P \leq 0.01$ and $P \leq 0.05$ were adopted to find out the statistical differences for the study samples.

Results and Discussion

Genotypes of the polymorphisms of the study genes:

Humans possess a large polymorphism in different parts of the gene, through which the genetic predisposition to infection in many diseases such as cancer can be diagnosed, as the polymorphism of the gene can be identified and measured (Sharma *et al.*, 2020). It is clear from the results of Table (2) of the distribution of genotypes in the *GSTM1* and *GSTT1* genes to patients and healthy persons, as it appears from the results of this table for the *GSTM1* gene that the persons carrying the *GSTM1*$^-$ genotype from the group of patients appeared by 47 (94.0%) and in the healthy group they were 48 (96.0%) of the group of patients with the genotype (GSTM1$^+$) were 3 (6.0%) and the healthy 2 (4.0%), and the results of the statistical analysis did not prove any significant correlation between the genotypes of the *GSTM1* gene and prostate cancer for both patient and control groups.

As for the *GSTT1* gene, the results of the table showed that the *GSTT1*$^-$ genotype was present in 45 (90.0%) people in patients and 36 (72.0%) of the healthy group, while the genotype (*GSTT1* $^+$) represented 5 (10.0%) of the group Patients 14 (28.0%) of the healthy group, the results of the statistical analysis did not show a significant correlation between the *GSTT1*$^-$ genotype and between the healthy and patient group, while it showed a statistically significant relationship with a high significant correlation between the *GSTT1* $^+$ genotype with the healthy and patient group, as well as showed a high significant correlation between The two genotypes *GSTT1*$^-$ and *GSTT1*$^+$ and the occurrence of prostate cancer in both healthy and patients groups.

But for the genotype (*GSTM1*$^-$*, GSTT1*$^-$) it appeared in 43 (86.0%) people of the patients group and 40 (80.0%) people of the healthy group, while the genotype (*GSTM1* $^+$ and *GSTT1*$^+$) was present in 7 (14.0%) people Of the group of patients and 10 (20.0%) individuals of the healthy group, the results of the statistical analysis did not prove any significant association between the two genotypes (*GSTM1*$^-$- *GSTT1*$^-$ and *GSTM1*$^+$ -*GSTT1*$^+$) with the two groups of patients and healthy individuals. The results of the current study agreed with the results of the study (Zhang *et al.*, 2019; Medjani *et al.*, 2020b) in the absence of an association between prostate cancer and the *GSTM1* gene for both *GSTM1*$^+$ and *GSTM1* $^-$ and the study also agreed with our study regarding the existence of an association between prostate cancer with the *GSTT1* gene and its genetic (*GSTT1*$^+$, *GSTT1*$^-$) for both healthy and patient groups.

The study (Benabdelkrim, and Berredjem, 2018)also agreed with the current study in the genotypes (*GSTM1*$^+$ and *GSTT1*$^+$ and (*GSTM1*$^-$, *GSTT1*$^-$), as they were not associated with disease in the healthy and patients groups. While the results

of the current study disagreed with the two studies (Drozdz-Afelt *et al.*, 2020; Santric *et al.*, 2021)., as they did not prove any significant association between the two genes *GSTM1* and *GSTT1* and the occurrence of prostate cancer and for all their genotypes.

The large discrepancy in the results of studies on the relationship of the two genes *GSTM1* and *GSTT1* with prostate cancer may be caused by the influence and interaction of risk factors such as race, smoking and place of residence on the polymorphism of the genetic group *GSTs* (Tcheandjieu *et al.*, 2020).Several individuals may have the same genetic mutation or genotype that causes infection However, the disease appears on some individuals without the other, and this is due to several factors that prevent penetration or gene expression, including modified genes, which are other genes that affect the expression of the gene containing the mutation, or the effect of the factor on genes such as lifestyle and food (Hanany, and Sharon, 2020).

Table 2
Shows the distribution of genotypes in samples of patients and healthy subjects

gene	genotype	Number & ratio	sample		p.v
			patient	healthy	
GSTM1	*GSTM1*$^{-}$	Number	47	48	0.92
		ratio	94.0%	96.0%	
	GSTM1^{+}	Number	3	2	0.66
		ratio	6.0%	4.0%	
P.V of genotypes (+ and -) of the two groups of patients and healthy subjects			0.65		
GSTT1	*GSTT1*$^{-}$	Number	45	36	0.32
		ratio	90.0%	72.0%	
	GSTT1^{+}	Number	5	14	0.03
		ratio	10.0%	28.0%	
P.V of genotypes (+ and -) of the two groups of patients and healthy subjects			0.02		
GSTM1 & *GSTT1*	*GSTM1*$^{-}$, *GSTT1*$^{-}$	Number	43	40	0.74
		ratio	86.0%	80.0%	
	GSTT1^{+} $^{+}$, *GSTM1*	Number	7	10	0.53
		ratio	14.0%	20.0%	
P.V of genotypes (+ and -) of the two groups of patients and healthy subjects			0.42		

* (-) indicates a genotype deletion
* (+) indicates the presence of the genotype

References

Benabdelkrim, M., Djeffal, O. and Berredjem, H. (2018) 'GSTM1 and GSTT1 polymorphisms and susceptibility to prostate cancer: A case-control study of the Algerian Population', Asian Pacific Journal of Cancer Prevention, 19(10), pp. 2853–2858. doi: 10.22034/APJCP.2018.19.10.2853.

Drozdz-Afelt, J. M. et al. (2020) 'Polymorphism of glutathione S-transferase in the

population of Polish patients with carcinoma of the prostate', Environmental Science and Pollution Research, 27(16), pp. 19375–19382. doi: 10.1007/s11356-020-08435-7.

Galvin, K. T., Garland, S. N. and Wibowo, E. (2021) 'The Relationship between Sleep Hygiene , Mood , and Insomnia Symptoms in Men with Prostate Cancer'.

Hanany, M., Rivolta, C. and Sharon, D. (2020) 'Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases', Proceedings of the National Academy of Sciences of the United States of America, 117(5), pp. 2710–2716. doi: 10.1073/pnas.1913179117.

Hasan, O. M. (2018) 'Polymorphism of GSTM1 and GSTT1 gene in prostate cancer Osamah Mohammed Hasan Mohammed Ibrahem Nader', (May).

Jawad, Z. N. et al. (2018) 'ASSOCIATION OF MDM2 (T309G) GENE POLYMORPHISM WITH INTERSTITIAL URINARY BLADDER CANCER', 18(2), p. 5075.

Jawad, Z. N. and Kamal, A. R. (2020) 'Association of Additive Risk of Pioglitazone Use with the Presence of CYP1A1 Polymorphisms in the Occurrence of Bladder Cancer', 11(5), pp. 375–376. doi: 10.31838/srp.2020.5.53.

Jawad, Z. N., Kamal, A. R. and Awad, W. (2020) 'The effect of montelukast on CD14 gene expression in asthmatic patient in Kerbala', AIP Conference Proceedings, 2290(December), pp. 10–13. doi: 10.1063/5.0029590.

Jemal, A. et al. (2021) 'Prostate cancer incidence 5 years after us preventive services task force recommendations against screening', Journal of the National Cancer Institute, 113(1), pp. 64–71. doi: 10.1093/JNCI/DJAA068.

Khan, M. H. et al. (2014) 'Association of rs1042522 polymorphism with increased risk of prostate adenocarcinoma in the Pakistani population and its HuGE review', Asian Pacific Journal of Cancer Prevention, 15(9), pp. 3973–3980. doi: 10.7314/APJCP.2014.15.9.3973.

Kohestani, K. (2021) Magnetic Resonance Imaging as a Screening Tool for Prostate Cancer.

Medjani, S. et al. (2020a) 'Association of CYP1A1, GSTM1 and GSTT1 gene polymorphisms with risk of prostate cancer in Algerian population', African Journal of Urology, 26(1). doi: 10.1186/s12301-020-00049-2.

Medjani, S. et al. (2020b) 'Association of CYP1A1, GSTM1 and GSTT1 gene polymorphisms with risk of prostate cancer in Algerian population', African Journal of Urology, 26(1). doi: 10.1186/s12301-020-00049-2.

Rögner, N. et al. (2021) 'A sensitive LC–MS/MS method for the quantification of 3-hydroxybenzo[a]pyrene in urine-exposure assessment in smokers and users of potentially reduced-risk products', Separations, 8(10). doi: 10.3390/separations8100171.

Santric, V. et al. (2021) 'Polymorphisms in Genes Encoding Glutathione Transferase Pi and Glutathione Transferase Omega Influence Prostate Cancer Risk and Prognosis', Frontiers in Molecular Biosciences, 8(April), pp. 1–8. doi: 10.3389/fmolb.2021.620690.

Sharma, S. et al. (2020) 'The impact of human genetic polymorphisms on rotavirus susceptibility, epidemiology, and vaccine take', Viruses, 12(3). doi: 10.3390/v12030324.

Tcheandjieu, C. et al. (2020) 'Role of GSTM1 and GSTT1 genotypes in differentiated thyroid cancer and interaction with lifestyle factors: Results from case-control studies in France and New Caledonia', PLoS ONE, 15(1), pp. 1–14. doi: 10.1371/journal.pone.0228187.

Zhang, M. et al. (2019) 'Association of Hsa-miR-23a rs3745453 variation with prostate cancer risk among Chinese Han population: A case-control study', Medicine (United States), 98(52). doi: 10.1097/MD.0000000000018523.