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Comparative Expression Genes of *GSTM1* and *GSTT1* Genes among Benign and Malignant Prostate Cancer

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ABSTRACT

Background: *Prostate cancer is one of the malignancies that affects men and significantly contributes to increased mortality rates in men globally. Patients affected with prostate cancer present with either a localized or advanced disease.* ***Objective:*** *The present study aimed to determining the relationship between gene expression of GSTM1 and GSTT1 genes in the sera of patients and compared it with healthy.* ***Materials and methods:*** *A case control study consist of three group included was in this study . The first group involves 50 patients with PC were observation in Al- Amal Oncology Hospital in the period from April 2021 to April 2022 under the supervision of oncology specialists was included in this study. Second group consist of 30 patients. They have benign hyper plaisa (BHP), this group has been collected from urosergical department . Third group was include 20 healthy volunteers (non prostate cancer and non BHP). The RNA extraction from whole blood of both the patients and healthy by using protocol in TransZOl Up Plus RNA kit (Transgen- China) to determining gene expression of GSTM1 and GSTT1 genes in study groups.* ***Results:*** *Results of the present study showed the 60-69 years age group scored highest percentage in benign (56.7%), malignant (54.0%), compared to control (healthy) (50.0%), while >69 years scored least percentage in these groups (3.3%, 14.0%, and 25.0%) respectively with significant different ($p<0.05$). Additionally, it is found the GSTM1 and GSTT1 genes levels scored highest mean levels in malignant group (8.67±2.17 and 8.80±3.65) and least mean level in healthy (1.00±0.00 and 1.00±0.00) compared to benign group with significant different ($p<0.05$).*

Conclusions: *We concluded the GSTM1 and GSTT1 genes levels are play major roles in pathogenesis patients with prostate cancer. PCa is high prevalence in elderly population.*

Keywords; prostate cancer, GSTM1, GSTT1 genes.

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INTRODUCTION

Prostate cancer is not only one of the most frequently diagnosed cancers in males but also one of the leading causes of cancer deaths worldwide (Rawla, 2019). Prostate cancer may be asymptomatic at the early stage and often has an indolent course, and may require minimal or even no treatment. However, the most frequent complaint is difficulty with urination, increased frequency, and nocturia, all symptoms that may also arise from prostatic hypertrophy (Holm et al., 2022). It is estimated that 1.6 million men are diagnosed, and 366,000 men die of prostate cancer each year (Pernar et al., 2018). Prostate cancer is the second most frequent malignancy (after lung cancer) in men worldwide, counting 1,276,106 new cases and causing 358,989 deaths (3.8% of all deaths caused by cancer in men) in 2018 (Ferlay et al., 2020).

The incidence and mortality of prostate cancer worldwide correlate with increasing age with the average age at the time of diagnosis being 66 years. Of note, for African-American men, the incidence rates are higher when compared to the White men, with 158.3 new cases diagnosed per 100,000 men and their mortality is approximately twice as White men (Panigrahi et al., 2019). In 2018 more

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than 10 000 new cases of prostate cancer were diagnosed in Sweden, making prostate cancer the most common malignant disease in Swedish men and roughly as common as breast cancer (Lundgren, 2021).

The GST polymorphisms combination showed an increased risk for carcinogenesis, and studies with larger casuistry can contribute to the clarification of the role in individual patient differences for the response to chemotherapy and/or radiotherapy and identify biomarkers of susceptibility (Maniglia et al., 2020). Homozygous deletions of the GSTM1 and GSTT1 genes are common and result in a complete loss of enzymatic activity. Hence, individuals are at greater risk toward the development of malignancies (Medjani et al., 2020). Recent study showed a reduced GSTM1 gene expression in prostate tissue was observed in PCa patients with some environmental chemical exposures. Intriguingly, physical activity might play a protective role against PCa development, possibly as a result of increasing GSTM1 gene expression in prostate tissue (Gómez-Martín et al., 2019). However, only a few studies have addressed the role of GST polymorphisms with regard to the survival of PC patients, proposing the important catalytic and regulatory roles as the potential underlying molecular mechanisms involved in PC progression (Santric et al., 2021).

The present study aimed to determining the relationship between gene expression of *GSTM1 and GSTT1 genes* levels in sera of patients and compared it with healthy.

MATERIALS AND METHODS

1.Population studies

A case control study consist of three group included was in this study. The first group involves 50 patients with PC were observation in Al- Amal Oncology Hospital in the period from April 2021 to April 2022 under the supervision of oncology specialists was included in this study. Second group consist of 30 patients. They have benign hyper plaisa (BHP), this group has been collected from urosergical department . Third group was included 20 healthy volunteers (non prostate cancer and non BHP).

2.Quantitative gene expression of *GSTM1 and GSTT1 genes*

2.1 RNA Extraction from Blood Samples

A blood samples (100) were collected from patients and healthy. The blood sample (3 ml) was collected from study groups, and then mixing in EDTA tube, then immediately transferred 200µl of EDTA-blood in a tube containing 600 µl of Tizol and mixed it well and stored at -20 C until use for RNA extraction to study the gene expression of *GSTM1 and GSTT1 genes*. The RNA extraction from whole blood of both the patients and healthy by using protocol in TransZOl Up Plus RNA kit (Transgen- China).

2.2 Two-step quantitative Real Time-PCR for gene expression

A- gDNA Elimination and cDNA production

The complementary DNA (cDNA) production was performed by utilize protocol within EasyScript® One-Step gDNA deleted and complementary DNA generation Super-Mix (Transgen- China).

Procedure

1- Transferred 3-5 µL of extracted RNA to RT-PCR tubes containing 1 µL Anchored Oligo (dT) Primer (0.5µg/µl) with 1 µL Random Primer (0.1µg/µl).

2- Incubated in thermo cycler (65 C for five minutes and 4 C for ten minutes).

3- After incubation, it was added10 µL EX Reaction Mix,1 µL gDNA remover, 1 µL Easy Script®RT/RI Enzyme Mix and 3 µL RNase-free water to complete the final volume to 20 µL

4- Incubated in thermo cycler as shown below:

- Step1; temperature (25°c), time (10min), and purpose (Random Primer (N9) (primer binding).

- Step2; temperature (42°c), time (15min), and purpose (Anchored Oligo (dT)18 (extension).
- Step3; temperature (85°c), time (5seconds), and purpose (Inactivate reverse transcriptase enzyme (inactivation).

B- Amplification of cDNA

The amplification done by using TransStart® Green qPCR SuperMix kit with specific primers (Transgen- China). This process including several primers includes; ***GSTM1*** (Forward- TTCCCAATCTGCCCTACTTG and Reverse- ATGGTTGTCCATGGTCTGGT) **(Medhi et al., 2011**), ***GSTT1*** *gene* (Forward- TTGCTGACTCCCTCTGGTTT and Reverse- TCGAAGGGAATGTCGTTCTT) (designed in this study), and ***ACTB*** (*Housekeeping genes*) (Forward- CTGTGGCATCCACGAAAC and Reverse- CAGACAGCACTGTGTTGG) (**Medhi et al., 2011).**

The following approach was used to carry out the amplifying in a reaction volume of 20 µl:

1-The (Syper Green qPCR SuperMix) were defrosted at ambient temperature (25°C).

2-The components volume required to prepare the required number of reactions were calculated as below;

Master mix Syper Green (10 µl),Forward primer (1 µl),Revers primer (1 µl), cDNA (3 µl),Nuclease free water (N.F.W) (5 µl).

3- All of the components were placed in a tube that was quickly spun to tumble down the material and remove unwanted bubbles.

4-The The qRT-PCR program conditions includes several stages;

Stage 1; step (initial denaturation), temperature (94ºC), time (30 minute), and cycles (1 cycle).

- Stage 2; steps and temperature (denaturation (94ºC), annealing (58ºC), and extension (72ºC), time (5 , 15, 20 second) respectively, cycles (40 cycle).
- Stage 3; step (dissociation), temperature (55-95ºC), time (1 minute), and cycles (1 cycle).

3. Statistical Analysis

GSTM1 and GSTT1 levels were first tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test). Parameters that fit both tests (no significant difference) were given as mean ± standard deviation (SD), where student t test used to comparative between two groups and F test used to comparative more than two groups. The parameters that did not fit the normality tests (significant difference) were given as median and range, and significant difference between median was assessed by Mann-Whitney (for comparison between two groups). The other parameters (nominal and ordinal) were given as percentage frequencies, and significant differences between frequencies were assessed by Pearson-Chi-square test or two-tailed Fisher exact probability (p). The statistical package SPSS version 25.0 and Graph pad prism version 6 were employed to carry out these analyses.

RESULTS AND DISCUSSION

1. Distribution of study groups according to age groups

Results of the present study showed there is significant differences ($p<0.05$) among age groups and among study groups, where the 60-69 years age group scored highest percentage in benign (56.7%), malignant (54.0%), compared to control (healthy) (50.0%), while >69 years scored least percentage in these groups (3.3%, 14.0%, and 25.0%) respectively (table 1).

Table 1: Distribution of study groups according to age groups.

Ages Groups			Study groups			Total	P value
			Benign (n=30)	Malignant (n=50)	Healthy (n=20)		
	50-59	n	12	16	5	33	P<0.05*
		%	40.0%	32.0%	25.0%	31.0%	
	60-69	n	17	27	10	54	P<0.05*
		%	56.7%	54.0%	50.0%	47.0%	
	>69	n	1	7	5	13	p>0.05
		%	3.3%	14.0%	25.0%	10.0%	
P value			P<0.001***	P<0.001***	p>0.05	P<0.001***	

The present study showed ahigh occurrence of prostate cancer with age progression, where we found the highest percentage of these diseases appeared in elderly patients due to lifestyle, chronic diseases, organ dysfunction, immune system dysfunction, and genetic disorders. Increasing incidence of prostate cancer has been observed worldwide, particularly in Asia, and Northern and Western Europe (Wong *et al*., 2016). The prevalence of prostate cancer also increases with age, with an estimated rate of 5% in men aged less than 30 years, to 59% in men aged more than 79 years upon autopsy studies (Bell *et al*., 2015). According to the data from the World Health Organization, the mean life expectancy of male increased from 64.1 years in 2000 to 69.1 years in 2015 (WHO, 2017).

Prostate cancer is a disease that is expected to become more prevalent in an aging population. Previous results showed that the aging-adjusted incidence rates of prostate cancer in the Asian region, as well as the developing Western countries, had been increasing, whereas the aging-adjusted incidence rates of prostate cancer in the developed Western countries had already plateaued and even decreased with time (Teoh *et al*., 2019).

Many organs experience a loss of tissue mass and a decline in regenerative capacity during aging. In contrast, the prostate continues to grow in volume. In fact, age is the most important risk factor for prostate cancer. However, age-related factors influence the composition, morphology, and molecular features of prostate epithelial progenitor cells, the cells of origin for prostate cancer (Freeland *et al*., 2021).

Prostate cancer is more likely to develop in older men and in non-Hispanic Black men. About 6 cases in 10 are diagnosed in men who are 65 or older, and it is rare in men under 40. The average age of men at diagnosis is about 66 (Hussein *et al*., 2015).

The previous study showed the age effect on incidence and mortality showed sharp increasing trends from 40 to 79 years, and the period effect showed both of them continuously increased with advancing period, but cohort effect showed substantial decreasing trends from 1917–1921 to 2002–2006 birth cohort (Liu *et al*., 2019).

In 2021 year in Australia , it is showed the age-specific mortality rate for males aged 45-49 years diagnosed with prostate cancer was estimated to be less than one, meaning less than one person per 100,000 people diagnosed in that age group died. Contrastingly, the estimated age-specific mortality rate for males aged over 90 years old diagnosed with prostate cancer is over 314 per 100,000 people (Franco *et al*., 2021).

2. Relationship of *GSTM1 and GSTT1 genes* with study groups

Results of present study showed there is significant differences ($p<0.05$) between gene expression of *GSTM1* and *GSTT1* genes with study groups, where it is found the *GSTM1* and *GSTT1* genes scored highest mean levels in malignant group (8.67±2.17 and 8.80±3.65) and least mean level in healthy (1.00±0.00 and 1.00±0.00) (table 2 and figure 1).

Table 2: comparative gene expression of *GSTM1 and GSTT1 genes* among study groups were calculated by F test.

Genes		N	Mean	SD	Statistics
GSTM1	Benign	30	5.73	3.22	P<0.001*** LSD=1.50
	Malignant	50	8.67	2.17	
	Healthy	20	1.00	0.00	
GSTT1	Benign	30	3.76	1.50	P<0.001*** LSD=1.65
	Malignant	50	8.80	3.65	
	Healthy	20	1.00	0.00	

Glutathione S-transferases (GSTs) involve a superfamily of multifunctional and ubiquitous phase II metabolic enzymes. It catalyzes the conjugation of electrophilic substrates to soluble glutathione to facilitate their cellular excretion. Additionally, *GSTs* are able to detoxify noxious products of the cellular metabolism, such as reactive oxygen and nitrogen species through their glutathione peroxidase activity (Dar *et al*., 2017). *GSTs* constitute the major antioxidant defensive system against oxidative stress by reducing reactive oxygen species, which are generated by many toxic xenobiotics (Huang *et al*., 2015).

Homozygous deletions of the *GSTM1* and *GSTT1* genes are common and result in a complete loss of enzymatic activity. Hence, individuals are at greater risk toward the development of malignancies (Medjani *et al*., 2020).

Recent study showed a reduced GSTM1 gene expression in prostate tissue was observed in PCa patients with some environmental chemical exposures. Intriguingly, physical activity might play a protective role against PCa development, possibly as a result of increasing GSTM1 gene expression in prostate tissue (Gómez-Martín *et al*., 2019).

In the case of PC, a growing body of evidence suggests that GSTs might be involved not only in the development but also in the progression of the disease. However, only a few studies have addressed the role of GST polymorphisms with regard to the survival of PC patients, proposing the important catalytic and regulatory roles as the potential underlying molecular mechanisms involved in PC progression (Santric *et al*., 2021).

Recent studies have been reported regarding the genetic status of *GSTM1* and *GSTT1* polymorphism and prostate cancer development. But the effect of polymorphisms of these two genes on PCa is still unclear because of inconsistent results among different populations (Medjani *et al*. 2020).

Previous study suggested that GSTM1 null allele was a low-penetrant risk factor for PCa among Asians (Wei *et al*., 2012). Another

study showed The GSTM1 null genotype may increase individual susceptibility to prostate cancer. On the other hand, the null-activity genotype of GSTT1 did not appear to contribute to the risk of prostate cancer in Algerian population (Benabdelkrim *et al*., 2018). Previous study showed the *GSTM1* null genotype may increase individual susceptibility to prostate cancer. On the other hand, the null-activity genotype of *GSTT1* did not appear to contribute to the risk of prostate cancer in our population (Benabdelkrim *et al*., 2018).

In previous studies, it has been observed that the lack or decreased expression of key enzyme genes that help reduce cellular oxidative stress leads to an increased risk of PCa (Santric *et al*., 2020). This has been observed in the correlation study between *GST*s gene polymorphism and PCa. GSTP1, for example, can increase the risk of PCa through methylation modification, whereas increased GSTM1 gene expression protects against the occurrence and progression of PCa (Ashtiani *et al*., 2011). This is consistent with our results that *GSTT1* null genotype, *GSTM1* null genotype, and *GSTP1* (AG + GG) genotype are positively correlated with the susceptibility and malignancy of PCa. In addition, previous studies almost only studied *GST*s gene as independent predictors to draw corresponding conclusions. For *GSTT1* null genotype, it is often considered to be related to the occurrence of PCa only when interacting with environmental factors (Pyo *et al*., 2015). Recent study confirm that *GSTT1* null genotypes may play an important role in the development of PCa in patients with Mets. This may also be the problem with the contradictory conclusion that there is a contradiction between the *GSTT1* gene and the occurrence and development of PCa, that is, the effect of Mets is not taken into account (Liu *et al*., 2022).

Recent study showed the role of GSTO and GSTP class in redox regulation, especially glutathionylation/deglutathionylation cycle, might be suggested as a potential mechanism in the process of PCa adaptation to oxidative stress. Our data on the effects of both *GSTO1**A/A and *GSTO2**G/G variant genotypes on the risk of PCa have the potential to improve the susceptibility biomarkers development in the field of urologic oncology. Besides the results on the effect of novel *GSTP1* polymorphism on the overall survival of patients with PCa, it would be beneficial to investigate its potential association with cancer-specific survival in a larger cohort. Moreover, future functional investigation and interconnection of *GSTP1* polymorphisms and HIF-1α regulation could provide better outcomes and therapeutic chances for men with PCa (Santric *et al*., 2021).

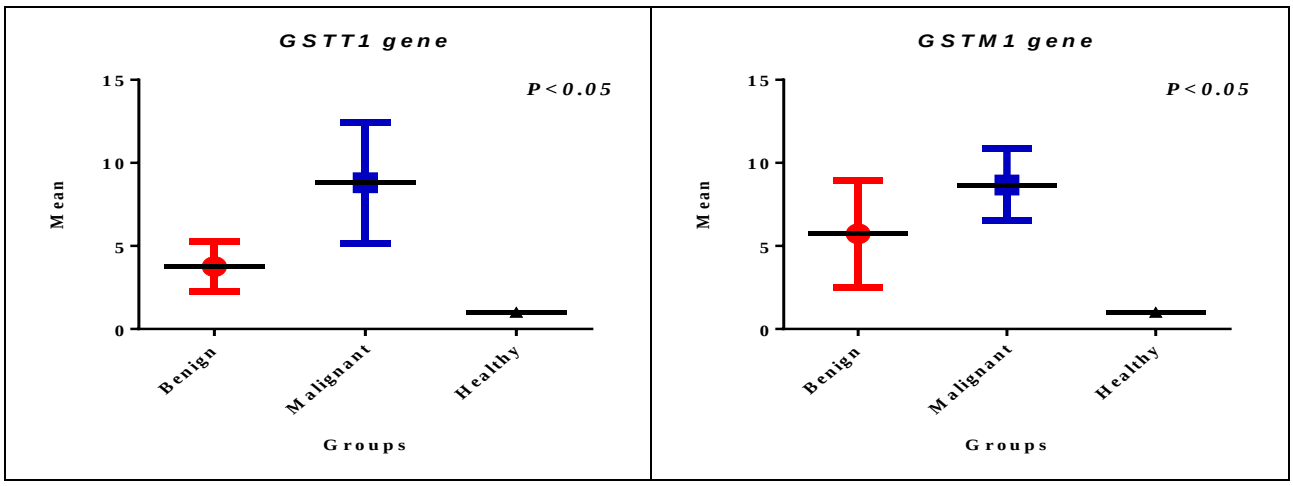

Figure 1: comparative gene expression of *GSTM1 and GSTT1 genes* among study groups.

CONCLUSION

We concluded the *GSTM1* and *GSTT1* genes levels are play major roles in pathogenesis patients with prostate cancer. PCa is high prevalence in elderly population.

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