RESEARCH ARTICLE

Study of promotor -21 A/T polymorphism of catalase CAT (rs7943316) gene in patients with breast cancer

Salah Hashim Shaheed[1, 2], Yasir Haider Al-Mawlah[3, *], Sahar Dakhil Yonis[4], Asaad Abd Alhussain Al-Shouk[4]

[1]College of Applied Medical Sciences, University of Kerbala, Kerbala, Iraq. [2]Department of Medical Laboratory Technique, Al-Safwa University College, Kerbala, Iraq. [3]DNA Research Center, University of Babylon, Babylon, Iraq. [4]Department of Anatomy, College of Medicine, University of Al–Qadisiyah, Al Diwaniyah, Qadisiyyah, Iraq.

Received: February 1, 2024; **accepted:** March 18, 2024.

Oxidative stress is implicated in many forms of cancer, and catalase is one of the most critical enzymes involved in the organic body's defensive mechanism against stress on antioxidation. Catalase shows a vital role in the body's primary defense versus oxidative stress. Several studies have indicated that CAT gene polymorphism plays an essential role in the pathogenesis of cancer. This study aimed to recognize the influence of the CAT (rs7943316) gene polymorphism on breast cancer progress using the collection of blood samples from breast cancer patients. After the extraction of genomic DNA, the single-nucleotide polymorphism (SNP) of rs7943316 gene was analyzed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The results showed that the highest genotype in the control group was AT genotype (63.3%), followed by TT genotype (36.7%) and AA genotype (0%). In the breast cancer (BC) group, AT genotype was the highest one (55.7%), followed by TT genotype (34.3%) and AA genotype (10.0%). Individual carriers of the A/T and T/T genotypes were less expected to develop BC with OR = 0.135, 95% CI = 0.0073-2.4882, *P* = 0.178 and OR = 0.1420, 95% CI = 0.0075-2.70, *P* = 0.1943, respectively. In addition, there were no significant differences in frequencies of the T allele of the CAT gene (rs7943316) between breast cancer patients and control groups with OR = 0.67, 95% CI = 0.4002-1.4459, *P* = 0.4039. The results suggested that there was no correlation between rs7943316 polymorphisms of CAT gene and the development of BC. The genotypes AA, AT, and TT had no potential risk for breast cancer in females.

Keywords: CAT (-21 A/T); rs-7943316 gene promoter; single nucleotide polymorphism; catalase.

[*]**Corresponding author:** Yasir Haider Al-Mawlah, DNA Research Center, University of Babylon, Babylon 51001, Iraq. Email: Yasser.almawla@uobabylon.edu.iq.

Introduction

Breast carcinoma (BC) is the dominant illness in women, representing about 33% of all malignant tumors in females, and is the world's second leading cause of death in the health sector, preceded by lung cancer [1]. Because of the generation of DNA damage and the influence of such superoxide dismutase on intracellular signal pathways, oxidative stress plays an essential role in carcinogenesis processes [2]. Reactive oxygen species (ROS) may play an important role in cancer formation and the maintenance of oncogenic characteristics [3]. To counteract the negative effects of ROS, cells possess an antioxidant defense system comprised of enzymes such as myeloperoxidase (MPO), glutathione peroxidase (GPx), catalase (CAT), and mitochondrial manganese superoxide dismutase (MnSOD) [4, 5]. These enzymes work together to

neutralize or scavenge ROS, preventing cellular damage.

Catalase is a principal antioxidant enzyme that catalyzes hydrogen peroxide into O_2 and H_2O, thus counterbalancing the harmful effects of ROS [6]. The catalase gene, which is pinpointed on chromosome 11p13, has 12 introns and 13 exons [7]. Catalase is the main enzyme that is activated by ROS, specifically H_2O_2 [8]. Previous study showed that catalase gene had several single nucleotide polymorphisms (SNPs) in breast cancer specifically, and the SNPs might occur or be induced by miRNAs [8, 9]. The polymorphism A-21T (rs7943316) (Gene ID: 847) in the CAT gene is significant owing to its proximity to the transcription promoter site. Also, some studies alluded to its proximity to the place where the transcription factor attaches [10, 11]. Catalase is one of the most important enzyme groups in the cell's oxidative stress defense. It is also speculated that the A-21T CAT gene polymorphism diminished the capacity of antioxidant stress activity that was linked to illness [12]. In genetics, promoter typically refers to a specific DNA sequence located upstream of a gene, which plays a crucial role in initiating gene transcription, essentially "turning on" the gene's expression. However, the term "promotor" likely refers to a broader concept, which the catalase gene polymorphism can increase susceptibility to cancer, not necessarily by directly affecting its expression levels [13]. It is important to note that, although there are some potential applications, catalase gene polymorphisms cannot be used routinely in clinical settings before more research has been done [13, 14]. As many studies deemed the CAT to be one of the most important enzymes in intracellular detoxification, multiple studies found that variation in the CAT enzyme was strongly linked to multifactorial features including cancer diseases [8, 12]. This study aimed to assess the relationship between breast cancer susceptibility and catalase gene (rs7943316) variants in Iraqi women by employing a case-control design and genotyping analysis. We investigated this association to elucidate potential genetic risk factors specific to the Iraqi population and to contribute to the understanding of breast cancer development. The results of this study could be valuable for developing targeted preventive strategies for women at higher risk.

Materials and methods

Population of Study.

The study comprised 70 breast cancer patients from Merjan University Hospital (Hillah, Babylon, Iraq) aged from 26 to 80 years old and a control group of 30 healthy females aged from 20 to71 years old. The study period was from September 2021 to January 2022. All procedures of this study were approved by Research Ethics Committee of the University of Babylon (Hillah, Babylon, Iraq) (Approval number: DSM-5324). All participants were informed about the study objectives and procedures, and written informed consent was obtained according to the Declaration of Helsinki.

Collection of blood samples

Four (4) mL venous blood was obtained from each participant using EDTA-containing blood collection tubes. The blood samples were stockpiled in Eppendorf tubes and stored at -20°C for future use [14, 15].

Extraction of genomic DNA and genotyping

Genomic DNA was extracted from blood sample using the Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid Biotech Ltd, New Taipei City, Taiwan) following manufacturer's instructions. The quality of a DNA sample was assessed by the optical density (OD) at 260 and 280 nm wavelengths obtained using NanoPhotometer NP80 (Implen Co., Schatzbogen, München, Germany). All DNA samples were stored at -20°C [16, 17]. The polymerase chain reaction (PCR) primers specifically for CAT gene (rs7943316) were designed and synthesized by Macrogene Co., Gangnam, Seoul, Republic of Korea) with the forward primer sequence of 5'-AAT CAG AAG GCA GTC CTC CC-3 and the reverse primer sequence of 5'-TCG GGG AGC ACA GAG TGT AC-3'. The PCR reaction volume was 20 µL containing 3 µL of

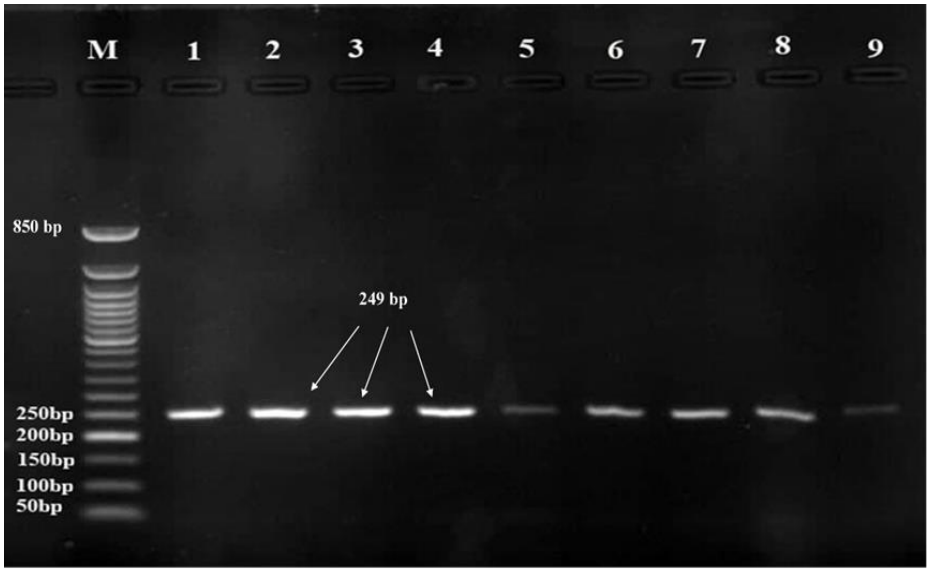

Figure 1. PCR product of the CAT gene (rs7943316) digested with HinfI. M: DNA marker. Lanes 1-7: samples of patients. Lanes 8-9: samples from control group.

DNA template, 1 µL of each primer, 12.5 µL of PCR master mix, and 2.5 µL of DNase-free water. The PCR amplification was carried out using Biometra TOne Series thermal cycler (Analytik, GmbH. Co, Göttingen, Germany) with the program as 94°C for 2 mins followed by 30 cycles of 94°C for 30 s, 60°C for 25 s, 72°C for 30 s, then 72°C for 5 mins.

Restriction fragment length polymorphism (RFLP).

HinfI was used for PCR product restriction digestion. To determine the optimal digestion time, aliquots of PCR products were digested with 2 units of the HinfI enzyme (Promega, Madison, WI, USA) at 37°C for varying durations ranging from 1 to 4 hours. Following digestion, the enzyme was inactivated by incubation at 65°C for 20 minutes. The agarose gel electrophoresis was employed to check the restriction digestion results of PCR products.

Statistical Analysis.

SPSS version 23.0 (IBM, Armonk, New York, USA) was employed for statistical analysis. The dependent samples t-test was applied to compare sample means from two related groups. The whole number of incidences of the tested allele in the population was divided by the whole number of alleles to compute allele frequencies. The odds ratio (OR), 95% confidence intervals, and *P* values of genotype distributions and allele frequencies were calculated using the Hardy-Weinberg equilibrium assumption and a Chi-square test. *P* value less than 0.05 was deemed as significant difference [19].

Results and discussion

Polymorphism of CAT (rs7943316) gene (genotyping)

The results of gene polymorphism showed the existence of a single band (249 bp) of the catalase gene CAT (rs7943316) (Figure 1). PCR amplified CAT (rs7943316) products were digested with HinfI restriction enzyme with the restriction site DNA sequence of 5'-G^ANTC-3'. Depending on the presence or absence of the rs7943316 polymorphism, various digestion patterns were observed. Genotyping was classified into three categories according to presence/absence of polymorphism including TT homozygote with one band of 249 bp, AT heterozygote with three bands of 249, 175, and 74 bp, and AA homozygous with two bands of 175 and 74 bp fragments (Figure 2).

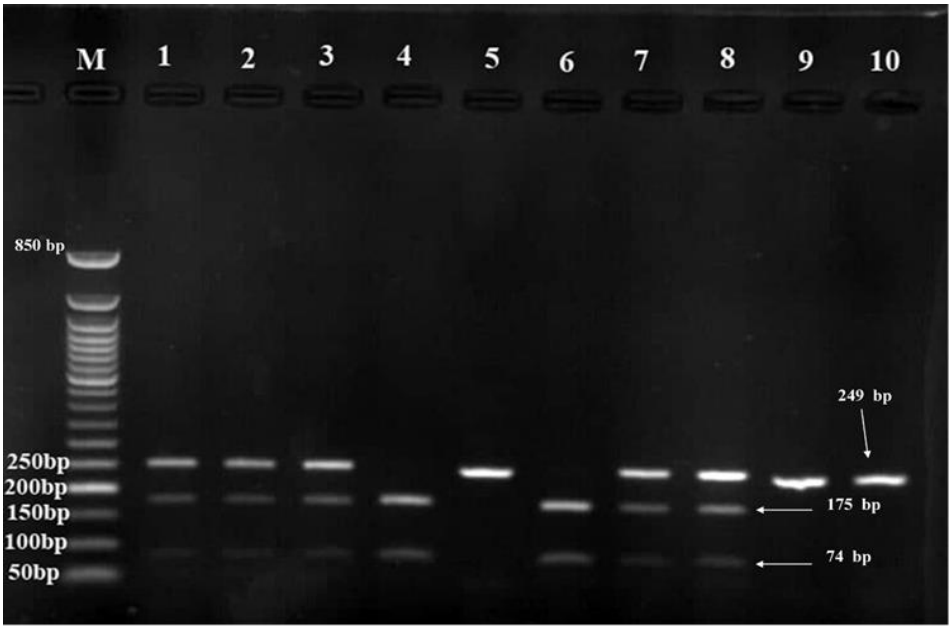

Figure 2. RFLP results of CAT gene (rs7943316) allelotyping. M: DNA marker. Lanes 1-3 and 7-8: AT heterozygous allele (three bands at 249, 175, 74 bp). Lanes 5, 9, and 10: TT homozygous allele (single band at 249 bp). Lanes 4 and 6: AA homozygous allele (two bands at 175 and 74 bp).

Distribution of CAT (rs7943316) polymorphism genotype with allele frequency between patients and control group

The distributions perceived in the Catalase gene (rs7943316) polymorphism in the BC group and control group were listed in Table 1. The higher genotype in the healthy control group was AT heterozygote genotype with 19 out of 30 cases (63.3%) followed by 11 cases of homozygote mutant genotype (TT) (36.7%). There was 0% for homozygote genotype AA in the healthy control group. In the BC group, the highest genotype was AT heterozygote genotype with 39 out of 70 cases (55.7%) followed by 24 cases of the mutant homozygote genotype (TT) (34.3%) and 7 cases of homozygote genotype (AA) (10.0%). Individual carriers of the A/T and T/T genotypes were less expected to develop BC with OR = 0.135, 95% CI = 0.0073-2.4882, *P* = 0.178 and OR = 0.1420, 95% CI = 0.0075-2.70, *P* = 0.1943, respectively. Also, there were insignificant differences between BC patients and healthy groups in frequencies of the T allele of the CAT gene (rs7943316) as OR=0.67, 95% CI=0.4002-1.4459, *P* = 0.4039.

To comprehend the mechanisms underlying the pathologic process of BC and link pathological effects to the clinic applications to improve the protection, diagnosis, and management of BC patients in Iraq, detecting genetic variations affecting BC is a prominent focus of research. According to the results of this research on the catalase gene (rs7943316) polymorphism, individuals with the mutant homozygous genotype (TT) and mutant heterozygous genotype (AT) demonstrated lower rates of BC than those with the wild type (AA). These results vigorously implied that the CAT gene (rs7943316) polymorphism could lower the chance of BC in Iraqi patients. The genes of antioxidant enzymes contain numerous polymorphisms which may affect the efficiency of their detoxification of ROS. Anaerobic metabolism naturally produces ROS [20]. To stop the hazardous buildup of these species, the human body creates a complex collection of antioxidant molecules [21]. All aerobic cells have the antioxidant molecule CAT, and the liver, kidney, and erythrocytes contain the largest concentrations of the enzyme [22]. By converting H_2O_2 into H_2O and O_2, the heme enzyme CAT is critical in avoiding hydrogen

Table 1. Distribution of CAT (rs7943316) genotype and odd ratio among patients and control.

Genotype allele	Patient group (n = 70)		Control group (n = 30)		*P* value	OR	CI (95%)
	No.	%	No	%			
Codominant							
AA[a]	7	10	0	0			
AT	39	56	19	63	0.178	0.135	0.0073 - 2.4882
TT	24	34	11	37	0.1943	0.142	0.0075 - 2.70
Dominant							
AA	7	10	0	0			
AT+TT	63	90	30	100	0.1813	0.1388	0.0077 - 2.5103
Recessive							
AA+AT	46	66	19	63			
TT	24	34	11	37	0.8191	0.9012	0.3695 - 2.1981
Over dominant							
AA+TT	31	44	11	37			
AT	39	56	19	63	0.48	0.7284	0.3022 - 1.7554
Alleles							
A	53	38	19	32			
T	87	62	41	68	0.4039	0.7607	0.4002 - 1.4459

Note: [a] reference.

peroxide accumulation and preserving cells from the damaging effects of oxidative stress [23]. Allelic variations of the catalase gene may influence oxidative DNA damage and disease risk by lowering CAT enzyme activity and increasing susceptibility to ROS, changing detoxification of ROS, and increasing oxidative stress [24]. Many studies reported that the CAT gene came with three genetic polymorphisms in the promoter region including -21T (rs7943316), C-262T (rs1001179), and C-844T (rs769214) [25–27]. Several studies have reported associations between CAT gene polymorphisms and the risk of breast cancer, along with links to other multifactorial diseases [18, 28]. Another study suggested that the main reason for breast cancer was regions that mutated and contained many sites that served as binding sites for transcriptional factors [29]. Additionally, by changing the binding affinity of transcription factors, the polymorphism in the promoter region of catalase gene (rs7943316) might result in changed promoter activity, alterations in gene expression, and reduced catalytic activity of the catalase enzyme [30]. This study is the first time to link the catalase gene (rs7943316) variants to the BC in the Iraqi population. The results of this study showed an insignificant association between CAT -21T genotypes and the risk of breast cancer disease. There were several studies on the relationship between catalase polymorphisms and the risk of various cancers [31]. However, there were very few studies on the correlation between the CAT-rs7943316 polymorphism and cancer risk. Several studies have proved that rs7943316 polymorphisms are unassociated with the risks of papillary thyroid carcinoma [32] or hepatocellular carcinoma [33]. Numerous researchers have suggested that catalase polymorphisms may be linked with the possibility of many cancers such as cervical cancer, prostate cancer, pancreatic cancer, and colorectal cancer [7, 34–36].

There were several limitations to this study. First, the CAT gene contains a variety of other polymorphisms. Further study of the impact of CAT polymorphisms on mRNA levels should be conducted concurrently. Second, it has been noted that certain environmental factors such as

electromagnetic fields and medications were linked to the mRNA levels of some antioxidant genes including catalase, SOD2, and SOD1 [37].

Conclusion

This study did not find a correlation between CAT rs7943316 polymorphisms and the development of BC. The genotypes AA, AT, and TT in the CAT A-21T gene (rs7943316) did not show the potential risk for breast cancer in female patients. The CAT A-21T gene (rs7943316) polymorphism is involved in reducing the risk of BC. Further research with larger sample sizes in other ethnic groups, another SNP of the catalase gene, and functional investigations are needed to confirm the significance of the rs7943316 polymorphisms in the BC pathophysiology.

References

1. Huober J, Schneeweiss A, Hartkopf AD, Müller V, Lux MP, Janni W, *et al*. 2020. Update breast cancer 2020 Part 3–Early breast cancer. Geburtshilfe und Frauenheilkunde. 80(11):1105-1114.
2. Frederiks WM, Bosch KS, Hoeben KA, van Marle J, Langbein S. 2010. Renal cell carcinoma and oxidative stress: The lack of peroxisomes. Acta Histochem. 112(4):364-371.
3. Gargouri B, Lassoued S, Ayadi W, Karray H, Masmoudi H, Mokni, *et al*.2009. Lipid peroxidation and antioxidant system in the tumor and in the blood of patients with nasopharyngeal carcinoma. Biol Trace Elem Res. 132(1-3):27-34.
4. Rajaraman P, Hutchinson A, Rothman N, Black PM, Fine HA, Loeffler JS, *et al*. 2008. Oxidative response gene polymorphisms and risk of adult brain tumors. Neuro Oncol. 10(5):709-715.
5. Wu SH, Lee KW, Chen CH, Lin CC, Tseng YM, Ma H, *et al*.2010. Epistasis of oxidative stress-related enzyme genes on modulating the risks in oral cavity cancer. Clinica Chimica Acta. 411(21-22):1705-1710.
6. Geybels MS, Van Den Brandt PA, Van Schooten FJ, Verhage BAJ. 2015. Oxidative stress-related genetic variants, pro-and antioxidant intake and status, and advanced prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 24(1):178-186.
7. Tefik T, Kucukgergin C, Sanli O, Oktar T, Seckin S, Ozsoy C. 2013. Manganese superoxide dismutase Ile58Thr, catalase C-262T and myeloperoxidase G-463A gene polymorphisms in patients with prostate cancer: Relation to advanced and metastatic disease. BJU Int. 112(4):E406-E414.
8. Borgstahl GE, Parge HE, Hickey MJ, Johnson MJ, Boissinot M, Hallewell RA, *et al*. 1996. Human mitochondrial manganese superoxide dismutase polymorphic variant Ile58Thr reduces activity by destabilizing the tetrameric interface. Biochemistry. 35(14):4287-4297.
9. Karunasinghe N, Han DY, Goudie M, Zhu S, Bishop K, Wang A, *et al*. 2013. Prostate disease risk factors among a New Zealand cohort. J Nutrigenet Nutrigenomics. 5(6):339-351.
10. Ding G, Liu F, Shen B, Feng C, Xu J, Ding Q. 2012. The association between polymorphisms in prooxidant or antioxidant enzymes (Myeloperoxidase, SOD2, and CAT) and genes and prostate cancer risk in the Chinese population of Han nationality. Clin Genitourin Cancer. 10(4):251-255.
11. Panduru NM, Moţa E, Moţa M, Cimponeriu D, Serafinceanu C, Cheţa DM. 2010. Polymorphism of catalase gene promoter in Romanian patients with diabetic kidney disease and type 1 diabetes. Rom J Intern Med. 48(1):81-88.
12. Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P. 2011. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol Vis. 17:2325.
13. Wang CD, Sun Y, Chen N, Huang L, Huang JW, Zhu M, *et al*. 2016. The role of catalase C262T gene polymorphism in the susceptibility and survival of cancers. Sci Rep. 6(1):26973.
14. Majeed SR, Omara AM, Al-Koofee DA. 2021. Association of interferon-induced helicase (IFIH1) gene polymorphism rs1990760 with type two diabetes mellitus in Iraqi population. Meta Gene. 30:100952.
15. Mawlah Y, Naji M, Imari M, Abdulabbas H. 2022. Micro-RNA evaluation, specification, and stabilization study in mixed/non-mixed body fluids as a specific molecular marker. Journal of Advanced Biotechnology and Experimental Therapeutics. 5(2):347.
16. Al-Kashwan TA, Algenabi AHA, Omara AM, Kaftan AN. 2021. Association of vitamin D receptor gene polymorphisms BsmI (rs 1544410) and TaqI rs (731236) with the type 2 diabetes mellitus in Iraqi patients from the middle Euphrates region. Meta Gene. 28:100854.
17. Kaftan AN, Hussain MK, Algenabi AHA, Omara AM, Al-Kashwan TA. 2021. Association of sunshine vitamin receptor gene polymorphisms (rs 2228570) and (rs7975232) with the type 2 diabetes mellitus in Iraqi patients from the middle Euphrates region. Gene Rep. 22:100977.
18. Al-Mawlah YH, Alasadi YF, Al-Darraji MN. 2021. Association between genetic polymorphisms of (Cu/ZnSOD and CAT C262T) and the risk of breast cancer. Gene Rep. 25:101401.
19. Stanković A, Kolaković A, Živković M, Djurić T, Bundalo M, Končar I, *et al*. 2016. Angiotensin receptor type 1 polymorphism A1166C is associated with altered AT1R and miR-155 expression in carotid plaque tissue and development of hypoechoic carotid plaques. Atherosclerosis. 248:132-139.
20. Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, *et al*. 2010. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chem Biol Interact. 188(2):334-339.
21. Finkel T. 2003. Oxidant signals and oxidative stress. Curr Opin Cell Biol. 15(2):247-254.
22. Crawford A, Fassett RG, Geraghty DP, Kunde DA, Ball MJ, Robertson IK, *et al*. 2012. Relationships between single

nucleotide polymorphisms of antioxidant enzymes and disease. Gene. 501(2):89-103.
23. Bauer G. 2012. Tumor cell-protective catalase as a novel target for rational therapeutic approaches based on specific intercellular ROS signaling. Anticancer Res. 32(7):2599-2624.
24. Forsberg L, De Faire U, Morgenstern R. 2001. Oxidative stress, human genetic variation, and disease. Arch Biochem Biophys. 389(1):84-93.
25. Glynn SA, Boersma BJ, Howe TM, Edvardsen H, Geisler SB, Goodman JE, *et al*. 2009. A mitochondrial target sequence polymorphism in manganese superoxide dismutase predicts inferior survival in breast cancer patients treated with cyclophosphamide. Clin Cancer Res. 15(12):4165.
26. al Balawi IA, Mir R, Abu-Duhier FM. 2018. Potential impact of vascular endothelial growth factor gene variation (-2578C > A) on breast cancer susceptibility in Saudi Arabia: A case-control study. Asian Pac J Cancer Prev. 19(4):1135.
27. Polonikov AV, Ivanov VP, Solodilova MA, Kozhuhov MA, Panfilov VI. 2009. Tobacco smoking, fruit and vegetable intake modify association between -21a> t polymorphism of catalase gene and risk of bronchial asthma. J Asthma. 46(3):217-224.
28. Al-Mawlah YH, Al-Darraji MN, Al-Imari MJ. 2022. Study of small non-coding RNA (miRNA) expression pattern of fertile/infertile male semen. Acta Informatica Medica. 30(3):205-212.
29. Kodydková J, Vávrová L, Kocík M, Žák A. 2014. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. Folia Biologica (Czech Republic). 60(4):153.
30. Nawab SN, Zehra S, Fawwad A, Azhar A. 2017. A study on catalase gene promoter polymorphism-21 A/T (rs7943316) in healthy Pakistani population. Pak J Med Sci. 33(6):1521.
31. Jamhiri I, Saadat I, Omidvari S. 2017. Genetic polymorphisms of superoxide dismutase-1A251G and catalase C-262T with the risk of colorectal cancer. Mol Biol Res Commun. 6(2):85.
32. Salimi S, Harati-Sadegh M, Eskandari M, Heidari Z. 2020. The effects of the genetic polymorphisms of antioxidant enzymes on susceptibility to papillary thyroid carcinoma. IUBMB Life. 72(5):1045-1053.
33. Liu Y, Xie L, Zhao J, Huang X, Song L, Luo J, *et al*. 2015. Association between catalase gene polymorphisms and risk of chronic hepatitis B, hepatitis B virus-related liver cirrhosis and hepatocellular carcinoma in Guangxi population. Medicine (Baltimore). 94(13):e702.
34. Castaldo SA, da Silva AP, Matos A, Inácio Â, Bicho M, Medeiros R, *et al*. 2015. The role of CYBA (p22phox) and catalase genetic polymorphisms and their possible epistatic interaction in cervical cancer. Tumor Biol. 36(2):909-914.
35. Jansen RJ, Robinson DP, Stolzenberg-Solomon RZ, Bamlet WR, Tan X, Cunningham JM, *et al*. 2013. Polymorphisms in metabolism/antioxidant genes may mediate the effect of dietary intake on pancreatic cancer risk. Pancreas. 42(7):1043.
36. Chang D, Hu ZL, Zhang L, Zhao YS, Meng QH, Guan QB, *et al*. 2012. Association of catalase genotype with oxidative stress in the predication of colorectal cancer: Modification by epidemiological factors. Biomed Environ Sci. 25(2):156-162.
37. Su S, He K, Li J, Wu J, Zhang M, Feng C, *et al*. 2015. Genetic polymorphisms in antioxidant enzyme genes and susceptibility to hepatocellular carcinoma in Chinese population: a case-control study. Tumor Biol. 36(6):4627-4632.