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Role of Glutathione S-transferase Polymorphism in Susceptibility to Breast Cancer in Sudanese Women

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Background: *Glutathione S-transferase (GST) encoded by the polymorphic GSTM1 and GSTT1 gene is one of the major enzymes in human breast tissue that have been associated with several drug-induced toxicities and cancer in various tissue. The study was aimed to evaluate the association between GSTM1 and GSTT1 variants and breast cancer (BC) risk in Sudanese women.*

Subjects and Methods*: 118 patients and 100 controls were recruited in this study. 5 ml of the blood sample were collected from each participant. DNA was extracted and GSTM1 and GSTT1 gene polymorphisms were genotyped by Polymerase Chain Reaction (PCR).*

Results: *Significant differences for the risk factors between cases and controls were observed, such as menopausal status (P= 0.001), age at menarche (P= 0,036), marital status (P= 0.013), and parity (P= 0.023). GSTT1 null genotype had an increased risk factor of (BC) <0.0001, OR 7.24, and 95%CI (2.9067-18.0688). GSTM1 genotypes were not significantly associated with (BC) (P = 0.251). Also, the combination of GSTT1 null and GSTM1 present via GSTT1 present and GSTM1present genotypes had an increased susceptibility to (BC) 14.67 times (OR= 14.67, 95%CI= 5.22-41.19, p> 0.0001).*

Conclusion and Recommendation: *In conclusion, the GSTT1 gene was associated significantly with (BC); thus the use of the GSTT1 gene may be useful for screening of the earlier investigation for (BC) should be recommended.*

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Introduction

Breast cancer (BC) institutes as a core of public health problem with a cosmopolitan concern. Annually around a million new cases are diagnosed. Thereinafter, consequentially about 4.4 million women are still alive with the ailment and practically 400,000 subjects were passing away in the year 2002. [1]

(BC) is defined as the reciprocal site-specific malignancy that affecting women of different ages, and influenced by multicultural, multiethnic, and other diverse genetic- environmental components.

Therefore, the utmost collective reasons cause abundant mortality incidences in women diagnosed with breast tumors as diverse and international hygienic measures. [1-5]

Nevertheless, (BC) always remains as the first or the second most common malignancy of females' type invades in African societies. The incidences and mortality rates are predicted to be increased rapidly. [6-9] Most African (BC) patients die at a young age through diminutive treatment and inadequacy of medical supply and lack of good therapeutic supervision as it was validated by several investigators. [10, 11]

In Sudan, (BC) is a multifaceted disease and his etiology remains indistinguishable and the peril factors are elucidating merely a minor proportion of cases.[12-14]

Recently, many studies were performed to investigate the hazard factors for the occurrence of (BC) such as age, exogenous hormones, lifestyle, ionizing radiation environmental factors, genetics and reproductive events (pregnancy, breastfeeding, menarche, and menopause). [14]

Glutathione S-transferases (GSTs) are usually contributed to the metabolism of environmental factors such as carcinogens, reactive oxygen species, and chemotherapeutic agents. [15-17]

They are generated catalyzing reactions against electrophilic bonds through glutathione. [15-17] The GSTs are made up of four major groups: GST, GST, GSTμ, and GST. Moreover, GST-M1 and GST-T1 are isolated isoforms of glutathione transferase enzymes which are contributed to the metabolism of the most chemicals compound and may act as carcinogens. [18]

GSTM1 and GSTT1 are allocated or sited on chromosome q11and1p13correspondingly.[19]The obliteration of these genes is allied with a lack of enzyme activity and amplified the vulnerability to cancer.[20] A recent study indicated that the GSTT1 erasure was connected with augmented recurrence risk, while GSTM1 interconnected with nastiest prognosis limitations at diagnosis. [21] Previous studies reported that both GSTM1- and GSTT1-null polymorphisms are associated with an increased risk of (BC) in the Asiansan and Portuguese populations. [22, 23] Recently, in the Sudanese population many studies have reported that, the prevalence of GSTM1 and GSTT1 deletion homozygosity were reached 54.7% and 42.1% respectively. Furthermore, these studies have ended that 24.6% of Sudanese women lack both copies of the studied genes. [24, 25]

The existing study is aimed to investigate the presence or deletion of one or both of *GSTM1* and *GSTT1* gene photocopies and their risk factors associated with (BC) among Sudanese women.

Subjects and methods

Subjects:

A total of 118 blood samples were collected in EDTA-containing tubes from (BC) diagnosed females who were participating in this study. Hundred contest- aged females were used as a control group. Each patient filled a standardized questionnaire at the time of blood donation. The questionnaire containing data on family history of (BC), weight, height, reproductive histories age, menstrual, age, and others (first degree relatives; only mother, sister or daughters, and smoking status). A written consent was obtained from each participant.

Methods:

Genotyping: Genomic DNA was isolated from blood samples using the QIAGEN DNA extraction kit. The PCR was performed in 50 μl volume. The Sequences of the GSTT1 forward and reverse primers were

5`-**TTCCTTACTggT CCTCACATCTC-3`** and 5`-**T C A C C g g A T C A TggCCAgCA-3`**. Whereas, the sequences for the GSTM1 forward and reverse primers were;

5`-gAACTCCCTgAAAAgCTAAAgC-3` and **5`-g TT ggg CT CA AA TA T A CggTgg-3`** .`[26]

Undoubtedly the null genotype was due to the absence of GSTT1 and GSTM alleles rather than a fiasco in the PCR analysis. Moreover, the co-amplification of human β-globin was prepared by using

5`-CAACTTCATCCACgTTCACC-3`and 5`-g AA g A g C C A A g g A CAggT AC-3

Primers used as an internal control. The PCR reaction was performed and completed in an applied biosystem 9700 thermocyclers containing: 50-100 ng of genomic DNA; 200 nM of dNTPs; 100 pmol of each, GSTT1, GSTM1, and bglobin primer; 1.5 units of Taq polymerase; 50 mM KCl 10 mM Tris/HCI (pH 9.2) and 2 mM MgCl2. Cycling circumstances was an initial 4 min at 94°C for denaturation, shadowed by 34 cycles of denaturation 94°C for 1 minute 61°C for 45 s and 72°C for 90 s. produces A 268 base pair band was amplified with β-globin primers indicating that the PCR reaction is reliable. The presence of a 215 bp and 480bp bands were indicated that GSTT1, and GSTM1 heterozygote form or in an image of homozygote correspondingly. While, their absence along with the presence of the internal control band indicated the Null GSTT1 together with GSTM1 consecutively.

Statistical analysis:

The data was analyzed by using SSPS version 20 IBM. Descriptive measures such as the chi-square ($X2^{)}$ test. This test was used to evaluate the associations between different genotypes of GSTM1and GSTT1 micro- variant isolates. In the current existing study, the outcomes of the comparison apprehended between the breast carcinoma versus control subjects, were considered to stand significant if the $P \leq 0.05$. Odds ratios (ORs) and CI 95% band (β-globin gene length is 268 bp) figure 1.

Results:

Potential risk factors for (BC) are listed in (Table: 1). Menopausal status and parity were showed significant differences between cases and controls (P= 0.001 and 0.023 respectively). A higher frequency of (BC) patients was reported among women with age at menarche of less than 13years p= 0.036 (Table: 1).

Genotype of GSTT1 and GSTM1 were assessed by multiplex PCR in 118 patients and 100 healthy controls. The presence of genotypes of GSTT1 and GSTM1 was indicated by a band of 480 bp and 215 bp respectively. The null genotype was indicated by the presence of the internal control band (β-globin gene length is 268 bp) in (Fig: 1.)

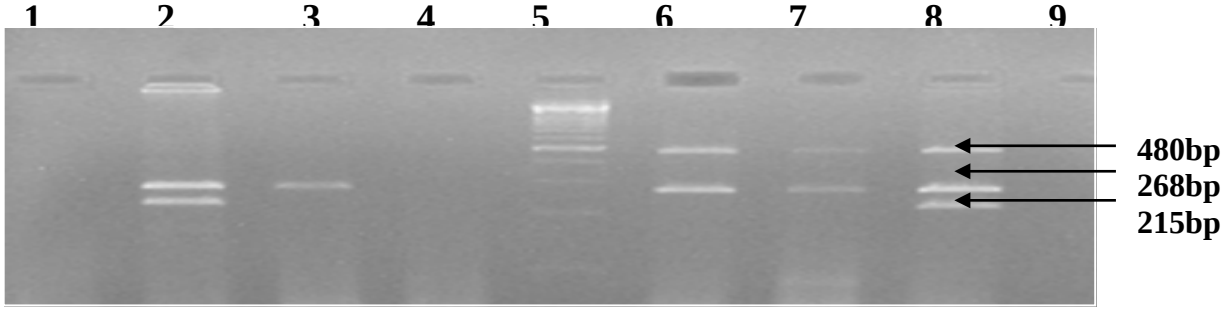

Figure 1: Detection of GSTT1&GSTM polymorphism using PCR method. PCR-Lane1: Control negative PCR, lane2: Control ß globin +GSTM1, lane3: ß globin: lane5: 100-bp DNA marker, lane6&7: Control ß globin+GSTT1, lane8: Control ß globin+GSTM1+GSTT1

In the contemporary study, the frequency of the GSTT1 null genotype was 51.7% in cases and 16% in controls. GSTT1 null genotype was associated with an increase in BC risk (P <0.0001) (Table: 2). While the frequency of the GSTM1 null genotype was 56.8% in cases and

49% in control subjects (Table: 2). GSTM1 null genotypes disclosed no significant difference between (BC) patients and controls (P = 0.251) as presented in (Table: 2).

The combination of the GSTT1& GSTM1 present and GSTT1& GSTM1 null genotypes were revealed significant differences between cases and control (p <0.0001), OR 7.24, and 95%CI (2.9067-18.0688).The GSTT1& GSTM1 presence via a combination of GSTT1 null and GSTM1 presence genotypes were found to be significantly different among cases and controls (p<0.0001), OR 14.6667, and 95% CI (5.2218-41.1948). Additionally, cases harboring GSTT1 null and GSTM1 were being as seven times as the risk of control groups (Table: 3).

Logistic regression was used to weight and clarifies the most explanatory variable that contributing significantly to the susceptibility of (BC). Cases and control were used as dependent variables while GSTT1 and GSTM1 genotypes, parity, and age at menarche as explanatory variables. The result explained that the GSTTI genotype was the only descried as a significant factor contributed to (BC) susceptibility, while others were excluded from the model (Table:4).

Table 1 Characteristics (Risk factor) of breast cancer patients (N=118) and co

Factors/ *P* values	**Cases**	**Control**
Main age ± SD	44.5 ±11.9	40.2 ± 8.6
Age group ***P* = 0.05**		
25-35	25	28
36-45	36	47
46-55	29	22
≥56	11	3
Age at menarche ***P* = 0.01**		
<13yrs	38	17
≥13yrs	80	83
BMI ***P* = 0.04**		
Underweight	10	2
Normal	39	31
Overweight	42	36
Obese	27	31
Family history of cancer ***P* = 0.43**		
Yes	22	15
No	39	42
No response	57	43
Menopause status ***P* = 0.001**		
Postmenopausal women	53	23
Pretmenopausal women	65	77
Age at first full term pregnancy ***P* = 0.48**		
No pregnancy	54	42
<29	37	39
≥29	27	19

Table 2: GSTT1 and GSTM1 genotypes frequencies and breast cancer risk

Genotypes	Cases	Controls	*P*
GSTM1 Present	51(43.2%)	51(51%)	**0.251**
GSTM1 Null	67(56.8%)	49(49%)	
GSTT1 Present	57(48.3%)	84(84%)	**<0.0001**
GSTT1 Null	61(51.7%)	16(16%)	

Table 3: combination of GSTT1 and GSTM1 genotypes and breast cancer risk

Combination	Cases	Controls	*P value*	OR	95% CI
GSTT1 + (+) GSTM +	17	44	**<0.0001**	**7.24**	**2.9067-18.0868**
GSTT1 - (+) GSTM -	28	10			
GSTT1 + (+) GSTM +	17	44	**0.0109**	2.5235	1.2374-5.5463
GSTT1 + (+) GSTM -	39	40			
GSTT1 + (+) GSTM +	17	44	**<0.0001**	**14.6667**	**5.2218-41.1948**
GSTT1 - (+) GSTM +	34	6			
GSTT1 + (+) GSTM -	39	10	**0.0145**	2.73	1.1714-6.3624
GSTT1 - (+) GSTM +	28	10			
GSTT1 - (+) GSTM +	34	6	0.22	2.024	0.6544-6.2587
GSTT1 - (+) GSTM -	28	10			

Table 4: GSTT1 the main factor causes susceptibility to breast cancer

Covariate included in the model	***P*** value	OR	95%CI
GSTT1 present	>0.0001	0.137	0.062 - 0.306

Variables removed from the logistic regression equation are GSTM genotype, Parity, Age at menarche, and Menopause P values are 0.923, 0.301.0.137, and 0.332 respectively.

Discussion

The current study was indicated a higher incidence rate of (BC) among the ages ranging from (36 - 45 years) of age class. This finding designated an increase in infection degrees and prevalence rates of (BC) among young and premenopausal women. Moreover, certain risk factors were associated significantly with (BC), and they incorporated with the tribal stocks that exhibited a significant difference between cases and controls (P<0.001).

In menopausal women a significant difference was recorded among cases and controls, with the highest frequency registered in postmenopausal women among cases (P =0.001). This finding shared the same conformity with some previous studies. [27-30]

The parity showed a significant difference between (BC) patients and control subjects. A higher frequency of (BC) ailment was registered among multiparous women (P=0.023). This finding is in the same consistency with the aforementioned studies publicized by voluminous researchers.[27-30]

Remarkably, the existent study bared a significant difference between cases and their controls. A higher frequency of (BC) was verified in age being less than 13years (P = 0.036) at menarche. A previous study was conducted to study the menarche age among African Americans and white skin nations, The study authenticated the difference of around 6 months in ages for African Americans when compares for those of white pedigrees.[6-9, 14]

This finding is potentially has a great impact on (BC) risk perils. Besides; this outcome may refer to the combination of early age at menarche with postmenopausal. Not only has the standing result attributed to explain higher rates of (BC) among women but also, maybe due to long periods of exposure to the sexual hormones.

Interestingly, to our knowledge, this is the first genetic study completed at the molecular basis with the associations of GSTs with (BC) in the Sudanese population and being the baseline data for further studies regarding (BC) and their therapeutic complications.

Copious studies regarding the factors that have had an important role in the disease susceptibility are reported with the association of genes encoding phase II enzymes such as GSTT1 and GSTM1. [21, 31, 32] In the current study, we investigate the role of GSTT1 and GSTM1in susceptibility to (BC) in patients and healthy controls. The prevalence of GSTT1 null homozygote was higher in (BC) patients than control (51.7% to 16% respectively). The distribution of GSTM1 null homozygote was higher in Sudanese women, but no significant differences existing between (BC) patients and controls (56.8% to 49% respectively).

The frequencies of homozygote null genotypes of GSTT1, and GSTM1 in the present study were initiated normally in GSTM1, while there was a frequent GSTT1 null genotype among cases when compared with controls. Our controls GSTT1 null genotype was in agreement with the frequencies presented and reported in the

literature for the Caucasians population.[33-35] However, in (BC) patients the GSTT1 null genotype was comparable to Asians and higher than Arabs and African populations.[36] This finding indicated that GSTT1 null genotype has a significant effect in increasing the (BC) risk in Sudanese women. This outcome shared the same line with the result of both meta-analysis studies.[37, 38] This verdict might be biologically explained by the fact that a decrease in GST enzyme activity could resultant in an inefficient detoxification of carcinogens which could lead to genetic damage and increased (BC) risk.

In conclusion the contemporaneous study abstracted that the Polymorphism of GSTT1 null was significantly contributed to the susceptibility of the (BC) in Sudanese women, while GSTM1 null genotype is not subsidized or promoted the hypothesis. Further studies are critically needed to refute or authenticate this assumption.

References

1. Van Ourti, T., et al., *Effect of screening mammography on breast cancer mortality: Quasi-experimental evidence from rollout of the Dutch population-based program with 17-year follow-up of a cohort.* International journal of cancer, 2020. **146**(8): p. 2201-2208.
Available from:https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.32584.
2. Bai, L., et al., *Exposure to ambient air pollution and the incidence of lung cancer and breast cancer in the Ontario Population Health and Environment Cohort.* International journal of cancer, 2020. **146**(9): p. 2450-2459.
Available from:https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.32575.
3. Shin, D.W., et al., *Breast cancer screening disparities between women with and without disabilities: A national database study in South Korea.* Cancer, 2020,
Available from:https://www.sciencedirect.com/science/article/abs/pii/S0002961020300453.
7. Joko-Fru, W.Y., et al., *Breast cancer survival in sub-Saharan Africa by age, stage at diagnosis and human development index: A population-based registry study.* International journal of cancer, 2020. **146**(5): p. 1208-1218.
Available from:https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/cncr.32693.
4. Jenkins, C., et al., *Experiences of accessing and using breast cancer services in Vietnam: a descriptive qualitative study.* BMJ open, 2020. **10**(3): p. e035173.
Available from:https://bmjopen.bmj.com/content/10/3/e035173.abstract.
5. Nisker, J., *Arrogance of 'but all you need is a good index finger': A narrative ethics exploration of lack of universal funding of PSA screening in Canada.* Journal of Medical Ethics, 2020. **46**(4): p. 249-252.
Available from:https://jme.bmj.com/content/46/4/249.abstract.
6. Bayard, S., et al., *Brief report: Global health initiatives and breast oncology capacity-building in Africa.* The American Journal of Surgery, 2020
Available from:https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.32406.
8. Rebbeck, T.R., *Cancer in sub-Saharan Africa.* Science, 2020. **367**(6473): p. 27-28.
Available from:https://science.sciencemag.org/content/367/6473/27.summary.

9. Kizub, D.A., et al., *Patient Advocacy Approaches to Improving Care for Breast and Cervical Cancer in East and Southern Africa.* JCO Global Oncology, 2020. **6**: p. 49-55.
Available from:https://ascopubs.org/doi/full/10.1200/JGO.19.00219.
10. Hirschfeld, M., et al., *Urinary Exosomal MicroRNAs as Potential Non-invasive Biomarkers in Breast Cancer Detection.* Molecular Diagnosis & Therapy, 2020, https://link.springer.com/article/10.1007/s40291-020-00453-y: p. 1-18.
Available from:https://link.springer.com/article/10.1007/s40291-020-00453-y.
11. Ritter, A., et al., *Circulating noncoding RNAbiomarker potential in neoadjuvant chemotherapy of triple negative breast cancer?* International Journal of Oncology, 2020. **56**(1): p. 47-68.
Available from:https://www.spandidos-publications.com/10.3892/ijo.2019.4920.
12. Saeed, I.E., et al., *Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010.* Cancer medicine, 2014. **3**(4): p. 1075-1084.
Available from:https://onlinelibrary.wiley.com/doi/full/10.1002/cam4.254.
13. Idris, H.M., et al., *Genetic polymorphism of GSTP1, GSTM1 and GSTT1 genes and susceptibility to chronic Myeloid leukaemia.* Asian Pacific Journal of Cancer Prevention, 2020. **21**(2): p. 499-503.
Available from:http://journal.waocp.org/article_88949.html.
14. Sutherland, E., *Subjective psychosocial experiences of South African breast cancer patients receiving diagnosis and treatment*. 2018, Stellenbosch: Stellenbosch University,
Available from: http://scholar.sun.ac.za/handle/10019.1/103326Retrieved
15. Uno, Y., et al.,*Systematic characterization of glutathione S-transferases in common marmosets.* Biochemical Pharmacology, 2020. **174**: p. 113835.
Available from:https://www.sciencedirect.com/science/article/abs/pii/S0006295220300459.
16. Kirtonia, A., G. Sethi, and M. Garg, *The multifaceted role of reactive oxygen species in tumorigenesis.* Cellular and molecular life sciences: CMLS, 2020,
Available from:https://link.springer.com/article/10.1007%2Fs00018-020-03536-5.
17. Mijatović, S., et al., *The Double-Faced Role of Nitric Oxide and Reactive Oxygen Species in Solid Tumors.* Antioxidants, 2020. **9**(5): p. 374.Available from:https://www.mdpi.com/2076-3921/9/5/374.
18. Chen, X., et al., *Recent advances in heterocyclic aromatic amines: An update on food safety and hazardous control from food processing to dietary intake.* Comprehensive Reviews in Food Science and Food Safety, 2020. **19**(1): p. 124-148.
Available from:https://onlinelibrary.wiley.com/doi/full/10.1111/1541-4337.12511.
19. Haiman, C.A., et al., *Benzene uptake and glutathione S-transferase T1 status as determinants of S-phenylmercapturic acid in cigarette smokers in the Multiethnic Cohort.* PLoS One, 2016. **11**(3).
Available from:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4784986/.
20. Goldberg, G.S. and R. Airley, *Cancer Chemotherapy: Basic Science to the Clinic*. John Wiley & Sons
Available from:https://books.google.com.sa/books?h

l=en&lr=&id=fADMDwAAQBAJ&oi=fnd&pg=PR11&dq=2020:

21. Campos, C.Z., et al., *Glutathione S-transferases deletions may act as prognosis and therapeutic markers in breast cancer*. Clinical and experimental medicine, 2018. **18**(1): p. 27-35.
Available from:https://link.springer.com/article/10.1007/s10238-017-0461-6.
22. Song, Z., et al., *Association of glutathione S-transferase T1, M1, and P1 polymorphisms in the breast cancer risk: a meta-analysis.* Therapeutics and clinical risk management, 2016. **12**: p. 763.
Available from:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4869650/.
23. Rauch, A., *Portuguese Society of Human Genetics.* Medicine, 2019. **98**(26): p. e15772.
Available from:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6620740/.
24. Malik, S.S., et al., *Genetic polymorphism of GSTM1 and GSTT1 and risk of prostatic carcinoma-a meta-analysis of 7,281 prostate cancer cases and 9,082 healthy controls.* Asian Pacific Journal of Cancer Prevention, 2016. **17**(5): p. 2629-2635.
Available from:http://journal.waocp.org/article_32450.html.
25. Mansoori, A.A. and S.K. Jain, *ADH1B, ALDH2, GSTM1 and GSTT1 gene polymorphic frequencies among alcoholics and controls in the Arcadian population of Central India.* Asian Pacific journal of cancer prevention: APJCP, 2018. **19**(3): p. 725.
Available from:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5980848/.
26. Pemble, S., et al., *Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism.* Biochemical Journal, 1994. **300**(1): p. 271-276.
Available from:https://doi.org/10.1042/bj3000271.
27. Majeed, A.I., et al., *Screening, diagnosis and genetic study of breast cancer patients in Pakistan.* Pakistan Journal of Medical Sciences, 2020. **36**(2).
Available from:http://www.pjms.org.pk/index.php/pjms/article/view/1059.
28. Akram, M., et al., *Awareness and current knowledge of breast cancer.* Biological research, 2017. **50**(1): p. 33.
Available from:https://link.springer.com/article/10.1186/s40659-017-0140-9.
29. Awadelkarim, K., et al., *Pathological, clinical and prognostic characteristics of breast cancer in Central Sudan versus Northern Italy: implications for breast cancer in Africa.* Histopathology, 2008. **52**(4): p. 445-456.
Available from:https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2559.2008.02966.x.
30. Awadelkarim, K.D., et al., *BRCA1 and BRCA2 status in a Central Sudanese series of breast cancer patients: interactions with genetic, ethnic and reproductive factors.* Breast cancer research and treatment, 2007. **102**(2): p. 189-199.
Available from:https://link.springer.com/article/10.1007/s10549-006-9303-z.
31. Kimi, L., et al., *Relevance of GSTM1, GSTT1 and GSTP1 gene polymorphism to breast cancer susceptibility in Mizoram population, Northeast India.* Biochemical genetics, 2016. **54**(1): p. 41-49
.Available from:https://link.springer.com/article/10.1007/s10528-015-9698-5.

32. Kong, X., Z. Li, and X. Li, *GSTP1, GSTM1, and GSTT1 polymorphisms as predictors of response to chemotherapy in patients with breast cancer: a meta-analysis.* Cancer chemotherapy and pharmacology, 2016. **78**(6): p. 1163-1173. Available from:https://link.springer.com/article/10.1007/s00280-016-3173-9.

33. Moini, M., et al., *Association study of Glutathione S-transferases Gene Polymorphisms (GSTM1 and GSTT1) with ulcerative colitis and Crohn's disease in the south of Iran.* Advanced biomedical research, 2017. **6** .Available from:https://www.karger.com/Article/Abstract/475978.

34. Qian, J., et al., *Glutathione S-transferase T1 null genotype is associated with susceptibility to inflammatory bowel disease.* Cellular Physiology and Biochemistry, 2017. **41**(6): p. 2545-2552. Available from:https://www.karger.com/Article/Abstract/475978.

35. Jourenkova, N., et al., *Effects of glutathione S-transferases GSTM1 and GSTT1 genotypes on lung cancer risk in smokers.* Pharmacogeneticsand Genomics, 1997. **7**(6): p. 515-518. Available from:https://journals.lww.com/jpharmacogenetics/Citation/1997/12000.

36. Strange, R. and A. Fryer, *The glutathione S-transferases: influence of polymorphism on cancer susceptibility.* IARC scientific publications,1999, https://europepmc.org/article/med/10493261(148): p. 231-249. Available from:https://europepmc.org/article/med/10493261.

37. Sergentanis, T.N. and K.P. Economopoulos, *GSTT1 and GSTP1 polymorphisms and breast cancer risk: a meta-analysis.* Breast cancer research and treatment, 2010. **121**(1): p. 195-202. Available from:https://link.springer.com/article/10.1007/s10549-009-0520-0.

38. Chen, X.-X., et al., *Glutathione S-transferase T1 polymorphism is associated with breast cancer susceptibility.* Cytokine, 2011. **56**(2): p. 477-480. Available from. https://www.sciencedirect.com/science/article/abs/pii/S1043466611001992