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Analysis of GSTA1 Genetic Variation in Healthy Women Versus Breast Cancer Patients in a Local Cohort

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Abstract
GSTs are critical in the detoxification of drugs, carcinogens, and reactive oxygen species, The genetic polymorphisms of GSTs enzymes have an etiological role in breast cancer. The aim of this study was to evaluate relation of glutathione S-transferases in the pathophysiology of breast cancer disease. The genetic investigation was done PCR, Gel electrophoresis and RFLP. The frequencies of GSTA1 genotypes in breast cancer patients were 76 % AA (mutant type) and 24% GG (wild type) while in healthy subjects 55% GG (wild type) and 45% AA (mutant type). Mutant type genetic polymorphism is related to Breast Cancer Patients.

Keywords: GSTA1, Breast Cancer, Genetic variation, Genetic Study, Genetic Polymorphisms

INTRODUCTION

According to WHO 2024, 670,000 deaths globally were caused by breast cancer in 2022. Breast cancer prevail in every country and it is most common cancer in 84% countries. Different metbolic enzymes involve in detoxification of potential harmfull agents that may cause cancer. The mutagenic variation or absence of these enzymes may be the cause of carcinigenesis. These mutations are seen in different populations. Breast cancer is the most widely recognized growth among ladies, after skin malignancy. One out of eight ladies in the United States (about 12%) will have breast cancer disease in her lifetime.New instances of breast cancer are around 100 times more typical in ladies than in men, however men can also get breast cancer growth as well. Male breast cancer is uncommon, however anybody with breast tissue can create breast malignancy (WHO) .The distribution of GSTA1 promoter haplotypes and diplotypes exhibits considerable variation among different human populations. In the African superpopulation, three unique promoter haplotypes were identified. (Vid Malakar et al., 2021) . In human populations, GSTs exhibit diverse and well-established polymorphisms. These polymorphisms produce proteins encoded by distinct alleles, which differ in their ability to metabolize carcinogens and anticancer agents. The interplay between GST polymorphisms and variability in responses to cancer treatments highlights their association with the risk of various cancers. Additionally, these polymorphisms are influenced by ethnicity, with certain patterns being more prevalent in specific ethnic groups, thereby shaping environmental sensitivity. Glutathione S-transferases (GSTs), a group of phase-II metabolic enzymes, play a vital role in detoxifying a wide range of harmful substances, including pesticides, carcinogens, chemotherapeutic agents, and pollutants, by conjugating them with glutathione. (Zielinska.M.S.,et al, 2015). The compounds affectability is diminished by converting into water soluble and then they are efficiently removing from the body. To environment-induced breast cancer, the magnitude to mobilize and detoxify ex``ogenous toxins can also interact with an individual's susceptibility (Saxena, 2012). It has been hypothesized that in some individuals, polymorphisms in genes that is convulated within DNA reformation, growth cause receptors and carcinogen metabolism, raise the danger of cancer. GSTs are dominant phase II detoxifying enzymes and they detoxify an extensive field of electrophilic substances as well as carcinogens, pharmaceuticals, pesticides and herbicides, che- motherapeutics, industrial pollutants and chemicals, compounds of oxidative damage and natural plant toxins and to make their analogous glutathionyl conjugates, they all are conjugated to glu- tathione, which usually develops into slightly reactive and higher water-soluble substances (Adnan, 2012). GSTs having two super families, one is membrane bound microsomal and other is cytosolic GSTs. The GSTs family is divided into seven clases: α (Alpha /A), μ(Mu/M), π(Pi/p) K(kappa/k),and θ(Theta/t) each encoded by specific gene . The alpha class for instance contain five active genes. (GSTA1-GSTA5)and seven pseudo genes on chromosome P12. Among the clases GSTs in the A,P,M groups exibit the highest affinity for binding glutathione with GSTA showing the strongest catalytic efficiency. (Attia *et al*., 2020). In human populations, GSTs polymorphisms are studied. Distinct alleles having proteins that exhibit different skills to metabolize mutagens. Through accelerating the degradation of compounds by its conjugation with glutathione, the glutathione S-transferase (GSTs) enzymes detoxify chemotherapeutic drugs and their metabolites. With heterozygotes displaying intermediate activity, Val homozygotes had a decreased conjugating activity than Ile homozygotes

(Sailaja, 2010). GSTA1 gene, is highly expressed in the liver (hepatocytes), kidney (proximal tubules), adrenal glands, pancreas, testis, and breast tissues, whereas its expression is relatively low in other tissues. Notably, aberrant overexpression of alpha class GSTs has been linked to malignancies such as colorectal and lung cancers, while decreased expression has been reported in stomach and liver tumors.In the liver. GSTA1, in particular, has been shown to interact with JNK, thereby influencing apoptosis development. From a pharmacogenomic perspective, GSTA1 is significant in oncology due to its involvement in the metabolism of key chemotherapeutic agents, including busulfan, thiotepa, doxorubicin, cyclophosphamide, and chlorambucil. Its high expression in the liver further positions it as a primary candidate gene for regulating drug clearance, underscoring its importance in cancer treatment and drug metabolism research.(Vid Malakar., et al 2021).

Materials and Methods

Chemicals: Taq polymerase, DNA Ladder 50bp, DNA Ladder 1kb, Agarose 100g, Tris base, Boric Acid, EDTA, Ethidium Bromide stain and DNA Extraction Kit.

Instruments: PCR, Gel Electropherosis, Eppendrofs 1.5ml, Pipete Sucker, Blue Tips, Yellow Tips, Eppendrofs 100ul .

Study Subjects

The project is designed according to the good clinical practice (GCP), the international conference on harmonized (ICH) tripartite Guideline and the ethical principles laid down in declaration of Helsinki (W.H.O. 2022). Written consent forms signed by all subjects will be collected. A total of 200 hundred local patient subjects will take part in this study. The physical examination and laboratory tests will be cnducted for each subject to check their health status. The subjects who have any other type of abnormalities in their history, physical examination or clinical laboratory investigation will be excluded from the study. All subjects will be above 18 years, non-alcoholics, non-smokers, on normal diet and no one will be on any medication.

Collection of blood samples

Blood was collected after an overnight fasting from both healthy and Patient subjects. All subjects did not have taken any antioxidant supplement from one week. The blank blood sample (10 mL) collected from each volunteer (Healthy and Patients) was used for biochemical parameters, complete blood picture and isolation of DNA. Blood sample was collected in EDTA containing and non EDTA containing centrifuge tubes for DNA extraction, plasma and serum separation and samples were stored at -20°C till analysis.

DNA isolation and quantification

Prepare PBS Solution: 150 Mm Sodium Chloride, 50 Mm Potassium Phosphate, pH=7.2. Buffer ACL in the kit is not required for blood genome in DNA isolation.

Polymorphism OF GSTA1

GSTA1 is known to have genetic polymorphisms in exon 5 guanine to adenine transition in exon 5, the PCR and restriction fragment length polymorphism (RFLP) studies will be performed by using methods described. A PCR method was used with few modifications to simultaneously amplify regions of GSTA1 in genomic DNA(Rubae'I et al 2021) . Briefly, the assay for theexon 5 variant uses the primer pair (5'-GTAGTTTGCC CAAGGTCAAG-3') and (5'- AGCCACCTGAGG GGTAAG-3'). The DNA will be separated from blood sample of each volunteer and subjected to PCR by applying denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, elongation at 72°C for 60 seconds, followed by 25 cycles of amplification. The PCR products will be digested for 2 hours at 37°C with 5 units of.

Results and Discussion

Mean age of the breast cancer patients was 52 years. Between the groups, no significant gender- or age-related differences were detected. The frequencies of GSTA1 genotypes were 76 % AA (mutant type) and 24% GG (wild type). PCR-RFLP (Restriction Fragment Length Polymorphism) method figured the GSTA1 gene. With annealing at 55°C, DNA fragments were amplified for 37 cycles and then amplification products were exposed to Alw26I restriction enzyme digestion where existence of the G allele resulted in the generation of 148 and 189bp fragments and the A allele fragments remained uncut.

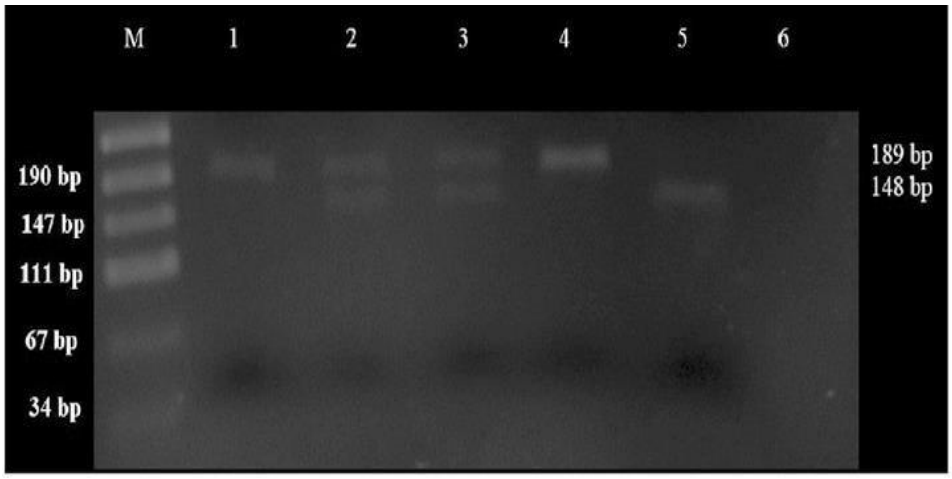

Fig 5: Digestion products of GSTA1 by Alw26I restriction enzyme. Lane M was the DNA Ladder, Lanes 1 and 4 was the mutant type AA, Lane 2 was heterozygous positive control for digestion, Lane 3 was AG, Lane 5 was wild type GG and Lane 6 was negative control.

Table 1 showed the genotype distribution and allelelic frequency of GSTA1 gene in breast cancer patients and healthy individuals.

Genotypes /alleles	**BC Cases (%)** n = 100	**Controls (%)** n = 100	**OR (95% CI)**	***P*-value**
Wild type Ile/Ile or G/G	24 (24%)	55 (55 %)	1.00	
Mutant type Val/Val OR A/A	76(76%)	45(45%)	1.55(1.00-2.42)	0.050

Allele frequencies of GSTA1 in the breast cancer groups showed in the Table 1. In this study, GSTA1 polymorphisms were analyzed in 200 subjects, including 100 healthy control subjects and 100 breast cancer. There was A and G allele frequencies of GSTA1 in the breast cancer patients. Significant difference was also found between these two groups. In the breast cancer groups, there were frequencies of two different genotypes, one was wild type and the other was mutant genotype of GSTA1. Between these two groups, significant difference was observed. All subjects in the breast cancer were genotyped for GSTA1 exon 5. The goodness of fit of the GSTA1 genotype distribution in the healthy control group to Hardy-Weinberg equilibrium was tested. Between the actual and expected distributions, significant difference was observed.The GST superfamily, a group of enzymes involved in phase II metabolism, exhibits significant polymorphism. Variations in these enzymes can affect their activity, potentially contributing to an individual's susceptibility to various diseases like cancer.(Şahinoğulları, Z. U. 2021).Over the past 28 years, 40% death rate caused by braest cancer have been decreased in the united state This decline in mortality can be atributed to enhance comprehensive treatment approaches, breast cancer prevention and early detection effort (Teslenko 2022)However, it is estimated that one in eight women will be diagnosed with breast cancer at some point during their life time (Rojas and Stuckey, 2016). Several studies have indicated that various risk factor contributed to development of breast cancer (Loibl et al., 2021). One of the significant risk factors is age, as over the 70% of all breast cancers are diagnosed in women older than 50 (Varghese and Wong, 2018). Morover, in the United States, Approximately 10% of all breast cancer cases are associated with genetic predisposition or family history, with Variation observed across different ethnicity and racial groups. (Teslenko 2022).Regarding the relationship between GSTA1 genotypes and various factors, no significant associations were identified between GSTA1 genetic polymorphisms and in each variables such as smoking, alcohol consumption, age groups, or gender.(Rubae'i SA et al 2021).Activity of GSTA1 was significantly lower with the mutant genotype than with the wild genotype, the mutant type has a decreased ability to detoxify mobilized carcinogens and thus their carriers have an elevated susceptibility to breast cancer. Respectively G/G genotype illustrated the non-A/A genotype. Subjects with the A/A genotype were remarked as a reference group to estimate the danger of breast cancer. Overall, 76 % of the patients in the breast cancer group carried the A/A genotype of GSTA1, which was higher than G/G genotype. The risk of breast cancer in the subjects with the A/A genotype was 3.16 -fold higher than that of the patients with the G/G genotype.Key approaches to preventing breast cancer involve reducing risk factors, undergoing routine cancer screenings, and using chemoprevention therapy. Regular screenings play a crucial role in identifying breast cancer at an early stage, whether at the primary tumor phase or the onset ofmetastasis. Early detection has significantly lowered mortality rates, as more than 90% of cancer-related deaths are attributed to tumor metastasis (Sun et al.,2017).

CONCLUSION

This research was designed to determined the polymorphisms in hereditary polymorphism of isozyme of Glutathione S-Transferase (GSTA1) in Healthy volunteers and Breast Cancer patients. The hereditary polymorphism was observed by PCR and gel electrophoresis. Total 200 subjects were studied in which 100 normal and 100 breast cancer subjects included. GSTA1 genetic studies show t`he frequencies of GSTA1 genotypes in breast cancer patients were 76 % AA (mutant type) and 24%

GG (wild type) while in healthy subjects 55% GG (wild type) and 45% AA (mutant type). Aparently it is observed that mutant genotype ismore prevelant in breast cancer patients as compared to healthy individuals. A study on broader scale may needed for accurate description. PCR-RFLP (Restriction Fragment Length Polymorphism) method figured the GSTA1 gene with annealing at 55°C, DNA fragments were amplified for 37 cycles and then amplification products were exposed to Alw26I restriction enzyme digestion where existence of the G allele resulted in the generation of 148 and 189bp fragments and the A allele fragments remained uncut. This research was designed to determined the polymorphisms in hereditary polymorphism of isozyme of GSTA1.

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