Genetic Polymorphism of Glutathione S-Transferase Mu 1 (GSTM1) and Its Association with Asthma Susceptibility: A Comprehensive Analysis

Jyoti Sharma[1*], Divya Sharma[2], Noopur Khare[3]

[1] Assistant Professor, Department of Biotechnology,
Bhai Gurdas group of institutions, Sangrur, Punjab.
[2] Assistant Professor, Department of Medical Laboratory Sciences,
Bhai Gurdas group of institutions, Sangrur, Punjab.
[3] Assistant Professor, Bhai Gurdas group of institutions, Sangrur Punjab.

Corresponding Author

Jyoti Sharma*

***E*-mail :** *jyoti6339@gmail.com*
Assistant Professor,
Department of Biotechnology,
Bhai Gurdas group of institutions, Sangrur, Punjab.

Abstract

Glutathione S-transferases and its genetic variants that change their activity, It was discovered that play a role affecting susceptibility and development of several pulmonary diseases. Glutathione S-transferases, in addition to their role in phase II metabolism, are important mediators, according to the study. According to the research, Glutathione S-transferases appear to be critical mediators of healthy lung growth. Glutathione S-transferases perform some functions in the genesis of asthma. Its GSTM1 null genotype appears to be associated to an elevated chance of inflammatory pulmonary ailments, include asthma. Our research project aimed to identify the genetic variation associated with the Glutathione S-transferase Mu 1 (GSTM1) gene in asthma patients and controls, which was accomplished using multiplex PCR genotyping of all subjects the results were obtained after employing multiplex PCR for GSTM1 genotyping to analyze blood samples from asthma sufferers and controls. All procedures were used, including DNA isolation, multiplex PCR, and gel electrophoresis. No significant changes were seen in GSTM1 null genotype rates between asthma patients as well as controls. Only in asthma patients were both GSTM1 along with GSTT1 genotypes are null found together (12.50%). However, there was no link between it and asthma susceptibility. There was no indication that GSTM1 was linked to asthma susceptibility. The lack of a link could be due to our randomized trial or other genetic-environmental interactions. However, the study's findings are limited to the demographic type and number of participants.

Keywords “Asthma”, “GSTM1 polymorphism”, “GSTT1”, “multiplex Polymerase chain restion”, “gel electrophoresis”.

Introduction

"Asthma constitutes a long-term inflammatory condition illness of the lungs where Numerous cells along with cellular components contribute Specifically, mast cells, neutrophils, eosinophils, T lymphocytes, macrophages, including epithelial cells," reported by the National Institute of Health Guidelines on Asthma. Although the word "asthma" is more accurate than "chronic inflammation that affects the upper respiratory tract," it nevertheless refers to a wide range the clinical diseases which vary on intensity, occurrence, adverse outcomes, triggers, therapeutic response, heredity, and natural history [1].

Asthma is a widespread condition in both children and adults, with significant morbidity, fatality, and economic burdens around the world. Asthma is increasingly recognised as a diverse condition, with new clinicopathological research elucidating the basic immunology of asthma. The Global Initiative for Asthma (GINA) has identified five unique asthma phenotypes based on population, medical, and physiological characteristics: allergic asthma, non-allergic asthma, delayed asthma, asthma with permanent airway obstruction, and asthma with adiposity [2]. Because it is such a complicated disorder, it could be caused by a number of variables, both environmental and hereditary. It is a disorder in which the lungs' airways narrow or become entirely obstructed, preventing regular breathing.

This pulmonary blockage, on the other hand, is reversible, either naturally or with treatment. Because of a better knowledge of asthma's basic heterogeneity, the paradigm of asthma as an unified entity is no longer valid. Asthma is today regarded as a catch-all term encompassing a variety of disorders with differing phenotypes, which together present with symptoms ranging from coughing and breathlessness to wheeze and tightness of the chest, and are followed by varied degrees of airway blockage.

Until several years ago, all asthmatics received the same treatment. The disease's diversity, on the other hand, results in a wide range of therapeutic approaches [3]. Even though there have been significant advancements in the management of asthma, mortality from this airway illness still occurs in a small percentage of people.

Asthma is an illness which impacts respiratory narrow the lungs have airways composed of a number regarding tubes, which seek to divide, eventually resulting in little air bags (called alveoli) whereby gas exchange occurs. Together the cartilaginous rings along with soft tissue maintain the major artery passages throughout the lungs, particularly the trachea and major bronchus. These tiny channels are known as bronchi as well as bronchioles.

By minimizing the dimensionality of the tiny airways, Smooth muscular contraction is dramatically increase airways resistance as well as decrease airflow towards the lungs [4]. Asthma is an autoimmune disorder. A sequence of events known as inflammation is generated in response to a number of stimuli, some of which are outside the environment, such as irritants, and others which respond to changes inside the body.

Mucous lumps also clog air passages. Mucus-producing cells appear wider and much more abundant in asthmatic patients than in non-asthmatic people. The bronchi are also enlarged, with an enhanced variety of inflammatory cells, which is assumed to be attributable to ongoing airway inflammation [5]. Because previously mentioned, asthma includes three major features: respiratory inflammation, pulmonary hyperresponsiveness, and mucosal hypersecretion.

All the three characteristics that produce bronchoconstriction are airway limitation when treating an asthmatic patient manifest such as wheezing as well as discomfort. When it comes to asthma, genes is proven to play a major role. Numerous several genetic variants associated with an increased risk about developing the disease have been discovered in massive genomic research [6].

Respiratory tract diseases, particularly infections caused by viruses childhood, raise the dangers of the diseases, specifically if your symptoms remain severe. Nicotine smoking, pollution, as well as ozone are all instances of airborne exposures that lead to higher rates of asthma [7]. Asthma is associated with atopic illnesses and allergies sensitivity to inhaled allergens. Additional factors, like the microbiome, vitamin D, and exposure to chemicals, and metabolites, have been proposed to involve with asthma progression [8,9]. Type 2 T assistance cell lymphocytes are present throughout the majority of asthmatics.

Certain cytokines compositions (Interleukin [IL]-4, IL-5, along with IL-14) and Type 2 inflammation is associated with inflammatory cells, including mast cells, eosinophils, basophils, which type 2 T helper lymphocytes, including immunoglobulin E [10]. Asthmatics who do not have a significant preference for type 2 inflammation frequently have a low or no response to corticosteroids and are harder to cure. Epithelial enlargement and goblet cell metaplasia with enhanced secretion of mucus are pathogenic alterations in the mucosal.

A variety of immune mediators released by inflamed and fundamental cells influence inflammation cells and immunological response.

Asthma causes the airways membranes to thicken, as evidenced by numerous diagnostic and pathology studies. Following airway smooth muscle contraction, elevated airway layer thickness adds to pulmonary constriction [11,12].

Thickening of the mucous membrane and smooth muscle of the airways amplifies the Increased airway resistance because almost any defined amount of their airway smooth muscle shrinkage. The elasticity strain of the linked lung is reduced by expanding the airway wall outside of the airways smooth muscle, lowering the pressure by which the airway smooth muscle must shrink [13]. Airway membrane dynamics may be affected by higher tissue mass and changing composition of the airway wall, specifically of the extracellular matrix, which can lead to airway hyperresponsiveness and irreversible airway constriction [14].

Glutathione S-transferase Mu 1 (GSTM1) is an enzyme that converts glutathione to glutathione [15]. The Mu 1 genes for a mu class cytoplasmic glutathione S-transferase. By conjugating electrophilic molecules with glutathione, the mu group of processors aids in the elimination of carcinogens, medicinal medicines, environmental contaminants, and cellular redox byproducts. Irritable bowel syndrome [16] and atherosclerosis coronary heart disease are linked to GSTM1 polymorphisms [17]. The GSTM1 as well as GSTT1 null mutants have also been related with the evolution of chronic obstructive pulmonary disease [18].

GSTM1 along with GSTT1 null genotypes include linked to an elevated risk of asthmatic, A current meta-analysis suggests that [19]. Removal of the GSTM1 gene leads to reduction in the elimination of harmful toxins, causing toxins to build up in the body which causes damage to healthy bodily functions, increasing the chance of developing diseases such as cancer and asthma.

The goal of this study was to find out how GSTM1 genotype was distributed in asthma patients and controls. Also, to see if having a overall GSTM1 deficient genotype raises the risk developing asthma patients when compared to controls.

Material and method

Study population

The current study was conducted in Patiala, Punjab. A total of 16 asthma patients and 15 healthy volunteers participated in this study. We aimed to minimize some apparent lifestyle and cultural biases by enrolling control volunteers from that very same geographic location. Written informed consent forms in the participants' original languages were obtained from all participants.

Sample collection

A licensed lab assistant collected all samples of blood (about 3ml per patient) in EDTA coated tubes and processed them as soon as possible to avoid damage on storage.

DNA isolation from whole blood sample

The salting-out and boiling procedures were employed in combination. For DNA extraction, Sucrose, NaCl, tris base, Triton X-100, and Na2EDTA, MgCl2, chloroform was also utilised. Buffers were made in accordance with the protocol [20]. Following that, DNA was extracted using the following methodology:

1. One millilitre of distilled water and one millilitre of blood were blended in a 2mL microtube during 30 seconds. Then it was centrifuged for 5 minutes at 7000 rpm for 5 minutes. The supernatant was disposed. This cycle was continued twice.
2. 1 mL buffer was combined with the above mixture, and vortexed. Thereafter, The tubes subsequently centrifuged for 5 minutes at 7000 After 5 minutes of spinning, the supernatant remained decanted.
3. Add one millilitre of buffer B as well as 100 µL of 10% SDS to the tube and vortexed. Afterwards, the tubes were subsequently incubated for fifteen minutes at 65°C for 15 minutes.
4. After 3 minutes of placing on ice, 400 µl aqueous chloroform as well as 400 µl of saturated NaCl consisted used, mixed for 30 seconds, then centrifuged for 10 minutes approximately 5000 rpm and 4°C.
5. The uppermost phases were transferring to a fresh tube, which was then filled with an equivalent amount of cold ethanol and agitated. The tube assemblies were centrifuged until 10 minutes around 13000 rpm at 4°C, with the supernatants being discarded.
6. 1 ml 70% ethanol was added and Centrifuged for ten minutes around 10000 rpm.
7. After discarding the supernatants, and the DNA was dried in the open air. After adding 50-100 µl of ddH2O, the mixture was kept at-20°C.

Optical density measurement

The optical density measurement was done using a Spectrophotometer.

Multiplex-PCR method

A reaction mixture containing Taq Buffer, dNTPs, and Taq DNA Polymerase was produced in a sample vial and then aliquoted into individual tubes to run numerous parallel reactions. After that, distilled water and template DNA were introduced. As a positive control, albumin was added [21].

Reaction mixture

i) ***DNA Template*** An appropriate DNA proportion should be used for good amplification. For this process, ideal DNA scales chosen were 2.0 MICROLITER. In the PCR procedure, too much DNA can result in unspecific results. When choosing a DNA isolation method, special care must be taken. Any contaminants in the extracted DNA, such as a trace of (EDTA), can disrupt the PCR process (FAR).

ii) ***Primers*** Primers are small oligonucleotides in range (18-30) nucleotides that are elucidated in details in Table 1.

Table 1 : Showing primer sequence and product size.

Gene	Primer sequence		Size of PCR product
GSTM1	F	5'-GAACTCCCTGAAAAGCTAAAGC-3'	215bp
	R	5'-GTTGGGCTCAAATATACGGTGG-3'	
Albumin	F	5'-GCCCTCTGCTAACAAGTCCTAC-3'	350bp
	R	5'-GCCCTAAAAAGAAAATCGCCAATC-3'	

iii) ***Taq DNA polymerase*** It is one of the most common chemicals used to improve the PCR process. It promotes the creation of DNA in the 5'-3' orientation. Polymerase concentrations of 0.3 l was utilized. In electrophoresis, more than polymerase might result in a large number of non-specific amplification output bands [22].

iv) ***Deoxynucleotide triphosphates (dNTPs) levels*** They are dNTPs, which are used in the PCR process to format new DNA strands. The optimal dNTP levels in a PCR solution; equal levels of each dNTP must be maintained. The concentration of dNPT used was 0.7 l [23].

v) ***Setting up reaction mixture*** The original DNA, primer pairs, DNA polymerase, dNTPs, a buffer (0.3 l), Albumin (Fp) (1.0 l), Albumin (Rp) (1.0 L) and Distilled water- 13µl were all mixed together in the first phase of PCR. The combination was then subjected to a temperature cycle that allows for denaturation, annealing, and extension. The product was subsequently placed on an appropriate gel and yields [24].

PCR programming

Steps of PCR programming

Table 2 : Steps of PCR programming.

Step	Programme	Temperature	Time
STEP 1	Initial denaturation	95ºC	Five minute
STEP 2	Final denaturation	95 ºC	One minute
STEP 3	Annealing	72 ºC	One minute
STEP 4	Extension	72 ºC	One minute
	Step 2 to Step 4 were repeated 35 times		
STEP 5	Final extension	72 ºC	Seven minute
STEP 6	Hold	4 ºC	Several hours

Agarose gel electrophoresis

The following dyes were used to stain and visualise DNA in gels: etidium bromide, SYBR Gold, along with SYBR Green 1. DNAs separating through agarose gel migration are detectable by colouring them using dyes having a relatively the fluorescence is modest greater affinity for DNA, as well as a higher quantum efficiency of fluorescence upon adhering into nucleic acids [25]. The method employed was given below:

a) To make a mould, the corners of a clean, dry glass plate tray were taped that came with the electrophoresis apparatus. Placed the mould on the bench's horizontal part [26].
b) Prepared enough electrophoresis buffer to refill the electrophoresis tank and cast the gel (typically 1 TAE or 0.5 TBE).
c) Prepared an agarose solution in electrophoresis buffer at a volume adequate for segregating the DNA sample's predicted size fragments [27].
d) In an Erlenmeyer flask, combine the dried agarose powder with a certain amount from electrophoresis buffer.
e) The neck of an Erlenmeyer flask was loosely plugged.
f) The slurry was heated in the water bath until the agarose dissolves.
g) Transferred insert the glass vessel into a 55°C water bath using gloves and tongs.
h) Added Ethidium bromide beginning at an ending value of 0.5 g/mL once the molten gel has cooled. By slowly whirling the gel solution, it was mixed thoroughly [28].
i) Selected an suitable comb during creating the specimen chambers through the gel.
j) Placed the pommel of the comb was 0.5-1.0 mm above the surface of the plate.
k) The heated agarose solution was poured into the mould.
l) Poured the lesser gel immediately Upon top of the supportive gel after allowing it to solidify the glass plate is at normal temperature.
m) Allowed use the gel on settle thoroughly (thirty to forty-five minutes) at ambient temperatures), then gradually removed the comb and poured a modest amount of electrophoresis solution on top.
n) Scraped away the tape after pouring out the electrophoresis buffer mounted the gel inside the electrophoresis container [29].
o) Covered the gel with enough electrophoresis buffer.
p) 0.20 mL of the required Six gel-loading buffers was added to the DNA samples.
q) used a micropipette to load the samples solution right towards the chambers in the immersed gel.
r) Sealed in the polymer gel tank cover and connected an electrical wires for the DNA to travel to the positive anode [30].
s) A power of voltage is 1-5 V/cm was utilised.
t) This gel developed let to run till FF contained bromophenol blue containing xylene cyanol travelled a sufficient distance.
u) The current flow was switched off and the wires and cover from the gel tank were removed once DNA samples and dyes had travelled a significant distance travelled through the gel-like substance [31].

Visualization

The gel was visualized under UV trans-illuminator and the results were recorded by taking a photograph using a gel documentation system.

Statistical analysis

The statistical comparison of results from asthmatic and healthy control individuals were performed using the chi-squared test. The Chi-squared test originally performed manually using the formulae described below [32].

Chi-square ($\chi2$) Test

The aforementioned $\chi2$ test is among the most frequently utilised non-parametric analysis. Its quantity describes The extent of the difference among theory along with observation. The formula for the calculation involving Chi-square is as follows:

$\chi2 = \Sigma$ (O-E)2/E

Where O = observed frequency

E = expected frequency

Expected frequency for any cell can be calculated as

E = RT×CT / N

Where E = expected frequency

RT = the row total for the row containing the cell

CT = the column total for the column containing the cell

N = total number of observations

The designed value of $\chi2$ when contrasted against the Table 2 values at a specified degree of independence (d.f.) and a defined extent of significance. The above degree of freedom was obtained as (column number-1) with (row number-1). While the estimated $\chi2$ value was more than the Table 2 value at the stated in terms of importance, differences were regarded as statistically significant.

Results

The study found there had been no statistically there are substantial variances GSTM1 null genotype frequencies in the spaces between group of asthma patients along with the control group. Notably, the presence of both GSTM1 as well as GSTT1 null genotypes was observed in 12.50% of the asthma patients. However, this co-occurrence of null genotypes did not appear to be connected with a greater vulnerability towards asthma.

Conclusion

In conclusion, the study did not find any evidence of a direct link comparing the possible GSTM1 null genotype to susceptibility to asthma. The absence of a significant association might be attributed to various factors, including the study's design, the relatively small sample size, and potential genetic-environmental interactions. It is It is vital to emphasise that the investigation's findings include constrained by the specific demographic characteristics and the limited number of participants. Further research with a larger and more diverse population, as well as exploration of additional genetic and environmental variables may be required to offer a more full knowledge of the role of GSTM1 in asthma susceptibility.

References

1. Falk NP, Hughes SW, Rodgers BC. Medications for chronic asthma. American Family Physician. 2016 Sep 15;94(6):454-62.
2. Becker AB, Abrams EM. Asthma guidelines: the Global Initiative for Asthma in relation to national guidelines. Current opinion in allergy and clinical immunology. 2017 Apr 1;17(2):99-103. https://doi.org/10.1097/ACI.0000000000000346
3. Kuruvilla ME, Lee FE, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clinical reviews in allergy & immunology. 2019 Apr 15;56:219-33. https://doi.org/10.1007/s12016-018-8712-1
4. Lee AJ, Lim JW, Kim H. Ascorbic Acid Suppresses House Dust Mite-Induced Expression of Interleukin-8 in Human Respiratory Epithelial Cells. Journal of cancer prevention. 2021 Mar 3;26(1):64. https://doi.org/10.15430/JCP.2021.26.1.64
5. Mohanan S, Tapp H, McWilliams A, Dulin M. Obesity and asthma: pathophysiology and implications for diagnosis and management in primary care. Experimental biology and medicine. 2014 Nov;239(11):1531-40. https://doi.org/10.1177/1535370214525302
6. Patil VK, Holloway JW, Zhang H, Soto-Ramirez N, Ewart S, Arshad SH, Karmaus W. Interaction of prenatal maternal smoking, interleukin 13 genetic variants and DNA methylation influencing airflow and airway reactivity. Clinical epigenetics. 2013 Dec;5:1-6. https://doi.org/10.1186/1868-7083-5-22
7. Bønnelykke K, Coleman AT, Evans MD, Thorsen J, Waage J, Vissing NH, Carlsson CJ, Stokholm J, Chawes BL, Jessen LE, Fischer TK. Cadherin-related family member 3 genetics and rhinovirus C respiratory illnesses. American journal of respiratory and critical care medicine. 2018 Mar 1;197(5):589-94. https://doi.org/10.1164/rccm.201705-1021OC
8. Hachim MY, Alqutami F, Hachim IY, Heialy SA, Busch H, Hamoudi R, Hamid Q. The Role of Systems Biology in Deciphering Asthma Heterogeneity. Life. 2022 Oct 8;12(10):1562. https://doi.org/10.3390/life12101562
9. Das T, Nandy S, Dey A. Asthma-Induced Inflammatory Responses and Reversal by Botanicals. Medicinal Plants for Lung Diseases: A Pharmacological and Immunological Perspective. 2021:103-25. https://doi.org/10.1007/978-981-33-6850-7_4
10. Khairullah ZA, Rashid BA, Shwiehk RS. Client Characteristics and Satisfaction with the Quality of Primary Health Care Centers Services in Babylon Governorate, Iraq. International Journal of Pharmaceutical Quality Assurance. 2021;12(2):1-5.
11. Fahy JV. Type 2 inflammation in asthma—present in most, absent in many. Nature Reviews Immunology. 2015 Jan;15(1):57-65. https://doi.org/10.1038/nri3786
12. James AL. Relationship between airway wall thickness and airway hyperresponsiveness. InAirway wall remodelling in asthma 2020 Oct 28 (pp. 1-27). CRC Press.
13. James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, Bai TR, Abramson MJ, McKay KO, Green FH. Airway smooth muscle hypertrophy and hyperplasia in asthma. American journal of respiratory and critical care medicine. 2012 May 15;185(10):1058-64. https://doi.org/10.1164/rccm.201110-1849OC

14. Prakash YS, Halayko AJ, Gosens R, Panettieri Jr RA, Camoretti-Mercado B, Penn RB. An official American Thoracic Society research statement: current challenges facing research and therapeutic advances in airway remodeling. American Journal of Respiratory and Critical Care Medicine. 2017 Jan 15;195(2):e4-19. https://doi.org/10.1164/rccm.201611-2248ST
15. Khatri SB, Iaccarino JM, Barochia A, Soghier I, Akuthota P, Brady A, Covar RA, Debley JS, Diamant Z, Fitzpatrick AM, Kaminsky DA. Use of fractional exhaled nitric oxide to guide the treatment of asthma: an official American Thoracic Society clinical practice guideline. American journal of respiratory and critical care medicine. 2021 Nov 15;204(10):e97-109. https://doi.org/10.1164/rccm.202109-2093ST
16. Pourkeramati A, Zare Mehrjardi E, Dehghan Tezerjani M, Seifati SM. Association of GSTP1, GSTT1 and GSTM1 gene variants with coronary artery disease in Iranian population: a case–Control Study. International journal of general medicine. 2020 May 28:249-59.
17. Maris K, Prasetyo AYE, Subandi, Suharti, Analysis of Molecular Modeling and Molecular Docking of Beta-glucanase from Metagenomic Expression Library as Candida Antibiofilm Candidate. International Journal of Drug Delivery Technology. 2022;12(3):929-935.
18. Liu C, Ran R, Li X, Liu G, Xie X, Li J. Genetic Variants Associated with Chronic Obstructive Pulmonary Disease Risk: Cumulative Epidemiological Evidence from Meta-Analyses and Genome-Wide Association Studies. Canadian Respiratory Journal. 2022 Jun 9;2022. https://doi.org/10.1155/2022/3982335
19. Lee JU, Jeong JY, Kim MK, Min SA, Park JS, Park CS. Association of GSTM1 and GSTT1 null genotypes with toluene diisocyanate-induced asthma. Canadian Respiratory Journal. 2022 Feb 11;2022. https://doi.org/10.1155/2022/7977937
20. Ghaheri M, Kahrizi D, Yari K, Babaie A, Suthar RS, Kazemi E. A comparative evaluation of four DNA extraction protocols from whole blood sample. Cellular and Molecular Biology. 2016 Mar 31;62(3):120-4.
21. Wysocki K, Park SY, Bleecker E, Busse W, Castro M, Chung KF, Gaston B, Erzurum S, Israel E, Teague WG, Moore CG. Characterization of factors associated with systemic corticosteroid use in severe asthma: data from the Severe Asthma Research Program. Journal of Allergy and Clinical Immunology. 2014 Mar 1;133(3):915-8.
22. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. Discovery medicine. 2013 Apr 26;15(83):243-9.
23. Kadiwala AI, Patel PH, Chakraborthy G. Formulation of Generic In-Depth Investigation of Analytical Methods for the Determination of Bempedoic Acid and Ezetimibe in Biological Fluid and Pharmaceutical Dosage Form: A Review. International Journal of Pharmaceutical Quality Assurance. 2021;12(2):45-51.
24. Wenzel SE. Emergence of biomolecular pathways to define novel asthma phenotypes. Type-2 immunity and beyond. American journal of respiratory cell and molecular biology. 2016 Jul;55(1):1-4. https://doi.org/10.1165/rcmb.2016-0141PS
25. Shivram Sagar DR, Sudharsan S. A study on the correlation between clinical features, spirometry and ABG in acute exacerbation of obstructive lung diseases. Cough. 2021 Oct;4:46. https://doi.org/10.22271/27069567.2022.v4.i1b.325.
26. Todkar PP, Hamrapurkar PD. Development and validation of a stability-indicating reversed-phase high-performance liquid chromatography method for elagolix sodium using quality by design approach. International Journal of Pharmaceutical Quality Assurance. 2020;12(2):6-13.

27. Varghese N, Komala M. Determining the Influence of Breadfruit Mucilage on the Disintegration and Dissolution of Losartan Fast-Dissolving Tablets. International Journal of Drug Delivery Technology. 2023;13(3):780-791.
28. Piacentini S, Polimanti R, Simonelli I, Donno S, Pasqualetti P, Manfellotto D, Fuciarelli M. Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. Molecular biology reports. 2013 Apr;40:3299-313. https://doi.org/10.1007/s11033-012-2405-2
29. El Rifai N, Moustafa N, Degheidy N, Wilson M. Glutathione S transferase theta1 and mu1 gene polymorphisms and phenotypic expression of asthma in Egyptian children: a case–control study. Italian Journal of Pediatrics. 2014 Dec;40(1):1-6. https://doi.org/10.1186/1824-7288-40-22
30. Karam RA, Pasha HF, Amal S, Rahman HM, Gad DM. Impact of glutathione-S-transferase gene polymorphisms on enzyme activity, lung function and bronchial asthma susceptibility in Egyptian children. Gene. 2012 Apr 15;497(2):314-9. https://doi.org/10.1016/j.gene.2012.01.059
31. Tamer L, Calikoğlu M, Ates NA, Yildirim H, Ercan B, Saritas E, ÜNLÜ A, Atik U. Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology. 2004 Nov;9(4):493-8. https://doi.org/10.1111/j.1440-1843.2004.00657.x
32. Saadat M. Association between null Genotypes of glutathione S-transferase M1 and T1 and susceptibility to systemic lupus erythematosus: A meta-analysis. Scripta Medica. 2024 Feb 23;55(1):97-105. https://doi.org/10.5937/scriptamed55-48004