



FREQUENCIES OF GSTP1(Ile105Val) POLYMORPHISM AND ITS ASSOCIATION WITH CML IN SUDANESE PATIENTS

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ABSTRACT

Background: Glutathione S-transferases (GSTs) are enzymes belong to phase II metabolic isozymes, best known for their ability of detoxification of xenobiotic. Several studies reported that gene Polymorphisms within GSTs family conferred a significant association with increased risk of CML. There is no published data about the association of gene polymorphism of these enzymes to CML Sudanese patient's, So the present study aimed to find out if there was any association between GSTP1 gene polymorphism and CML in Sudanese patients.

Materials and methods: This was case control study conducted between January 2016 and June 2017 in which a total of 115 patients with chronic myeloid leukaemia attended the Radiation and Isotope Centre-Khartoum and 104 un related apparently healthy individual as control group were involved. DNA extracted from collected EDTA venous blood samples and analysed by RFLP (Restriction fragments length polymorphism) to detect the presence of GSTP1 (Ile105Val). **Results:** A total of 58.2% (67) of CML patients had heterozygous (Ile/Val) type of GSTP1 Ile105Val polymorphism, 4.35% (5) patients had the homozygous type (Val/ Val) and 37.3% (43) had the wild genotype of GSTP1 (Ile / Ile). In contrast there were 40.3% (42) of the control group with heterozygous (Ile/Val) type, 1.9% (2) with homozygous type (Val/ Val) and 57.6% (60) with wild genotype of GSTP1 (Ile / Ile). GSTP1 Ile105Val polymorphism frequency was found to be significantly elevated in patient with CML 62.6% (72) compared to control group 42.3% (44) (OR = 2.28, 95% confidence interval (CI) 1.33–3.91, p value = 0.002).

Discussion and Conclusion: GSTP1 Ile105Val polymorphism was found to be significantly elevated in patient with CML and were associated with CML development and progression, also CML patients in advanced phase (accelerated and blast crisis) had higher frequency of GSTP1 Ile105Val polymorphism compared to chronic phase and this was agreed with most of the published studies.

KEYWORDS: GSTP1: Glutathione S-transferase Pi, **Ile105Val:** Iso Lucin 105 Valin, **CML:** Chronic Myeloid Leukaemia.

INTRODUCTION

GSTs (Glutathione-S-transferase) is a family which belong to phase II metabolizing enzyme, The main function of GSTs is depend on of production of GSH from the synthetic enzymes gamma-glutamyl-cysteine-synthetase and glutathione synthetase and specific transporters that remove conjugates of GSH from the cell, That will lead to the main role of GSTs in detoxification of xenobiotics by catalyzing the nucleophilic attack by GSH on electrophilic carbon,

sulfur, or nitrogen atoms of nonpolar xenobiotic substrates, so preventing their interaction with the crucial cellular proteins and nucleic acids.^{[1][2]}

Protein sequence and structure are important additional classification criteria for the three super families of GSTs (cytosolic, mitochondrial and MAPEG). Human cytosolic GSTs belong to the alpha, zeta, theta, mu, pi, sigma, and omega classes.^{[1][3]}

GSTs play important roles in cancer development and chemotherapeutic drug resistance, also they implicated in a variety of diseases by virtue of their involvement with GSH. Although the evidence is minimal for the influence of GST polymorphisms of the alpha, mu, pi and theta classes on susceptibility to different types of cancer, many studies have implicated such genotypic variations in asthma, atherosclerosis, allergies and other inflammatory diseases.^[2] Nasopharyngeal carcinoma and NHL were the most common tumors in Sudanese male in 1984 and in the last 20 years CML became the predominant cancer, while lymphoma remained the second most common cancer in men.^[4] In women, breast, cervical and ovarian cancers remained the three most common cancers over both time period, but there was also an increase in the incidence of CML among women.^[4] The registered number of the CML prognostic patients attending Radiation and isotope center-Khartoum is 249 patients in 2015. Recently several studies Worldwide examined different genes polymorphism inside GSTs family and they found significance association between the polymorphism and chronic myeloid leukaemia regarding risk factors and prognosis of the disease^{[5],[6],[7]} and it has been recommended as protocol for monitoring patients prognosis. So the present Study aimed to find out if there was any association between GSTP1 gene polymorphism and CML in Sudanese patients.

MATERIALS AND METHODS

This was cross sectional case control study conducted in the Radiation and Isotope Center Khartoum (RICK) in the duration between January 2016 and June 2017, a total of 115 patients (50 female and 65 male) diagnosed with chronic myeloid leukaemia attended (RICK) for prognosis, their age ranged between (15-81 years old), the patients whom had history or diagnosis with other malignancy beside CML were excluded from the study. The control group consists of 104 matched un related apparently healthy Sudanese individual with a negative history for previous malignancies.

DNA Extraction

DNA was extracted from EDTA blood samples by Chelix (100) method protocol^[8] (modified method) in which 500 µl of blood sample was placed in Eppendorf's tube and 1000 µl of red cell lysis buffer (RCLB) was added, Mixed well and centrifuged at 12000 rpm for 2 minutes, then supernatant is discarded and the WBCs pellet was washed again with 1000 µl of (RCLB), washing repeated 3 to 4 times until clear pellet is obtained (to remove the haem which inhibit PCR), after that the pellet was washed with 1000 µl of barbitone buffersaline (PBS), centrifuged at 12000 rpm for 2 minutes and the supernatant was removed, then 200 µl from chelex was added and mixed well by vortexing, after that Poiling of content using heat block at 100°C for 20 minute was done (vortex every 5 minute), then centrifuged at 14000 rpm for 2 minute and the supernatant was transferred into clean Eppendorf's tube.

Samples were stored at -20°C until analysis, Gene quant device (Amersham bioscience– Biochrome LTD-Cambridge CB4 of J. England) used to detect the quantity of (DNA & Protein) and quality (Purity & ratio) of DNA.

Genotyping of GSTP1 (Ile105Val) polymorphism

GSTP1 (Ile105Val) polymorphism was determined with a polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP]. The PCR primers were: 5'-GTA GTT TGC CCA AGG TCA AG-3' (F) and 5'-AGC CAC CTG AGG GGT AAG-3' (R). PCR was carried out in a total volume of 25 µl. It consist of 5µl of genomic DNA, 1µl from each primer ready to load master mix (Maxime PCR premix series) and 13µl distilled water. PCR was initiated, initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 61°C for 1 minute, 72°C for 1 minute and last extension at 72°C for 7 minutes. PCR products were analyzed on a 2% Agarose gel stained with 0.5 µg/mL ethidium bromide, and visualized by gel documentation system (to check the presence of 436 Pb of GSTP1). Then the PCR product was digested with the restriction endonuclease Alw26I restriction enzyme {Thermo Scientific Alw26I # ER0031 (onebio)} as followed, For each 10 µl of PCR product a 7.5 µl nuclease free water, 2 µl from 10X Tango buffer (#B19) and 0.5 µl from Alw26I restriction enzyme were added, then incubated at 37°C for 16 hrs; followed by incubation at 65°C for 20 minute to inhibit the enzyme activity. The products are then resolved on 2% agarose gel electrophoresis containing ethidium bromide, then visualized using UV transilluminator.

The amplified fragment after digestion with Alw26I restriction enzyme, will give rise to 2 fragments at 329 bp and 107 bp indicating the presence of wild type (Ile/Ile), while the appearance of 2 fragments at 222 bp and 107 bp would indicate the presence of homozygous mutant type (Val/Val), moreover the presence of 3 fragments at 329 bp, 222 bp and 107 bp indicates the presence of heterozygous mutant type (Ile/Val). Figure (1). For quality control, genotyping of 21 (9.59 %) of the samples were repeated blindly and they were identical to the initial results.

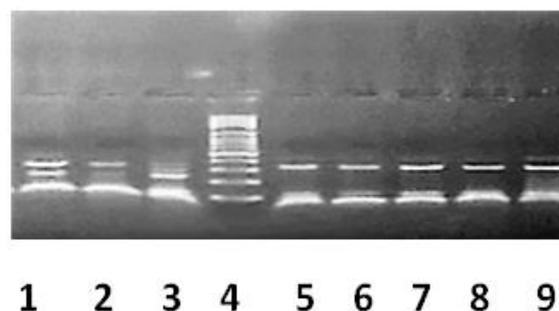


Figure (1): Show amplified DNA fragment after digestion with Alw26I restriction enzyme, Lane 4 DNA ladder: MW 100-1500 bp fragments, lane 5- 9:

fragments at 329 bp and 107 bp indicates the presence of wild type (Ile/Ile), lane3: 2 fragments at 222 bp and 107 bp indicates the presence of homozygous mutant type (Val/Val), lane 1 and 2 show: 3 fragments at 329 bp, 222 bp and 107 bp indicates the presence of heterozygous mutant type (Ile/Val).

Statistical Analysis

All data was entered and analyzed using statistical analysis soft were SPSS (statistical package for social sciences) version 21. Statistical analysis included descriptive statistics of mean, standard deviation. Odds ratio (OR) with a confidence interval (CI) of 95% was calculated. The Pearson's chi-square test was used to compare the genotype distribution between patients and control. P-value less than 0.05 were considered as statistically significant. mann-whitney U tests was used to compare mean for the abnormal distributed data.

RESULT

A total of 93 CML patients were in chronic phase, 20 in accelerated phase while 2 in blast crises. 58.2% (67) of CML patients had heterozygous (Ile/Val) type of GSTP1 Ile105Val polymorphism, 4.35% (5) patients had the

homozygous type (Val/ Val) and 37.3% (43) had the wild genotype of GSTP1 (Ile / Ile). In contrast there were 40.3% (42) of the control group with heterozygous (Ile/Val) type, 1.9% (2) with homozygous type (Val/ Val) and 57.6% (60) with wild genotype of GSTP1 (Ile / Ile) Table (1). In the present study, GSTP1 Ile105Val polymorphism (Ile/Val) (heterozygous) and (Val/ Val) (Homozygous) genotypes frequency was found to be significantly elevated in patient with CML 62.6% (72) compared to control group 42.3% (44) (OR = 2.28, 95% confidence interval (CI) 1.33–3.91, p value = 0.002).

With respect to clinical phase, CML patients in advanced phase (accelerated and blast crisis) had higher frequency of GSTP1 Ile105Val polymorphism 77.2% (17/22) compared to chronic phase 63.4% (59/93). In 80% (16/20) of the patients in accelerated phase had GSTP1 mutations.

Considering gender difference; there was insignificant association between GSTP1 (Ile105Val) and gender, in which the mutation were detected in 53.9% (41) male with CML versus 46.1% (35) in females. {Odds ratio (OR) 1.37, 95% confidence interval (CI) 0.62-3, p value =0.557}.

Table (1): Frequency of GSTP1 variant among case and control groups.

Population			GSTP1			Total
			Heterozygous (I/Val)	Homozygous (Val /Val)	Normal (I/ I)	
Study groups	Case	Count	67	5	43	115
		%	58.26%	4.35%	37.39%	100%
	Control	Count	42	2	60	104
		%	40.39%	1.92%	57.69%	100%

DISCUSSION

Exogenous and Endogenous toxin may lead to alterations inside different genes, so can increase susceptibility to cancer development, like CML. Alteration in metabolizing enzymes like GSTP1 may alter their normal functional activity that responsible for protection of the cells from any toxin.^{[5],[6]}

In the present study strong association of CML with GSTP1 (Ile 105 Val) was observed in the studied patients and this finding was in concordance with several other studies.^{[5],[7],[9]} Table (2).

Regarding phases of CML, this study found that higher frequency of GSTP1 Ile105Val polymorphism in advanced disease (accelerated and blast crises) compared to chronic phase and this was agreed with sheerin, etal in which (57.1% homozygous mutant, 28.6% heterozygous mutant and only 14.3% with wild type; p = 0.03).^[5], also this result was agreed with Kagita Sailaga, etal, whom

found that CML patients in advanced phase (accelerated and blast crisis) had higher frequency of heterozygous (Ile/Val) genotype (47.62%) compared to chronic phase (36.5%).^[9]

CONCLUSION

This study suggests that GSTP1 Ile105Val polymorphism were associated with CML development and progression. Hence the study of this polymorphism would be helpful in assessing the risk for disease occurrence and progression.

Table (2): Agreement between different published data and present study regarding association between GSTP1 Ile105Val polymorphism and CML.

Agreed								
Study	Place of study	No of cases studied	Date	OR	95% CI	P.value	Percent of GSTP1 Ile105Val in case	Percent (%) of GSTP1 Ile105Val in control
Recent study	Sudan	115	2017	2.28	1.33–3.93	0.002	62.6	42.3
1- Sheerin, etal ^[5]	Egypt	40	2014	3.9	1.5 – 9.7	0.004	67.5	35
2-Claudia Banescu, ^[7]	Romania	168	2014	2.5	1.08 – 5.7	0.02	22.9	17.4
3-Kagita ^[9]	India	206	2010	-----	$\chi^2=9.57$ df = 2	0.008	45.7	43.5

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REFERENCE

1. Josephy PD. "Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology". *Hum Genomics Proteomics*. 2010; 2010: 876940.
2. Hayes JD, Flanagan JU, Jowsey IR. "Glutathione transferases". *Annu. Rev. Pharmacol. Toxicol.* 2005; 45: 51–88.
3. Oakley A. "Glutathione transferases: a structural perspective". *Drug Metab. Rev.* May 2011; 43(2): 138–51.
4. H.M.A. Hamad. Cancer initiatives in Sudan. *Ann Oncol.* 2006; 17(supp__8): 32-36.
5. Shereen Elhoseiny, Mohamed El-Wakil, Mohamed Fawzy, Aya Abdel Rahman. GSTP1 (Ile105Val) Gene Polymorphism: Risk and Treatment Response in Chronic Myeloid Leukemia. *JCT*. January 2014; 5(1): 1-10.
6. Ovsepyan VA, Vinogradova Elu, Sherstneva ES. Cytochrome P4501A1, Glutathione-S-Transferase M1 and T1 Gene polymorphism in Chronic Myeloid Leukemia. *Genetika*. 2010 Oct; 46(10): 1360-2.
7. Claudia Bănescu, 1 Adrian P. Trifa, 2 Septimiu Voidăzan, 3 Valeriu G. Moldovan, 1 Ioan Macarie, 4 Erzsebeth Benedek Lazar, 5, *etal.* CAT, GPX1, MnSOD, GSTM1, GSTT1 and GSTP1 Genetic Polymorphisms in Chronic Myeloid Leukemia: A Case-Control Study. *Oxidative Medicine and Cellular Longevity*. 2014; 2014 (2014): Article ID 875861, 6 pages.
8. P. Sean Walsh, David A. Metzger and Russell Higuchi Cetus Corporation and Illinois State Police. *Bio Techniques*. March 2013; 54(3): 134–39.
9. Kagita Sailaja, D Surekha, D Nageswara Rao, D Raghunadha Rao, S Vishnupriya. Association of the GSTP1 gene (Ile105Val) Polymorphism with Chronic Myeloid Leukemia. *Asian Pacific J Cancer Prev*. 2010; 11: 461-64.