ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

GSTP1 Gene Mutation as a Risk Factor for Development of M2 and M4 Subtypes of Acute Myeloid Leukemia.

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ABSTRACT

Glutathione S-transferases , are family of metabolizing enzymes, with distinguishable role in the detoxification of procarcinogens metabolites . Polymorphisms in Glutathione S-transeferase P1 impose a risk to acute leukemia development. to bridge links between Glutathione S-transeferase P1 gene polymorphism (Isoleucine 105 Valine) and susceptibility to acute myeloid leukemia. Glutathione S-transeferase P1 genotypes were determined in a total of 100 denovo acute myeloid leukemia adult patients. The frequency of the three different genotypes of Glutathione S- transferase P1 in our patients were Isoleucine/Isoleucine ,Isoleucine/Valine and Valine/Valine to be 39% ,45% and 16% respectively. Valine /Valine genotype of Glutathione S-transferase P1 gene is involved in risk development of M2 and M4

Keywords: Genotype - GSTP1 - PCR

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ISSN: 0975-8585

INTRODUCTION

Acute myeloid leukemia, a hematopoietic neoplasm that is characterized by the presence of a malignant clone of myeloid cells in the bone marrow with maturation arrest at the level of blast **(1)** .

Glutathione S- transeferase (GST) enzymes , master the detoxification of chemicals found in the environmental , considering the role of these enzymes in protecting tissues from oxidative damage so it is obvious to figure out that genetic polymorphisms within GST enzymes are linked to an increase or decrease in the prevelance of cancer **(2)** . The first single nucleotide polymorphism (SNP) identified in the coding DNA sequence of the GSTP1 gene maps to exon 5. This SNP shows an adenine to guanine A→G transition at nucleotide 313, translating an isoleucine → valine substitution at codon 105 (Isoleucine 105 Valine) . The different GSTP1 allelic proteins differ significantly in their ability to metabolize anti-cancer agents **(3)** . The Val allele is associated with a decreased enzyme activity, when compared to the Ile allele **(4)** .

PATIENTS AND METHODS

Study Design and Sample Collection

A cohort of 100 denovo AML patients compromising M2 and M4 subtypes were recruited . All patients were subjected to complete blood count and bone marrow examination , FAB classification, cytochemistry and immunophenotyping

Genotyping

The genotypes of GSTP1(Ile 105 Val) were determined for all patients using PCR based restriction fragment length polymorphism (RFLP) technique . DNA was extracted according to manufacturer's protocol. DNA was used to amplify 442 bp fragment

A yield of 442bp fragments was produced , this PCR product was subjected to restriction digestion
The presence of wild type (Ile/Ile) was detected by 329 and 113 bp fragments. Homozygous mutant genotype (Val/Val) was confirmed by 216 and 113bp fragments .Heterozygous genotype (Ile/Val) was characterized by fragments consisting of 329, 216 and 113 bp

Statistical Analysis

Data were coded and entered using the statistical package SPSS version 22. Data was summarized using mean, standard deviation, median and range for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. P-values less than 0.05 were considered as statistically significant .

RESULTS

This study encompassed one hundred patients with de novo M2 and M4 subtypes of acute myeloid leukemia. The AML patients group showed a mean of 39.89 ± 12.94 years for age .The clinical and haematological characteristics of AML patients are represented in tables 1 and 2.

The frequency of the three different genotypes of Glutathione S- transferase P1 in our patients was Isoleucine/Isoleucine ,Isoleucine/Valine and Valine/Valine was noted to be 39% ,45% and 16% respectively as shown in table 3

Combined GSTP1 Ile/Val heterozygous and Val/Val homozygous mutant genotypes were detected in 61% of patients

ISSN: 0975-8585

In an attempt to correlate GSTP1 genotypes to different patient characteristics, significant statistical difference was noticed between GSTP1 Val/Val homozygous genotype among M2 and M4 subtypes Val/Val homozygous genotype was present in only 7.14% of M2 cases but accounted for 27.27% of M4 cases (p= 0.019) .

Table 1: Clinical data of AML patients

Variables			Cases (N=100)	
			Count	%
Organomegaly	Hepatomegaly	+ve	39	39.0%
		-ve	61	61.0%
	Splenomegaly	+ve	22	22.0%
		-ve	78	78.0%
Lymphadenopathy		+ve	20	20.0%
		-ve	80	80.0%

Table 2:Hematological data of AML patients at diagnosis

Variables	Cases (N=100)			
	Mean	SD	Median	Range
TLC x10^9/L	61.07	51.32	48.10	1.34-214.80
PB Blasts %	46.92	18.98	44.00	14.00-90.00
BM Blasts %	61.04	16.22	62.00	23.00-89.00

Table (3) :The frequencies of GSTP1 genotypes in AML patients

Variable		Cases (N=100)	
		Count	%
GSTP1 genotype frequency	Wild type (Ile/Ile)	39	39.0%
	Heterozygous (Ile/Val)	45	45.0%
	Homozygousmutant (Val/Val)	16	16.0%
	Both mutant genotypes (Ile/Val&Val/Val)	61	61.0%
Allele frequency	Allele Ile	123	61.5%
	Allele Val	77	38.5%

* P < 0.05 is significant

DISCUSSION

Acute myeloid leukemia is characterized by infiltration of the bone marrow by clonal poorly differentiated haematopoietic cells. **(5)** .

DNA damage is an important risk factor in the development of acute leukemia **(6).** The capacity to detoxify intermediates is genetically controlled and variable between individuals, which may explain differences in

leukemia risk **(7).** GSTs control the detoxification of xenobiotics .The GSTs genes polymorphisms yield a lack of enzyme activity with subsequent reduction in their function .

Our results are consistent with the study conveyed by Dunna et al. in 2012 **(8),** emphasizing the association of GSTP1 gene (Ile105Val) polymorphism with acute leukemia among 290 acute leukemia . However, our results were inconsistent with two studies, by He et al. and Tang et al. in 2014 **(9)**. They found no association between GSTP1 (Ile 105Val) polymorphism and the risk of AML .

No statistically significant differences were observed between GSTP1genotypes and patients ' clinical presentations including: hepatomegaly (p= 0.327), splenomegaly (p= 0.231) and lymphadenopathy (p= 0.844). Dunna et al., in 2012 **(16)** , observed significant increases in the mean WBCs count and the mean bone marrow blast percentage of AML patients with Val/Val genotype compared to those with Ile/Ile genotype ($p < 0.05$).

The present study suggests that Glutathione S-transferase P1 (Isolucine 105 Valine) polymorphism confer increased risk development of M2 and M4 subtypes of AML
Future studies should be done to investigate the role of GSTP1 gene (Ile105Val) polymorphism in development of the other subtypes of AML.

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