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Title:

CYP1A1 and CYP2D6 polymorphisms and susceptibility to chronic myelocytic leukaemia

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Abstract

Background: CYP1A1 and CYP2D6 are both xenobiotic metabolizing enzymes belonging to the CYP450 enzyme family. Polymorphisms in these genes vary between individuals, resulting in dissimilar patterns of susceptibility to the effects of carcinogenic substances and drugs. **Objective**: In a prospective study, the influence of CYP1A1*2C and CYP2D6*4 gene polymorphisms on the susceptibility to chronic myelocytic leukaemia (CML) were investigated. **Method:** Prevalence of CYP1A1*2C and CYP2D6*4 was detected in blood specimens from three hundreds participants - two hundred patients and a hundred healthy individuals as a control group, using PCR-RFLP. **Results:** CYP1A1 Ile/Val and Val/Val genotype frequency in our study population was 82% & 15% in CML patients and 55% & 8% in controls, respectively. This suggests carriers had an elevated risk (OR=18.38, 95% CI=7.364-45.913, p value; =0.000 and OR=23.125,95 % CI=7.228-73.980, p value=0.000, respectively). Individuals carrying the CYP2D6 heterozygous genotype (IM) were notably fewer in number within the CML group at 43.5%, as opposed to 93% of the controls. This suggests the IM genotype may have a prophylactic function in lowering CML risk (OR=0.036, 95% CI=0.005-0.271, *p value* =0.001). In spite of the distribution of the homozygous mutant (PM) genotype being higher in cases with CML (87% as opposed to 6% in the control), this difference was deemed non-significant (OR=0.558, 95% CI=0.064-4.845, *p value* =0.597). **Conclusion:** These findings indicate that polymorphic CYP1A1 and CYP2D6 genes affect the susceptibility to CML.

Key words: Leukaemia, CML, CYP1A1, CYP2D6, polymorphisms, Sudan.

1. Introduction

In approximately 90% of cases, chronic myelocytic leukaemia (CML) is a clonal abnormality of myeloid precursors typified by the positive Philadelphia chromosome (Ph). Ph arises as a reciprocal translocation of chromosomes 9 and 22 [1]. Most cancers including CML result from a complex interaction between factors such as exposure to radiation and/or environmental carcinogens such as benzene, smoke and pesticides. These factors may affect the biotransformation of xenobiotics, causing DNA damage in haematopoietic cells [2].

Xenobiotic metabolism is catalyzed by a variety of enzymes classified into two phases, namely Phase I and Phase II. The former constitutes the theme of this study and involves hydrolysis (i.e. in which a polar group is added to the xenobiotic or alternatively released by enzyme activity), oxidation, and reduction. In Phase II a series of conjugate reactions link the metabolite formed in Phase I to a polar endogenous molecule [3]. Additionally, polymorphisms within the gene coding for both Phase I and Phase II enzymes may alter their expression, function and activity. Several studies have been conducted on the relationship between genetic polymorphisms of these genes, especially CYP450 and GST, regarding susceptibility to various types of cancer including CML [1, 2]. Conflicting results were observed in different ethnic groups and in response to Tyrosine Kinase Inhibitors (TKIs) treatment [4, 5, 6]. Cytochrome P450 (CYP450) enzymes are Phase I, and are responsible for the metabolism of both endogenous and exogenous compounds, which may convert xenobiotics or procarcinogens into DNA reactive metabolites [7]

The CYP1A1 gene is polymorphic and encodes an enzyme which metabolizes several drugs and dietary compounds. It also plays a crucial role in the bioactivation of procarcinogens, such as arylamines and polycyclic aromatic hydrocarbons [8]. The most common polymorphisms of CYP1A1 are T3801C (*2A), A2455G (*2C) and C4887A (*4) [5]. These have been studied in association with various cancers such as lung cancer [9] and oesophageal cancer [10]. CYP1A1*2C (A2455G) is a substitution of Valine (Val) for Isoleucine (Ile) at position 462 of the CYP1A1 enzyme [11]. It elevates enzyme hydrophobicity and creates a two-fold increase in catalytic activity and mutagenicity [4]. Elevation in the activity of the CYP1A1 enzyme is accompanied by higher levels of adduct formation, which elevates the risk of some cancers [12] and haematological malignancies, including CML [5, 13].

CYP2D6 is located on chromosome 22q13.1 and encodes the CYP2D6 enzyme [14]. Its dormancy is the foundation of adverse reactions for patients under treatment with certain antidepressants, antiarrhythmics, opioids and lipophilic β-adrenoceptor blockers [15]. It also metabolizes several endogenous substances such as hydroxytryptamines, neurosteroids, and tyramine [16]. This enzyme performs several

biochemical functions and influences more than 10,000 inter-individual differences in metabolism levels [17].

The CYP2D6 polymorphism can be classified into four categories according to its activity: ultra-rapid metabolisers (UMs), extensive metabolisers (EMs), intermediate metabolisers (IMs) and poor metabolisers (PMs) [18]. This polymorphism has been associated with certain cancers [17, 19], Parkinson's disease [20] and systemic lupus erythematosus [21]. The CYP2D6*4 (1846G>A) polymorphism [20], which results from a defect in splicing at the junction of the 3rd intron and 4th exon, is a main source of the CYP2D6 poor metaboliser phenotype. This polymorphism may cause a reduction or absence in the quantity and activity of CYP2D6 [13].

This study was aimed at the relationship between CML and genetic polymorphisms in CYP1A1, and CYP2D6 within the Sudanese population. The population is genetically varied because of its multi-ethnic lineage i.e. Arab, African and others and lack of research has limited an understanding of the subject.

2. Materials and method

2.1. Subjects and sample collection

This study was carried out on 200 diagnosed CML patients (34% females, 66% males) who presented themselves to the Radiation and Isotopes Centre (RICK) in Khartoum-Sudan. CML diagnosis was according to standard hematologic and cytogenetic criteria. All patients were in chronic phase of the disease except one patient, who was in the accelerated phase. 100 controls were included in the study too. Individuals here amounted to 51 male and 49 female, and all were unrelated healthy volunteers with no history of cancer.

All participants were interviewed using a prepared questionnaire with special consideration for age, residence, occupation and any family history of cancer. Individuals were made aware of the nature of the study and gave written informed consent and approval was obtained by The Institutional Ethical Committee from Al-Neelain University.

2.2. DNA extraction

For each subject, 3 ml of venous blood was collected in a vacuum tube containing EDTA. DNA was then isolated using the Guanidine Chloride DNA extraction method and then stored in Tris EDTA at – 20ºC.

2.3. Genetic Polymorphism

The mapping of genetic polymorphisms for CYP1A1*2C and CYP2D6*4 was performed by PCR/restriction fragment length polymorphism (RFLP). The primers used were CYP1A1*2C and CYP2D6*4 synthesized by Macrogen. PCR mapping was a standardised three step process using a Sensoquest thermocycler, as described by Taspinar *et al* and Sailaja *et al* [1, 22].

Amplification products were visualized with 2% agarose gel and subjected to restriction enzyme analysis, using BsrD1 for CYP1A1*2C and BstN1 for CYP2D6*4 (New England BioLabs Inc). For each reaction; 0.2 µl of the respective enzyme, 2 µl of the corresponding enzyme buffer, 5 µl of the PCR product and 2.8 µl of double distilled water were incubated overnight at 37°C. Banding patterns were then observed on 3% agarose gel stained with ethidium-bromide.

Banding patterns for CYP1A1*2C were as follows; 204 bp presence indicates the homozygous mutant (Val/Val, G/G), 149 bp and 55 bp presence indicates the homozygous wild type (Ile/Ile, A/A) and presences of 204 bp, 149 bp and 55 bp indicates the heterozygous (Ile/Val, A/G). For CYP2D6*4, a banding presence of 334bp indicates the poor metabolizer (A/A, PM), 230bp and 104bp indicates the extensive metabolizer (G/G, EM) and 334bp, 230bp and 104bp indicates the intermediate metabolizer (G/A, IM).

2.4. Statistical analysis

Results were examined using the Statistical Package for the Social Sciences (SPSS) Version 21. Genotype distribution between CML patients and the control were compared using Pearson's chi-square test and the risk of disease development was evaluated by odd ratio (OR) with a 95% confidence interval (95% CI). We considered p-values of less than 0.05 to be statistically significant.

3. Results

Table 1 shows CYP1A1*2C and CYP2D6*4 polymorphism genotype distribution for CML patients and the control. The frequencies of CYP1A1 Ile/Ile, Ile/Val and Val/Val genotypes were 3%, 82%, & 15% in CML patients and 37%, 55%, & 8% in controls, respectively. It was observed that variations in the distribution of CYP1A1 Ile/Val heterozygous and Val/Val mutant genotypes were notably greater in patients with CML. It was also significant that CML patients had a 23.125-fold increased presence of the Val/Val homozygous variant genotype (95% CI=7.228-73.980, p value=0.000) and a 18:38 fold increase in Ile/Val heterozygous variant presence (95% CI=7.364-45.913, p value =0.000).

Distribution of CYP2D6 heterozygous genotype (IM) was significantly lower in CML cases at (43.5%) as opposed to 93% in the control, suggesting that this genotype may play a protective role in reducing CML risk. (OR=0.036, 95% CI=0.005-0.271, *p value* =0.001). Distribution of CYP2D6 homozygous mutant (PM) genotype was higher in patients with CML, (87% against 6% in the control), but this difference was not considered statistically significant (OR=0.558, 95% CI=0.064-4.845, p value =0.597). Furthermore, the homozygous wild type (EM) genotype distribution was 13% in CML patients as opposed to only 1% of the control.

The combined polymorphisms of CYP1A1*2C and CYP2D6*4 were also considered with a significant difference observed only in individuals with a combined AA and IM genotype (OR: 0.02, 95% CI: 0.001-0.293, *p value* = 0.004), see **Table 2**.

Table 1: Distribution of CYP1A1*2C and CYP2D6*4 polymorphism genotypes in CML patients and in control individuals.

Genotypes/Allele frequency		CML N (%)	Control N (%)	OR	95%CI
CYP1A1*2C	AA (Ile/ Ile)	6 (3)	37 (37)	Reference	-
	AG (Ile/Val)	164 (82) [a]	55 (55)	18.38	7.4 - 45.9
	GG (Val/Val)	30 (15) [a]	8(8)	23.125	7.2 - 73.9
	A(WT)	176 (44)	129 (64.5)	-	-
	G (MUT)	224 (56)	71 (35.5)	-	-
CYP2D6*4	GG (EM)	26 (13)	1 (1)	Reference	-
	GA (IM)	87 (43.5) [a]	93 (93)	0.036	0.01 - 0.27
	AA (PM)	87 (43.5) [b]	6 (6)	0.558	0.06 - 4.85
	G (WT)	139 (34.8)	95 (47.5)	-	-
	A (MUT)	261 (62.3)	105 (52.5)	-	-

Key: N= total number; OR= Odd Ratio; CI= Confidence Interval; WT= Wild Type allele; MUT= Mutant allele; PM= Poor Metabolizer (homozygous mutant status); EM= Extensive Metabolizer (homozygous wild type status); IM= Intermediate Metabolizer (heterozygous status).
Statistical significance; (**P-value**) is shown in superscript parenthesis; (a) = <0.05, (b) = >0.05.

Table 2: Combination effect of CYP1A1*2C and CYP2D6*4 genotypes on CML risk.

Genotypes		CML N (%)	Control N (%)	OR	95%CI
CYP1A1*2C	CYP2D6*4				
AA	EM	3(1.5)	1(1)	Reference	
AA	IM	2 (1) [a]	33(33)	0.02	0.001-0.29
AA	PM	1(0.5) [b]	3(3)	0.11	0.005-2.73
AG	EM	18 (9) [b]	1 (1)	6.00	0.30 - 124.1
AG	IM	71 (35.5) [b]	50(50)	0.47	0.048 - 4.68
AG	PM	75(37.5) [b]	3 (3)	8.33	0.66 - 105.71
GG	EM	5 (2.5) [b]	1 (1)	1.67	0.074 - 37.73
GG	IM	14 (7) [b]	7 (7)	0.67	0.06 - 7.64
GG	PM	11 (5.5) [b]	1 (1)	3.67	0.173 - 77.5

Key:- N= Total Number; OR= Odd Ratio; CI= Confidence Interval; PM= Poor Metabolizer (homozygous mutant status); EM= Extensive Metabolizer (homozygous wild type status); IM= Intermediate Metabolizer (heterozygous status). Statistical significance (**P-value**) is shown in superscript parenthesis; (a) = <0.05, (b) = >0.05

4. Discussion

Exposure to xenobiotics can lead to changes in genetic makeup and increase the risk of cancer development and haematological malignancy [23]. The CYP family which

constitutes the theme of this study, has an important role in the detoxification of environmental factors through increased expression in lymphoblastic and myeloblastic lines [24]. Therefore, CYP1A1 and CYP2D6 enzymes may well be responsible for carcinogenesis in haematopoietic cells.

Within this study, frequency of CYP1A1 Ile/Val and Val/Val genotypes were significantly higher in patients (82% and 15%) than the control (55% and 8%, respectively), indicating an increased risk of CML with carriers of these genotypes. It was also found that Ile/Val genotype presence elevates CML risk 18.38 fold (OR=18.38, 95% CI=7.364-45.913, *p value*=0.000), and the Val/Val genotype 23.125 fold (OR=23.125, 95% CI=7.228-73.980, *p value*=0.000). Interestingly, CML risk elevations for the two genotypes are the highest so far reported.

Other studies using controls also agree with our results in linking heterozygous genotype distribution (CYP1A1 Ile/Val) with an elevated risk of CML. Taspinar and co-workers confirm this relationship in Turkey [22] and Achkar and co-workers likewise in Syria [11]. Additionally, under meta-analysis, Lu *et al* found an association between the CYP1A1*2C polymorphism and the risk of CML among Caucasians [5]. This polymorphism appears to also influence susceptibility to acute leukaemia [13, 25] and solid tumours, such as cancers of the breast [12], head or neck [26]. Studies conducted in Russia and Iran however, have reported no association between the CYP1A1*2C polymorphism and CML [25-27]. Additionally, a study conducted in India by Lakkireddy and co-workers contradicts our findings with a significant increase in Ile/Val heterozygous genotype presence in their control, indicating this genotype as playing a protective role against CML development [4].

Some studies elucidate the association of CYP1A1 polymorphism with responsiveness to TKIs. In this, a recent study concluded that one polymorphic allele of CYP 1A1 seemed to be frequent in Nilotinib responders and may have influence on drug response [6]. Additionally, Lakkireddy et al suggested the heterozygous (Ile/Val) genotype of CYP1A1*2C polymorphism may be a reliable predictor of response to imatinib therapy [4].

Some studies have also raised the possibility of an association of the CYP1A1*2C polymorphism with both tumour suppressor genes and oncogene mutations, through an increased formation of DNA adducts [11, 22]. The presence of the CYP1A1 Val allele increases enzyme activity and leading to an uncontrolled proliferation of haematopoietic cells, decreased differentiation, and reduced apoptosis of malignant haematopoietic blast cells [4]. Such incidences affect the ability to activate procarcinogens and are more likely to develop various cancers, including CML.

In this study, frequency and distribution of the CYP2D6 IM was significantly lower in cases with CML (OR=0.036, 95% CI=0.005-0.271, *p value* =0.001), suggesting that this genotype may have a prophylactic role in lowering risk. The CYP2D6*4 polymorphism is characteristic of the PM phenotype in reducing or eliminating CYP2D6 protein activity which, in turn, decreases procarcinogense activation and genotoxic metabolites formation, and consequently lowering the risk of CML development [13, 28]. However, there is statistically no significant difference in the homozygous mutant genotype (PM) presence between patients and the control but this may be due to the low frequency of control individuals carrying this genotype (6%). CYP2D6 genotypes were also reported to have a protective effect against other types of cancer including breast carcinoma [28] and papillary thyroid cancer [29].

Previous studies have reported that a variation in CYP2D6 enzyme activity contributes to a susceptibility to haematological malignancy, yet Chen *et al* showed no association between this polymorphism and the susceptibility to CML [7]. Earlier studies conducted in UK and Portugal however, associate the PM genotype and EM genotype with an increased risk of CML [30, 31]. The CYP2D6 PM has also been associated with an elevated risk of acute leukaemia, including AML and ALL [32]. And Sailaja *et al,* also found a slight increase of IMs in their CML group compared to their control [1].

The variation in findings between these genotypes can be attributed to differences in ethnicity, environment, lifestyle and variability among individuals in the metabolism of xenobiotic. It is universally understood however, that subjects with genotypes that alter the metabolism and detoxification of carcinogens are more likely to develop cancer.

The combined effect of CYP1A1*2C and CYP2D6*4 polymorphisms and CML risk were also studied. We found a significant interaction between CYP1A1*2C AA and CYP2D6*4 IM genotypes, indicating that carriers of this combination may have a protection against CML, as shown in **Table 2**.

It will be remembered that all patients in this study were in the chronic phase of the disease except one patient being in the accelerated phase. Thus, the effect of genetic polymorphism on the clinical phase was very difficult to confirm.

5. Conclusion

Our study suggests that CYP1A1*2C may increase CML susceptibility whereas the CYP2D6*4 variant may play a protective role among the Afro Arabic population of Sudan. Our current data is considered as the first published study to report the association between CYP1A1 and CYP2D6 polymorphisms and CML in Sudan, and it may provide a basis for further research and a more extensive study in this country. Further studies considering genetic polymorphisms in both phase I and phase II metabolizing enzymes have to be done in order to investigate the combined effect on the susceptibility to CML which may provide biomarker for early diagnosis/ prognosis and new target for therapy.

List of Abbreviations

ALL = Acute Lymphoid Leukaemia

AML = Acute Myeloid Leukaemia

CI= Confidence Interval

CML = Chronic Myelocytic Leukaemia

CYP1A1= Cytochrome 1A1

CYP2D6= Cytochrome 2D6

DNA = Deoxyribonucleic acid

EDTA= Ethylenediamine tetraacetic acid

EMs= Extensive Metabolisers

Ile = Isoleucine

IMs = Intermediate Metabolisers

MUT= Mutant allele

OR= Odd Ratio

PCR= Polymerase Chain Reaction

Ph = Philadelphia Chromosome

PMs= Poor Metabolisers

RFLP= Restriction Fragment Length Polymorphism

RICK = Radiation and Isotopes Centre

SPSS= Statistical Package for the Social Sciences

TKIs = Tyrosine Kinase Inhibitors

UM = Ultra-rapid metabolisers

Val =Valine

WT= Wild Type allele

Conflict of interest

No conflict of interest

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