DEBRISOQUINE HYDROXYLASE GENETIC POLYMORPHISM AND NEUROLEPTIC INDUCED ACUTE DYSTONIC REACTION AMONG PSYCHIATRIC PATIENTS, MUHIMBILI NATIONAL HOSPITAL

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine (Psychiatry) of the University of Dar–es-Salaam,

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar-es-Salaam a dissertation entitled: -

Debrisoquine hydroxylase genetic polymorphism and neuroleptic induced acute dystonic reaction among psychiatric patients, Muhimbili National Hospital, in partial fulfilment of the requirement for the degree of Masters of Medicine (Psychiatry).

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DEDICATION

This work is dedicated to my wife Agnes, my daughter Neema, my son Baraka and my parents the late Mr. Ramadhani Magimba and the late Halima Chambuso. Thanks parents you made me what I am today.

ABSTRACT

Acute dystonic reactions (ADR) are the earliest and most dramatic extra-pyramidal side effects resulting from neuroleptic therapy due to inhibitory effects on dopaminergic receptor activity in the limbic system. They usually occur within the first few days of starting treatment and are characterised by involuntary tonic and/or clonic contractions of muscle groups, especially those of the head and neck, producing intermittent or sustained abnormal postures. Common motor presentations of ADR are torticollic, trismus, blepharospasm and dystonias of the trunk and limbs. Metabolism is the major determinant of the pharmacological and toxicological effects of administered neuroleptic drugs that is in turn determined by cytochrome P450 (CYP450) status. There is an association between CYP450 genotypes and the clinical responses to neuroleptic drugs (phenotype) including occurrences of extra-pyramidal side effects, which are more pronounced in persons with poor metaboliser (PM) status.

To verify whether poor metaboliser (PM) of debrisoquine hydroxylase (CYP2D6) are susceptible to develop ADR in an in-patient sample of 98 persons with mental disorders (64 males and 34 females), allele status for CYP2D6*1, *2, *3, *4, *5 and *17 as well as geno duplication was determined by allele-specific PCR, long-PCR and restriction fragment length polymorphism analysis (RFLP). All the patients were screened and enrolled to the study if they developed ADR within two weeks of admission (N=49) and a sample matched by age, sex,

previous neuroleptic drug use and dosage that did not develop ADR in this time frame was enrolled as controls

The results revealed a predominantly young population of mean age 28.73±SD 6.95, a large proportion (n=60) clinically diagnosed to have schizophrenia, while 38 were suffering from bipolar affective disorder. A significantly higher proportion of patients with ADR had PM status (allele variants: CYP2D6*4/*4, *4/*5 and *5/*5) compared to those that did not develop ADR (chi. sq. = 9.5-19.39; p=0.00). On the other hand a higher proportion of patients that did not develop ADR had extensive metaboliser status (CYP2D6*1/*1 allele variant), compared to patients that developed ADR this variation being marginally significant at a chi. sq of 12.3 (p=0.05). The alleles noted to be most significantly associated with ADR were CYP2D6*17 and CYP2D6*5

CONCLUSION:

Patients that develop ADR were more likely to have mutant variants of the CYP2D6 gene that confers poor metaboliser status through decreased capacity/ activity of the enzyme, which may explain their increased susceptibility to adverse effects of neuroleptic drugs. Extensive metabolizer status (CYP2D6*1/*1) was protective against developing side effects of neuroleptic drugs. These findings suggest need to explore neuroleptic drug doses best suited to the Tanzanian context to allow for the best therapeutic effects with the smallest degree of discomfort due to drug side effects that is a major barrier to drug treatment adherence. It is also recommended that knowledge of the genotype and phenotype status of CYP2D6 that only needs to be done once in a patient's life can go a long way towards individualising therapy. Analysis of the polymorphic status of drug metabolising enzymes coupled with pharmacokinetic studies in Tanzania populations can be a good basis for optimal individualised therapy, which may in turn increase adherence by patients to prescribed drugs.

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ABBREVIATIONS

ADR	- Acute dystonic reaction
AIMS	- Abnormal involuntary movement
BPRS	- Brief Psychiatric Rating Scale
CYP450	- Cytochrome P450
CTP2D6	- Debrisoquine hydroxylase
DNA	- Deoxy neuleoic Acid
EMS	- Extensive metabolisers
GST	- Glulathione-s-Transfarase
IMS	- Intermediate metaboliser's status
MNH	- Muhimbili National Hospital
NAT	- N-acetyl transferase
PMs	- Poor metaboliser's status
PCR	- Polymerase chain reaction
RFLP	- Restriction fragment length polymorphism
SHRS	- St Hans Rating Scale

1.0 Introduction

Acute dystonic reactions (ADR) are the earliest and most dramatic extra-pyramidal side effects resulting from neuroleptic therapy due to inhibitory effects of dopaminergic receptors (activity) in the limbic system[1]. They usually occur within the first few days of starting treatment and are characterised by involuntary tonic and/or clonic contractions of muscle groups, especially of the head and neck, producing intermittent or sustained abnormal postures. Common motor presentations of acute dystonic reaction are torticollis, trismus and blepharospasm, while dystonia of the trunk and limbs, which are less common[1]. An imbalance or critical relationship between increased dopamine release and turnover and dopamine receptor blockage may account for ADR[2]. In normal clinical use, the prevalence of acute dystonic reactions in Western populations has been reported to range from 2% to 10%[3,4], but higher frequencies have been described in small groups of high-risk patient(s). The occurrence of acute dystonic reactions is influenced by patient parameters of age and sex as well as drug factors of neuroleptic type, potency and dose[2]. Drugs acting on the central nervous system show great inter-individual and inter-ethnic variation in effect. Factors responsible for this variability include the extent of drug-protein binding; metabolism to active and inactive metabolites; and variability in amount or sensitivity of drug-target receptors.

2.0 Statement of the problem

Acute dystonic reactions usually occur within the first few days of starting neuroleptic drug treatments. They occur at a significant prevalence at drug doses in use in normal clinical settings. This often generates amongst the mentally ill a negative view of drugs and their effects that can be detrimental to adherence to prescribed drugs, a factor that is important for the prevention of relapses of psychotic conditions in the future. The occurrence of acute dystonic reactions is influenced by amongst other factors inter-ethnic variations in effects of neuroleptic drugs. Factors responsible for this variability range from extent of drug-protein binding, metabolism to active and inactive metabolite and variability in amount of or sensitivity of drug-target receptors. Metabolism is the major determinant of the pharmacological and toxicological effects of administered drugs that is, in turn, determined by cytochrome P450 (CYP450) status, a major hepatic enzyme in the metabolism of neuroleptic drugs. Few studies in the African context make associations between CYP450 genotypes and the clinical responses to drugs (phenotype) including occurrence of extra-pyramidal side effects. Results of associations such as these can assist mental health care providers working with African patients to more suitably titrate neuroleptic drug dosages for the Tanzanian population; dose optimisation for therapeutic effect with minimal side effects.

3.0 Literature review

3.1 Metabolism, pharmacological and toxicological effects of drugs

Metabolism is the major determinant of the pharmacological and toxicological effects of administered drugs. Most psychotropic drugs are lipophilic which enables them to cross blood-brain membrane barriers to reach their target receptors. Metabolism to more water-soluble products is necessary for their elimination and failure to do so can lead to drug accumulation, which is associated with the intensification of adverse effects such as neuroleptic induced ADR.

The major enzyme systems involved in the bio-transformation of drugs are cytochrome P450 (CYP), Glutathione-S-transferase (GST), UDP-glucoronyl transferase, sulfanotransferases and N-acetyl transferases (NAT) which are mainly found in the liver although they are also present in other tissues at lower amounts. Environmental and genetic factors affect the activities of these enzymes, which can lead to enhanced or decreased drug biotransformation and disposition[6].

Considerable international interest, shown by growth of the field of pharmacogenetics has focused in the past 10-20 years on genetic factors affecting the efficacy of drugs. Mutations in gene coding for a drug metabolising enzyme can give rise to enzyme variants with higher, lower or no activity. If the mutant allele occurs with a frequency of at least 1-2% in the normal population and causes a different drug response or phenotype this phenomenon is termed a pharmacogenetic polymorphism[6]. Polymorphisms

in drug metabolism divide the population into at least two phenotypes, extensive and poor metabolisers[6].

Polymorphism has been detected in many drug metabolising enzymes at both genotypic and phenotypic levels. Two of the most well studied and best-characterised polymorphisms are those for debrisoquine-4-hydroxylase (CYP2D6) and S-mephenytoin hydroxylase (cytochrome CYP2C19)[8], both being very important enzymes for the metabolism of neuroleptic and other psychoactive drugs.

CYP2D6 phenotype investigations in Caucasians have consistently shown that 5-10% of this population are poor metabolizers (PMs) [12]. Brosen[15] noted a two to five times difference between poor and extensive metabolizers in the capacity to metabolise CYP2D6 substrates; hence PMs obtain the same steady state serum levels as extensive metabolizers (EMs) at doses which are 50-80% lower than those given to EMs. Studies comparing the status of these enzymes in Caucasians and Orientals have clearly demonstrated inter-ethnic genetic differences, which could explain the higher dose requirements to achieve therapeutic responses for CYP2D6 substrates in the former compared to the latter[9, 10].

Fewer studies have been conducted to determine CYP450 polymorphism in the black African populations of sub-Saharan Africa. In a review of studies conducted in the continent, it is evident that the African population is heterogeneous, with regards to these polymorphisms with a prevalence of

CYP2D6 PMs that ranges from 0-19 %[11]. While the allelic variants CYP2D6*4,*5, and 10 (poor metabolizer alleles), are common across most ethnic groups; variants more common for the African continent are CYP2D6*5 and 10, while the allelic variant CYP2D6*17 has only been detected in black African populations[11], with prevalence rates of 9% (n=122) in Ethiopian populations[23], 20% (n=212) and 30% (n=176) amongst normal healthy Tanzanian subjects and Tanzanian psychiatric patients respectively, and 34%(n=104) in Zimbabwean subjects[22].

3.2 Implications of pharmacogenetic polymorphism in use of neuroleptics

Cytochrome P450 2D6 (CYP2D6) has been shown to metabolise most neuroleptics and antidepressants currently in use[6]. Studies on the clinical implications of differences in the rate of metabolism of neuroleptics in Caucasoid (Europeans and white North Americans) and Oriental (Chinese, Japanese and Koreans) populations[9, 13,14], indicate important clinical implications with a lower therapeutic effect being evident for ultra-metabolizers (predominantly Caucasians) and moderate to severe side effects for poor metabolizers (predominantly Orientals).

Antipsychotic-induced extra-pyramidal adverse effects continue to be a serious problem in the treatment of psychotic disorders. Acute dystonic reactions, which occur during the first days of treatment, are often distressing and frightening for the patient, and can be life threatening with laryngeal involvement, which fortunately can be reversed with anticholinergic drugs. These effects have long term implications on adherence to treatment that also warrants attention to ensure relapse prevention in patients that are required to remain on treatment long-term. Much recent research has focused on improving the ability to use available pharmacotherapy in the most effective and least toxic manner[16]. This remains the best option in many low-income countries where the advantages of the growing use of the new atypical antipsychotic drugs in reducing the rate of acute dystonic reactions cannot be fully appreciated due to the price tag attached to these newer drugs.

Aguilar et al[17] noted 23 out of 62 (37%) first-episode psychotic patients who were receiving neuroleptics developed dystonia after treatment with haloperidol, this reaction being significantly associated with young age, severity of illness and negative symptoms at baseline. A more extensive study of 646 (41% female) consecutive admissions[18] yielded an overall prevalence of ADR of 5.3%; the prevalence being higher in patients on neuroleptics alone (8.5%) versus those on neuroleptics combined with anticholinergic (anti-Parkinsonian) medication (2.5%). The use of prophylactic anticholinergic drugs in patients on neuroleptics is controversial. Although their efficacy in treating extra pyramidal side effects is not questionable, controversy surrounds when to initiate therapy and for how long. A review of the few double-blind, placebo controlled trials on the value of concurrent anticholinergic medication use at the outset of treatment with neuroleptics, revealed evidence to support the use of these agents to prevent ADR. Methodological deficiencies in many of the studies, prevents them from being conclusive[19].

To determine whether poor metabolizers are more susceptible to acute dystonic reactions, Armstrong et al[20], in a descriptive study of 76 schizophrenics noted a lack of difference in genotype frequencies between schizophrenics and a control group, suggesting CYP2D6 genotype was not a factor in determining susceptibility to the disease. They however noted that four of the five PMs were suffering from a movement disorder at the time of study. In a larger sample of 112 schizophrenics, 52.9% experienced extra-pyramidal side effects; 4 of the schizophrenics (3.4%) that carried two

detrimental alleles, were classified as PMs, and all had a history of extra pyramidal side effects. There was no significant difference in the allele frequencies of extensive metabolizers (54.6% homozygous for a functional CYP2D6*1 allele and 37% heterozygous for detrimental alleles) and ultra-rapid metabolizers in those that experienced side effects compared to those that did not[21]. Because of the low numbers involved of PMs, in both of the reported studies, it is not possible for the authors to be emphatic on a conclusion that PMs have a greater susceptibility to neuroleptics induced extra pyramidal side effects.

3.3 Rationale of the study

There is less information on the neuro-pharmacotherapeutic implications of CYP2D6 polymorphism in African populations. There is no retrievable documentation of therapeutic or toxicological responses to drugs acting on the central nervous system, which are substrates of CYP2D6. Furthermore, there is no retrievable documentation on associations between phenotypic and genotypic characteristics in persons using such drugs. The proposed study hopes to address this imbalance by investigating the association between the development of acute dystonic reaction and polymorphisms in CYP 2D6.

3.4 Hypothesis

Poor metabolizers of neuroleptics classified on the basis of mutant alleles of CYP2D6 will have a higher prevalence of ADR.

3.5 Broad objective

To determine any association that exists between CYP2D6 genotype and its possible phenotypic expression using as a proxy of phenotype expression the presence or absence of ADR, amongst psychiatry patients receiving neuroleptic medication.

Specific Objectives

3.5.1 To determine debrisoquine-4-hydroxylase (CTY2D6) genotype among psychiatric patients who develop acute dystonic reactions following

treatment with oral neuroleptic drugs chlorpromazine, haloperidol and thioridazine.

3.5.2 To determine debrisoquine-4-hydroxylase (CTY2D6) genotype among age, sex, and drug dose matched sample of psychiatric patients who do not develop acute dystonic reactions following treatment with oral neuroleptic drugs, chlorpromazine, haloperidol and thioridazine.

4.0 METHODS AND MATERIALS

4.1 Study design

This was a hospital based cross-sectional comparative study which was conducted at the Psychiatry Unit of the Muhimbili National Hospital, Dar-es-salaam Tanzania, between October 2001 and April 2002. The total duration of the study was seven months.

4.2 Description of study area

Dar-es-Salaam is Tanzania's largest city and one of the commercial centres in East-Africa. The population has grown rapidly from around 450,000 in 1973 to 1.2 million in 1988, with a projected population of 3.6 million by the year 2000[42].

Dar-es-salaam City is divided into three administrative municipalities namely Ilala, Temeke and Kinondoni. The region has a hot and humid tropical climate with average temperatures around 23°C in the cold season (June to September) and 27°C in the hot season (December to March). There are two rainy seasons, the long rains from March to May, also known as the harvesting season and the short rains from October to December also known as the seeding period. While Kiswahili and English are official languages in the country, Kiswahili is more commonly used in daily communication by the larger majority of Tanzanians including residents or Dar-es-salaam. The Muhimbili National Hospital located in Ilala municipality is a tertiary referral hospital. It has a 70 bed psychiatry department with ongoing and active research programs. The major drugs available for treatment of psychotic symptoms in the clinical setting of the study include chlorpromazine, haloperidol and thioridazine.

4.3 Target population

The target population comprised of all patients admitted to the psychiatry unit of the Muhimbili National Hospital, with psychotic symptoms that required anti-psychotic medication. The facility admits on average 1,500 patients per annum, the majority having psychotic symptoms. The study sample was recruited from this population, using a consecutive admissions selection approach over a five-month period.

4.4 Sample size

The estimated sample size was computed using EPI-INFO Version 6 taking into consideration the following assumptions:

1) The prevalence of acute dystonic reactions in patients admitted with psychotic symptoms was estimated at 10% (as there is no prophylactic anti-cholinergic treatment policy in the setting of the study).
2) An allele frequency of CYP2D6*17 among patients with ADR of 60%.
3) An allele frequency of CYP2D6 among patients without ADR of 30 %.
4) A Power of 80% and Confidence interval of 95%.
5) An equal sample size for patients with ADR and patients without ADR.

Using these assumptions and EPI-INFO Version 6 a minimum sample size in each group of 49 was derived where by 49 patients would be those that develop ADR within two weeks of in-patient status and 49 patients were controls (patients that did not develop ADR within this time frame). With an assumed prevalence of ADR in psychotic patients on medication in a hospital setting of 10%, a minimum of 490 consecutively admitted patients would have

to be screened for movement disorder following neuroleptic medication, to achieve the study samples.

4.5 Instruments

4.5.1 Screening instruments:

Socio-demographic and drug related information: A structured interview schedule collected information on age, gender, and ethnicity, level of education, marital status, employment, and staple food in diet. Information was also collected on the duration since onset of illness, diagnosis of illness and first use of prescribed neuroleptic drugs; furthermore the types and doses of neuroleptic and anticholinergic drugs used in the two week period prior to admission and at time of interview were inquired into.

The Brief Psychiatric Rating Scale (BPRS): This is a standardized screening instrument that is used to rate psychopathology in patient populations[26]. The BPRS is a rating scale that has been used in various cultural settings, and has demonstrated good reliability (Cronbach's alpha range = 0.7 - 0.9), even when used by less experienced raters[27]. It also compares favourably with other measures of psychopathology such as the Brief Symptom Inventory[28], suggesting good construct validity.

The St. Hans Rating Scale (SHRS): This is a multidimensional rating scale for evaluation of neuroleptic induced hyperkinesias, Parkinsonism, akathesia and dystonia. Good convergent and divergent validity has been demonstrated with this instrument against the Abnormal Involuntary Movement Scale (AIMS). It

has demonstrated high inter-ratter reliability (percent agreement 82 – 97%) when used with experienced and less experienced interviewers[29].

Instrument translation: A back translation approach supported by a panel discussion following back translation from Kiswahili to English was used to ensure transfer of conceptual meanings on the various scales; during their translation from English to Kiswahili. The Brief Psychiatric Rating Scale (BPRS) and The St Hans Rating Scale (SHRS) were translated to Kiswahili by the researcher. Indigenous research assistants who would be familiar with the local nuances of the Kiswahili language and had no access to the original English versions of the instruments conducted the back translation to English. A panel of three residents met as a team and reviewed the two English versions of the instruments and used the ensuing discussion to refine the Kiswahili translation to ensure adequate transfer of the concepts contained in these two standardized screening instruments. Further adjustments in the translated Kiswahili versions were made after the instruments were piloted amongst 20 psychiatric patients who were not recruited to the study. These measures were taken to ensure both construct validity and comprehension of the questions that would be asked in the intended study population.

4.6 Data collection procedures

4.6.1 Interviews

Recruitment: A total of 500 consecutive psychiatry admissions of patients with psychotic symptoms who have previously been admitted to the psychiatry unit of the Muhimbili National Hospital in Dar es Salaam were recruited to the

study after being informed of the purpose of the study and agreeing to participate by signing a prepared informed consent forms (appendix 2).

Socio-demographic and neuroleptic drug use related a trained research assistant collected information at recruitment. Furthermore baseline ratings for movement disorder were conducted, using the SHRS and psychopathology assessed using the BPRS. The researcher administered the SHRS and BPRS.

Follow-up assessments: To avoid observer bias the researcher on the ward conducted follow-up ratings of movement disorder. Ratings for movement disorder and psychopathology were conducted on the third day post admission when or shortly after the patient was received to a general ward from the acute ward (patients are contained in the acute ward for 24-48 hours). Further follow-up movement disorder ratings occurred after five days, ten days and fourteen days from the first rating. A recruitment register was used to assist in keeping track of all required assessments for data collection.

Data quality checks were conducted between the researcher and research assistant initially on a daily basis (first month) and later twice weekly. Recruited patients were enrolled to the study on the basis of presence or absence of neuroleptic induced movement disorder at any point of movement disorder rating. The researcher conducted all the enrolments.

*Enrolment of patients with and without movement disorder: T*hose patients who screened positive for any neuroleptic induced movement disorder, at any

point of assessment for movement disorder as defined by the SHRS were enrolled into the study to derive a sample of patients with acute dystonic reactions following treatment with oral neuroleptic drugs. For each study subject enrolled, a control was selected from the recruitment register amongst those that screened negative for movement disorder at all points of assessment. The controls were matched as closely as possible for age, sex, duration of neuroleptic medication and where possible ethnicity. At enrolment, after obtaining verbal consent from the patients with and without ADR, 5mls of blood was drawn from the left (right for left handed individuals) ante-cubital vein and frozen at -20 degrees centigrade within 3-4 hours as per protocol for PCR.

4.6.2 Exclusion criteria

All patients on depot neuroleptic medication were excluded from recruitment to the study. Patients with severe kidney, liver, organic brain disease, neurological disease or chronic disease and those with smoking or alcohol drinking habits or drug abuse were also excluded at recruitment. Patients too disorganised to understand the risks and benefits of the study at recruitment were excluded for ethical reasons.

4.6.3 Laboratory procedures

4.6.3.1 Chemicals

Most of the biochemical reagents used in this project were bought from Sigma (St. Louis, MO, USA) and these include: ethylenediaminetetraacetic acid

(EDTA), N-lauroylsarcosine, bromophenol blue, ethidium bromide, orange green, xylene cyanol FF, Tris-HCl, Triton X-100 (t-octylphenoxypolyethoxyethanol), guanidin-isothiocyanate, ethanol, sodium chloride, sodium acetate, ammonium acetate, sodium dodecyl sulphate (SDS), phenol, chloroform, iso amylalcohol, potassium chloride (KCl), Trizma base (Tris [hydroxymethylaminomethane), agarose (low melting point), 8-hydroxyquinoline, boric acid, acetonitrile, nylon membrane (0.45 μm), phosphorus acid, ammonium sulphate, sodium azide, ammonia, acetic acid, ficoll, gelatin and mineral oil. National Diagnostics (Harare, Zimbabwe), Taeuber and Corssen (Pvt) Ltd (Harare, Zimbabwe), Harvest Medical and Scientific (Pvt) Ltd (Harare, Zimbabwe), supplied most other reagents including, sucrose, ethanol, sodium hydroxide, phosphoric acid, potassium di-hydrogen phosphate, di-sodium hydrogen phosphate, sodium chloride, phosphorus acid, ethanol, methylated spirit and phenol. Nujol mineral oil was bought from Perkin Elmer (USA) and agarose LMP was from Pharmacia Biotech AB (Uppsala, Sweden).

4.6.3.2 Molecular biology reagents

Molecular biology reagents used in this project were bought from Roche Molecular Diagnostics (Randburg, South Africa) and these include: proteinase K, RNase, glycerol, ethidium bromide, DNA molecular markers, RNase (DNase free), deoxynucleoside triphosphates (dATP, dCTP, dGTP, dTTP), Expand™ Long Template PCR system, agarose LE, mineral oil, restriction enzymes *Alu* I, *Sma* I, *Bam* HI, *Taq* I, *Asp* 718, *Rsa* I, *Msp* I and *Pst* I. The restriction enzyme *Alw26* I was obtained from Promega (Wisconsin, USA).

4.6.3.3 Extraction of DNA from frozen blood

The method was adapted from Gustafson *et al.*[30]. Each frozen blood samples was thawed at room temperature water bath and transferred to a clean polypropylene tube where one volume of phosphate-buffered saline (PBS – 1mM KH_2P0_4, 154 mM NaCl, 5.6 mM Na_2HP0_4, pH 7.4) was added. The mixture was centrifuged and the supernatant carefully poured off. The pellet was suspended in half the original volume of blood and mixed well. Each sample was digested with Proteinase K (500 µg/mL of original blood volume) and RNase A (75 µg/mL of original blood volume) in the presence of SDS (final concentration of 0.5%) at 37°C for 2 hours.

After incubation, an equal volume of phenol was added to each sample followed by centrifugation. The upper phase was transferred to a clean tube and phenol extraction repeated. An equal volume of chloroform (chloroform: isoamylalcohol, 24:1) was added to the aqueous phase and centrifuged. The upper aqueous phase was transferred to another tube. The volume of each sample was determined and sodium chloride added to give a final concentration of 0.1M.

Two volumes of cold (–20°C) 95% ethanol were added to each sample and gently mixed by inverting. Overnight incubation at 4°C of the sample facilitated precipitation of DNA. The samples were centrifuged, supernatants decanted and pellets washed with 70% ethanol. The pellets were air dried before being re-suspended in one-tenth the original blood volume of Tris-EDTA (TE-10mM Tris/ 1 mM EDTA). The DNA was kept at 4°C for PCR-RFLP analysis.

4.6.3.4 Genotyping of cytochrome P450 2D6

Allele specific PCR was used for the amplification of *CYP2D6**2, *CYP2D6**3, *CYP2D6**4. The exon 6 mutation (^{2938}C to T) was used to identify the presence of the *CYP2D6*2* allele by discounting all the samples with an additional mutation in exon 2 (^{1111}T to C) which is indicative of *CYP2D6**17. The PCR analysis of the exon 2 mutation identified presence of *CYP2D6**17[31], while PCR analysis of the CYP2D6*5 polymorphism was performed according to the method of Steen *et al.*[31].

The PCR incubation mixture constituents in a total volume of 25 μl were: 200-300 ng of genomic DNA (1-2 μl), 0.25 μM of each primer (0.625-1 μl each), 0.2 mM of each de-oxynucleotide triphosphates (total volume of 4 μl), 0.625 units of *Taq* polymerase (0.125 μl) and 2.5μl of X10 PCR buffer (Tris-HCl pH 8.3, 50 mM KCl). The amplification of different genes required specific $MgCl_2$ concentrations in a volume of 5 μl.

The general cycling conditions were: an initial denaturation step for 4 minutes at 94°C. A specific number of cycles with a denaturation step of 1 minute at 94°C, a specific primer annealing temperature from 0.5 to 1 minute, a specific extension temperature for 0.5 to 1 minute. The last cycle was followed by a 7-minute extension step at 72 °C. The PCR product (15 μL) was analyzed on a 2% agarose gel stained with ethidium bromide.

Identification of the *mEH* 139Arg mutant variant required the digestion of the PCR product with the restriction enzyme *Rsa* I. The digestion mixture consisted of 10 μl of the PCR product, 4 units of *Rsa* I (0.5 μl of the 10U/μl stock), 4 μl of the X10 restriction enzyme buffer and made up to 40 μl with water. The mixture was incubated at 37°C overnight. The mixture was concentrated by vacuum centrifugation, after which the sample was analyzed on 3% agarose gel stained with ethidium bromide.

The C^{-1496} G mutation (sequence numbering is according to Kimura *et al.*[32]) in the 5'-flanking region of the CYP2D6 gene[33]. Part of the 5'-flank and exon 1 of the CYP2D6 gene was amplified using the forward primer 5'-GAA TTC AAG ACC AGC CTG GAC-3' and reverse primer upr1669[33]. The amplifications were run in the presence of 1.0 mmol/L magnesium chloride and performed in a Perkin Elmer Thermocycler 2400 as described by Raimundo et al.[33]. This fragment was subsequently used as template in an allele-specific PCR reaction using the wild type forward primer 5'-ACT TGG AAG AAC CC-3', the mutant forward primer 5'-ACT TGG AAG AAC CG-3' and the reverse primer 5'-CGG AGA TTT CCT CT-3'. The amplifications were run as described for the detection of the G^{1747} to A[34]. The reactions were run in the presence of 1.4 mmol/L magnesium chloride.

4.6.3.5 Detection of CYP2D6*17

The procedures followed were those according to Masimirembwa et al.[35]. Exon 1 and 2 were amplified from genomic DNA using CYP2D6 specific primers Pr9B and Prexon2R. Thirty-five cycles were run with annealing

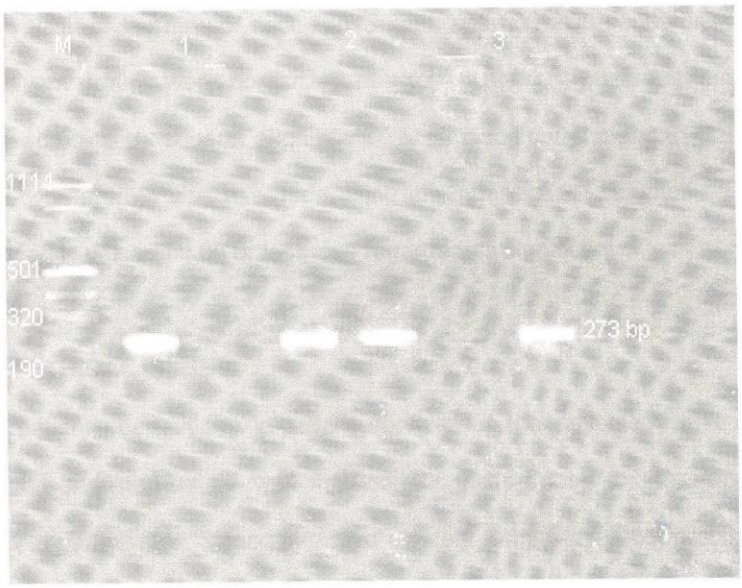
temperatures at 60 degrees and 0.8 mM magnesium chloride in the incubations. A second allele-specific set of PCRs was run on the product of the first PCR for 15 cycles at 60 degrees annealing temperature and 0.6 mM magnesium chloride. All PCR products were analyzed on 1.5% agarose gels stained with ethidium bromide. The primer sequences were:

Pr 9B 5' ACCAGGCCCCTCCACCGG – forward primer

Pr ex2R 5' CTCTCTGCCCAGCTCGG – reverse primer

Thermocycler type: Perkin Elmer DNA Thermocycler 480, Molecular weight marker VIII and X from Roche Diagnostics South Africa was used whereby the house keeping gene used was beta-atin.

Figure 1. Allele specific genotyping of the $C^{1111}T$ mutation indicative of *CYP2D6*17*.



Key: M, molecular weight marker; sample 1, *CYP2D6*1/*1*; sample 2, *CYP2D6**1/*17; sample 3, *CYP2D6**17/*17.

In the first PCR, a gene specific fragment of *CYP2D6* was amplified by PCR; the $C^{1111}T$ mutation was amplified in the second PCR using mutation specific and wild type primers. Each sample was run in duplicate both tubes having one common primer. In the first tube the second primer contained the wild type sequence at the site of mutation while in the second tube the second primer contained the sequence of the mutant.

4.6.4 Statistical analysis

Computerised data entry was conducted using EPI-Info Version 6. Data cleaning was conducted at the end of data entry. Data were converted to

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SPSS version 10 for further analysis. Frequency counts and preliminary cross tabulations of socio-demographic characteristics, percent endorsement (at any level of severity) of each BPRS item, and CYP2D6 genotypes for the two patient groups were conducted and significance of observed variations assessed using chi-square. Significance of variation in the mean BPRS score in the two samples was assessed using the independent samples t-test. A one tailed P-value of 0.05 or less was regarded as significant.

5.0 ETHICAL ISSUES

Each patient seen at the in-patient services of the psychiatry unit of the Muhimbili Medical Centre was informed of the purpose of the study and invited to participate. Signed informed consent forms were obtained from all subjects (attending relatives witnessed signature for patients who were symptomatic at the time of consent). Verbal consent sufficed for blood sample collections. It was made clear to all patients and relatives that refusal to participate in the study, would not jeopardise the quality of psychiatry care they received at the facility.

Aseptic techniques were ensured during the collection of all blood samples. Recruitment forms, which included patient's names and hospital case note number, were stored by the research team in a secure environment. All data source records were only used for purposes related to the study and kept in secure storage, where they will remain for at least five years after completion of data analysis before being destroyed, as a measure to protect confidentiality.

6.0 RESULTS

6.1 Patient demographic characteristics

From October 2001 to April 2002, a total of 500 patients admitted to th psychiatry general wards were recruited for the study.

Table 1: Distribution of patients with and without ADR by demographi characteristics

Demographic characteristic	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	X^2	P-value
Age:				
Below 24 years	15 (30.5)	13 (26.5)	0.20	0.67
Above 24 years	34 (69.4)	36 (73.5)		
Mean Age (±SD)	28.73 ± 6.95	28.69± 6.13		
Sex:				
Male	32 (65.3)	32 (65.3)	0.00	1.00
Female	17 (34.7)	17 (34.7)		
Marital Status:				
Single	35 (71.4)	33 (67.3)	0.19	0.66
Marriage Monogamy	14 (28.6)	16 (33.7)		
Education Status:				
No Education/ below Std VII	32 (65.3)	35 (71.4)	0.43	0.53
Above standard VII	17 (34.7)	14 (28.6)		
Employment Status:				
Employed and self-employed	22(44.9)	23 (46.9)	0.04	0.84
Unemployed	27 (55.1)	26 (53.1)		
Residential Locality:				
Urban	10 (20.4)	11 (22.4)	0.06	0.81
Rural/per urban	39 (79.6)	38 (77.6)		
Monthly average	60,750±40.4	54,269±35.8	0.05	0.82

Of these 98 (19.6%) were enrolled to the study, 49 of whom developed an acute dystonic reaction within two weeks of admission. The remaining 49 were age, gender and duration of previous antipsychotic drug use matched controls recruited during the same time frame.

Of the 98 participants enrolled seventy (71.4%) were males, the mean age for patients with ADR was 28.73 $\pm$ 6.95 (1 SD) and 28.69$\pm$ 6.13 (1 SD) for those without ADR. The demographic characteristics of the study population are summarised in Table 1. The whole sample was comprised of the Bantu ethnic group (not in table), were a predominantly a young adult population of low socio-economic status (as defined by average monthly income) and education level (just about a third having attained more than seven years of formal education). The ADR group of patients did not differ significantly from those that did not develop ADR in marital status, education level, average monthly income or residential locality in the year prior to admission.

6.2 Previous neuroleptic drug use and ADR

Past neuroleptic drug use was explored amongst patients with ADR and controls with respect to type and average dose of neuroleptic used in the two weeks prior to the current in-patient stay. Table 2 summarises the distribution of types of drugs used and mean doses amongst patients with ADR and controls in the two weeks prior to their admission to the psychiatry unit.

The commonest drug used in the two weeks prior to admission to hospital by both patients with ADR and controls was haloperidol, reported by 67.3% and

65.3% respectively. Chlorpromazine and thioridazine were the other two drugs reported to have been used. None of the patients that developed ADR or the controls reported concomitant use of more than one antipsychotic drug during this period of time. There were no significant differences in the distribution of drugs used between the patients that developed ADR or controls.

Table 2: Distribution of type of neuroleptic and anti-Parkinsonian drug used and mean daily doses amongst patients with ADR and controls in the two weeks prior to admission

Type of drug and mean daily dose	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	X^2 t-test	P-value
Haloperidol				
Frequency	33(67.3)	32(65.3)	0.05	0.83
Mean dose (mg)	8.38±1.92	8.29±1.96		
Chlorpromazine				
Frequency	10(20.4)	12 (24.5)	0.23	0.63
Mean dose (mg)	360±89.44	360±89.44		
Thioridazine				
Frequency	6(12.2)	7(14.3)	0.09	0.77
Mean dose (mg)	340±96.61	340±96.61		
Phenergan				
Frequency	6 (12.2)	7 (14.3)	0.09	0.77
Mean dose (mg)	50±0	50±0		

Daily drug doses for haloperidol were on average just over 8 mg for both patients that developed ADR as well as controls, while the average daily dose for chlorpromazine and thioridazine ranged between 340-360 mg in both

groups of patients. The differences in the average daily drug doses in the two weeks prior to admission between patients that developed ADR and controls for all the antipsychotic drugs were not statistically significant (independent t-test).

Phenergan was the only antiparkinsonian drug reported to have been used in the two weeks period prior to admission. 12.2% and 14.3% of patients with ADR and controls reported Phenergan use at an average daily dose of 50 mg respectively.

6.3 Duration since onset of mental illness, first use of antipsychotic drugs and past experiences with drug side effects

The duration since onset of mental illness and since first use of antipsychotic drugs as well as drug adverse effects experienced during the first episode of mental illness were inquired into. The summarised findings are reported in Table 3.

Table 3: Distribution of duration since onset of mental illness and first use of antipsychotic drugs and previous experiences of adverse drug effects amongst patients with ADR and controls

Past illness and drug use characteristic	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	X^2	P-value
Duration since onset of mental illness (years)				
0-2	21 (42.5)	22 (44.9)	0.04	0.84
> 2	28 (57.1)	27 (55.1)		
Duration since first use of antipsychotic drugs (months)				
36	24 (49.0)	27 (55.1)	0.54	0.37
> 36	25 (51.0)	22 (44.9)		
Previous experience with adverse drug effects				
Yes	47 (96.0)	1 (2.0)		
No	2 (4.0)	48 (98.0)		

The duration since onset of mental illness was defined as the duration in years since the patient was initially aware that they had suffered a mental illness. Duration was categorised into two categories 0-2 years and above 2 years. As can be seen in Table 3, more than half of both patients that developed ADR and controls had been aware they had a mental illness for a period of more than two years. Fifty seven percent of patients that developed ADR had been aware of the presence of mental illness for more than two years compared to 55.1% of controls though this variation was not statistically significant.

More than half of the patients with ADR had first used antipsychotic medication more than 36 months prior to interview, compared to 45% of the controls. This differences was however not significant. Almost all of the patients with ADR however reported previous adverse drug effects related to antipsychotic use compared to only 2% of the controls. The types of adverse effects of drugs reported are summarized in Table 4.

Table 4: Distribution of types of drug adverse effect experienced during the first episode of illness as a result of drug treatment amongst patients with ADR

Type of drug adverse effect	N=49 n (%)
Body stiffness	15 (30.0)
Dribbling of saliva	11 (22.4)
Tremors	8 (16.3)
General weakness	7 (14.3)
Tongue protrusion	8 (16.3)

The most frequent type of side effect reported to have been previously experienced as a result of antipsychotic medication use in the group of patients with ADR was body stiffness (30%) followed by dribbling of saliva (22.4%), general body weakness (14.3%) and tongue protrusion (12.2%). Only one of the controls reported an adverse drug effect that was body stiffness.

6.4 Current psychopathology amongst patients with ADR and controls

Based on clinical assessment the ICD-10 diagnosis was derived for each of the patients with ADR and controls. In addition, individually endorsed items on the BPRS as well as the total number of endorsed items for patients with ADR and controls were compared.

Table 5: Type of mental disorder based on clinical assessment for ICD – 10 criteria among patients with ADR and controls

Diagnosis	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	Chi sq (p-value=)
Schizophrenia	32 (65.3)	28 (57.2)	0.44 (0.51)
Bipolar disorder	16 (32.6)	20 (40.8)	
Major depression	1 (2.0)	1 (2.0)	

Table 5 summarizes the ICD-10 diagnoses in the patients with ADR and controls. Schizophrenia and bipolar disorder were the two most frequent functional psychotic conditions elicited, none of the patients having a diagnosis of an organic psychosis (This was also an exclusion criteria). A larger proportion (61.2%) of the patients across both groups had a schizophrenic illness, with bipolar disorder being the second most common diagnosis. There was no statistical difference in the variations in diagnosis amongst patients with ADR and controls.

Table 6: Distribution of BPRS items endorsed at any level of severity amongst patients with ADR and controls

BPRS items	Symptom/sign present at any level of severity		
	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	X^2; p-value
Abnormal movements (not EPS)	35 (71.4)	28 (57.1)	1.0; 0.5
Guilty feelings	32 (65.3)	26 (53.1)	1.0; 0.5
Elicited disorganised thoughts	30 (61.2)	30 (61.2)	0.0; 0.58
Observed tension	29 (59.2)	26 (53.1)	0.37; 0.34
Unusual thoughts	29 (59.2)	28 (57.1)	0.04; 0.5
Belief has special abilities	28 (57.1)	22 (44.9)	1.47; 0.16
Hostility	27 (55.1)	20 (40.8)	2.00; 0.11
Observed reduced emotional tone	27 (55.1)	26 (53.1)	1.0; 0.5
Depressed mood	26 (53.1)	20 (40.8)	1.48; 0.16
Hallucinations	26 (53.1)	28 (57.7)	0.17; 0.42
Decreased concentration	26 (53.1)	25 (51.0)	0.04; 0.5
Observed emotional withdrawal	24 (49.0)	25 (51.0)	1.0; 0.5
Suspicion	24 (49.0)	23 (46.9)	0.04; 0.5
Decreased Energy	23 (46.9)	28 (57.1)	1.02; 0.21
Worry, fear or anxiety	21 (42.9)	20 (40.8)	1.0; 0.5
Somatic concern	19 (38.8)	20 (40.8)	1.0; 0.5
Mean BPRS score (mean number of items endorsed)	8.71±5.23	8.04±5.17	0.04;0.84 [a] (-2.7-1.49)

Key: [a] Independent samples t-test F value; p-value-one-tailed and (95% CI of difference)

The BPRS items with the highest frequency endorsed amongst patients with ADR were abnormal movements (abnormal gestures and postures) that excludes movements that could be due to extrapyramidal side effects (EPS) or simple heightened motor activity; followed by guilty feelings and

disorganized thinking. Somatic concern was the least frequent symptom endorsed amongst both patients with ADR and matched controls. There was no statistically significant difference in the variations of individual BPRS items endorsement rates or in the mean number of items endorsed in the two groups of patients (patients with ADR and controls).

6.5 Type of extra-pyramidal side effects, severity of ADR and cyp2D6 phenotype and genotype

The most commonly elicited extrapyramidal side effect was ADR with Parkinsonism reported by 62% of patients with ADR; while the least frequently elicited was ADR with hyperkinesias reported by only 3 patients with ADR. While 26.5% of patients presented with mild/mild to moderate ADR severity, a larger proportion (73.4%) presented with moderate-severe/severe types of ADR. The types of extra-pyramidal side effects as elicited by the SHRS are summarized in Table 7.

Table 7: Frequency distribution of extra–pyramidal side effects (EPS) and severity of ADR in patients with ADR

Type of EPS and Severity of ADR	n (%); N = 49
Types of EPS	
ADR with parkinsonism	31 (62.3)
ADR alone	9 (18.4)
ADR with tardive dyskinesia	5 (10.2)
ADR with hyperkinesias	4 (8.1)
Severity of ADR	
Mild	2 (4.1)
Mild – moderate	11 (22.4)
Moderate – severe	32 (65.3)
Severe	4 (8.1)

The distribution of CYP2D6 alleles in patients with ADR and controls is summarized in Table 8. The most frequent CYP2D6 allele amongst patients with ADR was CYP2D6*4/*4 (44.9%) which confers poor metabolising status to persons carrying such a genotype[30]. The comparative rate for this allele amongst the controls was 6.1% and the difference in rates for this phenotype amongst the two groups was statistically significant (Chi sq = 19.39 [Yates correction]; p=0.00).

Table 8: Distribution of CYP2D6 genotype and phenotype in patients with ADR and controls

Cyp2D6 genotype CYP2D6*X[b] (X=alleles)	Known phenotypes	Patients with ADR N=49 n (%)	Patients without ADR N=49 n (%)	X^2	P-value
*1/*1	EM	3 (6.1)	17(34.7)	12.3[a]	0.05
*1/*2		16 (32.1)	21(42.8)	1.09	0.29
*1/*17		20 (40.8)	21 (42.8)	0.04	0.84
*1/*3		3 (6.1)	1 (2.0)	0.26[a]	0.60
*1/*4		1 (2.0)	4 (8.0)	0.84[a]	0.35
*1/*5		5 (10.2)	1 (2.0)	1.60[a]	0.21
*2/*2		2 (4.1)	3 (6.1)	0.00[a]	1.00
*2/*17	IM	1 (2.0)	4 (8.2)	0.84[a]	0.36
*2/*4		3 (6.2)	4 (8.2)	0.00[a]	1.00
*2/*5		2 (4.1)	3 (6.1)	0.00[a]	1.00
*17/*4		8 (16.3)	5 (10.2)	0.80	0.37
*17/*5		2 (4.1)	1 (2.0)	0.00[a]	1.00
*17/*17		5 (10.2)	1 (2.0)	1.60[a]	0.21
*4/*4		22(44.9)	3 (6.1)	19.39[a]	0.00
*4/*5	PM	18(36.7)	2 (4.1)	16.08[a]	0.00
*5/*5		11(22.4)	1 (2.0)	9.50[a]	0.02

Key: EM= Extensive metabolizer; IM= Intermediate metabolizer; PM= Poor metabolizer

[a] Yates corrected chi-square test used with cells where n< 5

[b] Represents CYP2D6 alleles and their fragmentation, more than one fragmentation may be present in a single sample.

The least frequent CYP2D6 alleles amongst patients with ADR were CYP2D6*1/*4 (2%) and CYP2D6*2/*17 (2%), both alleles conferring an intermediate metabolizing status to persons carrying the genotype. The comparative rates for these two genotypes amongst the controls were higher

at 8% for each, though the variation in rates between patients with and without ADR was not statistically significant.

In general, comparative findings summarised in Table 8 indicate a marginally significant association between extensive metabolizing status (CYP2D6*1/*1) and absence of ADR amongst patients (rate of 35.1% amongst controls) if the presence of this allele is compared to the rate derived in patients with ADR (6.1%; [chi sq. 12.3; p=0.05]).

There was however, a strong association between occurrence of alleles conferring poor metabolising status (CYP2D6*4/*4, CYP2D6*4/*5, CYP2D6*5/*5) amongst patients with ADR (44.9%, 36.7% and 22.4% respectively) compared to amongst controls (6.1%, 4.1% and 2.0% respectively; chi sq. = 19.39 [p= 0.00]; chi sq. = 16.08 [p=0.00]; and chi sq.=9.5 [p=0.02] respectively) (table 8)

Table 9: CYP2D6 allele frequencies in patient with ADR and patient without ADR

CYP2D6 allele[a]	Patients with ADR N=49 n (%)	Patients without ADR% N=49 n (%)	X^2	P-value
CYP2D 6*1	20 (40.1)	26 (53.1)	1.47	0.22
CYP2D 6*2	11 (22.4)	18 (36.7)	2.40	0.12
CYP2D 6*3	3 (6.1)	2 (4.1)	0.00	1.00
CYP2D 6*4	8 (16.3)	3 (6.1)	2.56	0.11
CYP2D 6*5	11 (22.5)	1 (2.0)	9.50	0.00
CYP2D 6*17	15 (30.6)	7 (14.3)	4.91	0.03

[a] Represents CYP2D6 alleles without the fragmentation

In order to assess which allele was more commonly associated with ADR, table 9 summarizes the distribution of each allele without its fragmentation in relation to ADR status of the two patient populations. The most frequently occurring allele amongst patients with ADR was CYP2D6*1 (40.1%) and CYP2D6*17 (30.6%); while amongst controls the most frequently occurring alleles were CYP2D6*1 (53.1%) and CYP2D6*2 (36.7%). The variation in presence of alleles conferring PM status (CYP2D6*5) and IM status CYP2D6*17 amongst patients with ADR and controls was statistically significant. (22.5% and 2%, Chi sq. 9.50, p=0.00; 30.6% and 12.3% Chi sq. 4.91, p=0.03 respectively). There was no significant differences in the distribution of the other alleles i.e. CYP2D6*1, *2, *3 and *4 between the two groups of patients.

7.0 DISCUSSION

The findings from analysis demonstrates a lack of association between selected socio-demographic characteristics that were not used in matching the control group (education level, marital status, employment status and average monthly income) amongst patients that developed ADR in the two week period after admission when compared to the control group. Further more there were no significant differences in the two groups with regards to the type of psychopathology present during the current episode, as well as there severity (as evidenced by the mean BPRS scores), duration since first episode of mental illness or the duration since first use of neuroleptic drugs. These observations suggest that with regard to these potentially confounding variables, the group of patients that developed ADR compared to those that did not develop ADR were relatively similar.

Some studies have noted young age to be an important predictor of extrapyramidal side effects in patient populations [17, 18]. Agular et al.[17] in a study to find predictors of acute dystonia in first episode psychotic patients reported that young, severely ill patients in their first psychotic episode who have never been treated with neuroleptics were at higher risk of developing dystonia. This was not a finding noted in this sample. However, the study reported here, matched controls to patients with ADR by age and did not focus on an incident (of mental disorder) population that had never been exposed to neuroleptics prior to the study. Further more, the sample comprised of predominantly young adults and hence the age range in the

sample may not have been sufficiently wide to allow for variations in ADR by age to be assessed.

There was a trend towards a greater proportion of patients that developed ADR to have an ICD-10 diagnosis of schizophrenia when compared to the control group; while a diagnosis of bipolar disorder (mania) was more frequent amongst patients in the control group. However, these variations were not statistically significant. It is unlikely that a diagnosis of schizophrenia necessarily predicts a greater likelihood of ADR or PM status. Armstrong et al[20] when comparing antipsychotic drugs induced movement disorders in individuals with schizophrenia in relation to CYP2D6 genotype, found that there was no difference in genotype frequencies between individuals with schizophrenia and a normal control population, suggesting that CYP2D6 genotype was not a factor in determining susceptibility to the disease. However, he found that four of the five poor metabolizers compared to 44% of the remaining subjects were suffering from a movement disorder at the time of the study. He concluded, as have other authors[41] that CYP2D6 genotype is not a determinant of susceptibility to schizophrenia but may be a contributory factor to anti-psychotic drug-induced movement disorder including tardive dyskinesia.

Bertilsson et al[9] and Lou et al.[10] when comparing status of these enzymes in Caucasians and Orientals have demonstrated inter ethnic genetic differences which explained the higher dose requirements to achieve therapeutic responses for CYP2D6 substrates in Caucasians compared with Orientals.

Few comparative studies of therapeutic or other responses to drugs that are substrates of CYP2D6 have been conducted that involve black African subjects and subjects of other ethnicity. However, observation in the Tanzanian context suggests amongst black Africans therapeutic responses to antidepressants at much lower than recommended doses[40]. In the reported study, assessment of ethnicity of the sample revealed all the subjects to be Bantu in origin.

During the last 20 years it has been shown that the metabolism of many drugs, including most tricyclic anti-depressants and antipsychotic drugs is catalysed by polymorphic CYP2D6[36]. Individuals heterozygous for the functional CYP2D6*1 and defective alleles have on average lower CYP2D6 activity than those homozygous for the CYP2D6*1 allele, while subjects homozygous for defective alleles (PM) lack CYP2D6 activity[37]. Furthermore, an allele variant, carrying multiple copies of a functional CYP2D6 gene (CYP2D6*1 or CYP2D6*2) and associated with extremely high CYP2D6 activity (ultra metabolizers, UM) has been described[38]. These findings may explain the large inter individual variability in the plasma concentrations of drugs metabolised by CYP2D6 at fixed dosage schedules and may contribute at least partially, to differences in therapeutic response. Different studies suggest that amongst individuals with genetically deficient CYP2D6 metabolic capacity (i.e. the PM status) the co-administration of inhibitors of this enzyme may play a significant role in the appearance of adverse reactions to psychotropic drugs[34].

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In this study, an association between debrisoquine hydroxylase genetic polymorphism and neuroleptic induced dystonic reactions is supported. The findings revealed a statistically significant association between poor metabolizer status and occurrence of acute dystonic reactions. The CYP2D6 alleles and their fragments noted to predict poor metabolizer status amongst those developing ADR included CYP2D6 4*/4*, CYP2D6 4*/5* CYP2D6 5*/5*. These results are in agreement with findings from a previous study, where although the number of patients with PM status was small, the study suggested that PM genotype might be a predisposing factor for neuroleptic induced extra-pyramidal side effects[21].

Should these observation be accurate the converse would be expected if extensive metabolizer status were compared in the two patient groups, and this was evidenced in the findings of this study by 35.1% of patient without ADR being extensive metabolizers (EM), while this was the case for only 6.2% of those with ADR, these variation in rates of EM status being marginally significant for the homozygous allele CYP2D6 1*/*1, that is predictive of extensive metabolizer status. Hence these findings are in agreement with reports that carriers of a homozygous CYP2D6 allele that confers an extensive metabolizer genotype are protected to a certain extent from the risk of developing extrapyramidal side effects[39].

A previous study in a sample of patients with mental disorders suggested a high prevalence (30.4%-34%) of CYP2D6*17 that is predictive of intermediate metabolizer status, among the Bantu (linguistically) populations of East and

Southern Africa[22]. While no association was found with ADR when CYP2D6*17 and its fragments were considered in the cross tabulation, the allele itself was noted to be significantly associated with ADR, occurring at higher rates in the ADR group compared to in controls. A possible explanation for this difference could be the higher number of repeated fragments in the control group compared to the ADR group, creating too few numbers per cell for conclusive chi-square results. Given the high proportion of the population carrying the CYP2D6*17, studies using larger patient population samples should be a consideration to replicate these findings.

8.0 CONCLUSIONS AND FUTURE PERSPECTIVE

8.1 CONCLUSION

- Genetic polymorphism of drug metabolising enzymes have been poorly characterised in African populations compared to western countries which are making advances in applying such knowledge to optimise drug therapy and minimise side effects. The findings of this study revealed that a significant proportion of black African Tanzanian patients that develop neuroleptic induced acute dystonia posses a mutant variant of the CYP2D6 gene (CYP2D6*5 and CYP2D6*17) which tends to decrease the activity of the enzyme conferring PM status and IM status respectively leading to increased susceptibility to drug adverse effect.

- Extensive metabolizer status amongst patients was protective against the development of side effects of the drugs, which are substrate for CYP2D6.

8.2 FUTURE PERSPECTIVE AND RECOMMENDATION

- This study highlights the heterogeneity of Tanzanian populations with regards to CYP2D6 genotype. This has implications in an environment where poor neuroleptic drug compliance is strongly associated with drug side effects, particularly when standard recommended doses are utilized.

- Further studies using larger samples of psychiatric patients are required to better understand the relationship between IM status and occurrence of ADR.

- Knowledge of the genotype and phenotype status of CPY2D6 which needs to be done only once in a patient's life-time, can go a long way to guide the titration of drug doses to achieve optimal therapeutic effects with minimal adverse effects, given the narrow therapeutic index and propensity for debilitating side effects of neuroleptic drugs. The clinical impact of this would be in improved compliance and hence better recovery of patients with functional psychotic disorders.

- Facilities can be developed in sub-Saharan Africa including Tanzania for carrying out phenotyping and genotyping of drug metabolising enzymes, not only for clinical purposes, but also for population studies to obtain a phenotype/genotype status map of the country. Panel studies should be done to assess the clinical impact of CYP2D6 variants unique to Tanzanian population like the CYP2D6*17 and CYP2D6*5.

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