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Drug Metabolic Variability of CYP3A4*2, GSTT1 and GSTM1 gene polymorphisms and its correlation with clinical phenotyping in South Indians

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Abstract

Drug metabolism studies associated with traditional medicine could provide a better insight for understanding the human body differential susceptibility to disease, drug response and even the complex interaction of genetic and environmental factors in disease pathogenesis. Thus, the present investigation is designed to identify the genetic variations in drug metabolizing genes and correlate them with different phenotypes (temperaments) in healthy volunteers according to Unani medicine of philosophy. Four hundred (400 – 100 sanguine (*Damwi*), 100 Phlegmatic (*Balghami*), 100 Bilious (*Safrawi*) and 100 Melancholic (*Saudawi*)) normal healthy adults of either sex within the age group of 25-55 years formed the subjects of the study. The peripheral blood samples of these individuals were analyzed for CYP3A4*2 (664 T>C), GSTM1 and GSTT1 gene polymorphisms. The frequency of mutant allele 'C' in CYP3A4*2 (664 T>C) gene polymorphism was found to be high in *Safrawi* individuals (14.5%) when compared to other temperament individuals. The GSTM1 null genotypes were high in *Balghami* (20%) and *Damwi* (20%) individuals whereas Cytochrome 1 null genotypes were high in *Saudawi* individuals (12%) when compared to other temperament individuals. In combination analysis both the GSTM1 \ GSTT1 null genotypes were observed high in *Saudawi* (8%) when compared to other temperament individuals. Based on the assorted polymorphic variations obtained from the study, it is evident that there were differences in the metabolic activity of different phenotypic clusters (temperaments). Thus; the study brings out the possible phenotype-genotype correlation which would be useful in future pharmacogenomics and personalized medicine concepts in traditional medicine.

Keywords: Drug Metabolism, Temperaments, Drug Metabolizing Genes, PCR-RFLP, Phenotype-Genotype

1. Introduction

Health is considered as wealth in most of the communities around the globe which imply to be highly precious for every human being. According to the World Health Organization, health is defined as an individual's total condition of physiological, psychological, socioeconomic, and spiritual well-being, in addition to the absence of any illnesses in the body [1]. From ancient period every community has their own view of health as part of its culture with traditional medicines for curing ill health or disease. Unani medicine is one such age-old traditional medicine with holistic values.

According to Unani philosophy of medicine, Akhlat Arba (four Humours) i.e. Dam (Blood), Balgham (Phlegm), Safra (Yellow Bile) and Sauda (Black Bile) provide sustenance to the organs in the human body. The nutritional components of ingested food and liquids form these humours in the liver. When the balance of these humours is disrupted, the body's processes become aberrant, resulting in disease. These four humours have four different temperaments/Mijaz they are *Damwi* (blood) hot and wet, *Balghami* (phlegm) cold and wet, *Safrawi* (yellow bile) hot and dry and *Saudawi* (black bile) cold and dry characteristics [2]. An individual's temperament lasts their entire life. Age, location, season, nutrition, and employment all impact an individual's temperament and

predispose them to a certain category of disorders. Different personalities may be found in people with different temperaments; for example, Damwi (Sanguine) people are whitish brown, muscular, active and mostly with balanced emotions, Balghami (Phelgmatic) persons are with whitish, obese, dull, sluggish, calm etc. Safrawi (Bilious) persons may be pale in colour, slightly muscular, hyperactive, angry whereas *Saudawi* (Melancholic) persons are blackish, very lean, less active, disturbed sleep, acentric and nervous. These are clinical phenotypes as per Unani Philosophy. A disease is characterized by a pathological shift in temperament; for example, a Hot &Dry man who becomes unusually Cold & Wet may get severe arthritis. Usage of pain relievers will only provide symptomatic alleviation and may have negative effects. Using a Hot & Dry medication to change his atypical Cold & Wet Temperament to the usual, Hot & Dry Temperament can safely heal his arthritis [3]. Person with *Safrawi* Mizaj could develop health issues like heat-stroke, acute fevers, dehydration, insomnia, indigestion etc. Likewise, a person with *Balghami* mizaj may be prone to develop diseases like asthma, female infertility, obesity, nervine diseases, etc. *Saudawi mizaj* humans might get Leprosy, Hummae ruba, Splenomegaly, Constipation, Anorexia, Arthritis, Neuromuscular, Psychiatric illnesses, and other ailments. *Damwi mizaj* people are more susceptible to infectious infections [4].

Till now for most of the molecular research studies human population is grouped based on their ethnicity and ancestry [5]. But categorization of human populations by phonemics i.e. phenotypic features compared with genetic make could yield better results in modern biomedical research. However, there is lack of such studies related to define phenotypic features to cluster individuals. As a result, categorization of human populations continues to be a major difficulty for biomedical sciences [6]. The temperament categorization in the Unani system of medicine is based on distinctions in biological, physiological, and psychological features. Thus, contemporary research including molecular biology techniques with Unani system of medicine would be beneficial for the future medicine.

One of the most important variables responsible for inter-individual and inter-ethnic diversity in medication response is gene mutations or polymorphism. The polymorphism of genes encoding various drug metabolizing enzymes (DME), drug transporters, deoxyribonucleic acid (DNA) biosynthesis and repair enzymes are responsible for inter-individual heterogeneity in drug metabolism. Previous research in Caucasians, Asians, and Africans revealed that there were considerable ethnic variations in the metabolism of several medicines [7]. It is thought that the four Unani temperament types are considered to have varied drug metabolisms which may be attributed to genetic basis and leading in variations in clinical responses. The bioactivation of xenobiotics or drugs by phase I enzymes (cytochrome p450s) results in the formation of reactive oxygen species (ROS). Phase II enzymes, such as glutathione S-transferases (GSTs), are engaged in the detoxification process via an antioxidant defense mechanism [8]. Polymorphisms in these two enzymes may responsible for varied drug metabolism activity.

Cytochrome P450 (CYP450) enzymes are a class of microsomal membrane-bound monooxygenases that generate imperative endogenous macromolecules and metabolize pharmaceuticals. There are about 57 functioning Cytochrome P450 (CYP) genes and 46 pseudo-genes in humans. CYP3A4 is a cytochrome p450 gene found on chromosome 7q21.1. About 50 % of current marketed drugs are metabolized by CYP3A4 enzyme [9]. Previous research has found a tenfold variance in CYP3A4-mediated drug metabolism [10] and a 90-fold variation in CYP3A4 protein expression. CYP3A4 also participates in the oxidative metabolism of a large variety of xenobiotics, including 45–60% of clinically used pharmaceuticals. [11].The CYP3A4*2 664 T>C (Ser222Pro) promoter SNP appears to be linked to diminished expression and activity (1.7 to 5-fold less) [12] and also related to be risk factor for several cancers [13] and oxidative stress related diseases [11].

GSTs are a class of phase II enzymes that are classified as cytoplasmic, membrane, mitochondrial, or leukotriene C4 synthases. Subtypes of cytoplasmic GSTs include α, μ, π, σ, θ, κ, ζ, and Ω subtypes [14].They are involved in the detoxification of potential carcinogens and provided of a strong antioxidant function by neutralizing electrophiles and free radicals with nucleophilic glutathione yielding less toxic and more water-soluble compounds, which are readily excreted *via* urine or bile [15]. Thus, they protect the cell, protein, and nucleic acid from free radical damage. A diminution of their activity can make an individual more susceptible to various diseases including cancers [16]. A partial gene deletion at the GSTM1 locus on chromosome 1p13.3 (GSTM1 null genotype) and GSTT1 locus on chromosome 22q11.2 (GSTT1 null genotype) results in the complete absence of functional enzyme activity. Though there are several studies relating the CYP3A4*2 (664 T<C) and GST polymorphisms with the altered drug metabolism [17], studies especially related to Unani medicine in association with such genetic variations are scanty. Therefore, the purpose of the present study has been laid to evaluate the possible association of drug metabolizing enzyme variation (CYP3A4*2 and GST gene polymorphisms) in healthy individuals of South Indian population with different Unani temperaments. So far to our knowledge this is the first kind of study to relate genetic variation with Unani temperament theory of Unani Philosophy.

2. Materials and methods

A total of 400 subjects were included for the study. All the subjects were examined by Unani physicians from the OPD clinic of National Research Institute of Unani Medicine for skin diseases (NRIUMSD, Hyderabad).

2.1 Subject selection

All the healthy individuals of either sex in the age group of 20-60 years who were free from any kind of ill health or disease were included for the study and Pregnant women, lactating women, cancer patients, any other organ complications, mentally ill and people with hereditary family history were excluded from the study. Appropriate biochemical tests including Hb, cholesterol, urea, creatinine, liver function tests were performed to ensure the subjects are healthy. 100 subjects of each one clinical phenotype or temperament i.e. 100 Sanguine (*Damwi*), 100 Phlegmatic (*Balghami)*, 100 Bilious (*Safrawi*) and 100 Melancholic (*Saudawi*) were selected.

2.2 Assessment of Temperament

A case record form (CRF) was designed as per the Unani classical text for assessment of humours. Written as well as verbal consent and information was obtained from all the subjects to take part in the research process. The subjects were clinically examined and data on their clinical histories, physiognomy, along with social, psychological behavior, habits, and physical fitness were noted in the CRF form based on the Unani classics and temperament was confirmed by Unani physicians.

Present study was approved by the Institutional Ethics Committee of NRIUMSD, following the principles of Helsinki Declaration for subject consent.

2.3 Blood Sample Collection

Two ml of whole blood was collected by vein puncture from all the 800 subjects (400 patients and 400 controls). Blood was collected in K2 EDTA vacutainers and stored at -4°c in refrigerator until further use. Informed consent was taken from the subjects prior to collection of blood samples.

2.4 DNA isolation

Genomic DNA was isolated from whole blood by using HiPurA™ blood genomic DNA Purification Kit a column-based DNA isolation kit. Quality and quantity of DNA were evaluated by nanodrop reading by using Multimode reader and 1% agarose gel electrophoresis. DNA was then stored at -20°C until further use.

*2.5 PCR amplification and genotype determination of CYP3A4*2 (664 T>C)*

The CYP3A4*2 664 T>C (rs55785340) genotyping was performed as illustrated by Suman and Jamil [17] using appropriate primer sequence. The experiment was performed with polymerase chain reaction (PCR) mix in a PCR (Fermentas Life Sciences, Bangalore, India) for 35 cycles. Later PCR products yielding a fragment of 366 bp were visualized on 2 % agarose gel stained with ethidium bromide before digestion to identify proper amplification (Figure 1). Later PCR products were subjected to Restriction digestion by Hind III Fast Digest restriction enzyme (Thermo Scientific, India). The samples were incubated for 20 min at 37°C and the restriction fragment length polymorphism (RFLP) products were separated by 3 % agarose gel electrophoresis (Figure 2).

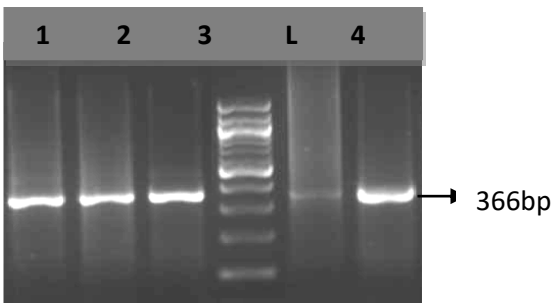

Figure 1 CYP3A4*2 (664 T>C) (rs55785340) gene polymorphism PCR products.
Lane 1, 2, 3, 4 & 5 PCR products 366bp.
Lane - L 100bp ladder.

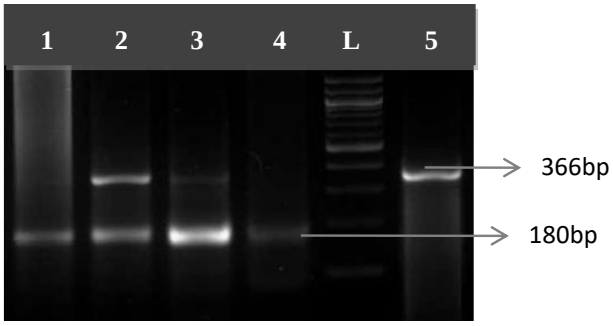

Figure 2 CYP3A4*2 (664 T>C) (rs55785340) gene polymorphism RFLP products.
Lane 1 & 4- TT genotype 180bp
Lane 2 & 3- TC genotype 366bp & 180bp
Lane 5 –CC genotype 366bp
Lane L 100bp ladder

2.6 PCR amplification and genotype determination of GSTM1 and GSTT1 genes

GSTM1 and GSTT1 null genotypes were determined using a customized multiplex PCR method for replication of both genes for molecular analysis [18]. Globulin gene was taken as an internal control for a successful amplification reaction. All the PCR reactions were carried out with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 1.2 units of Taq DNA polymerase under suitable PCR conditions. The amplified products were analyzed by 2% agarose gel electrophoresis. Presence of 215bp band indicates GSTM1 wild type and a 480 bp band represents GSTT1wild type. The absence of band was consistent with the null genotypes. Successful amplification was confirmed by presence of 350bp β-globulin gene (Figure 3).

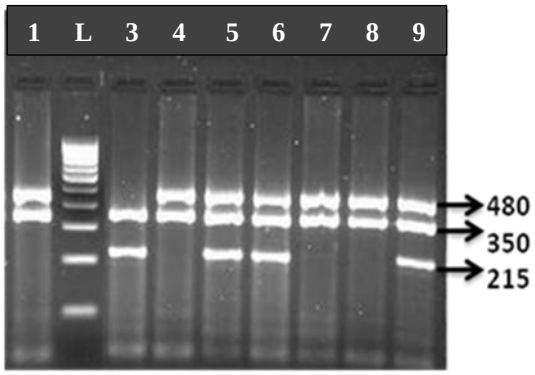

Figure 3 GST gene multiplex PCR products on gel electrophoresis.
L shows 100 bp ladder.
Samples 1, 4, 7 and 8 shows GSTT1 +,
Samples 3 show GSTM1+,
Samples 5, 6and 9 shows GSTT1 + and GSTM1 +.

2.7 Statistical Analysis

All the results are articulated as mean ± standard deviation. $\chi 2$ test was used to compare the differences in each genotype, allele, and combined genotypes frequencies. The risk analysis was performed by calculating Odds Ratio (OR) at 95 % confidence intervals (CIs). A two-tailed *p* value of <0.05 was considered to be significant. Statistical analysis was performed using openepi software (Version 3.01, April 2013; http://www.openepi.com).

3. Results

A total of 400 Healthy volunteers with 100 each from four different temperaments *(Balghami, Saudawi, Safrawi, and Damwi*) were selected by using Unani standard parameters of assessment of Mizaj for the current study. Age, gender wise distribution and biochemical parameters of subjects were stratified according to clinical phenotype or temperament wise and presented in table 1. All the basic biochemical parameters were within normal range indicating subjects to be healthy.

Table 1 General characteristics and biochemical parameters.

Characteristics	Healthy Volunteers No. of cases	Damwi/Sanguine Mean ± S.D (Range)	*Balghami*/Phlegmatic Mean ± S.D (Range)	*Safrawi*/Bilious Mean ± S.D (Range)	*Saudawi*/Melancholic Mean ± S.D (Range)
Male	150	49	14	48	39
Female	250	51	86	52	61
Age	400	38 ± 12 years (20 to 60 years)	38 ± 11years (20 to 60 years)	39 ± 11 years (20 to 60 years)	39 ± 11 years (20 to 60 years)
BMI	400	27 ± 4.9 (17 – 42)	29.2 ±5.2 (18 – 45.6)	26 ± 4.9 (17 – 44)	25.5 ± 5.39 (16 – 40.2)
Blood pressure (mm/hg) H	400	117 ±15 (80 – 180)	115.2 ± 16.2 (80 – 180)	118 ± 17 (80 – 190)	119 ± 17 (90 – 190)
Blood pressure (mm/hg) L	400	78 ±8.4 (60 – 100)	78.4 ± 7.7 (60 – 90)	80 ± 9.2 (60 – 110)	82 ± 8.1 (70-100)
Hb (gm %)	400	13.9 ± 2.3 (8.9 – 18.7)	12.74 ±1.88 (8.3 – 18.6)	13 ± 2.3 (6.5 –18)	12.5 ± 2.11 (7.2 – 16.5)
FBS (mg/dL)	400	94 ± 9.8 (63 – 113)	90.30 ±13.86 (54 – 131)	92.1 ± 12.9 (65 – 131)	96.1 ± 15 (59–130)
Cholesterol (mg/dL)	400	191 ± 40 (103 – 290)	199.11 ±38.85 (102 – 292)	164 ± 35.7 (87 – 241)	195 ± 50.1 (104 –320)
SGOT (IU/L)	400	24.3 ± 8.2 (12 – 45)	21.04 ±7.49 (10 – 42)	19.1 ± 7.38 (10 – 50)	21.6 ± 9.1 (10-50)
SGPT (IU/L)	400	23.3 ± 8.9 (10 – 48)	20.55 ±8.66 (10 – 47)	19.8 ± 9.99 (10 – 60)	20.18 ± 12.83 (10–62)
Bilirubin (mg/dL)	400	0.72 ± 0.28 (0.28 – 1.28)	0.58 ±0.24 (0.25 – 1.32)	0.72 ± 0.378 (0.11 – 1.4)	0.67 ± 0.32 (0.21–1.34)
Alkaline Phosphatase (IU/L)	400	75 ± 20 (28 – 119)	85.05 ±21.95 (41 – 141)	74 ± 18 (29 – 117)	79 ± 29 (36–149)
Urea (mg/dL)	400	25 ± 6.5 (11 – 23)	25.94 ±7.51 (10 – 26)	24.2 ± 7.69 (10 – 29)	24.8 ± 8.25 (10 –26)
Creatinine (mg/dL)	400	1.06 ± 0.17 (0.6 – 1.5)	1.02 ±0.18 (0.5 – 1.4)	1.15 ± 0.23 (0.53 – 1.6)	1.08 ± 0.24 (0.63–1.6)

SD= Standard deviation; BMI=Body Mass index; Hb=Hemoglobin; FBS=Fasting blood sugar; SGOT=Serum glutamic-oxaloacetic transaminase; SGPT =Serum glutamic pyruvic transaminase

*3.1 Analysis of CYP3A4*2 664 T>C gene polymorphism in different temperaments*

In the present investigation, 100 sanguine (*Damwi*), 100 Phlegmatic (*Balghami*), 100 Bilious (*Safrawi*) and 100 Melancholic (*Saudawi*) subjects CYP3A4*2 genotyping was carried out according to PCR-RFLP method and the results are presented in the table 2. The genotypic frequencies of CYP3A4*2 664 T>C gene polymorphism of *Balghami* temperament were TT 79%, TC 19% and CC 02%; *Damwi* temperament were TT 77%, TC 21% and CC 02%; *Safrawi* temperament were TT 75%, TC 21% and CC 04% and *Saudawi* temperament were TT 81%, TC 16% and CC 03% respectively. In the current investigation, there were no significant differences observed in the CYP3A4*2 polymorphism in four different temperaments.

According to the results observed the order of mutant allele percentage in different temperaments were *Safrawi* (14.5%) >*Damwi* (12.5%) >*Balghami* (11.5%) >*Saudawi* (11%). There were no significant differences among allelic distribution and the highest frequency of mutant alleles were observed in *Safrawi* temperament among them.

Table 2 Genotype and allelic frequencies of CYP3A4*2(T>C) gene polymorphism in four different clinical phenotypes based on Unani Philosophy.

Genotype /Alleles	*Balghami* n = 100	*Damwi* + *Safrawi* + *Saudawi* n = 300	p value	*Damwi* n=100	*Balghami* + *Safrawi* + *Saudawi* n = 300	p value	*Safrawi* n=100	*Balghami* + *Damwi* + *Saudawi* n = 300	p value	*Saudawi* n = 100	*Balghami* + *Damwi* + *Safrawi* n = 300	p value
TT	79	233	--	77	235	--	75	237	--	81	231	--
TC	19	58	0.97	21	56	0.74	21	56	0.65	16	61	0.43
CC	2	9	0.85	2	9	0.89	4	7	0.56	3	8	0.81
Allele												
T	177	524	--	175	526	--	171	530	--	178	523	--
C	23	76	0.75	25	74	0.95	29	70	0.35	22	77	0.57

$\chi 2$ test was performed; $p<0.05$ is considered significant

3.2 Analysis of GSTM1 and GSTT1 gene polymorphism in different temperaments

GSTM1 and GSTT1 gene polymorphism were carried out using multiplex PCR and results were presented in table 3. The genotypic frequencies of GSTM1 gene polymorphism of *Damwi* temperament were + 80% and – 20%; *Safrawi* temperament were + 94% and – 6%; *Saudawi* temperament were + 92% and – 8% and *Balghami* temperament were + 80% and – 20% respectively. GSTM1 null genotypes were significantly differed in *Balghami* (OR-1.95 CI: 1.06-3.58), *Damwi* (OR-1.95 CI: 1.06-3.58) and *Safrawi* (OR-0.33 CI: 0.14-0.81) temperaments.

The genotypic frequencies of GSTT1 gene polymorphism of *Damwi* temperament were + 90% and – 10%; *Safrawi* temperament were + 90% and – 10%; *Saudawi* temperament were + 88% and – 12% and *Balghami* temperament were + 91% and –9% respectively. GSTT1 null genotypes were observed high in *Saudawi* temperament when compared to other temperaments.

In combination analysis of GSTM1 and GSTT1 gene polymorphism the genotypic frequencies of *Damwi* temperament were ++ 80%, -–5% and others 15%; *Safrawi* temperament were ++ 83%, –3% and others 14%; *Saudawi* temperament were ++ 88%, -–8% and others 4% and *Balghami* temperament were ++ 76%, –4% and others 20% respectively. In combination analysis *Balghami* (OR-2.002 CI: 1.08-3.69) and *Saudawi* (OR-0.22 CI: 0.077-0.632) temperament significantly differed in terms of combined null genotypes compared to other temperaments.

Table 3. Genotype frequencies of GSTM1 and GSTT1 variants in four different clinical phenotypes based on Unani Philosophy.

Genotype	*Balghami*	*Damwi* + *Safrawi* + *Saudawi*	p value	*Damwi*	*Balghami* + *Safrawi* + *Saudawi*	p value	*Safrawi*	*Balghami* + *Damwi* + *Saudawi*	p value	*Saudawi*	*Balghami* + *Damwi* + *Safrawi*	p value
GSTM1 Present (+)	80	266	--	80	266	--	94	252	--	92	254	--
GSTM1 Null (-)	20	34	0.042	20	34	0.042	6	48	0.001	8	46	0.09
GSTT1 Present (+)	91	268	--	90	269	--	90	269	--	88	271	--
GSTT1 Null (-)	9	32	0.775	10	31	0.92	10	31	0.92	12	29	0.63
Combined genotypes												
GSTM1 (+), GSTT1(+)	76	251	--	80	247	--	83	244	--	88	239	--
GSTM (-) ,GSTT1(-)	4	16	0.95	5	15	0.83	3	17	0.43	8	12	0.313
Mixed genotypes	20	33	0.03	15	38	0.550	14	39	0.99	4	49	0.003

$\chi 2$ test was performed; $p<0.05$ is considered significant

4. Discussion

One of the outstanding aspects of Unani system of medicine is the idea that each human being has a unique constitution, which leads to the prescription of appropriate medications and dietary guidelines. This is comparable to pharmacogenomics and personalized therapy. While the use of race or ethnicity as a reason for phenotypic diversity is still debatable, we suggest an interdisciplinary approach which incorporates genetics with traditional medicine knowledge to yield better results. Biomedical researchers are in the terms of understanding some common diseases influenced by the environment and other physiological factors combined with certain gene polymorphisms in the treatment of illness [19].

Although several innovations were observed in scientific research of genomics and proteomics, but still very fewer studies have attempted to understand the association between traditional medicine constitutions and genetic polymorphism [20]. Unani Medicine maintains a greater emphasis on health maintenance and illness prevention than on treatment. Depending on the severity of the disease, particular diets, non-drug manipulations or regimens, and even drugs are given to preserve health and avoid disease. The physician prescribes medications based on the patient's temperament, the causative humour, the function of the organ affected, and the severity of the disease. Despite of similarities between most of the traditional medicines regarding the holistic and individual classification systems there are several internal differences in understanding and treating the disease. Detection of genetic disparities underlying drug metabolic variability in Unani temperaments may provide novel approach to Pharmacogenomics. We assume that humeral theory in Unani concept may have genetic connotation which might facilitate to classify human populations into broad phenotype clusters.

Every human being is unique in terms of genetic constitution; it is responsible for the drugs and environmental factors responses and risk for developing diseases. Chenna et al. [21] and Kumar and Jamil[22]

have analyzed the importance of polymorphic studies of drug metabolism genes and reported its influence in several chronic diseases including cancers. Thummel and Wilkinson [23] indicated that some mutations lead to the commonly observed adverse drug interactions during treatment. These studies enlighten the importance of polymorphic studies of xenobiotics or drug metabolism genes. Cytochrome P450 and GST genes are involved in the oxidation and detoxification processes of xenobitic compounds including several drugs. Among ChytochromeP450 gene there several variants, among them CYP3A4 genetic variants have been recognized to be involved in the metabolism of 50% drugs. CYP3A4 coding region have more than 20 variants and several of them have altered enzyme activity, ranging from a meager to a substantial loss in catalytic efficacy. From earlier pharmacogenetic and toxicogenetic studies CYP3A4 single nucleotide polymorphism (SNP) in the promoter region have been observed to be risk factor for several chronic diseases [13]. Suman and Jamil, [17] studied the role of CYP3A4*2 664T>C (Ser222Pro) SNP in drug metabolism of post chemo Breast cancer patients and observed that CYP3A4 polymorphism led to adverse drug reactions. Apart from steroids and carcinogens, common drugs metabolized by CYP3A4 enzyme include acetaminophen, cyclosporin, diazepam, erythromycin etc. [24].In the present exploration there were no significant differences observed in the genotype distribution of CYP3A4*2 genotypes but still *Safrawi* subjects with presence of high CC mutant alleles may show low metabolic activity and the *Saudawi* temperament individuals with high TT alleles may possess fast metabolic activity with respect to CYP3A4*2 gene polymorphism. These findings suggest that slow and fast metabolizers will require low and high dosages of CYP3A4 substrates, correspondingly.

GSTs are fundamental enzymes in xenobiotic-pathway. These enzymes reduce the ROS production and are involved in the conjugation of potentially mutagenic electrophilic compounds, with nucleophilic glutathione yielding less toxic and more water-soluble compounds which are easily excreted via urine or bile. GST polymorphisms single or in combination, are associated with altered metabolic capacity and may be considered as a risk factor for several diseases [15]. GSTM1 is expressed in human liver and other extra hepatic tissues such as stomach and brain; GSTT1 has been identified in human liver and at significantly high levels were also seen in normal gastric and colonic mucosa [16].

A partial gene deletion at the GSTM1 locus on chromosome 1p13.3 (GSTM1 null genotype) and GSTT1 locus on chromosome 22q11.2 (GSTT1 null genotype) results in the complete absence of functional enzyme activity [14]. The prevalence of the GSTM1 and GSTT1 null genotypes shows vast inter-ethnic variation: in Caucasians the GSTM1 null genotype is seen in 45–60% of individuals, while GSTT1 null genotype in 10–30% of the general population. Numerous studies on GST gene polymorphism indicate that loss of mu and theta GST genes increases susceptibility to inflammatory diseases such as asthma, autoimmune diseases, lung adenocarcinoma, development of Ischemia/Reperfusion (trauma) etc. [16,25]

In the present study GSTT1 genotype didn't showed association with any of the temperament whereas GSTM1 null genotypes were significantly associated with *Balghami*, *Damwi* and *Safrawi* and in combinational analysis *Balghami* and *Saudawi* showed significant association with mixed genotypes. On the whole *Balghami* phenotype individuals are showing susceptibility to diseases in terms of significantly high null GST genotypes whereas in contrast to them *Safrawi* individuals are showing defensiveness with significantly less GSTM1null genotypes. The results of the study indicate that there were differences in the metabolism rate of different phenotypic clusters according to the polymorphic variations.

5. Conclusion

Unani medicine and other conventional therapies, such Sasang constitutional medicine (SCM), Kampo medicine, and Traditional Chinese Medicine (TCM), have a lot in common, including holistic and person-centered system of classification. Analytical and biological investigations can help to strengthen the validity of these traditional remedies with hardly any side effects. The current investigation is the first and preliminary attempt to study and understand the metabolic variability of different Unani temperaments in relation with the metabolic gene polymorphism. Our findings suggest a possible genetic basis for metabolic variations associated with Unani temperaments. Extensive studies with large sample size and with additional drug metabolism gene polymorphisms such as CYP2D6, CYP2C9, CYP2C19, CYP2E1, other GST variants could attain better confirmed results and would have significant ramifications for pharmacogenomics, contemporary genetics, and Unani medicine.

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