Original Articles

PHARMACOGENETICS-ORIENTED THERAPEUTIC DRUG MONITORING OF DIGOXIN IN CRITICALLY ILL PATIENTS

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أجريت هذه الدراسة على عينة مكونة من سبعة وثلاثين مريضاً مصرياً وذلك لتحديد البنية الوراثية (genotypes) المختلفة لجين Multi-Drug Resistance-1 (MDR-1) ممن يعانون من الرفرفة الأذينية و أو الهبوط الأحتقاني للقلب والذين يستخدمون عقار ديجوكسين وذلك لمعرفة دور جين MDR-1 متعدد الأشكال Polymorphism في إحداث حالة مستقرة للمستويات العلاجية لعقار ديجوكسين في المصل، ولدراسة تطورات ذلك على حصيلة الحالة الإكلينيكية للمريض فقد تم سحب عينتين من الدم من كل مريض وتم أخذ العينة الأولى عند دخول المريض للمستشفى وذلك من أجل استخلاص الحمض النووي المنزوع منه ذرة الأكسجين (د.ن.أ)، ثم دراسة البنية الوراثية له. وأما العينة الثانية فقد تم أخذها بعد مرور ست ساعات من وصول الدواء إلى حالة تركيزية مستقرة من أجل دراسة تركيز مادة ديجوكسين فقد تم تقييم مستوياته في المصل وذلك باستخدام جهاز EMIT 2000 analyzer وأما تحديد البنية الوراثية لجين MDR-1 فقد تم ذلك باستخدام تقنية أطوال متعددة الأشكال بشظايا من سلسلة إنزيم بوليميراز محددة التفاعل Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) وقد ظهر أن عشرين مريضاً (54.1%) لديهم مستويات لدواء ديجوكسين في المصل في حدود المستوى العلاجي. كما تبين أن إثنا عشر مريضاً (32.4%) كان لديهم مستويات لدواء ديجوكسين في المصل تحت الحد الأدنى من المستوى التركيزي الفعال (أقل من 0.9 نغم/مل). بينما ظهر أن خمسة من المرضى (13.5%) كان لديهم مستويات لدواء ديجوكسين في المصل فوق الحد الأعلى للتركيز المأمون (2 نغم/مل) مع قيمة P مقدارها 0.0001 للمجموعات الثلاث. وقد أظهرت الدراسة الوراثية لجين MDR-1 لعشرة من المرضى (27%) أنهم ممن يحملون التركيبة الجينية المتماثلة TT وأن سبعة وعشرين مريضاً (73%) هم ممن يحملون التركيبة الجينية غير المتماثلة CT. ولم يظهر أي من المرضى أنه يحمل التركيبة الجينية المتوحشة CC. وقد أظهر التوزع الأللييلي أن 42% منها هي للأللييل النوع المتوحش C بينما 58% كان للأللييل المتجانس T. وقد أظهر المرضى الحاملين للجين المتجانس TT أن لديهم انخفاضاً معنوياً في مستويات عقار ديجوكسين في المصل مقارنة بأولئك الحاملون للجين غير المتجانس CT (قيمة P = 0.009). أما المرضى الذين ظهر لديهم تحسن معنوي فكانوا من الحاملين لجين CT وكان لديهم مستويات لعقار ديجوكسين في الحدود العلاجية. وعليه فإن المرضى الذين لديهم اختلاف في البنية الوراثية MDR-1 كان لديهم اختلافات في مستويات عقار ديجوكسين في المصل وأن التعرف على الاختلافات في MDR-1 قد وجد بأنه مفيد في التنبوء بالنتيجة العلاجية. نوصي بعمل دراسة مكثفة على عينات كبيرة وذلك من أجل دراسة الدور المهم لجين MDR-1 من حيث تأثيره على التخلص من المواد المختلفة الخضعة لتأثير الأنزيم disposition of different substrates حتى نتمكن من تصنيف ذلك حسب تفاصيل البنية الوراثية للمرضى وذلك من أجل تحسين مستوى العلاج وتقليل الفروق الفردية بين المرضى.

This study was performed to outline the different MDR-1 (Multi-Drug Resistance-1) genotypes in a sample of 37 Egyptian patients, suffering from atrial fibrillation (AF) and/or congestive heart failure (CHF) and are using digoxin, to assess the role of MDR-1 genotypes polymorphism in affecting steady state serum digoxin therapeutic levels, and studying the consequences on patients' clinical outcome. Two venous blood samples were drawn from each patient; the 1st sample was taken, on admission, for DNA extraction and genotyping and the 2nd was taken, 6 hours post dose after reaching steady state concentration, for serum digoxin assay. Serum digoxin levels were assayed using EMIT 2000 analyzer, and MDR-1 genotyping was done using a

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polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Twenty patients (54.1%) showed serum digoxin levels within the therapeutic range, 12 patients (32.4%) showed serum digoxin levels under the minimum effective concentration (< 0.9 ng/ml), while 5 patients (13.5%) showed serum digoxin levels over maximum safety concentration (> 2 ng/ml), with P value of 0.0001 among the three groups. MDR-1 genotyping revealed ten patients (27%) carrying the homozygous mutant TT genotype, 27 patients (73%) carrying the heterozygous mutant CT genotype, with no patient showing the wild CC genotype. Allelic distribution showed 42% for the wild type C allele while 58% for the homozygous mutant T allele. Patients carrying the homozygous mutant TT genotype showed significantly lower serum digoxin levels compared with those carrying the heterozygous mutant CT genotype (P value: 0.009). Patients with significant improvement carried the CT genotype and had serum digoxin levels within the therapeutic range. In conclusion, patients with different MDR-1 genotypes had variations in their serum digoxin levels and identification of MDR-1 variations was found useful in predicting therapy outcome. We recommend further extensive work on large samples to study the important role of MDR-1 gene in affecting the disposition of different substrates, to be able for individualizing them according to the patients' genetic profile in order to improve drug therapy and reduce inter-patient variability.

Key words: Digoxin, Therapeutic Drug Monitoring (TDM), gene polymorphism, p-glycoprotein, Multi-Drug Resistance-1 (MDR-1).

Introduction

Therapeutic drug monitoring (TDM) in the traditional sense involves the measurement and interpretation of drug concentrations in order to individualize therapy. TDM of the future will involve not only the mere measurement and interpretation of drug concentrations, but also will include a pharmacogenetics-oriented monitoring (1).

In traditional TDM, we assume that the plasma drug concentration mirrors the concentration at the site of action. As such, drug concentrations relate pharmacokinetics (time course of drug concentrations) to pharmacodynamics (concentration-effect relationship). On a molecular level, pharmacokinetics is controlled in part by the drug metabolizing enzymes, whereas pharmacodynamics is controlled by drug target proteins (2). Hence, with pharmacogenetics-oriented TDM, we can go beyond the pharmacokinetic interpretation involved in traditional TDM. With pharmacogenetics testing, we can evaluate genetic polymorphisms in drug metabolizing enzymes, transporters, and target or receptor proteins that are linked to inter-individual differences in the efficacy and adverse effect profile of many drugs (3).

The ultimate goal of pharmacogenetic monitorring is to personalize drug therapy, which is to determine the most effective and safest dosage of the best drug for a particular patient before the first dose given. As such, pharmacogenetic testing attempts to predict an individual's response to a given drug prior to its use and tailors a particular drug to the individual patient (4, 5).

In contrast to traditional TDM, which cannot be performed until after a certain drug is administered to the patient, pharmacogenetics oriented TDM can be conducted even before treatment begins (6). In traditional TDM, the interpretation of drug concentrations typically requires that the blood (or rarely, saliva) samples must be collected under steady-state conditions. Patient compliance with therapy is needed for the assay results to be interpretable. In contrast, genotyping can be performed not only with blood, but often it can also be determined less invasively from saliva, hair roots or buccal swab samples. Neither steady-state conditions nor patient compliance with drug therapy at the time of sampling are required for interpreting the genotyping results. Another unique difference is that a "drug level" in traditional TDM provides predictive value for only the single drug whose concentration is being measured (1), where as in pharmacogenetics-oriented TDM, the patient's genotype can provide predictive value for multiple drugs [e.g. a number of P-gp (Permeability-glycoprotein) substrates]. Furthermore, instead of the simple descriptive information provided by traditional TDM, pharmacogenetic testing could produce mechanistic information about why a patient may require a higher or lower dosage of the drug or a different drug altogether (7, 8). Also, genotyping has constant value over individual's lifetime (and not influenced by concomitant drug administration, alteration in hormonal levels or disease states) (4).

In current clinical practice, pharmacogenetic testing is performed for only a few drugs (e.g. mercaptopurine, thioguanine, azathioprine, trastuzumab and tacrine) and in a limited number of teaching hospitals and specialist academic centers. Other drugs (e.g. warfarin, phenytoin, codeine, oral hypoglycaemics, tricyclic antidepressants, aminoglycosides, digoxin, cyclosporin, cyclophosphamide, ifosfamide, theophylline and clozapine) are proposed to be potential candidates for pharmacogenetics-oriented TDM. However, prospective studies of pharmacogenetics-oriented TDM must be performed to determine its efficacy and cost effectiveness in optimizing therapeutic effects while minimizing toxicity (1).

In the current study we aimed at outlining the different MDR-1 genotypes in a sample of 37 Egyptian patients, suffering from AF and/or CHF and are using digoxin, to assess the role of MDR-1 genotypes polymorphism in affecting steady state serum digoxin therapeutic levels, and studying the consequences on patients' clinical outcome.

Patients and Methods

Patients:

Thirty seven patients suffering from CHF (17 patients), AF (11patients) and both CHF and AF (9 patients) were selected from the Critical Care Medicine Department, Cairo University Hospitals, to be enrolled in this study (which was approved by the Hospital Committee). Patients comprised 19 males and 18 females with a mean age of 54.4 ± 15.2 years. They were chosen from those patients who were admitted to the Critical Care Medicine Department, from April 2003 to December 2003. A patient is initially considered to be a candidate for this study when digoxin therapy was indicated. Patients were selected to have non-significant variations in their demographics and pretreatment clinical data. Medical history, careful physical examination, chemistry profile (with special focus on renal, liver and thyroid functions and electrolytes) were obtained upon enrolment into the study. Investigations also included standard 12-lead ECG using 3-channel direct writing recorder with a paper speed 25 mm/second on admission, and then daily through out the study. AF was diagnosed in patients relying on: the presence of irregular atrial activity occurring at rates over 300 beats/min, ventricular rates lying between 110-150 beats/min and ECG findings; absence of P waves, irregular undulation of the base line and totally irregular cardiac cycles (9). CHF was diagnosed in patients relying on: (a) Symptoms where all patients had symptoms of left sided heart failure, right sided heart failure or biventricular failure. These symptoms were consistent with NYHA class III and IV. (b) Physical signs included pulmonary crepitations, third heart sound, hepatomegaly, lower limb edema and ascites (10). Exclusion criteria (11) included: liver or renal impairment, electrolyte imbalance, abnormal thyroid function, severe hypo or hypertension, concomitant drugs that are known to potentially interact with digoxin (e.g. quinidine, verapamil, spironolactone, salbutamol), concomitant diseases increasing sensitivity to digoxin (e.g. early phase post-myocardial infarction, acid-base imbalance), or patients on previous digoxin therapy.

Methods:

Identification of the patient's MDR-1 gene polymorphism:

Five ml venous blood sample was drawn from each patient on admission on EDTA tube, and stored in the form of whole blood frozen at -30^{o}C till being used for DNA extraction and genotyping. DNA was extracted from blood sample, preserved with EDTA, using QIAGEN amp blood Mini Kit and buffer Al, ATL, AW1, AW2 and AE (QIAGEN Inc., Avenue Stanford, Valencia, CA, USA). Genomic DNA was subjected to amplification with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the thermal cycler for gene amplification (Perkin Elmer, Gene Amp PCR System 2400, Applied biosystem, USA). PCR amplification of exon 26 of the MDR-1 gene was carried out using the primers MDR-1 ex26f and MDR-1 ex26r. The PCR consisted of an initial denaturation steps at 94 ^{o}C for 2 min, followed by 35 cycles at 94 ^{o}C for 30 sec, 60 ^{o}C for 30 sec and 72 ^{o}C for 30 sec. the final extension step was performed at 72 ^{o}C for 7 min. MDR-1 gene alleles were identified from the amplicon yielded from PCR. The amplicon was subjected to 1-hour incubation with SAU3AI restriction enzyme, obtained from *Staphylococcus aureus* bacteria, and used for the digestion of PCR product (Amersham Biosciences, Amersham plc. LCB, England), and basal buffer (Amersham Biosciences, Amersham plc. LCB, England) for 37^{o}C using Jouan incubator (Jouan Inc., Jouan Global Center, Winchester, VA, USA). The

digestion products were separated, by electrophoresis using Gel-Electrophoresis Bath (Whatman, Biometra GmbH I.L. Goettingen, Germany), on 3% agarose gels containing ethidium bromide. The MDR-1 gene contains a recognition site for the restriction endonculease, SAU3AI, (GATC/CTAG) so the digestion of the PCR amplicon with SAV3AI yields 130 bp and 70 bp fragments. The MDR-1 gene T allele does not contain a recognition site for restriction endonu-clease, SAU3AI, so the 200 bp amplicon remains unaltered after incubation with SAU3AI. One unit of the enzyme is defined as the amount of the enzyme required to completely digest 1µg of Lambda DNA in 1 hour at 37°C in a total reaction mixture of 50 ml of the recommended assay buffer. To decrease genotyping error, twice determination was carried out for each sample.

Drug administration

Patients were given digoxin (Cardixene tables: each tablet contains 0.25 mg digoxin; Alexandria Company for Pharmaceuticals, Alexandria, Egypt). Digoxin dose was tailored for each patient according to his personal data, using Jelliffe's formula, utilizing the digoxin-dosing calculator (12).

Determination of serum digoxin level

For digoxin assay, 2 ml venous blood samples were drawn, 6 hours post dose of the 10th day, from each patient (through an indwelling venous catheter placed in either fore arm). Blood samples were immediately centrifuged after collection in order to separate the serum fractions. Severely lipemic and hemolyzed samples were avoided as they may cause poor quantitation. Sample collected from each patient was freshly assayed *using digoxin enzyme multiple immunoassay technique (EMIT 2000), supplied by Syva Company (*Dade Behring Inc., Cupertion, CA, USA). The collected samples were assayed, after calibration of the apparatus by duplicate determinations of the assay calibrators and construction of a standard curve that was validated and recalibrated by the control results. The calibrators ranged from 0 to 5 and contained digoxin concentrations of 0, 0.5, 1, 2, 3 and 5 ng/ml, respectively. Two controls were assayed in every 24-hour period. If controls were found to be within their concentration limits (X ± 25%), calibration was considered verified as was instructed by Syva company consultant. Serum digoxin levels were calculated automatically by the Emit 2000 analyzer (Syva Co, Dade Behring Inc., Cupertino, CA, USA).

Assessment of patient's outcomes:

Patient's outcomes were assessed in relation to digoxin levels and MDR-1 genotypes.

Statistical analysis:

Collected data were coded and verified prior to computerized data entry. Descriptive statistics were applied (Frequency, percentage, mean and standard deviation). Testing homogeneity of data was done using coefficient of variation. Tests of significance were applied to test hypotheses; these tests included the Chi-square and Student t-test (unpaired). Fisher Exact and Linear-by-Linear association tests were used instead of Chi-square test when a threat to validity of the test was evident (i.e., any cell contain a count less than 5), Mann-Whitney test was used for comparison between 2 independent groups when the mean values were violated. ANOVA test was used for comparison between more than two groups. P value < 0.05 was considered significant. Analysis was performed using statistical program SPSS version 10. Presentation was done using SPSS program, MS Word and MS Excel.

Results

Patients' demographics and clinical presentation:

Distribution of patients involved 11 suffering from AF (29.7%), 17 suffering from CHF (45.9%) and 9 patients (24.3%) suffering from both CHF and AF. Underlying risk factors for AF were: mitral valve stenosis in 3 patients; dilated cardiomyopathy in 2 patients; post coronary artery bypass graft in 2 patients; alcoholism in 1 patient; rheumatic heart diseases in 4 patients; with 8 patients showing lone AF.

Patients demographics and pretreatment clinical presentation showed that patients had a mean age of 54.4 $\pm$ 15.2 years (CV%: 27.9), mean body weight of 76.5 $\pm$ 10.2 Kg (CV%: 13.3), and their heights ranged from 155 cm to 180 cm with a mean of 166.4 $\pm$ 7.7 cm (CV%:4.4). All patients had normal liver, renal and thyroid functions, and normal electrolyte balance. All the study participants received digoxin dose calculated according to Jelliffe's formula, the mean daily dose of digoxin received was 0.23 $\pm$ 0.07 mg/day (C.V:30%) ranging from 0.125 mg to 0.375 mg. In addition to digoxin therapy, patients

were receiving a number of concomitant drugs, with different regimens, that are known to have no drug interaction with digoxin: 18 patients received ranitidine 150 mg tablets, 13 patients received ranitidine 50 mg ampoules, 10 patients received marevan 1 mg tablets, 7 patients received marevan 5mg tablets, 11 patients received furosemide 40 mg tablets, 1 patient received 40 mg, ampoules, 3 patients received clexane 40 mg ampoules, 5 patients received 20 mg ampoules, 2 patients received omperazole 20 mg capsules, 2 patients received aspirin 150 mg tablets, 1 patient received gentamycin 80 mg ampoules, 3 patients received dexamethasone 8 mg ampoules, 3 patients received amoxicillin /clavulinic acid 1.2 gm vials, 8 patients received cefoperazone 1.5 gm vials, 2 patients received vitamin B compex ampoules, 2 patients received clindamycin 600 mg ampoules, 3 patients received allopurinol 300 mg tablets, and 1 patient received metformine HCl 500 mg tablets

Serum digoxin levels:

Serum digoxin levels in the 37 patients ranged from 0.25 ng/ml to 4.62 ng/ml with a mean level of 1.57 ± 1 ng/ml (C.V:64%). There was a non-significant difference in mean serum digoxin levels between males and females (1.619 ng/ml and 1.528 ng/ml respectively, P value=0.79). Table (1) shows the distribution of patients within and outside the therapeutic range. Out of the 37 patients, 20 patients (54.1%) had serum digoxin levels within the therapeutic range (0.9 - 2 ng/ml (9,10)) with a mean of 1.6 ± 0.3 ng/ml, 5 patients (13.5%) had values greater than maximum safety concentration (MSC: more than 2 ng/ml) with a mean of 3.7 ± 0.8 ng/ml and 12 patients (32.4%) had values below the minimum effective concentration (MEC: less than 0.9 ng/ml) with a mean of 0.67 ± 0.17 ng/ml. A great variability in serum digoxin levels among patients was clear, where a statistically significant difference (P value < 0.0001) in serum digoxin levels was seen among patients falling within the therapeutic range and those falling either above the MSC or below MEC.

Genotyping of patients:

C3435T MDR-1 genotyping revealed 10 patients (27%) carrying the homozygous mutant TT genotype, and 27 patients (73%) carrying the heterozygous mutant CT genotype, with no patients showing the homozygous wild type CC. Allelic distribution in patients, showed that the wild type C allele composed 42% of the total number of alleles, while the mutant T allele showed 58%.The relation between different MDR-1 genotypes and serum digoxin levels in the patients' population are expressed in Table (2) and Figures (1). A highly significant difference in serum digoxin levels was found between patients having TT genotype and those having CT genotype, P value= 0.009; this is clear in Table (3).

Table 1: Distribution of patients within and outside the therapeutic range of digoxin.

Patients groups	Mean±SD (ng/ml)	Range (ng/ml)	P value
Group I: 20 patients (54.1%) within the therapeutic range (0.9-2 ng/ml)	1.6±0.3	1.02-1.98	
Group II: 12 patients (32.4%) under minimum effective concentration (< 0.9 2 ng/ml)	0.67±0.17	0.25-0.88	0.0001
Group III: 5 patients (13.5%) over maximum safety concentration (> 2 ng ng/ml)	3.7±0.8	2.44-4.62	

SD: Standard deviation, confidence limit: 95%.

Table 2: Relation between different MDR-1 genotpyes and serum digoxin levels.

Genotype Serum digoxin levels	CT Genotype		TT Genotype	
	No.	%	No.	%
Within therapeutic range (0.9-2 ng/ml)	16	43.2	4	10.8
Under minimum effective concentrations (< 0.9 ng/ml)	6	16.2	6	16.2
Over maximum safety concentrations (> 2 ng/ml)	5	13.5	0	0

No.: Number of patients;

Table 3: The difference in serum digoxin level between patients having different MDR-1 genotypes.

Para-meter	Group	No.	Mean Rank	Sum of ranks	P value
Serum digoxin levels	Patients with TT genotype	10	11.4	114	0.009
	Patients with CT genotype	27	21.81	589	

Mann-Whitney test No: Number of patients; confidence limit: 95%.

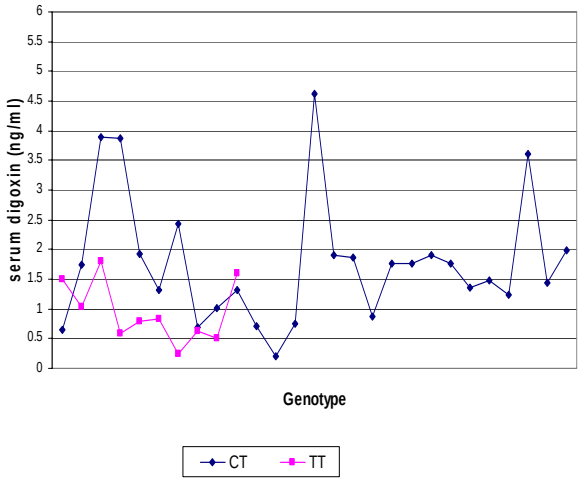

Figure 1. Serum digoxin levels in patients with the TT and the CT genotype.

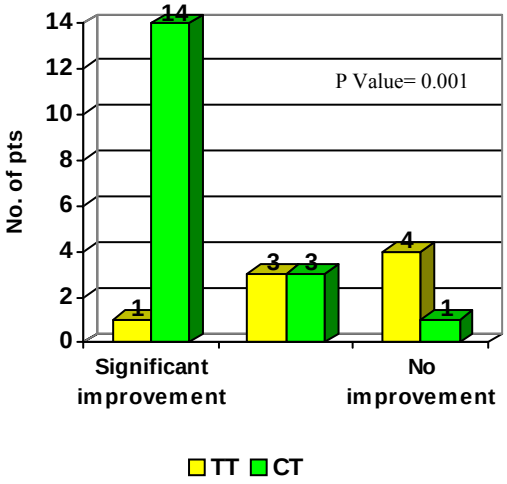

Figure 2. Relation between different MDR-1 genotypes and outcome of patients with CHF.

Patients' outcome:

By the end of the study, out of the 20 patients having AF, heart rate was controlled in 16 (80%), and was not controlled in 4 patients (20%), While in patients suffering from CHF, 15 patients (57.7%) showed significant improvement, 6 (23.1%) showed limited improvement whereas 5 patients (19.2%) showed no improvement,

Regarding patients with AF, a highly significant difference (P value= 0.005) was found in serum digoxin levels between patients who had their heart rates controlled (1.9 $\pm$ 0.7 ng/ml) and those whose heart rates were not controlled (0.7 $\pm$ 0.1 ng/ml). Most patients with controlled heart rates showed serum digoxin levels within the target therapeutic range. Also, regarding patients with CHF, a statistically significant difference in mean serum digoxin levels among patients with significant improvement (1.9 $\pm$ 1 ng/ml), those with limited improvement (0.9 $\pm$ 0.3 ng/ml) and those who showed no improvement (0.5 $\pm$ 0.19 ng/ml), P value = 0.004.

A non- significant relation in the outcome of patients having AF, with respect to their genotypes, P value= 0.72 (Fisher's exact test). However analysis the relation between different MDR-1 genotypes and outcome of CHF, using Linear-by-Linear Association, revealed a highly significant difference in the outcome of patients with respect to their genotypes, P value= 0.001*;* Figure (3).

Discussion

Considering the important role of MDR-1 gene in limiting oral absorption and target organ accumulation of many therapeutic agents, one may expect that patients with different MDR-1 genotypes will have variable expression of P-gp and thus will cause variations in the individual responses; either resistance or overreaction of some patients to drug therapy (14). In the current work we outlined the different MDR-1 genotypes in a sample of 37 Egyptian patients using digoxin, and studied the role of different MDR-1 genotypes in affecting serum digoxin therapeutic levels. Also, the consequences of gene polymorphism on patients' clinical outcome were taken into consideration.

Patients were selected on basis of having non-significant variations in their demographics and clinical data. This was done in order that, the only variations among patients would be those resulting from the difference in MDR-1 genotypes. Therefore, we avoided or minimized as possible physiological or pathophysiologic differences that may affect absorption, distribution, metabolism and elimination of digoxin (14), and avoided any abnormalities in conditions that may cause increased

sensitivity to digoxin, including electrolyte imbalance, renal failure, hepatic failure, hypertension, hypo-or hyperthyroidsm, where extremely large amounts of digoxin may be required in hyperthyroidism and smaller dosages may be required in hypothyroidism (11, 15). Two of our patients were suffering from dilated cardiomyopathy which may increase sensitivity to digoxin based on results obtained by Meissner *et al* (16) who observed that patients with cardiomyopathy had decreased p-glycoprotein expression in heart tissues. This sort of variation could be accepted in our patients since it resulted from variation in MDR-1 gene expression. Sex difference was not a source of variations because there was no significant difference in mean serum digoxin levels between males and females (1.619 ng/ml and 1.528 ng/ml respectively, P value= 0.79). This is supported by *Hudson and Pilote* (17) who presumed no sex-based differences in response to digoxin therapy. Also, it was expected that concomitant drug administration in this study would not produce variations in serum digoxin levels because none of the concomitant drugs administered to our patients have been reported to have significant clinical effects on digoxin pharmacokinetics (18). Furosemide or dexamethasone may increase the risk of digitalis toxicity in patients used any of them in this study because both can cause potassium loss, in addition dexamethasone can cause also sodium and water retention. Although these reactions are possible, these drugs are often used concomitantly with digoxin (19, 20). Patients' investigations, in the current study, therefore included special focus on electrolytes' monitoring. On the other hand, all of the study participants received oral digoxin dose calculated according to Jelliffe's formula (12), in order to optimize digoxin dose given to each patient. All patients followed a-once daily-dosage regimen, whereby digoxin in the form of oral tablets was given to the patient at the same time every day (21), by the nursing staff in order to ensure complete adherence to the received medication.

Serum digoxin levels:

Serum digoxin levels determination during the absorption and distribution phases is in general, not meaningful (22-24). Elevated digoxin serum concentrations during the distribution phase, might lead to the unnecessary actions of rapidly adjusting digoxin dose. It is usually not recommended to take a digoxin sample less than 6 hours after digoxin intake (25-27). We selected, day 10 for sampling to ensure steady state levels and 6 hours post dose administration as sampling time to ensure complete absorption and distribution.

A great variability in serum digoxin levels was observed in our patients' population, whereby 17 patients representing 45.9% of our study participants showed digoxin serum levels outside the therapeutic range. The important role played by P-gp in the absorption, distribution and excretion of a large number of structurally diverse drugs including the cardiac glycoside digoxin, (28-30) and the recently defined role of MDR-1 gene in determining the degree of expression of P-gp (31) render structural alterations, due to MDR-1 genetic polymorphism, an important factor that may contribute to the individual variability in the plasma or serum concentration of P-gp substrates. This may explain the observed variability in serum digoxin levels of our patients in spite of careful patient selection and optimization of all the classic co-variables known to affect digoxin concentrations, suggesting the presence of the recently evolving genetic factors that might contribute to this variability.

Gene-polymorphism:

C3435T MDR-1 genotyping revealed two different genotypes in our study participants. Ten patients (27%) showed the TT genotype, while 27 patients (73%) showed the CT genotype, with no patients showing the wild type CC genotype. In 2003, Hamdy *et al* (32) outlined the MDR-1 genotypes in a total of 200 unrelated Egyptian subjects and they revealed 34%, 51.5% and 14.5% for CC, CT and TT, respectively. Differences between our results of MDR-1genotyping and those of Hamdy *et al* (32) could be explained from different views. In Hamdy *et al* (32), the aim was principally genotyping and allele frequencies distribution in the Egyptian population. The selected subjects therefore, were unrelated Egyptian healthy subjects who were chosen randomly from students and staff members at Cairo University. This gives flexibilities in selection and consequently variations in genes. On contrast, our study populations were closely related patients because we focused on the effect of MDR-1 gene polymorphism on serum digoxin levels in patients with AF and CHF. Therefore we faced great difficulties in selecting patients, specially we tried to decrease variations among patients during the selection. This explains

the small sample size, the closely related subjects and may explain variations from Hamdy *et al* (32).

The prevalence of the TT genotype in our sample of Egyptian population was found similar to German Caucasians (31, 33), British Caucasians (34), Portuguese (34), Japanese (35), Chinese (34), Filipino (34), and Saudi Arabians (34). It differed significantly from South-West Asians (34), Ghanaians (34, 35), Kenyans, (34), Sudanese (34) and African Americans (34, 35). The frequency of distribution of the CT genotype, in this study, appeared to be different from the German Caucasians (31, 33), British Caucasians (34), Portuguese (34), Japanese (35), South-west Asians (34), Chinese (34), Filipino (34), Saudi Arabians (34), Ghanaian (34, 35), Kenyan (34), Sudanese (34), African Americans (34, 35). Our results revealed distribution frequency of 42% for the wild type C allele and 58% for the mutant T allele, which were similar to German Caucasians (31, 33), British Caucasians (34), American Caucasian (36), Portuguese (34), Japanese (35), South-West Asians (34), Chinese (34), Filipino (34), and Saudi Arabians (34). Results were different from Ghanaians (34, 35), Kenyans (34), Sudanese (34), and African Americans (34 - 36).

Effect of gene-polymorphism on serum digoxin levels:

Our data represent the first study to show the effect of variations in MDR-1 gene on serum digoxin levels in a sample of Egyptian patients suffering from AF and CHF. Current literature concerning the effect of different MDR-1 genotypes on serum digoxin levels is scanty (37). In the current study, TT genotypes showed significantly lower serum digoxin levels than CT genotypes. This is attributed to the level of expression of the MDR-1 gene encoded P-gp in the intestinal epithelial cells, since different MDR-1 genotypes control the amount of protein expressed in the intestine (38), and P-gp functions as an energy dependent efflux system which export its substrates from inside of the cells to the outside through cell membrane (37). Patients with lower P-gp expression therefore are expected to show higher digoxin levels and vice versa.

In agreement with our results, *Sakaeda et al.* (39), observed the same changes in serum digoxin levels in homozygous TT carriers compared to CC and CT carriers in a Japanese population. Different results were observed by *Hoffmeyer et al.* (31), *Kurata et al. (40),* and *Verstuyft et al. (38);* who found that homozygous TT subjects had significantly higher serum digoxin concentrations than CC and CT subjects.

Kim et al. (36), in their study on the H_1-antihistamine fexofenadine, reported significantly lower AUC of fexofenadine in the 3435TT group in comparison to the CC group. *Kerb et al.* (41), found a non-significant trend towards higher phenytoin plasma levels in TT subjects compared to healthy volunteers with CT or CC genotypes. Moreover, they found that the CC genotype was significantly more common in volunteers with low phenytoin plasma concentrations. Some other P-gp substrates, such as cyclosporine A, appear to be also affected by MDR-1 genetic polymorphism (42). In the study conducted by *Barlam et al.*, (42), they found that Chinese heart transplant patients carrying the CC genotype showed lower cyclosporine A plasma $AUC_{(0-4)}$ values when compared with TT genotypes. One can see that controversy still exists between investigators on which genotypes cause low P-gp expression and which cause high expression.

Patients' outcome:

Patients having rate controlled AF or those with CHF having significant improvement, in the present study, showed mean serum digoxin levels within the therapeutic range and more individuals caring the CT genotype while those with treatment failure showed serum digoxin levels under the minimum effective concentration. Data in the literature provide convincing evidence that serum digoxin levels within the target therapeutic range reduces heart failure-associated hospitalizations and emergency room visits (43-47).

So far, little information is available regarding the potential importance of MDR-1 polymorphisms on treatment outcome. In a study conducted by *Stanulla et al.* (48) the risk of CNS relapse in childhood acute lymphocytic leukemia (ALL) was significantly lower in patients TT or CT genotype in comparison to the CC genotype. In a second study carried out by *Fellay et al.* (49), on HIV-1-infected individuals with allelic variants of the MDR-1 gene, those with TT genotype had a significantly better immunological response to antiretroviral therapy containing nelfinavir than those having CC genotype, while those having CT genotype had a moderate response to therapy.

In conclusion, the discovery and characterization of variations in the MDR-1 gene and diagnostic tests for the discrimination of different MDR-1 alleles in human individuals may be an important tool for improving the therapy of diseases and individualizing doses of drugs that are substrates of the MDR-1 gene product. The identification of MDR-1 variations may also be useful to predict therapy outcome. Furthermore, the characterization of novel MDR-1 variants may be useful for the development of novel inhibitors that specifically modulate the activity of the individual types of MDR-1.

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