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Original Research Paper

Relationship Between EPHX1 Gene Polymorphism and Warfarin Resistance

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ABSTRACT:

Introduction: Warfarin is an oral anticoagulant with a major role in thrombosis prevention. In clinical practice, the warfarin anticoagulant effect should be regularly observed using the international normalized ratio (INRs) to ensure a proper, safe, and effective dose. Many factors can affect the variability of warfarin dose. Several studies confirmed the possible clinical use of genotype-guided warfarin starting dose to provide a safe anticoagulation therapy. **Aim**: This cross-sectional study aimed to identify the potential importance of EPHX1 gene polymorphism (rs2292566) in warfarin resistance. **Patients and Methods:** Thirty patients with warfarin resistance (>10 mg/ day) not reaching targeted INR (2-3) (Group 1) were enrolled in this study besides 10 patients with optimum response warfarin (Group 2). All patients were subjected to history taking, clinical examination, and laboratory investigation of EPHX1 gene polymorphism by PCR. **Results**: 27 patients (90%) of group1 were of GG genotype and 3 patients (10%) were heterozygous mutant (GA). While in group 2, 6 patients (60%) had (GG) genotype and 4 patients (40%) were heterozygous (GA). The (AA) genotype was not detected in both groups. The comparison between both groups regarding genotype distribution showed a statistically significant difference (P=0.031), The dose of warfarin required to achieve the treatable INR in GA was 12.09 ± 2.07 and in GG was 13.16 ± 2.61 with no significant difference between them (P=0.295). **Conclusion**: The incidence of EPHX1 gene polymorphism (rs2292566) is higher in patients with warfarin resistance with no effect on warfarin dose in the studied population.

Keywords: Oral anticoagulants, Warfarin resistance, EPHX1 gene.

INTRODUCTION:

Oral anticoagulants (OACs) are among the most prescribed drugs all over the world as a preventive tool for thrombosis. At present, both the avoidance and the treatment for thromboembolic conditions are mainly by using 4- hydroxycoumarin derivatives chiefly Warfarin. An additional group of oral anticoagulants (OAC) drugs, like Fluindione is now utilized in France [1]. The OAC maintenance dosage is affected by various factors, for example patient weight, sex, age, ethnicity and the pharmacogenetic effect of CYP2C9, EPHXI, and VKORC1 [2]. However, in certain patients, the excess dose of OAC may be needed and can't be clarified either by these factors or by hereditary genes variability [3]. Clinically, OAC resistance is defined by requiring higher dose of OA to reach the stable therapeutic International Normalized Ratio [INR]. While complete resistance is clinically defined by a very high dosage needed with the INR not reaching the stable therapeutic target [4]. Understanding of the molecular theoretical basis of personal liability to Warfarin resistance is challenging. Recent technique as next-generation sequencing has enabled the comprehensive profiling of genetic variation in Warfarin resistance. Molecular biology will improve our knowledge regarding the different genetic causes of Warfarin resistance and will help in the identification of patients with Warfarin resistance requiring targeted therapeutics [5]. Human EPHX1 gene located in the long arm of chromosome 1q42.1, consisting of 9 exons traversing roughly 35.48 kbps, and it encodes a protein of 455 amino acids. Some studies recommend that EPHX1 genetic polymorphisms influence the warfarin maintenance dose and the inter-individual variability in responses to warfarin may significantly affected [6,7]

This study aims to identify the potential importance of EPHXI polymorphism (rs2292566) in patients with Coumarin based oral anticoagulant resistance and its clinical significance.

Patients & Methods:

Study design: This retrospective cross-sectional study was carried out in the Hematology Unit of the Internal Medicine Department, Faculty of Medicine, Tanta university; on thirty patients with oral anticoagulant resistance (>10mg/day not reaching targeted INR (2-3)) (group1) besides the other 10 patients on oral

anticoagulant showing optimum response (group 2). This study was approved by the medical research ethics committee faculty of medicine, Tanta University (32183/03/18). The patients were 18 years old or older. Patients with prior blood transfusion (6-9 weeks), bone marrow or liver transplantation were excluded from the study. All participants were subjected to history taking including age, sex, occupation, residence, and special habits like smoking, family history, comorbid disease and any medication. Medical examination included body weight, height, body mass index, indication of anticoagulation dose, maximum warfarin dose taken and INR. The EPHX1 gene mutation (rs2292566) was detected by PCR-RFLP technique. Genomic DNA was extracted from blood samples collected on EDTA, using a Qiagen DNA blood mini kit (Hilden, Germany). The EPHX1 gene was amplified using the following primers f: ATGAAGGGGCGGCGGGGGCACTAAGGG, r: CTTGGCGAGGACGGGGCAGTTATGGAA (Invitrogen, USA). Each PCR reaction contained 10 Pmol of each primer and 40 ng DNA. After amplification of the EPHX1 gene (exon 4), the PCR product was subjected to an overnight digestion with RsaI restriction enzyme (ThermoScientific, USA). The fragments lengths were detected thereafter by agarose gel electrophoresis 2% with ethidium bromide staining.

Statistical Analysis:

The collected data were organized, tabulated and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 19, SPSS Inc. Chicago, IL, USA)

RESULTS:

The demographic and clinical data of the 2 studied groups are shown in table 1.

Table 1: clinical and demographic characters of the studied patients

		Group 1 (n=30)	Group 2 (n=10)	P value
Age (mean ± SD)		42.07±13.28	41.0±12.77	0.826
BMI (mean ± SD)		26.85±3.98	25.90±5.13	0.546
Sex (n & %)	Male	12 (40%)	18 (60%)	0.271
	Female	18 (60%)	12 (40%)	
Smoking (N & %)		7 (23.3%)	4 (40%)	0.307
Diabetes		4 (13.3%)	2 (20%)	0.609
Hypertension		4 (13.3%)	3 (30%)	0.230
Liver disease		5 (16.7%)	2 (20%)	0.810
Renal disease		3 (10%)	0.	0.298
Thyroid		3 (10%)	1 (10%)	1.0

Among the studied 30 patients (group 1), 90% were GG genotype and 10% were heterozygous mutant (GA) Among the studied 10 patients (group2), 60% were GG genotype and 40% were heterozygous mutant (GA) The (AA) genotype was not detected in any of group 1 and group 2. Comparison between the studied groups as regarded genotype distribution showed statistically significant value (P= 0.031) Table (2)

Table (2): Genotype distribution of the studied patients (group 1 and group 2).

Genotype	Group 1 (n=30)	Group 2 (n=10)	Total (n=40)
	N (%)	N (%)	N (%)
GG	27 (90%)	6 (60%)	33 (82.5%)
GA	3 (10%)	4 (40%)	7 (17.5%)
P-value	0.031*		

***Statistically significant (P<0.05)**

There was no statistically significant correlation between the maximum dose of warfarin and any of the clinical, pathological or demographic features of the studied groups. Table (3).

Table (3): Correlation between clinical, pathological demographic data of studied groups and maximum dose of warfarin.

Clinical and pathological data	Maximum dose of warfarin for the studied patients (n=40)	
	r	P
Age	- 0.197	0.297
BMI	- 0.198	0.295
Sex	0.734	0.138
Smoking	0.196	0.298
DM	0.261	0.609
HTN	1.443	0.230
Thyroid	0.000	1.000
Liver	0.062	0.810
Renal	1.079	0.298
Another drug intake	0.745	0.395

***Statistically significant (P<0.05)**

Although the mean of maximum warfarin dose was higher in GA than GG, there was no statistically significant difference between patients with GG genotype and those with GA genotype, regarding the maximum dose of warfarin, with mean values of maximum dose of warfarin 12.09±2.07 and 13.16±2.61 respectively (P=0.256) Table (4).

Table (4): Maximum dose of warfarin used for treatment in relation to the genotype of the studied patients.

Maximum dose of warfarin	Genotype of the studied patients (n=40)	
	GG	GA
Range	10 – 18	10 – 15
Mean ± SD	12.09 ± 2.07	13.16 ± 2.61
P-value	0.256	

***Statistically significant (P<0.05)**

DISCUSSION:

Warfarin is an anticoagulant prescribed for thrombosis and thromboembolism prevention [8]. In daily clinical practice, warfarin anticoagulant activity should be checked by the international normalized ratio (INRs) to ensure an accurate, safe, and appropriate dose. inaccurate dose administration may cause a potential high-risk bleeding and failure of inhibiting thrombosis [9]. Age, body mass index, food intake containing vitamin K, drug-drug interaction, and genetic variability are numerous factors that have been accounted to affect the changeability in warfarin dose [10]. Several studies verified that genotype-guided dosing of warfarin is a commonly recognized instance of pharmacogenetics, and clinical use of genetics-guided warfarin starting dose would provide safe and ideal anticoagulation treatment [11]. Recently, microsomal epoxide hydrolase 1 (EPHX1) has been suggested to affect the pharmacokinetics and pharmacodynamics of warfarin and to have a clinical impact on adjusting its therapeutic dose [12]. Given that, this study was performed to identify the potential importance of EPHX1 polymorphism in patients with coumarin based oral anticoagulant resistance and its clinical significance.

The current study showed no significant correlation between neither age or sex and maximum dose of

warfarin. In Suriapranata et al., 2011 [7] study, there was no significant correlation between sex and the dose of warfarin but, there was a significant negative correlation between age and the dose of warfarin. Moreover, Özer et al., 2013 [13] found a significant correlation between the age and the dose of warfarin. In another study by Khoury et al., 2014 [14], older patients required lower doses. However, there was no statistically significant difference in the warfarin doses according to gender. The high sensitivity to warfarin in elderly is not fully understood but it may be explained by decreased serum proteins level, different metabolic activities, and sub-optimal renal excretion. These physiological changes may lead to variability in the pharmacokinetics encouraging the persistence of the drug in the circulation [15,16]. In the current study, the mean BMI of both groups showed no statistical difference. And, there was no significant relation to maximum dose of warfarin. This agrees with Kabagambe et al., 2013 [16], who found that BMI does not significantly affect Warfarin dose. In contrast, Mueller et al., 2014 [17] who showed that BMI positively correlates with the total weekly warfarin dose. Their results showed that for each 1-point increase in BMI, the weekly maintenance warfarin dose will be increased by 0.69 mg and that the average warfarin weekly dose in these patients can be estimated using the formula: 12.34 + 0.69 × BMI. A more recent study of Tellor et al., 2018 [18] verified a statistically significant higher total warfarin dose (TWD) in the morbidly obese patients compared with underweights, normal/overweight and obese patients.

In the present study, there was no significant relationship between smoking and the maximum dose of warfarin. Similarly, Ozer et al., 2013 [12] didn't detect a significant relationship between smoking and warfarin maintenance dose. In contrary, Shahin et al., 2011 [19] observed that smoking was associated with a higher warfarin dosing. Regarding EPHX1 gene polymorphism (rs2292566), in this current study 90% of patients showing warfarin resistance were of GG genotype and 10% were heterozygous (GA). While in in patients with optimal response, 60% were of GG genotype and 40% were heterozygous and the (AA) genotype was not detected in any group. Almost similar results were found by Suriapranata et al. 2011 [13], who studied the same polymorphism in 205 patients and found that 78% of patients had GG genotype, 19% had GA genotype and 3% had AA genotype. The difference between the two studies may be explained by the different sample size.

The exact mechanism how EPHX1 genetic polymorphisms affect the requirement of warfarin dose is still unrecognized. One possible explanation could be that genetic mutations in the EPHX1 gene results in amino acid substitution and affect the EPHX1 enzyme activity, with resultant diminished warfarin metabolism and clearance, thus contributing to inter-individual dose variability [20].

The effect of genotype on the maximum dose of warfarin to reach the treatable INR was tested in the current study and there was no significant relationship between genotype and maximum dose of warfarin. Similar finding was reported by Ozer et al., 2013 [12] study; of 107 patients, 77 of them were of GG genotype, 25 of were GA and 5 patients were AA, and there was no significant difference between the groups regarding their warfarin doses. Similarly, in the study of Suriapranata et al., 2011 [13] formerly mentioned, there was no statistical difference between these groups regarding doses of warfarin.

CONCLUSION:

The incidence of EPHX1 (rs2292566) gene polymorphism is higher in patients with warfarin resistance. However current study failed to link this polymorphism to the warfarin maintain ace dose. The study was limited by the very small sample size, so further studies with larger sample size are recommended

Abbreviations: EPHX1: Epoxide hydrolase 1, INR: International Normalized Ratio, OACs: Oral anticoagulants, BMI: Body mass index, DM: Diabetes mellitus, HTN: Hypertension, TWD: Total Warfarin Dose, TTR: Time in Therapeutic Range.

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