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Original Research Article

Study of environmental and genetic factors determining warfarin toxicity

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ABSTRACT

Background: Warfarin is widely used anticoagulant in treatment and prevention of thrombosis. Despite its common use, warfarin can be associated with bleeding complications because of its narrow therapeutic index. A review of many studies show average yearly rates of warfarin related bleeding as high as 0.5% , 4.9% , 15% for fatal, major, minor bleeding complications. The study is to determine age, gender, pharmacogenetics, drugs influencing warfarin toxicity in Indian patients.
Methods: Observational and cross-sectional study was conducted over period of 1 year after obtaining institutional ethics committee permission. Written and informed consent was taken from patients admitted in tertiary care hospital who fulfilled inclusion and exclusion criteria.
Results: Most common age for Warfarin toxicity in our study was between 30 to 39 years (22.5%) with mean of 42.9 years. Bleeding risk was higher in elderly with 14 out of 26 patients with age >50 years had bleeding manifestations. Toxicity was more prevalent in female (60%). 40% patients were on drugs interacting with warfarin; NSAIDS (Nonsteroidal Anti-Inflammatary Drug) and antibiotics were the most common interacting drugs. In our study, 17.5% patients had acute liver disease and one patient had deranged creatinine (2.6). 40% of patients had VKORC1 variants and 35% of patients had CYP2C9 variants. Maximum patients developed toxicity within 15-30 days of initiation of warfarin.
Conclusions: Warfarin toxicity has multifactorial cause. Drugs and Genetic variation are most common factors influencing warfarin toxicity. Warfarin toxicity has low mortality rate, although it increases with (International Normalised Ratio) INR>10 and with increasing age.

Keywords: INR, Warfarin toxicity

INTRODUCTION

Warfarin is widely used anticoagulant in treatment and prevention of thrombosis, in chronic atrial fibrillation, mechanical valves, and pulmonary embolism. Despite its common use, warfarin can be associated with bleeding complications.[1-3] Achieving a safe therapeutic response can be difficult because of its narrow therapeutic index (1-20mg/d) and great variability in dose required, which is mostly a consequence of individual genetic variants and environmental factors like age, gender, diet, drug interactions. To maintain a therapeutic level, warfarin therapy requires intensive monitoring via INR. Bleeding is most common effect of warfarin toxicity.[4,5]

Major bleeding complications include GI haemorrhage, intracranial bleeding, and retroperitoneal bleeding. Minor bleeding complications include subconjuctival haemorrhage, haematuria, epistaxis, and ecchymoses. A review of many studies show average yearly rates of warfarin related bleeding as high as 0.5%, 4.9% , 15% for fatal, major, minor bleeding complications. In spite of

efforts to achieve tight control of the international normalized ratio (INR), warfarin accounts for more emergency room visits than any other drug excluding insulin.[6,7] Warfarin, the most commonly prescribed and trickiest to manage anticoagulant, is the key culprit of hospitalizations for the elderly patient population, according to a study published Nov. in the New England Journal of Medicine.

Warfarin was followed by insulin and oral-anticoagulants, and the researchers found that nearly two-thirds of hospitalizations were due to unintentional overdoses. Warfarin-related haemorrhages accounted for 21,010 hospitalizations, which represented 63.3 percent of all warfarin-related hospitalizations.[8,9] "Novel oral anticoagulants may be an alternative to warfarin, but until the role of these agents is better defined, and as long as warfarin remains the most common cause of emergency hospitalizations for adverse drug events, safety policies should address these harms. The study is to determine age, gender, pharmacogenetics, drugs influencing warfarin toxicity and to study association of deranged liver function tests and kidney function tests with warfarin toxicity in Indian patients.

METHODS

This is an observational and cross-sectional study. After obtaining institutional ethics committee permission and obtaining written and informed consent, patient admitted in tertiary care hospital who fulfilled inclusion and exclusion criteria were recruited over period of 1 year (2014- 2015).

The study included warfarin dependent patients more than 18 years of age, with INR>4, with or without bleeding complications. All the patients were maintained on strict warfarin diet as advised by dietician to eliminate influence of dietary factors on warfarin toxicity. Sample size was 80 (according to RAO software).

On the basis of INR and Warfarin genetic analysis tests and information from the questionnaire, toxicity of warfarin was studied. List of routine and specific investigations to be conducted for the study:

- Complete Blood Count
- PT INR
- Detailed Liver Function Tests
- Renal function test
- Warfarin gene study for CYP2C9 and VKORC1 variants

This was an observational study, no interventions was done. Patients requiring specific investigation were done according to standard protocol. Most of investigations were done in our institute at free of cost.

Patients who are Warfarin dependent, More than 18 years of age, INR >4 with or without bleeding, ready to give written informed consent were included and Patients with bleeding disorders were excluded from study.

Statistical analysis

The data was analysed using appropriate statistical tests. Chi square test and student t test was applied for comparison of variables like age, manifestations, and warfarin dose levels. The p value less than 0.05 is considered statistically significant.

RESULTS

We conducted study of 80 patients, out of which 60 % were female and 40 % were male (Figure 1). Mean age of patients were 42.9 years with maximum number of patients were between 30-39 years of age (22.5%), followed by 40-49 and more than 60 years (Table 1).

Table 1: Age distribution of patients.

Age [years]	No of patients	Percent
<20	4	5.0
20-29	16	20.0
30-39	18	22.5
40-49	16	20.0
50-59	10	12.5
>60	16	20.0
Total	80	100.0

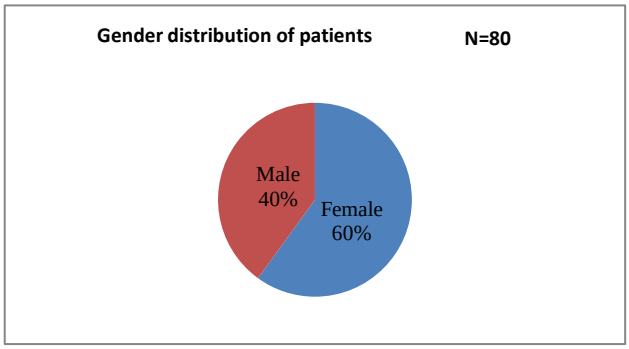

Figure 1: Gender distribution of patients.

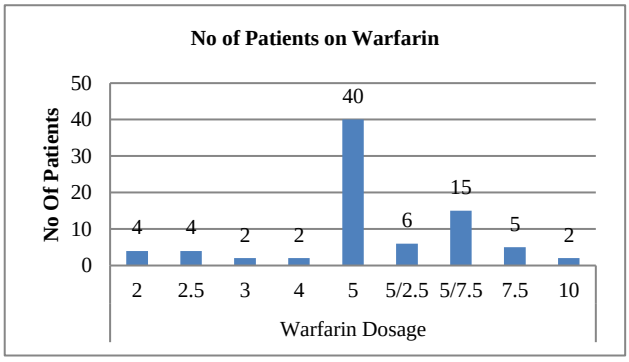

Figure 2: Warfarin dosage at which toxicity developed in study population.

Atrial fibrillation was the most common indication of initiation of warfarin, followed by cortical venous thrombosis, Prosthetic valve replacement and Deep vein thrombosis respectively. Maximum number of patients (68 i.e. 85%) were receiving higher doses of warfarin ≥5 mg when they developed warfarin toxicity. Out of these 50% patients were on warfarin dose of 5mg (Figure 2).

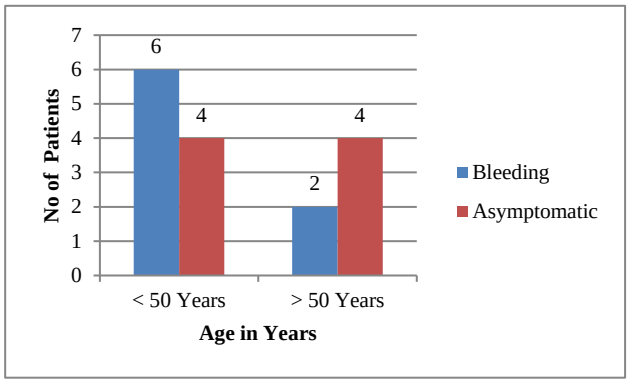

Figure 3: Correlation of warfarin dose <5 mg with age and bleeding manifestation.

When compared for age in years, warfarin dosage with risk of bleeding, it was found 8 patients out of 16 receiving warfarin <5 mg had bleeding manifestations (p value 0.301) which was not significant (Figure 3). However 26 out of 64 individuals who received warfarin of ≥5 mg developed bleeding manifestations (P value 0.033) (Figure 4).

Since p value is 0.033 for the student t test is less than that of 0.05 it indicates that there is significant association between age and bleeding manifestations when warfarin dose ≥5 mg/day (Table 2).

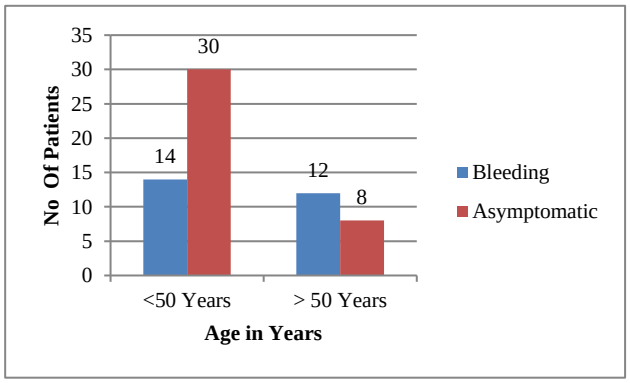

Figure 4: Correlation of age of patients with warfarin dose ≥5mg/d and bleeding manifestations.

Most of the patients in our study (65%) developed warfarin toxicity within one month of initiation of therapy followed after more than one year of warfarin therapy (30%) (Table 3). Out of these patients 52.5% of patients INR were between 4-9.9; while 47.5% patients INR was >10.

Table 2: Correlation of age of patients with warfarin dose and bleeding manifestations.

Warfarin dose	Age in years	Bleeding	No bleeding	P value
< 5	> 50	2	4	0.301
	<50	6	4	
>5	>50	12	8	0.033 (Signifi-cant)
	<50	14	30	

Table 3: Duration of warfarin prior to toxicity.

Duration of treatment	No. of patients	Percentage
<15 Days	24	30
15 days-1 month	28	35
1 month-1 year	4	5
>1 year	24	30
Total	80	100

Out of 80 individuals of warfarin toxicity in our study 46 (57.5%) were asymptomatic without any bleeding manifestations, 30 individuals had minor bleeding in the form of ecchymoses, menorrhagia, subconjuctival haemorrhage, streaky haemoptysis, haematuria, malena, bleeding per rectum. 4 of them developed life threatening bleeding of which 2 had sub-arachnoid haemorrhage and remaining 2 had subdural haemorrhage.

Table 4: Correlation of liver disorders with bleeding manifestations.

Liver Disorder	Warf dose	Bleeding	No bleeding	P value
Present	< 5 mg	6	6	0.18
	>5 mg	2	0	
Absent	<5 mg	2	2	0.65
	>5 mg	24	38	

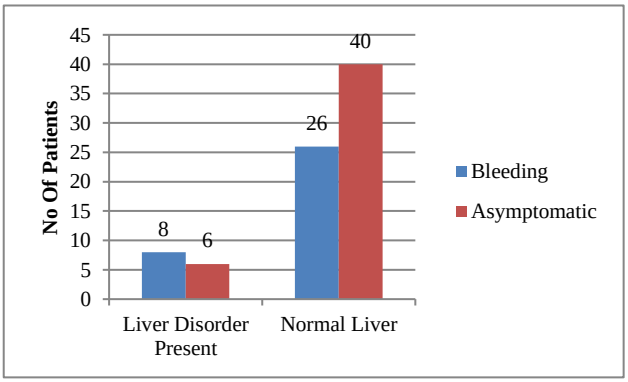

Figure 5: Correlation of liver disorders with bleeding manifestations.

In present study, 40% patients were on drugs interacting with warfarin. NSAIDS (12.5 %) and Antibiotics (15%)

were the most common interacting drugs, followed by Proton pump inhibitors (11.25%). 66 (82.5%) patients were not having any underlying liver diseases. Only one had deranged creatinine.

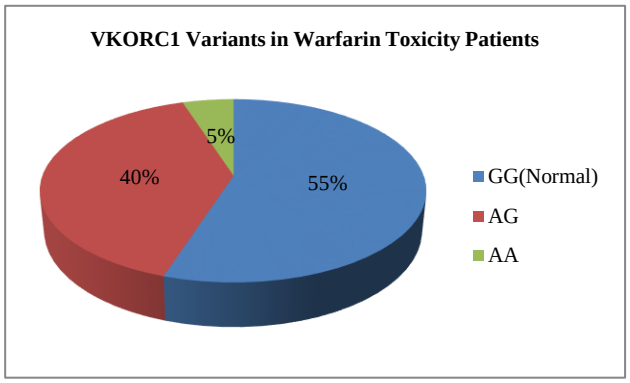

Figure 6: Role of VKORC1 polymorphism in warfarin toxicity.

Out of 14 individuals with underlying liver diseases 8 patients had bleeding manifestations while out of 66 patients without underlying liver diseases 26 patients had bleeding manifestations (Figure 5).

Table 5: Role of VKORC1 variants in warfarin toxicity patients.

VKORC1	No. of patients	Percentage
GG (Normal)	44	55
AG	32	40
AA	4	5

Table 6: Role of CYP2C9 in warfarin toxicity patients.

CYP2C9	No. of patients	Percentage
*1/*1(Normal)	52	65
Variants(*1/*2, *1/*3,*2/*2,*3/*3)	28	35

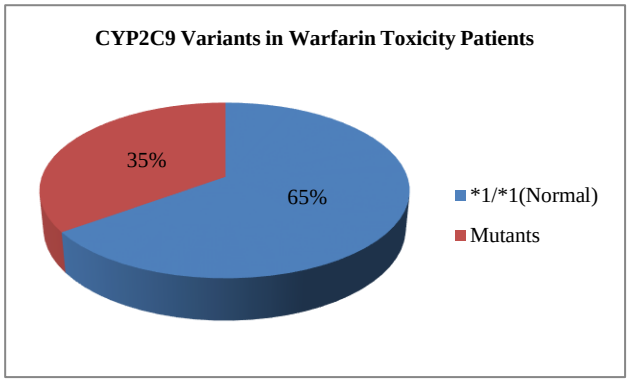

Figure 7: Role of CYP2C9 variants in warfarin toxicity.

When we analysed correlation between liver disorders and bleeding p value was 0.18. Since P value is more than 0.05 we concluded that there is no significant association between liver disorders and bleeding manifestations in warfarin toxicity patients (Table 4). In our study, 45 % of patients had VKORC1 variants (Figure 6) and 35% of patients had CYP2C9 variants (Figure 7).

DISCUSSION

Warfarin therapy is challenging because of substantial individual variations in dosage requirements that make over-anticoagulation common. In addition, because warfarin has a narrow therapeutic window, treatment frequently results in bleeding, sometimes major or life-threatening. Major bleeding, typically involving the gastrointestinal or urinary tracts or soft tissue, occurs in up to 6.5% of anticoagulated patients per year.

The incidence of fatal bleeding, primarily Intracranial Haemorrhage (ICH), is approximately 1% annually. The risk of haemorrhage increases with the intensity of warfarin anticoagulation; the variable most consistently associated with bleeding risk is elevation of the international normalized ratio (INR), a standardized prothrombin time. The relationship between the dose of warfarin and the response is influenced by genetic and environmental factors, including common mutations in the gene coding for cytochrome P450, the hepatic enzyme responsible for oxidative metabolism of the warfarin S-isomer.[2,3] Several genetic polymorphisms in this enzyme have been described that are associated with lower dose requirements and higher bleeding complication rates compared with the wild-type enzyme CYP2C9*.[4-6]

In addition to known and unknown genetic factors, drugs and various disease states can interfere with the response to warfarin.

Role of age in warfarin toxicity patients

In present study mean age of patients is 42.9 years with maximum number of patients were of age group between 30 to 39 years (22.5%) followed by 40 to 49 years and elderly patients more than 60 years (Table 1). Bleeding risk increases in elderly with increased warfarin dose. In our study, 14 out of 26 patients with age >50 years had bleeding manifestations. So in elderly population warfarin dose should be less than 5 mg/d with strict INR monitoring as p value is less than 0.03 (Table 2, Figure 4). Older adults are more sensitive to warfarin due to lower body weight, reductions in liver and renal function, and low dietary vitamin K intake.[7,8] A study by Annals of Internal Medicine done by Gurwitz JH, et al shows that Coumadin is the top drug landing elderly Americans in the emergency room, accounting for 17.3% of all adverse drug injuries.[9,12]

Gender distribution

In present study, warfarin toxicity was more prevalent in female (60%) (Figure 1). This is in concordance with a study done by Teklay G, et al, of the total 133 patients enrolled in the study, 78 (58.9%) were females.[10]

Drug interactions

In present study, 40% patients were on drugs interacting with warfarin. NSAIDS (12.5%) and Antibiotics (15%) were the most common interacting drugs. The finding of this study is consistent with previous studies.[11-14] One analysis in Norway showed that heparin, antibacterial and NSAIDs were common interacting drugs.[11] In parallel a retrospective cohort study, oral antibiotics (Azithromycin, Levofloxacin, and Trimethoprim / sulphamethoxazole (TMP/SMX)) were found to increase the incidence and degree of over anticoagulation.[14]

NSAIDs were also found to interact with warfarin. This finding is consistent with study by Kotirum, et al.[15] A double-blind, placebo-controlled, randomized study by Mahé I et al explain the in vivo interaction is that paracetamol (or its metabolites) interfere with enzymes involved in vitamin K-dependent coagulation factor synthesis. Paracetamol at 4 grams daily (a dose higher than that used in clinical practice) potentiates the anticoagulant response produced by warfarin.[24] A study of the interaction of omeprazole and warfarin in anticoagulated patients by Unge P, et evaluated 28 patients, omeprazole appears to be a partial inhibitor of enzymes within this subfamily of enzymes, P4502C, and the most probable explanation of the interaction of omeprazole with R-warfarin.[21]

Disease influencing warfarin toxicity

In present study, 17.5% patients had acute liver disease; however there was no statistically significant association between liver disorders and bleeding manifestations in warfarin toxicity patients (Table 4). Only one patient had deranged creatinine (2.6). Hepatic disease is often accompanied by coagulopathy due to a reduction in clotting factor synthesis.[16] These patients may appear to be "auto-anticoagulated" with baseline elevated INRs. Hepatic disease may reduce the clearance of warfarin. End stage renal disease is associated with reduced activity of CYP2C9 leading to lower warfarin dosing requirements.[17] Hypoalbuminemia associated with nephrotic syndrome increases the free fraction of warfarin, but there is also an associated increase in plasma clearance.[18]

Genetic factors influencing warfarin toxicity

In present study, 45% of patients had VKORC1 variants (Figure 6) and 35% of patients had CYP2C9 variants (Figure 7). A similar study done by Schwarz UI, et al and Stein CM, found that patients carrying VKORC1 haplotype A had significantly higher INR values in the first week than did non-A homozygotes.[23,25] Asians have the highest prevalence of VKORC1 variants, followed by Caucasians and African Americans. Polymorphisms in VKORC1 likely explain 30% of the variability in warfarin dose requirements. In a study by Voora D, et al analyzing the first few weeks of warfarin treatment showed that among carriers of the CYP2C9*2 or CYP2C9*3 allele, the proportion of patients with INR values of more than 3 was higher than that of patients who were not carriers.[19]

While several single-nucleotide polymorphisms of CYP2C9 have been reported, the CYP2C9*2 (Cysl44/Ile359) and CYP2C9*3 (Argl44/Leu359) polymorphisms have been identified as clinically relevant.[22] Both of these variants are associated with decreased enzymatic activity. Margaglione has also demonstrated bleeding rates as high as 27.9 per 100 patient-years in carriers of CYP variants.[20] In this study, findings were adjusted for other common variables associated with increased bleeding risk, such as increased age, drug interactions and abnormal liver function.

Several studies of the *2 and *3 CYP2C9 polymorphisms consistently show that patients with at least one CYP2C9 allele polymorphism have reduced warfarin requirements. Carriers of CYP2C9*2 and CYP2C9*3 require, on average, a 19% and 33% reduction, respectively, per allele in warfarin dose vs. those who carry the *1 allele. Carriers of the VKORC1 A allele require, on average, a 28% reduction per allele in their warfarin dose compared to those who carry none.

CONCLUSION

Warfarin toxicity has multifactorial cause. This study highlights that warfarin toxicity is more prevalent in females. The commonest age group is 30-39. Elderly are also more prone to toxicity. Warfarin toxicity in elderly has higher bleeding rates. Maximum patients developed toxicity at warfarin 5 mg/d dose within 15-30 days of initiation of warfarin. Most commonly NSAIDS, antibiotics interact causing warfarin toxicity. Genetic testing of CYP2C9 and VKORC1 helps to avoid warfarin toxicity by initiating such patients at low dose of warfarin. Patients of Warfarin toxicity have better outcomes and low mortality.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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