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THE IL-6 DEPENDENT EFFECT OF ORAL WARFARIN IN HEART VALVE REPLACEMENT PATIENTS BY MEASURING INTERACTING CLINICAL AND DEMOGRAPHIC VARIABLES

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ABSTRACT

Objective: To examine an inflammatory effect of warfarin and comparing with IL-6 levels along with different demographic and clinical variables.

Study Design: Qusai experimental study.

Place and Duration of Study: Center of Research in Experimental and Applied Medicine (CREAM), Army Medical College / National University of Sciences and Technology, Islamabad from Oct 2013 to Oct 2015.

Material and Methods: The study design was Quasi Experimental study. Samples were collected by Non-probability convenience sampling. Total 76 patients were included according to warfarin dose response in warfarin therapy patients, i.e. 32(42%) were taking <5mg / day, 37(49%) had been put on dose 5-10mg / day and 7(09%) were taking>10mg / day of warfarin dose. Patient's demographic and clinical variables were noted i.e. age, gender, BMI, duration of therapy, INR history, hepatic, gastrointestinal and diabetic complications. Human IL-6 ELISA assay was performed.

Results: The statistically significant difference was found between age groups (in years) and different levels of warfarin dose (p=0.046) along with IL-6 production. There is a negative correlation between warfarin dose and age group i.e. as age increases, the dose of warfarin decreases. Among the inter and intra-patient variability age and serum IL-6 levels were found to be statistically significant with warfarin dose response. BMI and warfarin dose were found to be weak positively correlated.

Conclusion: A marked immunomodulatory response of warfarin was noted by measuring IL-6 levels. IL-6 levels retained a significant association with warfarin dose.

Keywords: Warfarin, International Normalized Ratio (INR), IL-6, Clinical survey.

INTRODUCTION

Warfarin has been recognized as a therapeutic agent for the treatment of venous and arterial thromboembolic diseases[1]. Warfarin despite being a very effective drug has wide inter individual variability in dose requirements to attain efficient anticoagulation. Due to its narrow therapeutic range and interaction with various drugs and diseases makes its use complex and can lead to severe complications[2].

The warfarin dose is monitored by international normalized ratio (INR), which is involved in measuring the anticoagulant action of warfarin depending on prothrombin time (PT)[3]. Patients on anticoagulant drugs usually have a target INR of 2.0 to 3.0 under standardized conditions[4]. Interpretation of INR requires many considerations i.e, factors that may increase the INR are acute illnesses such as fever and diarrhea or that may decrease the INR are edema, high vitamin K intake and greater physical activity level[5]. Various factors fluctuated INR range during warfarin therapy including poor compliance, dosage error, and concurrent illness, concomitant use of other drugs, dietary interaction and aging. Together genetic and clinical factors contribute up to 47% of the dose variability[6]. Genetic factors are also known to affect the warfarin dosage requirements and

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anticoagulation therapy is affected by polymorphism in two genes, namely cytochrome vitamin K epoxide reductase complex subunit 1 (VKORC1) and P450 2C9 (CYP2C9)[7].

It is a challenging drug to use, because it requires an expert clinician and a well-educated patient[8]. The normal initiation dose of warfarin is 5mg/day, which is managed according to the patient's INR to get an appropriate maintenance dose[9]. The maintenance dosage of warfarin is the one required to achieve the INR within the therapeutic range. The range for maintenance doses of warfarin may be between 1-10mg/day[10]. The adjustments of dosage should be set through patient's prothrombin time. Usually, normal INR values (2.0-3.0) are considered sufficient for patients treated for bioprosthetic valve replacement and venous thromboembolism. For mechanical valve patients, they commonly require an INR range of 2.5 to 3.5[11].

Warfarin might influence inflammation independent from its effect as an anticoagulant. Kater and his colleagues proposed that warfarin could be involved in an inflammation pathway in addition to its anti-coagulant property[12]. Warfarin inactivates the inflammatory signal transduction at various concentrations, i.e. low warfarin concentrations, less than 20 mM inhibited IL-6 production and high concentration greater than 200 mM stimulated its release[13,14]. Various studies suggested that pro-inflammatory cytokines increase the cyclooxygenase enzyme expression while low dose of warfarin might down regulate this enzyme[15].

Keeping in mind the pleiotropy of warfarin, the purpose of this study was to investigate how various doses affected the IL-6 production in patients on warfarin therapy. The effective and safe dose response was evaluated in inter-patient and intra-patient variability with respect to patient's demography.

MATERIAL AND METHODS

The study design was Quasi Experimental study. It was conducted at Center of Research in Experimental and Applied Medicine (CREAM), Army Medical College/National University of Sciences and Technology, Islamabad Pakistan. Samples were collected by non-probability convenience sampling at AFIC/NIHD after formal institutional approval. Informed written consent was obtained from all the patients enrolled in the study. The data were collected on a proforma designed and assessed for its validity by the treating physician.

Total 76 patients, belonging to different regions of Pakistan, of heart valve diseases (congenital heart valve disease, mitral, aortic, pulmonary and tricuspid valve stenosis) who had undergone surgery (mitral, aortic, tricuspid valve replacements and repair) and were on warfarin therapy for management of anticoagulation were included in the study. The sample size was calculated according to different warfarin doses i.e. low dose taking patients, medium dose taking patients and patients receiving high doses.

Quantification of IL-6 by ELISA assay: Serum was obtained from patient's blood in a vacutainer tube by centrifugation at 3000 rpm for 5-10 minutes. IL-6 concentrations in serum samples were determined in triplicate by IL-6 EASIA kit (Invitrogen, Biosource Europe S.A) according to the manufacturer's instructions. The assay used a biotinylated antihuman IL-6 monoclonal antibody mixed with streptavidin horseradish peroxidase conjugate and human monoclonal antibody specific to IL-6 for detection at wave length 450 nm. The absorbance of standards were calculated by linear regression and the quantity of IL-6 in the samples was calculated on the basis of the formula R2=0.99. Patients were categorized among different age groups and compared with adequate warfarin doses i.e, (2.5 mg, 5 mg, 7.5 mg, 10 mg and 12.5 mg& 15 mg/day).

Statistical Analysis

Data were analyzed using SPSS version 22.0. Mean and S.D were calculated for quantitative a vertical categorical variables were presented by frequency and percentages Spearsman correlation coefficient test was done for

significant associations between warfarin dose, IL-6 levels, BMI and age of a patient. Pearson's chi square test is applied for significant difference. A *p*-value less than 0.05 considered as significant value.

RESULTS

Inter-patient variability between demographic variables associated with warfarin dose levels:

The baseline demographic variables analyzed for the study included age from 12 to 80 years. The mean ± S.D of age was 40.50 ± 12.6. Out of total number of patients,males were 44 (58%) and females were 32(42%). Thirty-two (42.1%) patients were on low dose of warfarin i.e. 2.5 or 5 mg/day,had low levels of IL-6, while seven (28.7%) patients, taking dose 12.5 mg/day & 15 mg/day had high IL-6 levels (table-I). Patients (65%) belonging to the age group 40-80 years had been prescribed to take <5mg/day of warfarin dose, resulted in low levels of IL-6. The Spearsman correlation between warfarin dose and age group was found to be -0.123 (table-II).

Table-I: Comparison of demographic and clinical characteristics associated with different warfarin dose levels (n=76).

Variables	Warfarin dose			*p*-value
	<5 mg/day (n=32) n (%)	**5-10 mg/day** (n=37) n (%)	**>10 mg/day** (n=07) n (%)	
Age groups				
15-40	10(31.3)	16(43.2)	3(42.9)	0.046
40-60	17(53.1)	07(18.9)	2(28.6)	
60-80	5(15.6)	14(37.8)	2(28.6)	
Duration of Warfarin Therapy				
< 2 months	6(18.8)	1(2.7)	-	0.170
2-6 Months	6(18.8)	7(18.9)	2(28.6)	
> 6 Months	20(62.5)	29(78.4)	5(71.4)	
Target INR of Patient				
< 2.0	13(40.6)	19(51.4)	3(42.9)	0.545
=2.0	6(18.8)	3(8.1)	2(28.6)	
>2.0	13(40.6)	15(40.5)	2(28.6)	
BMI of Patient				
Normal	9(28.1)	8(21.6)	1(14.3)	0.494
Over weight	13(40.6)	23(62.2)	5(71.4)	
Underweight	7(21.9)	3(8.1)	1(14.3)	
Obese	3(9.4)	3(8.1)	-	
Compliance of Medicine				
Poor	4(12.5)	6(16.2)	1(14.3)	0.909
Good	28(87.5)	31(83.8)	6(85.7)	
Status of Fever after taking Dose				
Normal	32(100)	37(100)	2(28.6)	0.001*
High	-	-	5(71.4)	

Table-II: Correlationof warfarin dose between age, BMI and IL-6 level (n=76).

Characteristics		Age of patient	BMI of patient	IL-6 level
	Mean ± SD	40.50 ±12.6	2.06 ± 0.83	92.5 ± 84.6
Warfarin Dose of a Patient	Spearsman Correlation	-0.123	.042	0.611
	Sig. (2-tailed)	0.0	0.722	0.001

Body mass index (BMI) was calculated as per WHO criteria and compared with warfarin dose and age group through correlation coefficient. Increased BMI was observed in patients with higher doses of warfarin. Spearsman value was 0.04 (table-II)[16].

Intra-patient variability by comparing clinical variables associated with different Warfarin dose levels:

Poor compliance of medicine was noted in patients presenting with gastrointestinal, hepatic and diabetic problems of high warfarin dosage requirements (12.5 mg/day and 15 mg/day)with increased IL-6 production. The compliance of medicine was good among patients taking warfarin dose 2.5 mg/day and 5 mg/day. Duration of therapy was an important factor to determine as it correlates the warfarin concentration in plasma with the inflammatory cytokines, beginning from less than two months, two to six months and greater than six months to years. Patients on long term warfarin therapy had greater levels of IL-6 because of the high plasma warfarin concentration. These patients were evaluated by inspecting their target INR weekly and fortnightly (fig). It was found that 35 (46.1%) patients having target INR <2 had high levels of IL-6 because of the maximum intake of dosage to maintain the therapeutic INR and 30 (39.5%) patients with target INR >2 had low levels of IL-6 (table-I). Rest of the patients had their target INR within the therapeutic range i.e. 2.0 - 3.0. In some cases, patient's blood had failed to clot and they were advised to stop the warfarin therapy for 2 to 3 days until their target INR would come within the therapeutic range.

Patients were advised to note their body temperature after taking the dose, the IL-6 production was then measured. Seventy one (93.4%) patients taking 2.5 mg/day, 5 mg/day and 7.5 mg/day had normal body temperature while patients belonging to group of high doses 12.5mg/day and 15mg/day had raised body temperature. IL-6 was observed with statistically significant value i.e. (p=0.001) (table-I).

DISCUSSION

Warfarin initiation and its long term treatment within the therapeutic INR range (2.0-3.0) is a challenging therapeutic stage, associated with the highest occurrence of thromboembolism[17].

We were concerned that warfarin might have action in inflammation affecting on IL-6 levels directly, independent from its action as vitamin K antagonist. Patients were observed and compared with controls. Our analysis included valuable results regarding IL-6 levels and warfarin interaction with different demographic and clinical variables of heart valve replacement patients. Inter and intra-patients variability was evaluated and their data was analyzed. Our results showed that elderly patients require low dose of warfarin which leads to the IL-6 inhibition with in normal therapeutic INR range. Fewer patients taking high doses, greater than 10 mg/day within the target INR, lead to IL-6 stimulation. This means that patients on initiation

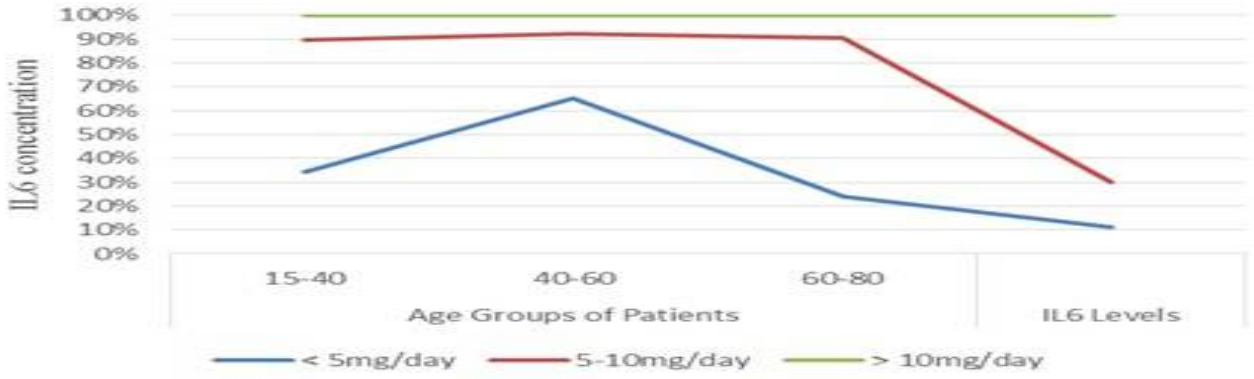

Figure: Effect of Warfarin dose on IL-6 concentration among age groups.

therapy must start with low dose in order to adjust their INR.

A significant difference was found between age and varied levels of warfarin dose (p=0.046) along with IL-6 production[18]. There is a negative correlation between age and warfarin dosage i.e, as age increases, the dose of warfarin decreases[12] and IL-6 production was suppressed as noted for age group between 40-80 years. This is called a negative correlation with statistically significant difference, sig. (2-tailed) value is 0.042 (table-I, II).

Most of the patients (53.9%) were overweight followed by normal weight (23.7%). Patients with higher dose of warfarin had increased BMI. The Spearsman correlation coefficient value was 0.04, that is warfarin dose and BMI were correlated with no significant difference (p=0.722). Our data supported the week positive association of Warfarin and BMI, as our work agreed with Mueller's proposal but found no direct interaction between patient's BMI and IL-6 levels.

CONCLUSION

Various demographic and clinical variables concluded in this study had greater influence on Warfarin dose response and IL-6 production. A marked immunomodulatory response of warfarin was noted by measuring IL-6 levels. IL-6 levels retained a significant association with warfarin dose. This study may help in decreased time of concomitant anticoagulant use or other anti-inflammatory drugs, which could eventually decrease healthcare costs. It may also help clinicians to plan for different strategy of treatment in Warfarin therapy patients and prevent the mortality and morbidity and hence save expenses of additional operative procedures.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Gomez-Outes A, Luisa Suarez-Gea M, Calvo-Rojas G, Lecumberri R, Rocha E, Pozo-Hernández C, et al. Discovery of anticoagulant drugs: a historical perspective. CurrDrug Discov Technol. 2012; 9(2): 83-104.
2. Horton JD, Bushwick BM. Warfarin therapy: evolving strategies in anticoagulation. AmFam Physician. 1999; 59(3): 635-46.
3. Baglin T, Keeling D, Watson H. Guidelines on oral anticoagulation (warfarin): –2005 update. Br JHaematol. 2006; 132(3): 277-85.
4. Schulman S, Crowther MA. How I treat with anticoagulants in 2012: new and old anticoagulants, and when and how to switch. Blood. 2012; 119(13): 3016-23.
5. Dumont Z, Mordasiewicz M, Kosar L, Schuster B. Warfarin Its highs and lows. Can Fam Physician. 2013; 59(8): 856-60.
6. Rouse M, Cristiani C, Teng KA. Q: Should we use pharmacogenetic testing when prescribing Warfarin? Clevel ClinJ Med. 2013; 80(8): 483-6.
7. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP, et al. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon Warfarin dose requirements: proposal for a new dosing regimen. Blood. 2005; 106(7): 2329-33.
8. Ghimenti S, Lomonaco T, Onor M, Murgia L, Paolicchi A, Fuoco R, et al. Measurement of Warfarin in the oral fluid of patients undergoing anticoagulant oral therapy. PloS One. 2011; 6(12): e28182.
9. Hillman MA, Wilke RA, Yale SH, Vidaillet HJ, Caldwell MD, Glurich I, et al. A prospective, randomized pilot trial of model-based Warfarin dose initiation using CYP2C9 genotype and clinical data. ClinMed Res. 2005; 3(3): 137-45.
10. Greaves M. Pharmacogenetics in the management of coumarin anticoagulant therapy: the way forward or an expensive diversion? PloS Med. 2005; 2(10): e342.
11. Massicotte P, Marzinotto V, Vegh P, Adams M, Andrew M. Home monitoring of Warfarin therapy in children with a whole blood prothrombin time monitor.J Pediat. 1995; 127(3): 389-94.
12. Qayyum A, Shafiq H, Najmi MH, Naveed AK. Effect of age on Warfarin dose requirement in pakistani population. Pak Heart J. 2014; 47(2). 61-66.
13. Kater AP, Peppelenbosch MP, Brandjes DP, Lumbantobing M. Dichotomal effect of the coumadin derivative Warfarin on inflammatory signal transduction. Clin Diag Lab Immunol. 2002; 9(6): 1396-7.
14. Saminathan R, Bai J, Sadrolodabaee L, Karthik GM, Singh O, Subramaniyan K, et al. VKORC1 pharmacogenetics and pharmacoproteomics in patients on Warfarin anticoagulant therapy: transthyretin precursor as a potential biomarker. PloS One. 2010; 5(12): e15064.
15. Popov A, Belij S, Subota V, Zolotarevski L, Mirkov I, Kataranovski D, et al. Oral Warfarin affects peripheral blood leukocyte IL-6 and TNF α production in rats. J immunotoxical. 2013; 10(1): 17-24.
16. Mueller JA, Patel T, Halawa A, Dumitrascu A, Dawson NL. Warfarin dosing and body mass index. Ann Pharmacother. 2014; 48(5): 584-8.
17. Botton MR, Bandinelli E, Rohde LEP, Amon LC, Hutz MH. Influence of genetic, biological and pharmacological factors on Warfarin dose in a Southern Brazilian population of European ancestry. BrJClin Pharmacol. 2011; 72(3): 442-50.
18. Whitley HP, Fermo JD, Chumney EC, Brzezinski WA. Effect of patient-specific factors on weekly Warfarin dose. TheraClin Risk Manag. 2007; 3(3): 499.