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The Effect of Serum Magnesium Level on Stable Anticoagulation in Patients Using Warfarin for Various Cardiac Indications

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Abstract
Warfarin is a vitamin K antagonist agent that inhibits clotting factors used for long-term anticoagulation. Time in therapeutic range (TTR) in patients using warfarin is one of the primary treatment effectiveness requirements. We aim to investigate the relationship between serum magnesium levels, the international normalized ratio (INR) values, and TTR values in people using warfarin for various indications. Our study is a single-center, cross-sectional, and retrospective study that included 169 patients between 18 and 70 who used warfarin for various indications. Demographic data, biochemical analysis, and coagulation parameters, including TTR calculation, were evaluated for all patients. Those with a TTR value below 60 were defined as labile INR, and those with 60 and above as stable INR group and compared. The mean INR value was higher in the labile INR group than the stable INR group (3.7 ± 2.9, 3.2 ± 0.3, respectively; $p = 0.030$). The Mg values are significantly lower in the labile INR group than the stable group (1.8 ± 0.2 mg/dL, 2.0 ± 0.1 mg/dL, respectively; $p < 0.001$). In binary multivariate logistic regression analysis, magnesium value was the most influential INR stabilization factor ($p < 0.001$). As a result of our study, it was concluded that magnesium levels are an influential factor in stabilizing INR. We can state that we have contributed to the literature and can be a reference for future studies.

Keywords Coagulation · Warfarin · Magnesium · Prosthetic valve disease · Atrial fibrillation

Introduction

Warfarin is a vitamin K antagonist agent that inhibits coagulation factors used for anticoagulation for a long time. It has a wide range of indications like preventing systemic embolism in patients with atrial fibrillation (AF), preventing and treating thromboembolic events in patients with mechanical heart valves, treatment, and prophylaxis of pulmonary embolism (PE) and venous thromboembolism (VTE). Although its use has decreased, especially in nonvalvular AF, with the introduction of direct oral anticoagulants (OAC), its importance continues to be the only oral anticoagulant for mechanical prosthetic valves. Warfarin blocks the gamma-carboxylation reaction by inhibiting the enzyme epoxide reductase, which converts inactive vitamin K to reduced vitamin K. Thus, it provides an anticoagulant effect by preventing the functionalization of factors 2, 7, 9, 10, and protein C-S. Its use and follow-up require great care because of its interaction with foods, other drugs, and other pathological conditions [1, 2]. The anticoagulant effect of warfarin is measured by prothrombin time (PT). PT is used to evaluate the extrinsic and common coagulation pathways, which would detect deficiencies of factors II, V, VII, and X, and low fibrinogen concentrations [3]. Because the reagents used for PT measurements and their sensitivities can vary between clinical laboratories, the international normalized ratio (INR) is used to standardize and express PT results. The INR represents the patient's PT's ratio divided by a control PT value obtained by using an international reference thromboplastin reagent developed by the World Health Organization (WHO) [4].

Bleeding is the most common and life-threatening side effect of warfarin. Current guidelines recommend using the HAS-BLED (hypertension, abnormal renal function and liver function, stroke, bleeding, labile INR, elderly [older

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than 65 years], drugs and alcohol) scoring tool to assess the risk of bleeding for patients with atrial fibrillation [5]. Labile INR was defined as time in therapeutic range (TTR) less than 60% in warfarin using patients [6].

Magnesium (Mg) is an intracellular cation that plays a significant role in many enzymatic reactions and hemodynamic processes. It also has some cardiac effects on vascular smooth muscle tone, peripheral vascular resistance, arrhythmia, blood flow dynamics, homeostasis, and coagulation cascade [7]. The effects of Mg on blood coagulation have been the subject of in vitro and in vivo studies. A decrease in Mg concentration was associated with platelet activation, and an increase in Mg concentration was associated with platelet inhibition [8]. Some studies have also shown that Mg therapy increases bleeding time in conditions such as preeclampsia, myocardial infarction, and pregnancy [9].

Mg can be found in the plastic lids of blood collection tubes and can sometimes be mixed with citrate solution. A shortening of prothrombin time was observed after Mg mixing into citrated plasma. This information has also been demonstrated in rat plasma in an animal experiment [10]. Butenas et al. showed that Mg ion could enhance the amidolytic activity of factor VIIa [11]. This situation has been considered as the underlying mechanism for prothrombin time shortening [12]. Besides, although it is stated that Mg is used to provide controlled hypotension and reduce bleeding during surgery, there are still some uncertainties in this regard. For example, in patients undergoing posterior lumbar surgery, it was shown that there was no significant difference in terms of hemoglobin, platelet count, PT, and fibrinogen between the group that received Mg infusion during the operation and the group that did not [13].

In our study, we aimed to investigate the effect of serum Mg level on INR stabilization in patients using warfarin.

Material and Methods

The study was designed as a single-center, cross-sectional, and retrospective. The study included 169 patients between 18 and 70 years who were using warfarin for various indications and applied to the cardiology outpatient clinic between February 2020 and August 2020. Age, gender, accompanying chronic diseases, medications, and warfarin usage indications were obtained from the hospital information system and recorded. Biochemical parameters, complete blood counts, and INR values in the last 6 months were retrospectively reviewed, and the data were recorded. During the biochemical analysis, the following parameters were measured: creatinine, sodium, potassium, aspartate transaminase, alanine transaminase, Mg, C-reactive protein (CRP), thyroid-stimulating hormone, total cholesterol, low-density lipoprotein, very low-density lipoprotein, high-density lipoprotein, and triglycerides. Complete blood count included red blood cell count, hemoglobin, hematocrit, red cell distribution width (RDW), platelet count, platelet distribution width, WBC count, neutrophil count, and lymphocyte count. Magnesium concentration were studied with spectrophotometric xylidyl blue method on Beckman Coulter AU 2700 device. PT and activated partial thromboplastin time (aPTT) were performed with photooptic turbidimetric method on ACL TOP 300 CTS device.

The exclusion criteria are as follows:

- Patients without periodic INR follow-up
- those with liver and kidney dysfunction
- thyroid pathologies
- active cancer histories
- nutritional disorders
- congestive heart failure
- Vitamin K deficiency
- hematological diseases
- neurological and psychiatric diseases that may affect regular drug use
- infectious diseases in the last six months
- alcohol use
- use of drugs that interact with the INR.

Patients were divided into two groups according to TTR values, with a cutoff value of 60%. A TTR below 60% was accepted as labile, and above %60 was accepted as stable. In the recommendation of the European Society of Cardiology (ESC) guidelines about AF, the optimal TTR value must be at least 70%. However, in the HAS-BLED scoring, the labile rate was defined as below 60%. Because of including a limited number of patients in our study and not to compare a third group, stable INR was accepted as 60% and above [14]. In addition, in previous studies, the accepted TTR threshold value as a 60–65% impacted our decision [15].

To calculate the TTR value, patients who had at least 4 measurements in the last 6 months were included, provided that they were not more frequent than 1 month and more prolonged than 2 months.

The effective therapeutic INR range was accepted as 2–3 for AF, venous thrombosis, pulmonary embolism, and mechanical prosthetic aortic valve. The 2.5–3.5 range was accepted as the therapeutic range in prosthetic mitral valve patients.

Statistical Analysis

Statistical evaluation was carried out with SPSS 23.0 package computer program (SPSS, Inc., Chicago, IL). Continuous variables were expressed as mean ± standard deviation or median (minimum–maximum) according to their distribution, while categorical variables were expressed as frequency and percentage (%). The distribution of data was evaluated using the Kolmogorov–Smirnov test. Comparison between groups was carried out using Mann–Whitney U or t-test according to the distribution of data. Similarly, correlation analysis was performed with the Pearson or Spearman correlation test. The chi-squared test was used to evaluate the differences of categorical variables between groups. A p-value of <0.05 was considered significant in the statistical analysis obtained.

Results

A total of 169 patients, 93 (55%) of whom were women. While INR follow-ups of 86 patients were in group TTR ≥ %60 (stable), 83 patients were in group TTR < %60 (labile) INR follow-ups. No significant difference was found in both groups regarding age, gender, accompanying pathologies: hypertension, hyperlipidemia, and coronary artery disease (all p values > 0.05). However, the rate of diabetes mellitus in the labile group was significantly higher than in the stable group ($p=0.011$). Comparison of demographic, clinical data, and the laboratory parameters of the patients are shown in Table 1.

Sixty-one patients with atrial fibrillation, 101 patients with prosthetic valve disease, 4 patients with PE, 3 patients with VTE prophylaxis, and 1 patient with factor V Leiden mutation, were using warfarin. TTR values were higher in patients using warfarin for mechanical prosthetic heart valve than those using warfarin for AF ($p=0.003$).

The mean INR value was higher in the labile INR group than the stable INR group (3.7 ± 2.9, 3.2 ± 0.3, respectively; $p=0.030$). The Mg levels are significantly lower in the labile INR group than the stable group (1.8 ± 0.2 mg/dL, 2.0 ± 0.1 mg/dL, respectively; $p<0.001$). There were no significant differences in hemoglobin, hematocrit, platelet levels, and other biochemical parameters in between the groups ($p>0.05$).In binary multivariate logistic regression analysis performed in terms of parameters that make a difference between groups, it was determined that magnesium value was the most influential factor in INR stabilization ($p<0.001$) (Table 2).

After determining that Mg was the most influential factor on INR stabilization, a Roc-curve analysis was performed. When the positive likelihood ratio was calculated to be 2.48

Table 1 Comparison of demographic, clinical data, and laboratory parameters

		TTR < %60 (Labile) ($n=83$)	TTR ≥ %60 (Stable) ($n=86$)	p values
Age (years)		63.9 ± 14.4	62.7 ± 10.2	0.506
Gender (female, n, %)		49 (59%)	44 (51.2%)	0.304
Warfarin indication, n, %	Metal prosthetic heart valve	38 (45.8)	63 (73.3%)	0.003
	Deep venous thrombosis	1 (1.2%)	1 (1.1%)	
	Factor V Leiden mutation	1 (1.2%)	-	
	Pulmonary embolism	4 (4.8%)	-	
	Atrial fibrillation	39 (47.0%)	22 (25.6%)	
Accompanying disorders				
Hypertension		44 (53.0%)	37 (43.0%)	0.194
Diabetes mellitus		25 (30.1%)	12 (14%)	0.011
Hyperlipidemia		22 (26.5%)	19 (22.1%)	0.503
Coronary artery disease		16 (19.3%)	10 (11.6%)	0.168
Laboratory parameters				
INR		3.7 ± 2.9	3.2 ± 0.3	0.030
Magnesium (mg/ dL)		1.8 ± 0.2	2.0 ± 0.1	< 0.001
Hemoglobin (g/dL)		12.1 ± 1.9	12.7 ± 1.8	0.053
Hematocrite (%)		36.5 ± 5.9	38.0 ± 5.4	0.074
PLT (/mm3)		283.6 ± 77.0	297.2 ± 66.1	0.219
Creatinine (mg/dL)		0.9 ± 0.3	0.9 ± 0.2	0.368
Sodium (mmol/ L)		137.7 ± 5.0	137.5 ± 4.0	0.690
Potassium (mmol/ L)		4.3 ± 0.6	4.3 ± 0.7	0.740
AST (U/L)		27.0 ± 20.5	24.1 ± 8.4	0.223
ALT (Ul/ L)		26.2 ± 18.6	23.4 ± 7.8	0.207

TTR time in therapeutic range, *PLT* platelet, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *INR* international normalized ratio

Table 2 Multivariate analysis of parameters that can affecting the INR value

	B	SE	95% CI	OR	*p* values
Constant	-55.453	44,095		0.000	< 0.001
Atrial fibrillation	0.975	1.544	0.129–54.682	2.651	0.528
Mechanical prosthetic heart valve	0.146	1.519	0.059–22.712	1.157	0.923
Diabetes mellitus	0.738	0.501	0.784–5.578	2.091	0.141
Magnesium concentration	7.026	1.228	101.3–122,496	1125.4	< 0.001

$n = 169$, $R2 = 0.475$ (Nagelkerke), $R2 = 0.357$ (Cox & Snell), Model: X2 (2) = 74.504, $p < 0.001$, *SE* standard error, *OR* odds ratio, *CI* confidence interval)

in the analysis with 95% confidence interval and area under the curve of 0.822, it was found that the cutoff value for Mg level was 1.91 mg/dL with 83.7% sensitivity and 66.3% specificity (Fig. 1). There was a significant relationship between the current cut off value of Mg and the INR value ($p < 0.001$).

Discussion

Mg is a cation that affects normal physiological functions like in the coagulation cascade [16]. Although Mg is not required for coagulation, it is critical to form fibrin via extrinsic and intrinsic pathways. Factor IXa activates factor X in Factor VIII, platelet membrane phospholipid and calcium, and helps form thrombin from prothrombin [17]. Thrombin has a vital role in fibrin formation from fibrinogen. Mg ions stabilizes the natural factor IX conformation and improves factor IXa to activate factor X [18]. Mg also increases factor VIII's affinity to factor IXa and thus the catalytic effect of factor IXa. Together with calcium, it accelerates factor Xa activity through tissue factor (TF)/factor VIIa, more than the effect of calcium alone [19]. Nevertheless, somehow in a study related to the blood compatibility and biodegradation properties of magnesium alloys, the blood compatibility of Mg alloys due to the enzyme activity suppression in the coagulation pathways by the eluted Mg+ + ion [20]. There are also data on the shortening of prothrombin time in coagulation tubes with magnesium contamination.

There are different studies and even conflicting findings on the homeostatic effects of Mg. Besides, our study was planned due to the lack of a study on INR interaction in patients using warfarin with Mg to date. In this study, the relationship between serum Mg level and INR

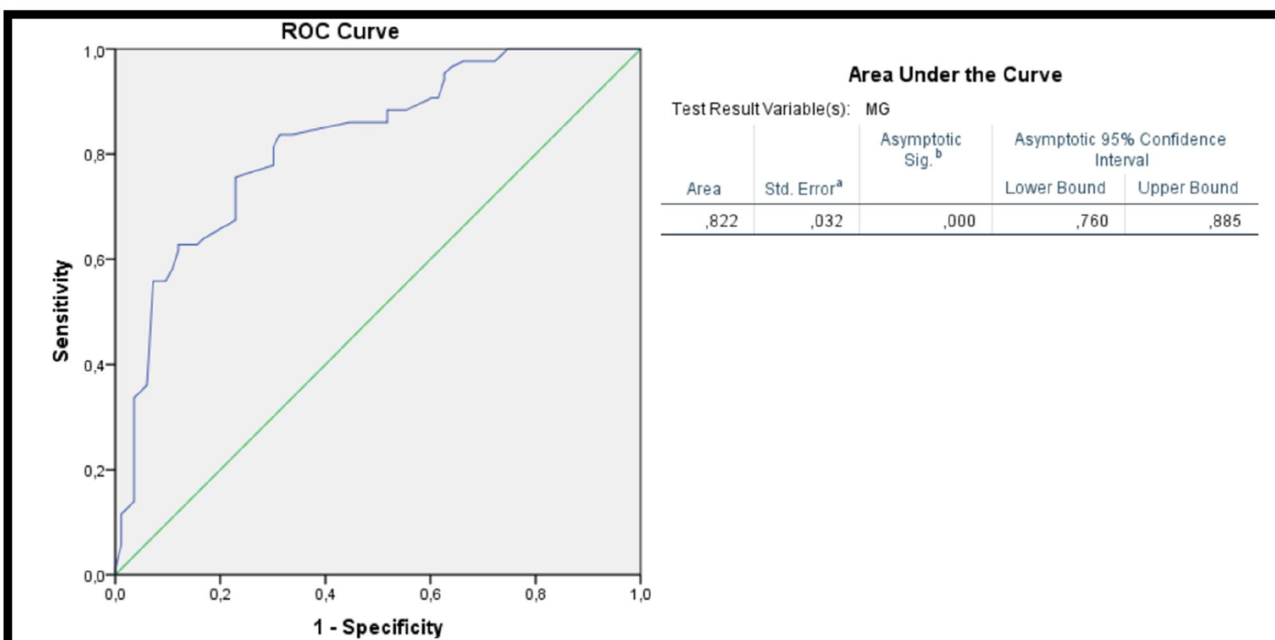

Fig. 1 Roc-curve analysis

was investigated in patients using warfarin for different indications.

In our study, the TTR rates of patients who had undergone valve surgery were significantly higher than those with AF ($p = 0.003$). This situation may be related to the previous cardiac operation history and the possibility of adopting more to the follow-up due to the changes in the quality of life they experienced during this period. Likewise, it may be influenced by social characteristics.

Another finding encountered in our study was that the rate of diabetic patients in the labile INR group was significantly higher than the other group ($p = 0.011$). Similar to our current findings, Nelson WW et al. showed that diabetes mellitus in patients with atrial fibrillation was independently associated with poor INR control [21]. Also, in the multivariate analysis of our study, it was stated that the main factor for TTR value to be in the optimal range was blood magnesium levels ($p < 0.001$). The magnesium level cutoff value was determined as 1.91 mg/dL, which is the accepted lower limit of the blood magnesium levels reference values in our hospital, to ensure optimal TTR value. In our clinic, the normal reference range for Mg is considered to be 1.9–2.5 mg/dL, but the normal range of values may differ slightly between different laboratories [22]. In a study, low TTR values have been associated with dementia and cognitive disorders [23]. However, patients with already known dementia and cognitive impairment were excluded from our study.

Our study found that the labile INR group's INR values were significantly higher than the stable INR group ($p = 0.030$). Studies in the literature showed shortenings in prothrombin time associated with magnesium levels. The relation of magnesium deficiency with high INR can be explained in the light of the findings in these studies in the literature [11, 12].

As a result, we found a significant relationship between TTR values and serum Mg level. With these findings, it is thought that our study can be a reference for future multicenter studies with a large number of patients and contribute to the literature.

Limitations

A limited number of patients are included in the study. As explained in the study's methodology, a labile INR was defined TTR value below 60%, and a stable INR was defined as TTR value of 60% and above. Although a correlation was shown between magnesium values and TTR values in the Roc-curve analysis and an Mg cutoff value was also found for the optimal TTR value, the differences due to the accepted TTR values may have influenced the results of the study. Besides, the differences in diabetes mellitus prevalence between the groups may also have affected the study results.

Conclusion

Magnesium is a crucial element that plays a significant role in many enzymatic, hemodynamic reactions, and coagulation processes. TTR in patients using warfarin is one of the basic requirements for the effectiveness of treatment. As a result of our study, it was concluded that magnesium levels are an influential factor in stabilizing INR. We can state that we have contributed to the literature and can be a reference for future studies.

Declarations

Ethics Approval and Consent to Participate Informed consent was obtained from all participants and the Institutional Ethics Committee approved the study (approval number: 016/2020–15.04.2020). The study was conducted in accordance with the Declaration of Helsinki.

Conflict of Interest The authors declare no competing interests.

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