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Possible Correlation between INR and Serum Calcium

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Abstract

Experimental and clinical studies have shown that long term anticoagulation therapy with warfarin can induce vascular calcification which is preventable by vitamin K. Osteoporosis has been shown to be associated with vascular calcification. In the present study, we wanted to see, whether INR (International Normalized Ratio), a measure of prothrombin time, and serum calcium (corrected by albumin) correlate in laboratory data of 94 anticoagulation patients on warfarin therapy. When adjusted on age and sex, there was an inverse correlation between the two variables. Clinical relevance of this observation and explanation of lowered calcium levels in blood parallel to increase in INR-values remain to be studied further.

Keywords

Warfarin, INR, Serum Calcium, Vascular Calcification

1. Introduction

An inverse correlation between the intake of vitamin K-2 (menaquinone) and vascular calcification has been reported in humans [1]-[3]. Warfarin, an antagonist of the vitamin K has been shown to induce arterial and cardiac valvular calcification presumably by inhibiting carboxylation and thus activation of two vitamin K-dependent proteins, Matrix Gla (MGP) and Growth Arrest Specific Gene 6 (Gas-6) proteins [4]-[6]. In murine models, warfarin treatment for a few weeks induces calcification of the artery wall and heart valves [7]-[9]. This process can be prevented or retarded in animals by high intake of vitamin K [10] [11] or transglutaminase inhibitors such as quercetin or KCC-009 [12]. Arterial calcification has been shown to coexist with osteoporosis [13]. Therefore we became interested in, whether during long lasting warfarin therapy INR values would be asso-

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ciated with serum calcium levels of which only one study on rhesus monkeys [14] and one in rats [15] have been published.

2. Materials and Methods

The material consisted of laboratory data from the Helsinki and Uusimaa District Hospital Clinical Chemistry data bank of patients of which both the INR measurement and serum calcium concentrations corrected by albumin had been requested by primary health care providers in the district area and sent to analysis in the same clinical laboratory Helsinki University Hospital Laboratory (HUSLAB) in the year 2012. Total plasma calcium levels were measured and subsequently corrected for albumin levels using a modified Payne's formula [16]. Only the gender and the age of the patients, but no other clinical data, were recorded. The therapeutic anticoagulation range for INR (International Normalized Ratio) indicating prothrombin time during warfarin therapy is 2.0 - 3.0. In patients with mechanical heart valve, the recommended range is 2.5 - 3.5. The reference range of calcium corrected with albumin is 2.15 - 2.51 mmol/L in the Helsinki University Hospital Laboratory. There were a total of 54,714 INR and 224 albumin corrected calcium measurements done during 2012 in the records. To be able to compare calcium and INR levels, we limited the analysis to samples where INR and Ca levels were analyzed within 2 days from each other. Thereafter 94 cases fulfilled this criterion.

The data are presented as means with standard deviations (SD), medians or as counts with percentages. Statistical significance for the hypotheses of linearity was evaluated by using bootstrap type analysis of covariance (ANCOVA) taking gender and age values as covariates. Ninety-five percent confidence intervals (95% CI) were obtained by bias-corrected bootstrapping. The bootstrap method is significantly helpful, when the theoretical distribution of the test statistics is unknown, or in the case of violation of the assumptions. Correlation coefficients were calculated by the Spearman method. STATA 13.1. StataCorp LP (College Station, TX, USA) statistical package was used for the analyses.

3. Results

From the 94 samples, one outlier with INR of 8.0 was excluded. Thus, results were analyzed from 93 patient samples. INR averaged 2.3 (median 2.4, SD 0.76), calcium averaged 2.42 mmol/L (median 2.42, SD 0.12). There was no direct correlation between INR and calcium levels (**Figure 1**). However, when adjusted to age and gender, calcium levels showed negative correlation with increasing INR ($p = 0.025$, adjusted for age and gender). Calcium levels lowered by 0.05 mmol/L when INR levels under the recommended treatment range 2.0 increased towards upper level of the treatment range of over 2.7 (**Figure 2**) and showed statistically significant difference ($p = 0.032$) between the highest and the lowest tertile.

4. Discussion

Vitamin K antagonists such as warfarin are effectively used in risk reduction of venous and arterial thrombosis. They inhibit not only post-translational activation of vitamin K dependent coagulation factors but also synthesis or activation of many extrahepatic proteins and thus cause poorly known adverse effects seen both in animal experiments and human materials [5] [6].

The calcification process is complex, but vitamin K seems to prevent vascular calcification at least in patients with chronic kidney disease [17] and dietary intake of vitamin K is associated with reduced risk and incidence of coronary artery calcification and coronary heart [1]-[3]. In addition to vitamin K dependent hepatic proteins (factors II, VII, IX, X, protein C and S) there are a number of extra-hepatic vitamin K dependent proteins (e.g. matrix Gla protein, osteocalcin, nephrocalcin, plaque Gla protein and proline rich Gla proteins [18] which might play, among other effects, a role in calcification. MGP knockout mice develop severe calcification [19]. Because warfarin inhibits the activation of MGP, this might be explanation for warfarin induced vascular calcification [4] [20] [21].

It is not surprising that INR, a secondary measure on warfarin effect was in a rather narrow therapeutic range and that serum calcium level in otherwise healthy subjects on normal diet did not vary too much. This might be the explanation for the absence of clear correlation, even though a tendency to negative correlation between these two variables was found.

Despite of the assumed and obvious complexity of the process we tried to use simple clinical laboratory data

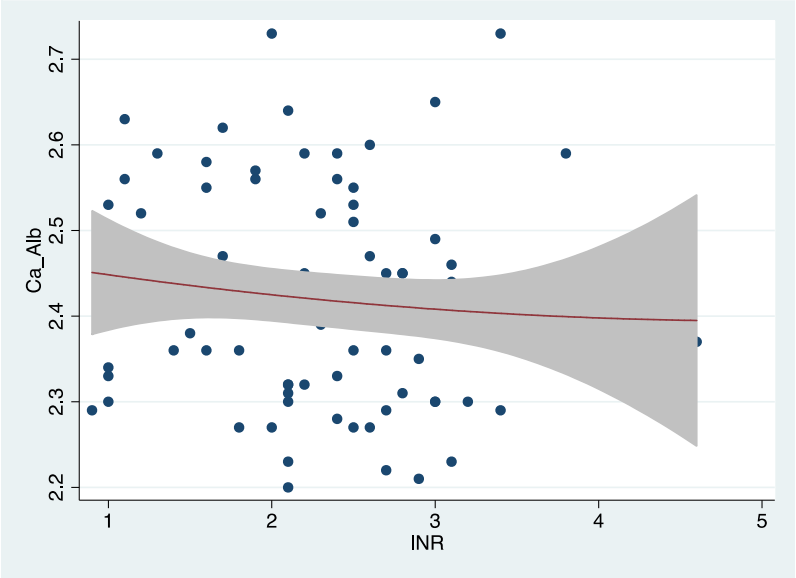

Figure 1. Distribution of individual serum albumin corrected calcium concentrations vs INR (International Standardized Ratio) values (n = 93). Correlation r = −0.12 (95% CI −0.3 to 0.09, p = 0.29).

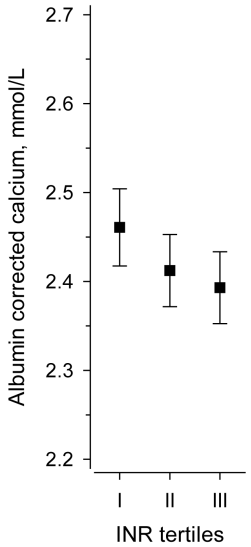

Figure 2. Albumin corrected serum calcium concentrations vs INR (international standardized ratio) divided in tertiles (I ≤ 2.0; II 2.1 - 2.6; III ≥ 2.7). Mean with 95 percent confidence intervals. Significances: I vs III p = 0.032; for linearity p = 0.025 (adjusted for gender and age).

on serum calcium and INR values of the same patients taken simultaneously (*i.e.* within a two-day interval) to find out possible relations between these two variables. Probably due to small physiological variations in serum calcium levels and the narrow therapeutic range of the INR values, no clear correlation could be found in the total material as such. However, when adjusted to the age and gender of the patients, a significant, inverse correlation was observed between these two variables. In some studies osteoporosis has been associated with vascular calcification [22]-[24]. On the other hand, Binkley and coworkers [14] could not confirm vitamin K deficiency and increase of osteoporotic markers in warfarin-treated rhesus monkeys.

It is difficult to explain, why “vitamin K deficiency” by warfarin (elevation of INR) tended to decrease serum calcium. Sokolnikov and coworkers [15] fed young rats with vitamin K deficient food, found prolongation of

prothrombin time, and *in vitro* in duodenal preparations reduced calcium absorption. Our recent work [25] showed that prolonged warfarin treatment with high doses did not change serum calcium concentrations in the rat, but increased calcium excretion in the urine by about 50%. Unfortunately, in the present laboratory data urinary excretion of calcium was not available.

5. Conclusion

Even though the present data based analysis is preliminary and has many shortcomings, it raises an important question, since vitamin K inhibitor warfarin is at present the most widely used oral anticoagulant. Yet, the possible adverse effect of vascular wall calcification is not widely known. This observation should stimulate further, more extensive epidemiologic or intervention trials.

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