

A Comparative Study Between the Effect of Hydrochlorothiazide and Furosemide on the Anticoagulant Activity of Warfarin

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Abstract

Background:It has been widely reported that diuretics therapy affect serum calcium level which is in turn may affect the coagulation pathways and thereby could affect the activity of anticoagulation therapy. This study was undertaken to evaluate the effect of simultaneous diuretic intake on the anticoagulant activity of warfarin.

Patients and Method: Twenty patients enrolled in this study divided to two groups, group 1 (N=10 patients) were taking furosemide 80mg/day plus warfarin 3 mg /day. Group 2 (N=10 patients) were taking hydrochlorothiazide 50 mg /day plus warfarin 3 mg/day. Blood samples were taken from each patient and used for measurement of serum calcium level, prothrombine time(PT) and international normalized ratio(INR).

Results: It has been found that there was a significant reduction ($P<0.01$) in calcium concentration in patients taking furosemide with warfarin (group 1) with respect to those who were taking hydrochlorothiazide in addition to warfarin(group 2). On the other hand both PT and INR weresignificantly increased in patients with group 1 with respect to those in group 2.

Conclusions:It may be concluded from this study that the combination of hydrochlorothiazide with warfarin may decrease the anticoagulant activity of the later.

دراسة مقارنة بين تأثير هيدروكلوروثيازيد وفوروسيميد على تخثر آخر من الوارفارين

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مفتاح البحث : وارفارين , تأثير الفيروسيمايد على فعالية الوارفارين , تأثير الهايدروكلوروثيازيد على فعالية الوارفارين , فروسيمايد , هايدروكلوروثيازيد

الخلاصة:

مقدمة: من المعروف ان العلاج بالمددرات له تأثير مباشر على تركيز الكالسيوم في الدم وهذا بدوره قد يؤثر على فعالية بعض الأدوية المضادة للتخثر. اجريت هذه الدراسة لتقييم ودراسة تأثير الاستخدام المشترك للوارفارين مع نوعين من المددرات هما الفيروسيمايد والهايديرولورثيازاييد.

المرضى وطريقه البحث: عشرون مريضا قد اشتركوا في هذه الدراسة ; عشرة منهم (المجموعة الاولى) كانوا يأخذون عقار الوارفارين 3 مغم يوميا بالإضافة الى الفيروسيمايد 80 مغم يوميا لمدة اكثر من سنة. اما العشرة الاخرين (المجموعة الثانية) فقد كانوا يأخذون عقار الوارفارين 3 مغم يوميا اضافة الى الهايديرولورثيازاييد 50 مغم يوميا لأكثر من سنة ايضا. تم اخذ عينات الدم من جميع المرضى لقياس مستوى الكالسيوم بالدم اضافة الى كل من PT & INR .

النتائج: لقد تبين ان هناك نزولا معنويا ($P<0.01$) بمستوى الكالسيوم بالدم لمرضى المجموعة الاولى الذين كانوا يتناولون الفيروسيمايد مع الوارفارين بالمقارنة مع اولئك الذين كانوا يأخذون الهايديرولورثيازاييد مع الوارفارين (المجموعة الثانية). من جهة اخرى فان كلا من PT & INR قد ازدادت معنويا ($P<0.05$) في المجموعة الاولى بالمقارنة مع المجموعة الثانية.

الاستنتاج: ممكن ان يستنتج من هذه النتائج ان الاستخدام المشترك للوارفارين مع الهايديرولورثيازاييد قد يقلل الفعالية المضادة للتخثر للوارفارين.

Introduction

Warfarin is the mainstay of oral anticoagulant therapy used for a variety of indications, including prevention of stroke in patients with atrial fibrillation and treatment of venous thromboembolism. Warfarin subject to several pharmacokinetic and pharmacodynamic drug-drug interactions. Many patients take simultaneous warfarin and diuretic therapy and since diuretics (both loop diuretic and thiazide diuretic) change serum calcium level, they may affect the anticoagulant activity of warfarin because of the important role of calcium in the coagulation pathways⁽¹⁾. In the extrinsic pathway, coagulation is activated in vivo, small amount of factor VIIa in the plasma bind to subendothelial tissue factor following vascular injury. Tissue factor accelerates activation of factor X by VIIa, phospholipids and Calcium by 30,000-fold. VIIa also can activate IX in presence of tissue factor providing a convergence between extrinsic and intrinsic pathway.⁽²⁾

Clotting by intrinsic pathway is initiated in vitro when XII, prekallikrein and high-molecular weight kininogen interact with kaolin, glass or another surface to generate a small amount of XIIa. Activation of XI to XIa and IX to IXa follows. IXa then activate X in a reaction that is activated by VIIIa, phospholipids and calcium⁽³⁾. Activation of factor X by IXa appear to occur by a mechanism similar to that for activation of prothrombin and may be accelerated by platelets in vivo⁽⁴⁾. Activation of factor XII is not required for hemostasis since patient with deficiency of

factor XII, prekallikrein or high-molecular weight kininogen do not bleed abnormally even though their aPTT are prolonged. Factor XI deficiency is associated with variable and usually mild bleeding disorder. The mechanism for activation of factor XI in vivo is not known, although thrombin activates factor XI in vitro.^(5, 6)

Loop diuretics (high-ceiling diuretics) such as furosemide , are the most potent diuretics and rapidly produce an intense dose-dependent diuresis of relatively short duration. Oral furosemide produces diuresis within 30–60 minutes of administration, with the maximum diuretic effect in 1–2 hours.⁽⁷⁾ Furosemide effect serum calcium concentration by increasing calcium loss in urine (calciurea) , so with long term use for this drug it lead to low calcium concentration in blood (hypocalcemia). And this may affect coagulation pathway by decreasing the activation of factor X to Xa by calcium , phospholipids and VIIa ,so the sequence of activation of clotting factors that end with fibrin formation is interrupted and this may lead to increase in bleeding tendency.⁽⁷⁾ Thiazide diuretic (hydrochlorothiazide) , are moderately potent and act by inhibiting sodium and chloride reabsorption at the beginning of the distal convoluted tubule. They produce diuresis within 1–2 hours of oral administration and most have a duration of action of 12–24 hours.⁽⁸⁾ Hydrochlorothiazide effect serum calcium concentration by decreasing calcium excretion in urine by increasing calcium tubular calcium reabsorption, so long term use of hydrochlorothiazide may increase serum calcium concentration (hypercalcemia) which may increase the activation of clotting factor X to Xa by calcium , phospholipids and VIIa.⁽⁹⁾

Warfarin is oral anticoagulant drug, inhibit the activation of clotting factors by inhibiting enzyme required for reduction of vit k (reduced form of vit k is required for activation of clotting factors).⁽³⁾ PT(Prothrombin time) is functional determination of the extrinsic (tissue factor) pathway of coagulation and is extremely sensitive to the vitamin-K dependent clotting factors (factors II, VII, and X). Tissue factor (factor III) is a transmembrane protein that is widely expressed on cells of non-vascular origin which activates factor VII during the initiation of the extrinsic coagulation pathway, a cascade mechanism results in fibrin production and clot formation.⁽¹⁰⁾ International normalized ration is the recommended result reporting for monitoring of oral anticoagulant therapy. A mean normal prothrombin time in seconds determined following World health organization recommendations accredited facility.⁽¹¹⁾ This

study was undertaken to assess the effect of both furosemide or hydrochlorthiazide on the anticoagulant activity of warfarin.

Patients and Method

This is a retrospective study carried out in AL –Sader hospital in Al-Najaf for the period of Jan. 2012 to Jan. 2013. Twenty hospitalized patients within the age range of 50-60 years were enrolled in this study under medical staff supervision. The patients were divided into two groups each of ten patients, patients of group 1 were taking furosemide 80 mg/day plus warfarin 3mg daily for about one year or more, while patients of the second group were taking hydrochlorthiazide 50 mg daily and warfarin 3mg daily for about one year or more. Based on the patients permissions, blood samples (about 3 ml) were taken from each one and sent to the hospital lab. for measurement of serum calcium concentration, prothrombin time and INR. Prothrombin Time (PT) is performed on platelet poor plasma prepared from blood collected into citrate anticoagulant. In the PT an aliquot of plasma is incubated at 37C with a reagent containing a phospholipid-protein extract of tissue (thromboplastin). CaCL₂ is then added and the time required for clot formation is the prothrombin time and measured by one of a variety of techniques (photo-optical, electromechanical) The result is reported in seconds (prothrombin time).^(11, 12)

INR is calculated by the following equation:

$$\left[\frac{\text{(patient prothrombin time in seconds)}}{\text{(mean normal plasma prothrombin time in seconds)}} \right]^{\text{ISI}}$$

The mean normal plasma prothrombin time = 12 Sec. Statistical analysis was done by using computer program (SPSS) version 10. Analysis of variance (ANOVA) was used for the comparison of differences between the two groups and P value <0.05 was considered to be statistically significant.

Results

Effect on serum calcium level: It has been found that there was a significant reduction (P<0.01) in calcium concentration in patients taking furosemide with warfarin (group 1) with respect to those who were taking hydrochlorthiazide in addition to warfarin (group 2) as illustrated in table (1).

Table (1): Means of serum calcium level in mg./dl of the two groups and the mean difference between the two groups by using variance analysis test (ANOVA).

Groups	Serum calcium(mg/dl)	Mean difference
Furosemide(80mg/day)warfarin(3mg/day)	8.02 ± 0.25	**
Hydrochlorothiazide(50mg/day)warfarin(3mg/day)	10.44 ±0.43	+2.42**

Data are expressed as mean ±SEM

** (P<0.01)

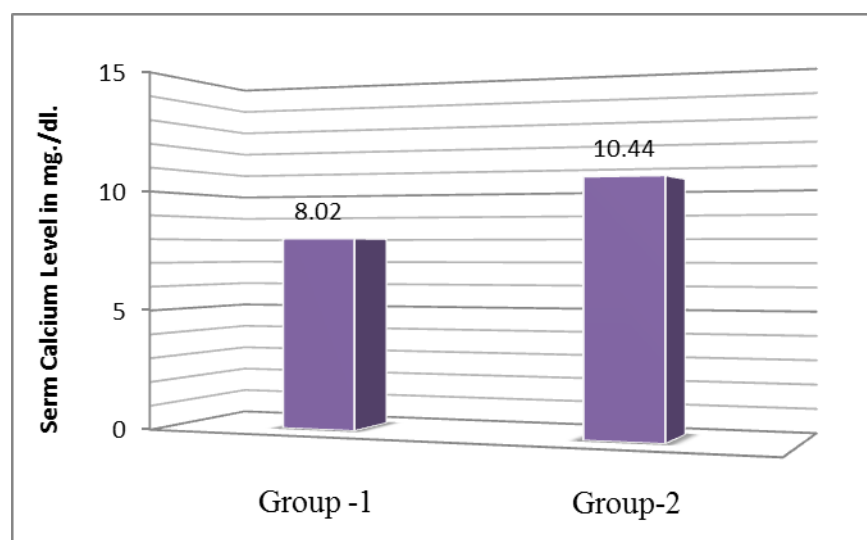


Figure (1): Comparative effect of hydrochlorothiazide and furosemide on serum calcium level in mg./dl.

Effect on prothrombin time: There was a statistically significant difference (P<0.05) in prothrombine time between the group of patients who were receiving furosemide plus warfarin(group1) and the group of patients who were receiving hydrochlorothiazide plus warfarin as illustrated in table(2)

Table (2) Means of prothrombine time in seconds of the two groups and the mean difference between the two groups by using variance analysis test (ANOVA).

Groups	PT (seconds)	Mean difference
Furosemide plus warfarin	19.36 ± 0.46	+1.45*
Hydrochlorothiazide plus warfarin	17.91 ± 0.29	- 1.45*

Data are expressed as mean ±SEM

* (P<0.05)

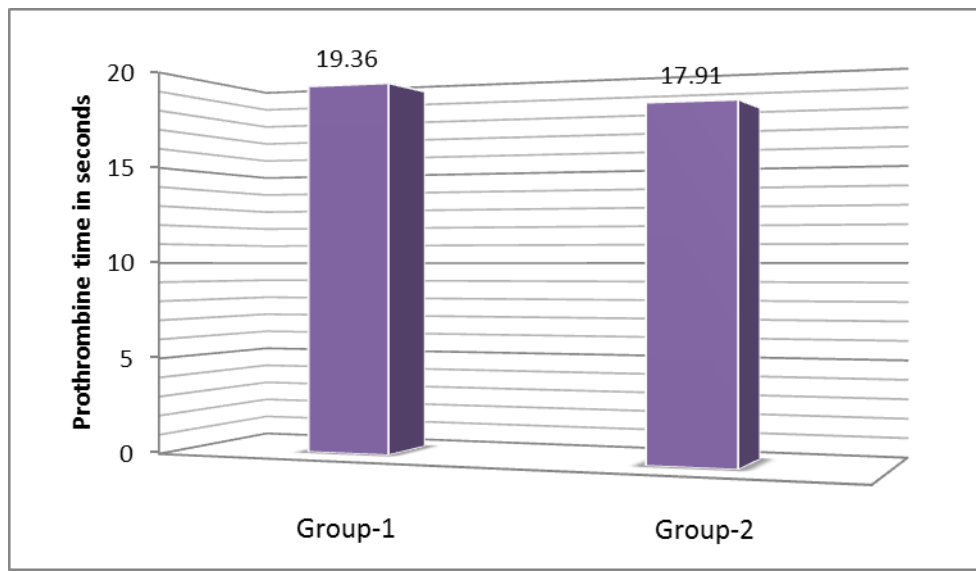


Figure (2): Comparative effect of hydrochlorothiazide and furosemide on prothrombine time

Effect on INR: There was a statistically significant difference (P<0.05) in INR between the group of patients who were receiving furosemide plus warfarin and when compared with those who were receiving hydrochlorothiazide plus warfarin as illustrated in table (3)

Table (3) Means of INR of the two groups and the mean difference between the two groups by using variance analysis test (ANOVA).

Groups	INR	Mean difference
Furosemide plus warfarin	2.53 ± 0.11	+ 0.48*
Hydrochlorothiazide plus warfarin	2.05 ± 0.07	- 0.48*

Data are expressed as mean ±SEM

* (P<0.05)

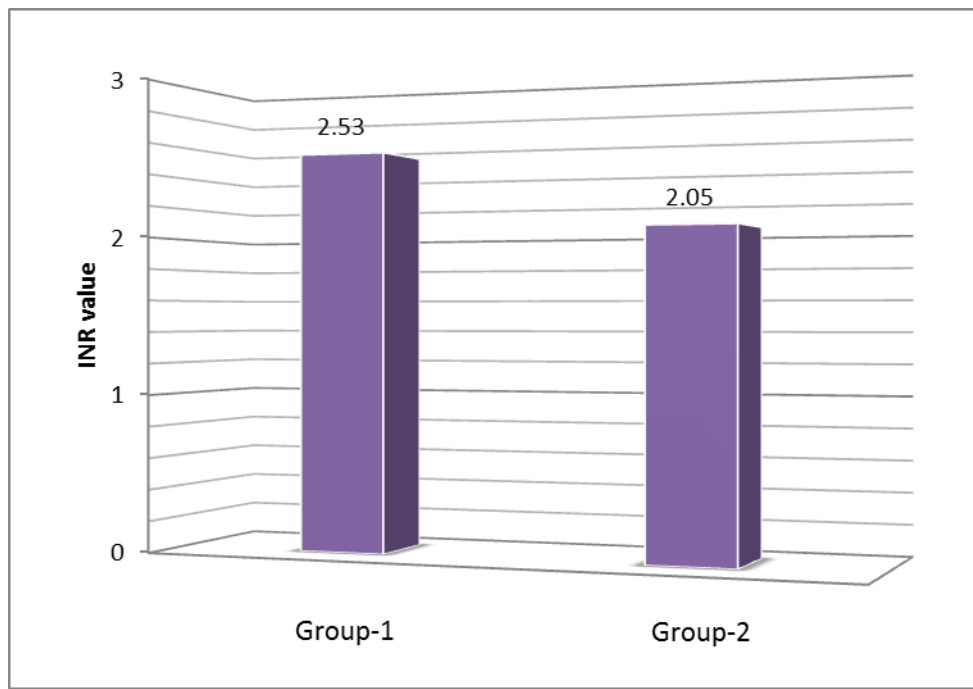


Figure (3): Comparative effect of hydrochlorothiazide and furosemide on INR

Discussion

It is clearly shown in this study that patients taking furosemide with warfarin shows a significant reduction in blood calcium level when compared with those patient who were taking hydrochlorthiazide with warfarin and this is actually may be explained by the fact that furosemide increase calcium excretion through the kidney by decreasing its reabsorption in the loop of Henle^(13,14,15), whereas thiazide diuretics decrease renal calcium loss by increasing tubular calcium reabsorption^(15,16,17).Furthermore, thiazide diuretics may alter the binding power of serum proteins

for calcium.⁽¹⁸⁾ Although the mean INR value in both groups was within the required therapeutic range (2-3), but its mean value was in the lower desired therapeutic range (2.05) in the group of patients who were receiving hydrochlorothiazide 50 mg/day with warfarin 3 mg/day. The mean INR value was in the middle required therapeutic range (2.53) in the group of patients who were receiving furosemide 80/ day plus warfarin 3 mg/day and such increment in INR value was statistically significant with respect to those who were taking hydrochlorothiazide with warfarin. Furthermore there was a statistically significant reduction in prothrombin time in patient taking hydrochlorothiazide with warfarin when compared with those who taking furosemide with this oral anticoagulant.

These results can be rationalized by the effect of thiazide diuretics on serum calcium level since thiazide diuretics increase serum calcium level, this lead to increase in activation of clotting factor X which will result in a decrease in the anticoagulant activity of warfarin which results in a reduction in prothrombin time and INR⁽²⁾. Furthermore diuretics may decrease the response to warfarin by reducing the plasma volume, with a subsequent increase in clotting factor activity⁽¹⁹⁾. On the other hand furosemide decrease calcium level which will improve the activation of clotting factor X, but this effect is neutralized by the fact that diuretics decrease plasma volume with a subsequent increase in clotting factor activity, so furosemide may not alter the response to warfarin.^(2,19)

Conclusions:

The results of this study may revealed that the concomitant use of hydrochlorothiazide with warfarin may results in a reduction in the anticoagulant activity of the later.

References

- 1- Koda- Kimble and Young's: Applied therapeutics,(Tenth edition) /section 1, p.207,(2013).
- 2-Goodman and Gilman's : Manual of pharmacology and therapeutics /section XI, p.951,(2008).
- 3- Rang'and Dale's pharmacology,(sixth edition) /chapter 21p.333,(2006).
- 4- Hultin MB, Nemerson Y,Activation of factor X by factors IXa and VIII ; a specific assay for factor Xa in the presence of thrombinactivated factor V Ill, *Blood*52:928-940, (1998).

- 5- Davie EW, Fujikawa K, The role of serine proteases in the blood coagulation cascade, *Annu Rev Biochem*44:779-829, 197, (2012).
- 6- Davie E, Fujikawa K, Kurachi K, Kisiel W, The role of serine proteases in the blood coagulation cascade, *AdvEnzymol* 48:277-318,(2009).
- 7-Lee CT, Chen HC, Lai LW, Yong KC, Lien YH ,Effects of furosemide on renal calcium handling.*Am J Physiol Renal Physiol*. Oct;293(4):F1231-7, (2007) .
- 8-H. Seitz and Z. F. Jaworski, Effect of Hydrochlorothiazide on Serum and Urinary Calcium and Urinary Citrate.*Can Med Assoc J*.February 8; 90(6): 414–420, (2010).
- 9-Sullivan JM, Dluhy RG, Wacker WE, Solomon HS, Williams GH, Samaha JK, Interrelationships among thiazide diuretics and calcium, magnesium, sodium, and potassium balance in normal and hypertensive man. *J ClinPharmacol*. Nov-Dec;18(11-12):530-43, (1978).
- 10- Khanin MA, Rakov DV and Kogan AE, Mathematical model for the blood coagulation prothrombin time test. *Thromb Res* 89: 227-232,(1998).
- 11- Kitchen S and Preston FE, Standardization of prothrombin time for laboratory control of oral anticoagulant therapy.*Thromb. Hemost.* 25: 17-25, (1999).
- 12- Kirkwood TBL, Calibration of Reference Thromboplastins and Standardisation of the Prothrombin Time Ratio. *Thrombosis Haemostasis*, 49 (3) 238-244, (1983).
- 13-Friedman PA, Gesek FA,Calcium transport in renal epithelial cells, *Am J Physiol*; 264:F181. (1993).
- 14-Hoenderop JG, Nilius B, Bindels RJ, Molecular mechanism of active Ca²⁺ reabsorption in the distal nephron, *Annu Rev Physiol*; 64:529., (2002).
- 15-Rejnmark L, Vestergaard P, Pedersen AR, Heickendorff L, Andreasen F, Mosekilde L, Dose-effect relations of loop- and thiazide-diuretics on calcium homeostasis.*Eur J Clin Invest*. Jan;33(1):41-50, (2003).
- 16.Brickman, A. S., S. G. Massry, and J. W. Coburn, (Changes in serum and urinary calcium during treatmentwith hydrochlorothiazide *J.Clin. Invest*. 51: 945-954, (1972).
- 17-Constanzo, L. S., and I. M. Weiner, The hypocalciuricaction of chlorothiazide. Proceedings of the6th Annual Meeting of the *American Society of Nephrology*.26,(1973).
- 18-Duarte, C. G., J. L. Winnacker, K. L. Becker, and A.Pace. (Thiazide induced hypercalcemia. *N. Engl.J. Med*. 284: 828-830,(1973).
- 19-Melmon KL, O'Reilly RA,Drug interaction involving oral anticoagulation.In: Melmon KL, editor. *Cardiovascular Drug Therapy, Philadelphia; FA Davis*.,:23–41, (1975).