

Control of oral anticoagulant therapy using EDTA plasma

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Abstract

Background: Prothrombin time test is traditionally used to monitor oral anticoagulant drugs given for various clinical conditions. Prothrombin time test is carried out on citrated plasma while most of the other hematological tests are carried on blood anticoagulated with Ethylenediamine tetra acetic acid (EDTA).

Objective: To evaluate the possibility of using EDTA plasma for the control of oral anticoagulant drugs.

Patients and methods: Thirty-six patients on different doses of Warfarin therapy were included in the study, and 21 normal healthy subjects were used as control group. The work was carried out in a private laboratory. Results were used to calculate the mean normal PT (MNPT) from both groups. Blood was withdrawn in 2 tubes: Citrate tube and EDTA tube. On each tube PT was determined in duplicate manually by Quick method using Biolabo thromboplastin reagents. For the patients group the international normalized ratio (INR) was calculated according to the thromboplastin manufacture kit instruction. SPSS version 9.0 was used to calculate the correlation and regression. MedCalc statistical program version 11.6.1.0 was used for assessing the clinical agreement using the Bland and Altman method.

Results: A linear relationship between citrate and EDTA samples for the INR and PT estimations was observed. The regression equations for INR and PT estimations with citrate plasma (y) and EDTA plasma (x) were:

$$\text{INR citrate} = (-0.4) + (1.25) \text{ INR EDTA}, R^2=0.97$$

$$\text{PT citrate} = (-3.14) + (0.89) \text{ PT EDTA}, R^2=0.96$$

Bland and Altman method was used to compare clinical difference of INR results of citrate and EDTA sample types which shows the mean difference (0.06) and the limits of agreement (0.46, -0.33) which represents the mean difference \pm 1.96 SD. Only four measurements were out of range.

Conclusion: Despite the very good correlation observed between results of citrate and EDTA samples, no clinical agreement was established between the two methods and the use of EDTA samples for performing PT tests by manual Quick method is not acceptable.

الخلاصة

المقدمة:- فحص زمن البروثرومبين هو الفحص الشائع في مراقبة الادوية المانعة للتخثر و التي تعطى لاجراض طبية متعددة. يتم اجراء الفحص باستعمال البلازما المعدة باستخدام مادة السترات, بينما تستخدم البلازما المعدة باستخدام مادة (الاي دي تي اي) في اجراء معظم فحوصات الدم الاخرى.

الاهداف:- تقييم امكانية استخدام البلازما المعدة باستخدام مادة (الاي دي تي اي) في اجراء فحص وقت معامل الخثرين لمراقبة الدوية المانعة للتخثر.

الطرق:- 36 مريضاً ممن يستخدمون جرعة مختلفة من دواء (وارفارين) تم ضمهم الى الدراسة بالاضافة الى 21 متطوع و بصحة جيدة تم استخدامهم كمجموعة سيطرة و نتائج هذه المجموعة تم استخدامها في قياس معدل زمن البروثرومبين الطبيعي (MNPT), من كلا المجموعتين تم سحب الدم في انبوين احدها يحتوي مادة السترات و الثاني يحوي مادة (الاي دي تي اي). تم قياس زمن البروثرومبين في الانبوين باستخدام طريقة (Quick) و باستخدام مادة الثرومبوبلاستين المصنعة في شركة (بابولابو). لمجموعة المرضى تم احتساب (INR) حسب تعليمات الشركة المصنعة. تم استعمال الاصدار التاسع لبرنامج SPSS لحساب درجة الارتباط و التراجع و تم استخدام برنامج (ميدكال) الاحصائي الاصدار 11.6 لتقييم درجة المقبولية السريرية بين الطريقتين بالاعتماد على طريقة (بلاند و التمان).

النتائج:- لوحظ وجود علاقة خطية بين نماذج السترات و نماذج (الاي دي تي اي) لكل من قيم زمن البروثرومبين و قيم (INR) وقد كانت معادلتا التقهقر كما يلي:

$$\text{INR citrate} = (-0.4) + (1.25) \text{ INR EDTA}, R^2=0.97$$

$$\text{PT citrate} = (-3.14) + (0.89) \text{ PT EDTA}, R^2=0.96$$

تم استخدام طريقة (بلاند و التمان) لمقارنة الفرق السريري في نتائج (INR) بين نماذج السترات و نماذج (الاي دي تي اي) والتي اظهرت ان معدل الفرق كان 0.06 وان حدود المقبولية هي (-0.33, 0.46) كما وجد ان اربعة قيم فقط كانت خارج الحدود .

الاستنتاج :- على الرغم من درجة الارتباط الجيدة جدا بين نتائج السترات و (الاي دي تي اي) فانه لم تكن هناك مقبولية سريرية لاستخدام (الاي دي تي اي) كبديل للسترات في قياس زمن البروثرومبين باستخدام طريقة Quick.

Key words: citrate, EDTA, INR, oral anticoagulants, PT.

Abbreviations: PT: prothrombin time; INR: international normalized ratio; EDTA: Ethylenediamine tetra acetic acid.

Introduction

Oral anticoagulant drugs have wide applications in clinical practice and Warfarin is the most popular one among them, it is rapidly absorbed from the gastrointestinal tract (within 90 minutes) with a half life of 36-42 hours. In the plasma it binds to plasma proteins (mainly albumin) and metabolized by the liver ⁽¹⁾.

Warfarin is a vitamin K antagonist, it acts by inhibiting vitamin K which is the cofactor in the γ . Carboxylation of glutamic acid residues in the vitamin K dependent coagulation factors, so by using warfarin there will be inhibition to the synthesis of factors II, VII, IX, X as well as protein C and S ^(2,3). Prothrombin time (PT) has traditionally been used to monitor oral anticoagulant drugs. The prothrombin time evaluates the extrinsic pathway factors ⁽⁴⁾.

The PT test is most commonly applied using the Quick method, based on the technique described by Quick in 1935. In this method an excess of thromboplastin and calcium is added to anticoagulated plasma, this test is easy to perform both manually and automatically. The Owren method was subsequently developed, it relies on using combined thromboplastin reagents (fibrinogen and factor V added to the reagent), this method has advantages over the Quick method since it does not measure factor V which is not decreased in oral anticoagulant therapy, both methods

are accepted for anticoagulant therapy ^(5,6). In Iraq the Quick method is the only one that is used.

The use of international normalized ratio (INR) to report the results of PT have minimized discrepancies in the results due to different thromboplastin reagents and greatly improved uniformity of anticoagulation and interpretation throughout the world ^(7,8).

Prothrombin time is traditionally measured in citrated plasma because factors V and VIII are more stable in citrated plasma ⁽⁹⁾; however most of other laboratory tests are performed using blood collected in Ethylenediamine tetra acetic acid (EDTA). EDTA is a polyprotic acid containing four carboxylic acid groups and 2 amine groups and a lone pair of electrons that chelate calcium. Calcium is necessary for a wide range of reactions in the coagulation cascade and its removal irreversibly prevents blood clotting within the collection tube ⁽¹⁰⁾.

EDTA has long been considered as unacceptable for the coagulation tests as it directly inhibits the coagulation process and interferes with end point determination; however it is used for other hematological tests because blood cells are preserved better in EDTA specimens ⁽⁵⁾.

The aim of the study is to evaluate the possibility of using EDTA as the anticoagulant in performing PT for monitoring oral anticoagulant therapy and to determine whether the results are

comparable in terms of PT in seconds and in terms of INR.

Patients and methods

Venous blood samples were obtained from 21 normal subjects (12 females and 9 males) age ranging from 27 year to 70 year; those samples were used as control group and used to calculate the mean normal PT for both EDTA and citrate samples.

Venous blood samples were obtained from 36 patients on Warfarin therapy at different doses ranging from (1-10 mg). Of the sample group 17 were females and 19 males with age ranging between 25-72 years. For each patient blood was collected in 2 tubes:-

- 1- Citrate tube containing 0.2 ml of 0.109 mol/ (3.2%) of sodium citrate and 1.8 ml of blood.
- 2- EDTA tube containing 0.072 ml of 75 g/l of K₃EDTA and 3 ml of blood providing an EDTA concentration of 1.5 mg/ml of blood.

Sample tubes were centrifuged at 2000g for 15 minutes at room temperature to prepare platelet poor plasma; samples were processed within 2 hours of collection.

On each tube prothrombin time was determined in duplicate manually by Quick method using Biolabo thromboplastin reagents (LOT NO 020810A1) with an ISI value of 1.66. The average is then taken and results were expressed in seconds. From the 21 normal healthy controls the mean PT was calculated for both citrate and EDTA samples and this mean normal PT (MNPT) was used to calculate the INR according to the thromboplastin kit manufacture instructions.

Statistical methods

SPSS version 9.0 was used to calculate the correlation and regression. MedCalc

statistical program version 11.6 was used for assessing the clinical agreement using the Bland and Altman method⁽¹¹⁾.

Results

INR and PT were measured for 36 patients on oral anticoagulants (warfarin) using citrate and EDTA tubes. Descriptive statistics of the values of INR and PT are summarized in table (1).

Regression analysis was used to establish the linear relationship between citrate and EDTA samples for the INR and PT estimations. The regression equations for INR and PT estimations with citrate plasma (y) and EDTA plasma (x) were:

$$\text{INR citrate} = (-0.4) + (1.25) \text{INR EDTA}, \\ R^2=0.97$$

$$\text{PT citrate} = (-3.14) + (0.89) \text{PT EDTA}, \\ R^2=0.96$$

A scatter plot of Citrate versus EDTA for INR and PT estimations with regression lines are shown in figure 1a and figure 1b respectively.

In order to verify the clinical acceptance of using EDTA sample interchangeably with citrate ones, Bland and Altman method was used to compare clinical difference of INR results of citrate and EDTA sample types. A plot of the differences in INR between the two sample types (citrate & EDTA) against their mean is shown in figure 2 which shows the mean difference (0.06) and the limits of agreement (0.46, -0.33) which represent the mean difference ± 1.96 SD. Only four measurements were out of range.

Discussion

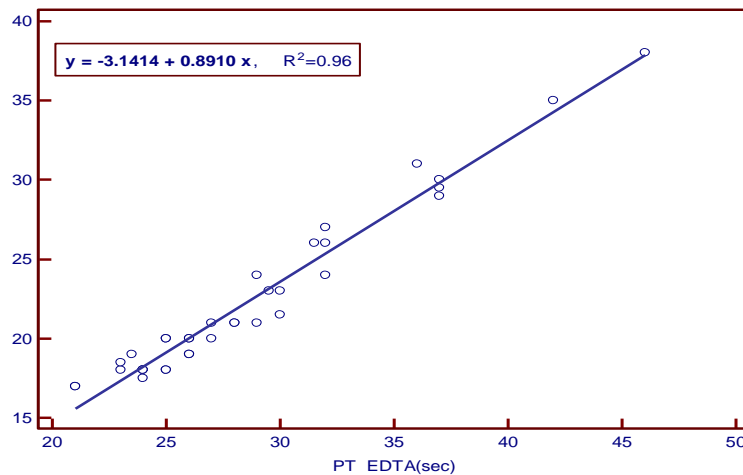
EDTA samples have long been used in many haematological measurements since they give the best preservation of blood cell morphology while the use of citrate samples is confined only to coagulation tests therefore the possibility of using EDTA blood for coagulation tubes seems

to be convenient, practical and save a lot of efforts and costs.

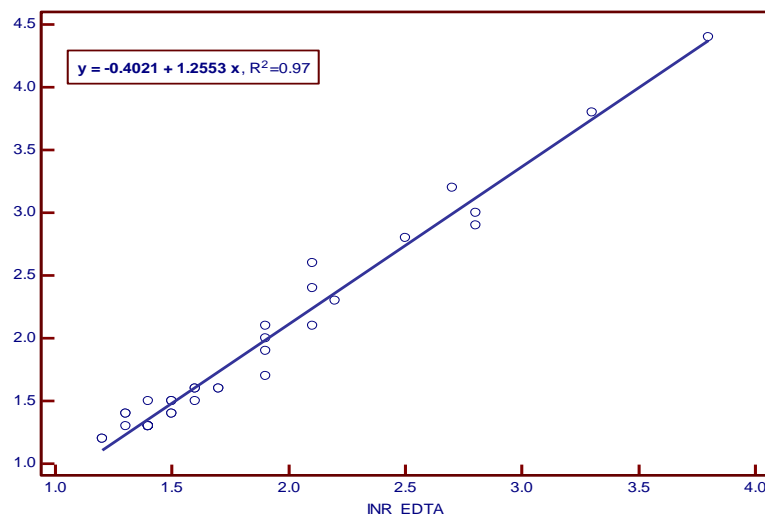
Table1. Descriptive statistics of the values of INR and PT using citrate and EDTA (n=36).

	INR		PT	
	citrate	EDTA	citrate	EDTA
Mean	1.88	1.825	22.38	28.65
SD*	0.78	0.61	5.25	5.78
Minimum	1.2	1.2	17	21
Maximum	4.4	3.8	38	46
Range	3.2	2.6	21	25

*: standard deviation



a



B

Fig 1: Linear regression between citrate and EDTA samples: (a) INR, (b) PT

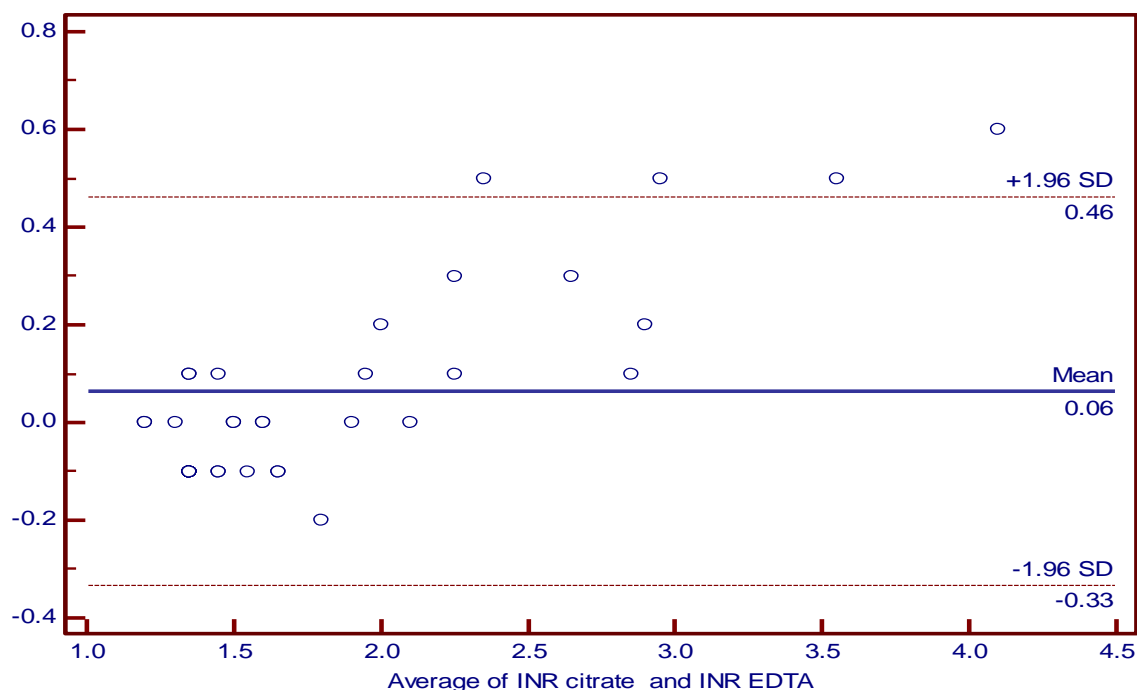


Fig 2: Differences in INR between citrate & EDTA samples against their means

This study aims to evaluate the possibility for using EDTA blood in performing the PT test for the control of oral anticoagulant drugs using the manual Quick method.

When the results of EDTA and citrate samples were directly compared a very good correlation for both PT in seconds and INR was demonstrated which offers the possibility of changing the results of PT in seconds and INR from EDTA samples to citrate samples and this is consistent with Horsti J. study⁽¹²⁾.

However, the presence of strong linear relationship between 2 methods does not mean that these 2 methods can be used interchangeably^(11, 13). When assessing the clinical applicability using the method of Bland and Altman, the mean difference was 0.06 and the limits of agreement were -0.33 to 0.46. These results differ from the study of Horsti⁽¹²⁾. Were the mean difference for the INR was 0.00 and the limits of agreement were -0.14 to 0.14. However, Horsti used in his study the automated Owren method and both could

be a possible cause for the difference in the results seen.

In practice any INR discrepancy between 2 results that is greater than 0.2 has a major influence on the clinical outcome and it may subject the patient to the risk of bleeding or thrombosis by over or under estimation of the results⁽¹⁴⁾. Furthermore the difference in the INR appears to increase when the INR increases which added further burden on the clinical applicability of the method as patients with high INR are those whom are at risk of bleeding and require accurate and precise determination of the INR.

Conclusions

Although some studies have proved the possibility of using EDTA blood in performing the PT test for the control of oral anticoagulant drugs using automated Owren method, in Iraq where only the Quick method is used and most of our hospitals and laboratories rely on the

manual rather than the automated method it seems that the use of EDTA samples for performing PT tests is not acceptable and may lead to serious discrepancies in INR estimation.

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