

## Differences in results of INR using reagents with different ISI in Sudanese patients on warfarin

RANIA SALAHALDIN DAFAALLA

Department of Hematology, Faculty of Medical Laboratory Science  
University of Al-Neelain, Khartoum, Sudan

ENAAM ABDELRAHMAN

Faculty of Medicine, University of Al Neelain  
Khartoum, Sudan

AHMED OMER AHMED

U of K, Faculty of Medicine, Khartoum, Sudan

MANAL M. ELTAHIR

Department of Pathology, Faculty of Medicine  
University of Science and Technology  
Khartoum, Sudan

### Abstract:

**Background:** *The Prothrombin Time (PT) in an individual vary with the type of thromboplastin (e.g. rabbit, human, bovine etc) used in the assay. This difference in sensitivities is known as the sensitivity index. Individual thromboplastins can be calibrated against an international WHO reference thromboplastin (INR). Oral anticoagulants (warfarin) cannot be prescribed at a fixed dose. For each patient the dose must be adjusted according to the result of INR conducted every four weeks. If the dose is low the patient is at risk of developing thrombosis. If the dose is high the patient is at risk of spontaneous bleeding. And this research is low published papers done on Sudan*

**Objectives:** *The purpose of this study was to evaluate differences in results by two different reagents with different ISI (international sensitivity index).*

**Method:** *This comparative study was done at (Sudan Heart Centre) , Khartoum state. We measured INR values of 50 patients on*

*warfarin with different ISI for each patient, using coagulometer technique type Bio bas 1, 1539 made in Spain. Clinical data were collected from patients' medical records.*

**Results:** *The ages were between 20-75 years. The mean INR values by using (ISI 1.01) were found to be lower than those obtained by using (ISI 1.30), (P value. < 0.05) and this was considered significant.*

**Conclusions:** *We have observed that INR results significantly affected by different ISI and that will have an impact on warfarin dose.*

**Key words:** International Normalized Ratio; Thromboplastin; ISI; oral anticoagulant therapy; warfarin.

## **INTRODUCTION:**

Oral anticoagulation therapy (OAT) is one of the most commonly used medications worldwide. The purpose of the treatment is to balance the risks of haemorrhage and thrombosis in the patient. Arterial and venous complications commonly are involved in morbidity and mortality in OAT patients globally. (Palareti G et al, 1996), (Odén A et al, 2002), (Ansell J et al 2004), (Hirsh J et al 2003) the vitamin K antagonists Coumadin and warfarin are inexpensive and the most widely used medicines in the prevention and treatment of thromboembolism in various clinical situations, and the benefit of OAT has been proved. (Turpie A et al 1997), (Hirsh J. et al , 2005), (Stein PD et al, 2001), (Hirsh J et al, 1998), (Schulman S et al, 2003), (Gage BF et al, 2000), (Wilson SE et al, 2004), (Dunn A et al, 2003) the major drawback with warfarin is a narrow therapeutic window and individually variable responses to the treatment. Thus frequent prothrombin time (PT) checks are required to ensure that anticoagulation remains within the therapeutic range. (which is 2.0–3.0 International Normalized Ratio (INR). )

Warfarin, also known by the brand name Coumadin (among others), is an anticoagulant normally used in prevention of thrombosis and thromboembolism i.e formation of blood clots in the blood vessels and their migration elsewhere in the body, respectively. Warfarin is used to decrease the tendency for thrombosis or as secondary prophylaxis (prevention of further episodes) in those individuals who have already formed blood clot (thrombus). Warfarin treatment can help prevent formation of future blood clots and help reduce the risk of embolism (migration of a thrombus to a spot where it blocks blood supply to a vital organ). ("coumadin". The American Society et al, 2011).

Warfarin is best suited for anticoagulation (clot formation inhibition) in areas of slow running blood (such as in veins and the pooled blood behind artificial and natural valves) and in blood pooled in dysfunctional cardiac atria. Thus, common clinical indications for warfarin use are Atrial fibrillation, prosthetic heart valves, deep vein thrombosis and pulmonary embolism (where the embolized clots first formed in veins). Warfarin is also used in anti phospholipids syndrome. It has been used occasionally after heart attacks (myocardial infarctions), but is far less effective in preventing new thrombosis in coronary arteries. Anti platelets drugs are usually used for Prevention of clots in arteries. Their mechanisms of action differ from warfarin (which normally has no effect on platelet function). (Hirsh J, Fuster V et al, 2003).

The PT/INR test evaluates the extrinsic and common pathways of the coagulation cascade, while the APTT test evaluates the intrinsic and common pathways. Using both tests will assess the integrated function of the coagulation factors. The World Health Organization (WHO) put forth the idea for prothrombin time standardization based on a mathematical formula known as The International Normalized Ratios (INR). Because manufacturers provide limited guidelines for

instrument; the laboratory should validate its values by performing local on-site International Sensitivity Index (ISI) calibration. The International Normalized Ratio (INR) /International Sensitivity Index (ISI) system was developed as a way to standardize the prothrombin time during the monitoring of patients on oral anti-coagulant therapy with vitamin K antagonists.

There is a natural variation in the response of patients towards anticoagulation therapy with vitamin K antagonists (VKAs). The biological variation of the International Normalized Ratio (INR) within patients treated with VKAs is between 9.1 % and 10.9 % (coefficient of variation [CV], in %) (Van G et al, 2009).

In addition to this natural variation, which cannot be influenced, measurement deviations can also be caused by external factors. These can lead to differences between measurements from different laboratories or between measurements performed with the Coagu- Chek® system and the lab. These deviations may be due to different sensitivities of the reagents used, different pre-analytics methods or variations in the calibration of laboratory reagents (determination and consideration of the deviation of a reagent/instrument to a reference). (Tripodi, A et al, 2003) The use of INR allows direct comparison of measured values, because the reagents used for measuring the prothrombin time (PT) are calibrated by a well-defined procedure and designated with a specific index called the International Sensitivity Index (ISI).

The ISI value indicates the degree of compliance to World Health Organization (WHO) reference thromboplastin, whereby an ISI of 1.0 means that the reagent has the same sensitivity as reference thromboplastin. (Tripodi, A. et al, 2013)

A high degree of comparability between INR values is achieved through calibration and standardization to ISI. However, the reagents have different sensitivities to the

activities of the clotting factors that are influenced by VKAs. We show that reagent sensitivity is heavily dependent on various factors:

The source of thromboplastin used (e.g. rabbit, bovine or human)\_(Smith, S et al , 2006) Phospholipids contained in the thromboplastin (e.g. natural mixture or synthetically produced) (Amukele et al, 2010) The reagent composition (e.g. with/without stabilizers and/or glycine; an aqueous or dry chemical substance)\_(Smith, S et al , 2006 ) .The sample (e.g. whole blood or plasma, undiluted or diluted) 20Each reagent is unique – there are no two reagents with absolutely the same properties. Even two WHO reference thromboplastins show a certain degree of deviation, which is larger for higher INR values.

Our aim was to investigate the degree of differences between INR values calculated using different ISI derived from warfarinised Sudanese patients and to ensure the Accuracy and precision of oral anticoagulants.

## **MATERIAL AND METHOD:**

This is comparative study .Was conducted in coagulation clinic in Sudan heart centre, Khartoum state. Many patients on wafarin with many different indications such thromboemobolic srtoke, congestive heart failure, myocardial infarction, artial fibrillation, deep vein thrombosis, prosthetic heart valve and pulmonary embolism attend the clinic for follow-up and to adjust their warfarin dose according to their INR result. The study was conducted from October 2015 to November 2015.

A total of 50 patients (21 Males and 29 Females) on warfarin from Khartoum city, in Sudan were included in this study. Their age range was between 20 to 75 years. Blood sample was collected from each subject into tri sodium citrate containers. The serum was separated and stored at 4C after

collection and immediately analysis to measurement of PT test by using coagulometer bio bas 1 made in Spain and then calculate the INR. The control was measure to calculate the INR according to ISI, the rule for calculation:

$$\text{INR} = (\text{patient PT (sec)} ) \div (\text{mean normal PT (sec)} )^{\text{ISI}} .$$

### **Techniques:**

Venous samples were obtained and collected in Tri sodium Citrate containers 3.8% in a ratio 1 part of citrate and 9 parts of blood anticoagulant used was Tri sodium citrate. Centrifuged quickly to obtain platelet poor plasma which transferred into clean plain test tubes. The samples were stored in the 4°C Proper sample handling was archived to ensure the validity of study.

### **Procedures:**

We placed the reaction cuvettes required for the samples to be run in the thermostat-

- We added magnetic beads into every cuvette and wait for the instrument to be at 37°C

It is very important to avoid air bubbles formation because they can stop the instrument before the clotting time. It is recommended to use a pipetting system that does not introduce bubbles.

- We added 50micoliter of sample in the cuvettes.
- We added 100micoliteof reagent
- We pressed the start key of bio bas coagulometre and read the prothrombine time
- After that we pressed reset to read next sample.
- We read every sample two times by reagent with ISI 1.01, and by reagent with ISI 1.30.
- We calculated the INR.

### **Statistical analysis:**

All information was analyzed by using statistical package for social science (SPSS) computer program. The correlation and comparison of the two results were tested by T-test.

### **Ethical considerations:**

This study was approved by faculty of medical laboratory science, Al Neelain University. The information and samples were collected from the patients after obtaining their consents.

## **RESULTS**

This study was conducted on 50 Sudanese patient on warfarin . Their genders were (21 males 42% , 29 females 58% ) ( fig1). The patients' ages were ranged from 20-75 years (Mean=43.0). (Fig 2).

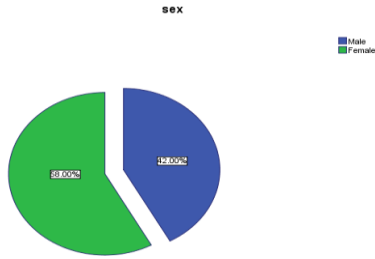
The statistical analysis of INR result by 1.01 (Mean 2.54) (Fig 3), INR result by 1.30 (Mean=9.33) (Fig 4).

Table (1) showed the comparison of results between two ISI values  $\leq 0.05$  was considered significant.

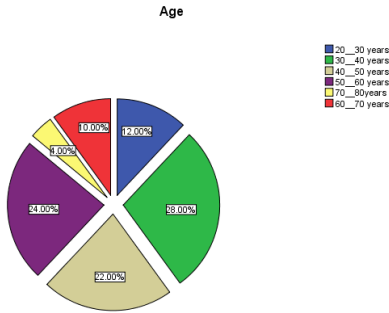
Table 2 showed the comparison of results according to the sex of the patients. There is no significant association between gender (P. value: 0.57) by ISI 1.01 and P.value: (0.64 by using 1.30).

(Fig 5) Ascatter plot shows the relationship between the INR by ISI1.01 & INR by ISI1.03 of the group ( $r=0.102$ ,  $P= 0.48$ ).

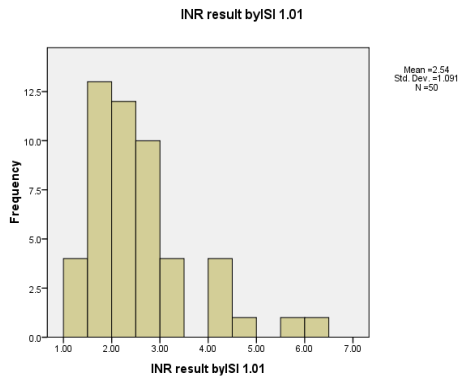
**Fig (1): Show distribution according to gender**



**Fig (2) : Show distribution according to Age**

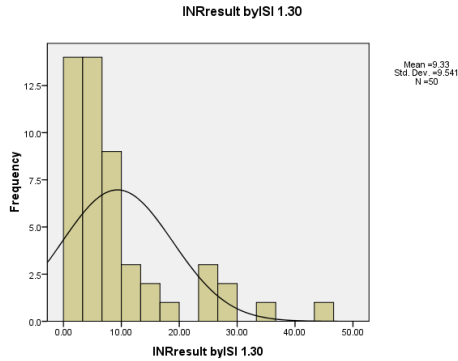


**Fig (3) showed the INR Result using ISI 1.01:**





**Fig (4) :** showed the INR result using ISI 1.30



**Table (1): Comparison between means of INR by ISI 1.01 and ISI 1.30**

Variable	Test group N=50	P value
INR by ISI 1.01	2.53± 1.09 (1.10_ _ 5.80)	< 0.000
INR By ISI 1.30	9.33± 9.54 (0.8_ _ 44.9S)	< 0.000

The table shows the mean ± SD (mini - max) and probability (P)

T-test was used for comparison.

P value ≤ 0.05 was considered significant.

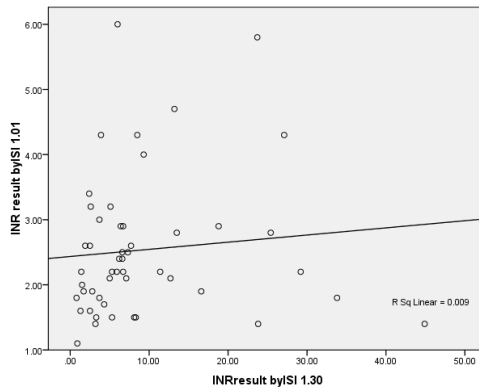
**Table (2): Comparison of means of INR by ISI 1.01 & INR by ISI 1.01 in males and females of the group**

Variable	Male N=21	Female N=29	P value
INR byISI 1.01	2.63 1.14 (1.14–5.5)	2.46± 1.016 (1.10–5.8)	= 0.57
INR byISI 1.30	10.095± 9.6 (1.4–44.9)	8.78± 9.6 (0.8–33.8)	= 0.64

The table shows the mean ± SD (mini - max) and probability (P)

T-test was used for comparison.

P value ≤ 0.05 was considered significant.



**Fig (5):** Ascatter plot shows the relationship between the INR by ISI 1.01 & INR by ISI 1.30 of the group ( $r=0.102$ ,  $P= 0.48$ ).

## **DISCUSSION:**

This comparative study was done on 50 patients on warfarin for different indications at Sudan Heart centre.

The results of the Prothrombin Time showed different values of INR results calculated by using two reagents with different of ISI ( 1.01 and 1.30) for the same sample with significant variation ( $P. value < 0.05$ ) - table (1). Comparison between ISI 1.01 and the ISI 1.30 showed significant difference. Table (2) comparison according to sex showed no significant difference. Similar results were obtained by Lind SE, Pearce LA, et... (1999) and Sermon AM,(2010)

## **CONCLUSION:**

The INR is an important clinical tool for monitoring Warfarin therapy. We concluded that the difference in reagent sensitivity in calculating INR will affect the patients' results and this will have an impact in warfarin dose.

Our results showed that low INR results will be obtained when using low ISI reagents the and high INR results

will be obtained by using high ISI reagents, so we recommend to use the same reagent for the same patient every time.

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