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Comparison of international normalised ratio and prothrombin time ratio resulted from different international sensitivity index reagents.

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Two hundred blood samples were studied for prothrombin time (PT) by three reagents with different International Sensitivity Index (ISI) 1.08, 1.25, and 1.90. The results were calculated to Prothrombin Time Ratio (PT ratio) and International Normalised Ratio (INR). The PT ratios were 1.3 ± 0.5, 1.3 ± 0.5, and 1.1 ± 0.3 and INRs were 1.36 ± 0.56, 1.43 ± 0.59, and 1.44 ± 0.96 respectively. The correlation studies expressed by the coefficient of correlation between two variable data (r) of the PT ratio and of the INR between reagents with ISI 1.08 and 1.25, 1.08 and 1.90, and 1.25 and 1.90 were 0.9, 0.9,0.7,0.8, and 0.8,0.9, respectively. The correlation study between PT ratio and INR of each reagent was 1.0 for all of them. According to the study results, the high sensitivity reagents (ISI near 1.00) had better correlation between them. Although higher ISI values produced less reliable PT ratios, the INR values enable the clinician to make a better comparison between PT results regardless of reagent, thus the system provides a reliable means in standardising for PT.

Key words : *International Normalised Ratio , Prothrombin Time, Coagulogram study.*

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นวพรรณ จารุรักษ์, ปราณี ไกรลาศศิริ, กัลยา เจนจบจริง. การเปรียบเทียบ โพรทรอมบินไทม์ เป็น อินเตอร์เนชั่นแนล นอแมไลส เรโฌ กับ โพรทรอมบิน ไทม์ เรโฌ ด้วยน้ำยาที่มีค่า อินเตอร์เนชั่นแนล เซ็นซิติวิตี้ อินเด็กซ ต่างกัน. จุฬาลงกรณ์เวชสาร 2536 กันยายน; 37(9) : 571-576

เลือดจำนวน 200 ตัวอย่างถูกนำมาหาค่า Prothrombin Time (PT) โดยน้ำยา 3 ชนิดที่มี International Sensitivity Index (ISI) ต่างกันคือ1.08,1.25, และ 1.90 รายงานผลเป็น Prothrombin Time Ratio (PT ratio) ได้ผลดังนี้ 1.3±0.5, 1.3±0.5,และ 1.1±0.3 ตามลำดับและInternational Normalized Ratio (INR) ได้ผลดังนี้ 1.36±0.56, 1.43±0.59, และ 1.44±0.96 ตามลำดับ ความสัมพันธ์ระหว่าง PT ratio กับ INR ของน้ำยาทั้งสามคือระหว่างน้ำยาที่มีค่า ISI 1.08 กับ 1.25, 1.08 กับ 1.90, และ 1.25 กับ1.90มีค่า r = 0.9, 0.9, 0.7, 0.8, and 0.8, 0.9, ตามลำดับ ส่วนความสัมพันธ์ระหว่าง PT ratio กับ INR ของน้ำยาแต่ละชนิดทั้งสามชนิดมีค่า r = 1.0 การศึกษานี้แสดงว่ากลุ่มน้ำยาที่มีความไวสูง (ISI ใกล้กับ 1.00) มีค่าความสัมพันธ์ดีกว่ากลุ่มน้ำยาที่มีความไวต่ำกว่า (ISI ไกลจาก 1.00) กลุ่มน้ำยาที่มีค่า ISI ต่างกันจะมีผลกับการรายงานผลเป็น PT ratio แต่จะไม่มีผลกับการรายงานผลเป็น INR ฉะนั้นการรายงานผลเป็น INR มีความน่าเชื่อถือ และสามารถใช้เป็นมาตรฐานในการศึกษาค่า PT โดยปราศจากผลกระทบจากการใช้น้ำยาต่างชนิดที่มีค่า ISI ต่างกัน

Quick , one-stage prothormbin time test (PT), originally introduced in 1935, remains the most commonly used test to monitor oral anticoagulant therapy. The principle of the test is that a plasma recalcification time is accelerated by the addition of tissue thromboplastin reagent.[1-8] As treatment is often extended over a peroid of months or even years, many patients may eventually be monitored by several laboratories, further compounding the need for PT standardisation.[1,3,8,9] In 1982, The World Health Organization (WHO) recommended a calibration scheme for thromboplastins to allow international standard of oral anticoagulant monitoring.[10] Instead of reporting patient values either directly in seconds, or as a ratio to the normal range mean Prothrombin Time Ratio (PT ratio), this scheme calls for expressing PT results in terms of an International Normalised Ratio (INR). By means of the calibration, all thromboplastins used routinely in any laboratory can be expressed by their relationship to the International Reference Preparation WHO/IRP 67/40 in terms of the International Sensitivity Index (ISI).[1,5,6,9-13]

The conversion of a PT ratio to an INR value is easily calculated. First,the PT ratio, R , of the patient PT divided by the PT of normal pooled plasma is determined. This ratio is then raised to the ISI power and the result is the INR. The conversion equation for transforming the results from PT ratio, to INR is given by $INR = (R)^{ISI}$. [1,5,6,9-13]

The INR system is believed to provide reliable and meaningful therapeutic values and help to minimise any differences that might occur from different laboratories, PT reagents, and test methodologies. [2,5,6,9,11,12,13] Since there are different ISI values of the commercial thromboplastins used in Thailand, the objective of this study is to compare the results of PT, reported in PT ratio and INR system, using different ISI reagents.

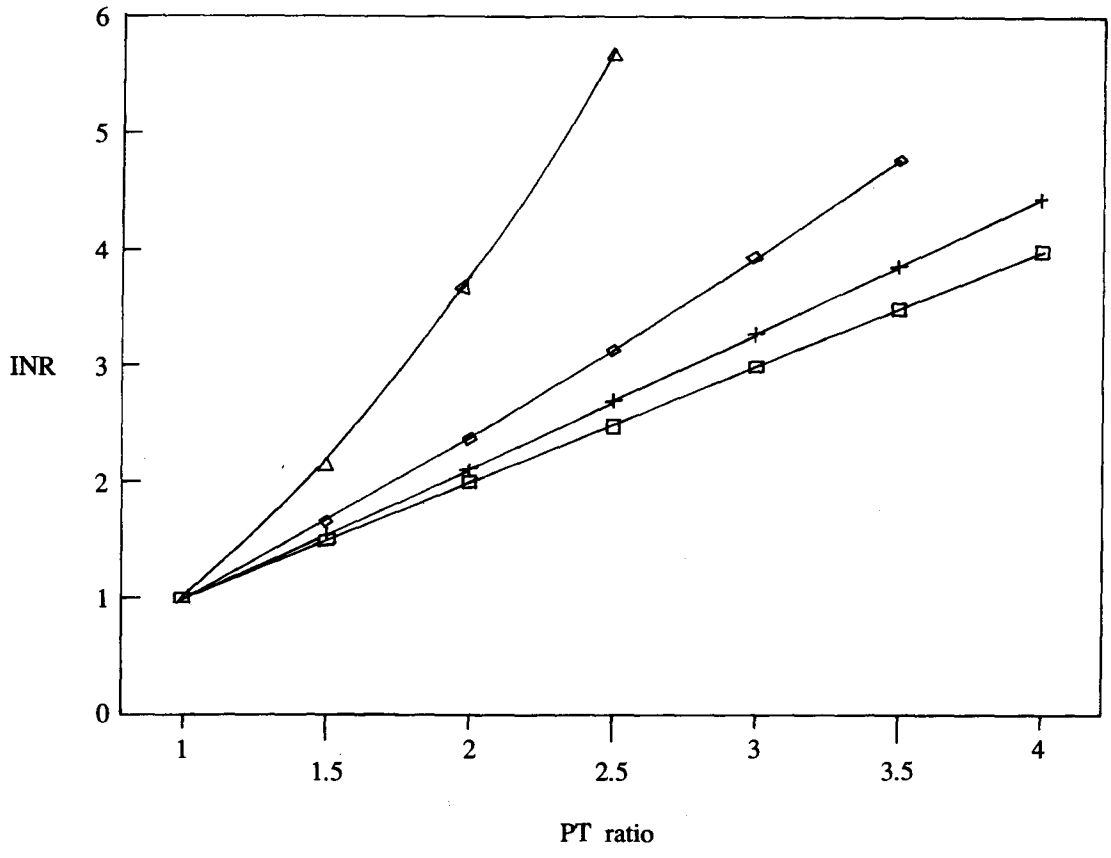
Materials and methods.

Two hundred blood samples regardless of normal or abnormal prothrombin time were collected randomly in evacuated tubes (Venoject) by using the last of the series of tubes collected. If only a coagulation specimen was needed, a "discard" tube had to be drawn first, the second tube collected for the coagulation studies to prevent contaminating tissue fluid in the collected specimens. All blood samples were anticoagulated with 3.8 % sodium citrate in which the ratio of blood to anticoagulant was 9:1. All the specimens were collected during 4th to 28th May 1993,by the experienced staff, at the Department of Laboratory Medicine, Chulalongkorn Hospital, to minimise trauma to the tissues. Specimens submitted in vacuum tubes containing less than 90% of expected volume which were not suitable for hemostatic testing had to be rejected. Visible hemolysis,severe lipemia, jaundice, anemia (Hct< 20%), polycythemia (Hct> 55%), and clotted specimens were also rejected. All the specimens were fractionated by refrigerated centrifuge (Omnifuge 2.0 RS, at 4° c, 1000 g,10 minutes) within 60 minutes from time of collection and the tubes remained capped during centrifugation to maintain pH, and were studied within 2 hours of the collected time.[6,14,15]

All the specimens were studied by three commercial working thromboplastins ; Thromborel S (Behringwerke AG, Germany,Human Placenta, Lot no.505429 A, ISI 1.08), Control plasma N (human) (Lot no. 502732) ; Thromboplastin IS (Baxter Diagnostics Inc., USA, Rabbit Brain, Lot no. TPS-5, ISI 1.25), Ci-trol Coagulation Control level I (Lot no. COL 1-653) ; and Thrombomat (bioMerieux, France, Rabbit Brain, Lot no. 608623 A, ISI 1.90), Uniplasmatol Normal (Lot no. 750732 A). All the specimens were analysed in duplicates by OVFY 03 Behring Fibrintimer, a photo-optical instrument, in accordance with the manufacturer's instructions for each reagent, and the average values were used. All the data were calculated as PT ratio which were used by previous reports (and is still used by some laboratories) and INR which was recommended by WHO. Ten sample results from these three reagents were randomly chosen and a graph was plotted to convert the PT ratio to INR by using the following formula:$INR = (R)^{ISI}$, R = PT ratio. Statistical analysis was performed by using unpaired student's t-test. The factors with p-values < 0.05 were considered statistically significant. The relationship between the two data were subjected to analysis by using the coefficient of linear correlation (r).[16,17]

Results

The PT ratios of the three reagents, Thromborel S, Thromboplastin IS, and Thrombomat, were 1.3 ± 0.5, 1.3 ± 0.5, and 1.1 ± 0.3respectively. The results of Thromboral S and Thromboplastin IS showed no significant statistical difference, but the results of the two reagents showed significant statistical difference with the result from Thrombomat ($p<0.05$). The results that had been calculated to INR for those reagents were 1.36 ± 0.56, 1.43 ± 0.59, and 1.44 ± 0.96 respectively, and there were no significant statistical difference (table1). Figure 1 illustrates the comparison of the PTratio and the INR which was obtained from using these three reagents. Ideally the reagent with ISI should be 1.00 while the calibration constant of the preparation is defined as 1.0. For the reagent with ISI nearest the ideal reagent, the slope of the line (calibration constant) is also near 1.0 which is shown in Figure 1.



❐ Ideal line obtained by using International Reference Preparation of thromboplastin, ISI = 1.00

\+ Line obtained by using Thromborel S, ISI = 1.08

◇ Line obtained by using Thromboplastin IS, ISI = 1.25

△ Line obtained by using Thrombomat, ISI = 1.90

Figure 1. Graphs for converting Patient's PT ratio in to INR. For a given PT ratio plotted on the X-axis, a vertical line is extended to the appropriate ISI curve. A horizontal line from this point is then drawn to the Y-axis to determine the INR value.

Table 1. Prothrombin time reported in PT ratio and INR by three different ISI reagents.

		Thromborel S (ISI=1.08)	**Thromboplastin IS (ISI = 1.25)**	**Thrombomat (ISI=1.90)**
PT ratio	(x ± SD)	1.3 ± 0.5	1.3± 0.5	1.1 ± 0.3
INR	(x ± SD)	1.36 ± 0.56	1.43 ± 0.59	1.44 ± 0.96

The relationship between the PT ratioand the INR from these reagents Thromborel S and Thromboplastin IS and Thrombomat was shown by a correlation study, r were 0.9, 0.9, 0.7, 0.8 and 0.8, 0.9 respectively. (Table 2)

Table 2. Correlation study in PT ratio and INR between Thromborel S and Thromboplastin IS (I), Thromborel S and Thrombomat (II), and Thromboplastin IS and Thrombomat (III).

		Reagents		
		I	II	III
PT ratio	(r)	0.9	0.8	0.7
INR	(r)	0.9	0.9	0.8

The relationship between the INR and the PT ratio of these individual three reagents also was shown by correlation study and r was 1.0 for all of them. (Table 3)

Table 3. Correlation study between PT ratio and INR of each reagent.

	r between PT ratio and INR
Thromborel S	1.0
Thromboplastin IS	1.0
Thrombomat	1.0

Discussion

The results from table 1 showed significant statistical difference for the thrombomat reagent which had a ISI value much higher than the others. (Thromborel: ISI = 1.08, Thromboplastin IS:ISI = 1.25, Thrombomat : ISI = 1.90), while the results reported by INR of those reagents showed no significant statistical difference. Thus reporting just the patient PT ratio alone is clearly an insufficient measure of the level of oral anticoagulant therapy. Several factors contribute to the differing degrees of responsiveness observed for various thromboplastin reagents ; among these are the species and tissue source of the thromboplastin, and the relative concentrations of other components of the reagent formulation, such as calcium. [1,2,3,5,9,13,14] In addition, thromboplastins vary in their responsiveness to proteins induced by vitamin K antagonists (PIVKAs), and this may be an important difference between thromboplastins from different species. Figure1, illustrates that, if we only use the PT ratio, with a reagent ISI=1.90 a patient would need to have more anticoagulant to achieve the same PT ratio obtained with a reagent ISI = 1.08 or from a reagent which had lower ISI or we can say that the less sensitive reagent (ISI much more than 1.0) the higher the dose of anticoagulant to reach the same PT ratio.

The results demonstrated in Table 2 and Table 3, showed the relationship between each reagent and between each reporting system. For each reporting system between these reagents, the results of the study showed that there is better relationship between the reagents with ISI nearer to 1.0 than those which ISI further from 1.0 , while the INR reporting system showed a better relationship between these reagents than the PT ratio reporting system.

Reporting patient results by INR can provide more reliable and meaningful therapeutic values than the PT ratio and improve interlaboratory correlation ; INR values enable the physician to make a better comparison between PT results regardless of the reagent. When a laboratory changes reagents for its PT assays, conversion to INR should help minimise the differences that may occur.[1,5,6,9-13]

The INR system offers the clinician advantages in standardising the monitoring of patients receiving oral

anticoagulant therapy. The system provides a reliable means for minimising variability between different laboratories, and PT reagents, thus potentially improving the consistency of the therapeutic monitoring. Globally, the INR system should promote communication and agreement on optimal therapeutic ranges by allowing direct comparison between clinical trials nationwide or even worldwide.

Summary

According to this study, the reagent with high sensitivity (ISI~1) had better correlation between the reporting systems especially in the PT ratio system than the reagent with low sensitivily (ISI much more than 1), In the INR reporting system the differentiation of the ISI of the reagents does not cause a significant statistical difference.

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