Laboratory Wise Variations in Prothrombin Time - INR - A Cross Sectional Study in Trivandrum, Kerala

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ABSTRACT

BACKGROUND

Prothrombin time (PT) is routinely used as a test of coagulation. Thromboplastin is the key ingredient in the reagent for this test. Prothrombin time international normalized ratio (INR) readings can vary according to the thromboplastin used in the reagent. The composition of thromboplastin reagents can influence the sensitivity of each batch of reagents. Various thromboplastin reagents having different international sensitivity index (ISI) values are available now. This study was intended to evaluate the effect of different thromboplastins on INR reading for mitral valve replaced patients under stable oral anticoagulant therapy.

METHODS

The study was conducted on the citrated plasma received from the mitral valve replaced patients having stable international ratio between 2 to 3 for three months. 62 samples were collected from the clinical pathology laboratory, Govt. Medical College, Trivandrum. Each sample was tested with different thromboplastin reagents having international sensitivity index 1.0, 1.1 and 1.6 by measurement of the prothrombin time and conversion into international normalized ratio. The INR obtained from the thromboplastin with international sensitivity index 1.0 was considered as the standard. INR results obtained from samples tested with thromboplastin reagents with ISI 1.1 and 1.6 were compared with the standard by using analysis of variance (ANOVA) and Dunnett's post hoc tests.

RESULTS

Sixty-two samples were tested with the thromboplastin reagent having ISI – 1.0, the mean INR is 2.42, for ISI – 1.1 mean INR value is 2.53 and for ISI 1.6, the mean INR value is 3.19. While comparing the mean value of INR for different reagents using ANOVA, the F value was 14.86, which was significant. P value less than 0.01. In the Dunnett post hoc test, the P value of difference between ISI 1.0/1.6 was < 0.01. Between ISI 1.1/1.6 also the P value is < 0.01. Both of these were significant. The P value of difference between the reagents having ISI 1.0 and 1.1 is 0.838 which denotes no significant difference.

CONCLUSIONS

In conclusion, the thromboplastin reagent with ISI 1.0 or nearest to 1.0 is most desirable for accurate INR report.

KEYWORDS

Prothrombin Time, International Sensitivity Index, International Normalized Ratio

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DOI: 10.18410/jebmh/2021/395

How to Cite This Article:
Meethal SJK, Jayakumar R, Sreenivasan SK, et al. Laboratory wise variations in prothrombin time-INR - a cross sectional study in Trivandrum, Kerala. J Evid Based Med Healthc 2021;8(24):2112-2116. DOI: 10.18410/jebmh/2021/395

Submission 17-02-2021,
Peer Review 27-02-2021,
Acceptance 30-04-2021,
Published 14-06-2021.

BACKGROUND

The prothrombin time is related to the extrinsic pathway of coagulation and is sensitive to the vitamin K dependent clotting factors (factors 2, 5, 7, 9 and 10). In the PT estimation, plasma is incubated at 37^0 C with a reagent containing a tissue factor, thromboplastin and calcium chloride. The time of clot formation is then estimated by manual, photo optical or electromechanical methods. The result is reported in seconds (prothrombin time), or as a ratio compared to the laboratory mean normal control.

The results of the prothrombin time test may vary from lab to lab, so a ratio called the INR was introduced by the world health organization (WHO) in the early 1980's for standardizing PT results. For this purpose, a batch of human brain extract was selected as the first international reference preparation (IRP), and a correction factor (international sensitivity index, ISI) was introduced to correlate the sensitivity of commercial thromboplastin preparations to the IRP. By definition, the ISI of the first IRP was 1. The demand for laboratory monitoring of oral anticoagulant treatment is increasing day by day.

We wanted to assess the change in international normalized ratio in relation to international sensitivity index (ISI) of different thromboplastin reagents on blood samples from mitral valve replaced patients under stable oral anticoagulant treatment.

METHODS

This cross-sectional study was conducted on the citrated plasma received from the mitral valve replaced patients who were under stable oral anticoagulant treatment in cardiothoracic surgery valve clinic, having stable international normalized ratio between 2 - 3 for 3 months. 62 samples were collected and analysed in the clinical pathology and haematology lab, Govt. Medical College, Trivandrum from August 2017 to January 2018 after ethical committee approval (IEC no – 08 / 12 / 2017 dated 28 / 07 / 2017). Each sample was tested with different thromboplastin reagent having international sensitivity index 1.0, 1.1 and 1.6 by measuring the prothrombin time and conversion into international normalized ratio. The international normalized ratio derived from the thromboplastin with international sensitivity index 1.0 was considered as the standard. Other thromboplastins were tested against this by ANOVA and Dunnett post hoc tests.

Sample Collection Technique

Blood was withdrawn from an antecubital vein or other visible veins in the forearm by means of a syringe and needle. Blood was collected in 2 ml safe lab tubes containing 3.2 % sodium citrate maintaining 9 : 1 ratio of blood and anticoagulant. After collection the containers were firmly capped to minimize the risk of leakage. Specimen was mixed by inverting the containers several times.

Sample Processing

The sample was centrifuged at 3000 rpm for 10 minutes. The supernatant plasma was collected for doing prothrombin time estimation.

Principle

The PT test measures the clotting time of plasma in the presence of an optimal concentration of thromboplastin. The test depends on reactions with prothrombin, factor V, factor VII, factor X, and on the fibrinogen concentration of the plasma.

Reagents

In our study, three reagents with different international sensitivity index (ISI) were used.

- Thromborel S (siemens, Health care Diagnostics products, Germany, Lot. 546984). Thromboplastin reagent which consists of recombinant thromboplastin [International sensitivity index (1.0)] and contains calcium chloride.
- Uni plastin (Tulip diagnostics (P) Ltd), India, Lot. No. 302710P) with an ISI value 1.1. Uni plastin is a ready to use liquid calcified thromboplastin reagent, which is derived from rabbit brain.
- Liquiplastin (Tulip diagnostics (P) Ltd, India, Lot. No. 301707P with an ISI Value 1.6. Liquiplastin is a liquid ready to use calcified thromboplastin reagent, which is derived from rabbit brain

Figure 1. Uniplastin, Liquiplastin & Thromborel

Procedure

- 0.1 ml of plasma is delivered in to a glass tube and placed in a water bath. Waited for 1 - 2 minutes and allowed it to warm.
- Added 0.2 ml of pre-warmed thromboplastin reagent. Mixed the contents and recorded the end-point.

Then the recorded time was converted in to INR by comparing with the PT of a reference plasma and by applying the value of international sensitivity index of the reagent in the test.

$$INR = \left[\frac{PT\ Patient}{PT\ Reference\ Plasma}\right]^{ISI}$$

Sample Size Calculation

The calculation of sample size was based on a previous study, evaluation of different thromboplastin and coagulometers on international normalized ratio. Readings for patients under stable oral anticoagulant therapy; J blood disorders and transfusion; Oct 2015[1]. The calculated sample size was 59. ANOVA and Dunnett Post hoc test were used for statistical analysis.

RESULTS

A total of 62 citrated plasma samples were tested. Using thromboplastins having ISI 1, 1.1 and 1.6 and compared the results between each group. The international normalized ratio obtained from the thromboplastin reagent with international sensitivity index 1.0 was considered as the standard. INR values obtained while testing with a reagent having ISI 1.1 was compared with ISI 1 sample. ISI 1.6 group was also compared with ISI 1 sample. ISI 1.1 group was again compared with ISI 1.6 group. Age group, sex distribution, brand of valve and type and duration of anticoagulation were also studied.

The predominant age group present in the study was between 41 and 50 years (40.3 %). Females were the predominant sex group present in the study. Samples were collected from mitral valve replaced patients. Out of these 62 cases, Medtronic mechanical valve was the most used one (69.4 %)

Brand of Valve	Frequency	Percent
TTK CHITRA	15	24.2
Medtronic	43	69.4
SJM	2	3.2
Starr - Edwards	2	3.2
Total	**62**	**100.0**

Table 1. Brand of Valve

Medication and Duration of Treatment

Out of 62 patients, 47 (75.8 %) were taking oral anticoagulant Nicoumalone (Acitrom) and 15 (24.2 %) were taking warfarin. About half of the patients were taking oral anticoagulant for more than 6 years

Duration	Frequency	Percent
< 2	11	17.7
2 - 4	10	16.1
4 - 6	9	14.5
6 - 8	13	21.0
8 - 10	9	14.5
➢ 10	10	16.1
Total	**62**	**100.0**

Table 2. Duration of Treatment

ISI and INR Results and Statistical Analysis

With thromboplastin reagent having ISI – 1.0, the mean INR is 2.42, for ISI – 1.1 mean values are 2.53 and 3.19 for ISI 1.6. There was significant difference between the mean INR results obtained when the same blood sample was tested with reagents having ISI 1 and 1.6 (P value less than 0.01 in Dunnett post hoc test). Similar significant difference was noted when reagents having ISI 1.1 and 1.6 were compared. But there was no significant difference between reagents having ISI 1 AND 1.1.

ISI Value	Mean	SD	N	F	P Value	Dunnett Post-Hoc Test Pair	p
ISI 1.0	2.42	0.73	62			ISI 1.0 & ISI 1.1	0.838
ISI 1.1	2.53	0.81	62	14.86	p < 0.01	ISI 1.0 & ISI 1.6	P < 0.01
ISI 1.6	3.19	1.00	62			ISI 1.1 & ISI 1.6	P < 0.01

Table 3. Comparison of INR Values Based on ISI

DISCUSSION

The clotting screen includes prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen assay. The prothrombin time and international normalized ratio are assays evaluating the extrinsic pathway of coagulation. Prothrombin time is the primary assay used in monitoring oral anticoagulant therapy. This test measures the clotting time of plasma in the presence of an optimal concentration of thromboplastin. The prolongation of PT depends on reduction in the vitamin K dependent clotting factors II, V, VII, IX and X concentration in the plasma.[2]

PT test requires the use of laboratory reagents called thromboplastins which are commercial products producing different clotting times for a particular blood sample. The naturally available thromboplastins are obtained from rabbit brain or lung. Recombinant thromboplastins are manufactured by using synthetic phospholipid and recombinant tissue factor produced in *E. Coli*.

The results of the prothrombin time can vary according to various thromboplastin reagent preparations. Hence such results cannot be directly compared with one another. To render coagulation times as comparable as possible, the World Health Organization approved a standard reference thromboplastin in 1983. Every manufacturer of thromboplastin must calibrate his reagent against the WHO standard. The value obtained is known as the international sensitivity index.[3] This enables the various sensitivities of the thromboplastins to be ascertained and it will be used to calculate the INR.

Various reagents having different ISI are commercially available now. This study aimed at evaluating thromboplastin reagents having different ISI values. Variation in prothrombin time and INR according to reagent used was tested. Reagents with ISI 1.0, 1.1, and 1.6 were used and ISI 1.0 is the recommended standard by WHO.

The samples were collected from mitral valve replaced patients. Among the 62 cases selected, 25 persons are coming under the age group 41 - 50 (40.3 %) and 32 are females (51.6). Medtronic mechanical valve is the most used one (69.4 %) for the mitral valve replacement in these patients. Most of these patients take oral anticoagulant Nicoumalone (Tab. Acitrom) (70.8 %). The duration of anticoagulant treatment ranged from six months to 20 years.

Rasha M. Ahmed and Abdel Rahim M. Muddathir conducted a study to evaluate different thromboplastins and coagulometers on international normalized ratio readings for patients under stable oral anticoagulant therapy. The study showed that some further efforts are needed to achieve

harmonization of INR results among different laboratories because variation would most probably induce the clinician to make a change in warfarin dose. In addition, standardization of instruments, reagents, and controls is also warranted to decrease this variation.[1]

The present study was carried out with three individual thromboplastin reagents having different ISI, to evaluate these reagents and the effect of variation in ISI on PT/INR value. When using reagent with ISI 1.0, the mean INR is 2.42 with 0.73 standard deviation. For ISI – 1.1, mean value is 2.53 with standard deviation of 0.81 and mean value of INR is 3.19 and standard deviation 1.0 for reagent having ISI 1.6. When comparing the mean values with each other by ANOVA test, the F value was 14.86 and the P value was less than 0.01. This shows that there is a significant change in INR, when using different ISI reagents. Dunnett post hoc test was employed to detect the exact significance of difference between each group. With the reagents having ISI 1.0 and 1.6, the P value is less than 0.01. A significant P value of less than 0.01 is also seen when comparing the mean INR values from reagents with ISI 1.1 and 1.6. There was no significant difference between ISI 1 and 1.1.

A UK study by DA Tuberner et al. suggested that the ISI value of thromboplastin can strongly influence the inter-laboratory variability of the INR obtained with it.[4] The study by David L McGlasson suggests local calibration of thromboplastin to eliminate the variability in PT/INR results.[5] There is an inverse relationship between sensitivity and ISI. When the ISI is low, the sensitivity of the reagent is more. There is a practical advantage in using a sensitive reagent with ISI of 1.2 or less. In some cases, the ISI is influenced by the use of coagulometers also. Hence the ISI should be assigned for the combination of reagent and endpoint detection system. A thromboplastin should not be used with techniques for which the ISI is unknown.[6]

Higher INR values have been resulted when thromboplastins containing recombinant tissue factor was used.[7] With more use of anticoagulant therapy, the methods of coagulation monitoring have also advanced. The introduction of anticoagulation clinics, the development of point of care INR devices, and patient self-testing methods are some of them.[8] In a study using Coaguchek device for patient self-monitoring, the number of hospital visits was less compared to laboratory monitoring. There was no compromise in safety also. However individual correlation between lab values and Coaguchek device was recommended.[9] There are reports of inconsistent INR results when a new lot of thromboplastin with identical ISI was used.[10] Hence ongoing calibration and external quality assurance are required to keep errors in INR calculation to the minimum.[11] Local calibration for ISI value of thromboplastins have standardized INR calculation Calibration using plasma from warfarinised persons can give erroneous results in calculation of INR of liver disease patients. Hence, local calibration is carried out using plasma from liver disease patients.[12] However, such variation is minimum even in liver disease patients when recombinant thromboplastin was used.[13]

CONCLUSIONS

The prothrombin time in an individual will vary with the type of thromboplastin 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 (International Reference Preparation or IRP) to assign them an international sensitivity index or ISI. The WHO recommended ISI is 1.0. But various reagents with different ISI are available in the market, and also, they are used in certain laboratories. Here we used three different reagents having ISI's 1.0, 1.1 and 1.6. By considering the thromboplastin reagents having ISI 1.0 as the standard, ISI 1.1 produced no significant change – P value 0.838. But with ISI 1.6 there is marked variation in the PT/INR values (P value less than 0.01).

ISI value strictly affects the sensitivity of the prothrombin time and also the INR. Undoubtedly, we can say that it will adversely affect the oral anticoagulation dosage. The clinician prescribes the dosage of anticoagulant treatment based on the INR value. Hence it is most important that we get the accurate report. On the basis of our study, we can obviously say that the sensitive and accurate monitoring of anticoagulation can be done by using a reagent having ISI 1.0 or of nearest to 1.0 while measuring prothrombin time.

Recommendations

There is significant variation in PT/INR values according to the particular thromboplastin used in the test. Reliable INR within target therapeutic ranges are essential for safe and effective oral anticoagulation. So, it is strictly recommended to use a thromboplastin reagent with ISI value 1.0 or nearest to 1.0 while doing PT/INR test.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.
We sincerely thank Prof. Abdul Rasheed, Former HOD, CTVS, Medical College, Trivandrum and Mrs. Indira K., Asst Professor, Dept. of Medical Laboratory Technology, Medical College, Trivandrum, for their guidance. We express our gratitude to Dr. Oommen P. Mathew, Statistician, Trivandrum for the statistical assistance.

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