Role of D-Dimer Assay in Diagnosis of Deep Vein Thrombosis

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ABSTRACT

Deep Vein Thrombosis (DVT) affects a significant proportion of population and if untreated timely leads to high mortality due to pulmonary embolism. Though color Doppler ultrasonography is the gold standard for diagnosis but non availability of experienced radiologist especially at night has led to alternative investigation for diagnosis of DVT. Plasma D-dimer levels which measure the degradation products of cross linked fibrin, are a valuable diagnostic test for the exclusion of deep venous thrombosis. D-dimer levels were analyzed in fifty patients suspected of deep vein thrombosis clinically and compared the results with color Doppler ultrasonography. A value of 250 ng mL\(^{-1}\) was chosen as cut off. For the purposes of analysis, results were expressed as either negative (<250 ng mL\(^{-1}\)) or positive (≥250 ng mL\(^{-1}\)). Patients were classified as low, moderate or high pretest probability for DVT as per Well’s scoring system. D-dimer assay had a sensitivity of 97.3% (CI 85.8-99.9%), a specificity of 38.5% (CI 13.9-68.4%), a positive predictive value of 81.8% (CI 67.3-91.8%) and a negative predictive value of 83.3% (CI 35.9-99.6%). In patients with a low or moderate pretest probability of DVT, the negative predictive value of this assay was 100% and this test can safely exclude the DVT in these patients. Thus in patients having low and moderate pretest probability automated latex D-dimer test can safely exclude DVT.

Key words: D-dimer, deep vein thrombosis, diagnosis, color Doppler

INTRODUCTION

Deep Vein Thrombosis (DVT) affects approximately 84 individuals per 100,000 every year (Anderson et al., 1991). The consequences of acute DVT and its treatment make the accurate and timely diagnosis of DVT particularly important. An untreated proximal DVT is associated with a 30-50% risk of Pulmonary Embolism (PE), with a concomitant 12% mortality rate, whereas, unnecessary treatment of DVT incurs the risk, inconvenience and expense of anticoagulation (Anderson et al., 1991). Unfortunately, the diagnosis of DVT based on clinical signs and symptoms alone is inaccurate in up to half of patients with the classical findings of pain, swelling and tenderness, if DVT is not confirmed by diagnostic testing (Barnes et al., 1975; Cranley et al., 1973; Haeger and Sjukhuset, 1969; Kakkar et al., 1969).

Duplex ultrasonography is currently the most widely used noninvasive tool for the diagnosis of DVT (Sumner and Mattos, 1993). Although duplex scanning is both noninvasive and inexpensive, it does have limitations, including a limited ability to assess the iliac and central venous circulation (Lewis et al., 1994; Vaccaro et al., 1990). However, only 15-28% of patients suspected of having deep venous thrombosis actually have a thrombosis and only 1% of patients
with an initially normal ultrasonography develop deep venous thrombosis within 1 week (Cogo et al., 1998; Birdwell et al., 1998; Bernstein, 1984; Lensing et al., 1989). This also increases the load on the radiologist in busy institutions. This has led to a search for a more efficient diagnostic strategy. Other approaches, notably the determination of pretest probability and measurement of plasma D-dimer levels, have been more recently incorporated into diagnostic strategies for acute DVT.

Plasma D-dimer levels which measure the degradation products of cross linked fibrin, has been reported to be a valuable diagnostic test for the exclusion of deep venous thrombosis (Birdwell et al., 1998). Hence, the present study was planned to study diagnostic value of D-dimer assay in patients with suspected venous thrombosis and to compare the usefulness of latex D-dimer assay with color Doppler ultrasonography in diagnosis of DVT.

MATERIALS AND METHODS

The study was carried out on 50 patients presenting to Outpatient Department of Surgery or Accident and Emergency Department with signs and symptoms suggestive of deep venous thrombosis between November 2009 and February 2011. The study conformed to the guidelines set forth by research ethics board and all patients provided written informed consent.

All the patients with clinical diagnosis of deep vein thrombosis, made on the basis of findings described by Bernstein (1984), were subjected to routine laboratory investigations including Activated Partial Thromboplastin Time (aPTT), Prothrombin Time (PT) and index (PTI) (Bernstein, 1984).

Color Doppler ultrasound of deep veins was done in all the patients to confirm the diagnosis (Lensing et al., 1989). Patients with normal color Doppler ultrasonography results underwent repeat color Doppler ultrasonography approximately one week later at follow-up. Patients were classified as venous thrombosis positive or negative according to the results of color doppler ultrasonography. Patients in whom DVT was diagnosed at presentation or during follow-up were treated with anticoagulants.

Patients were also assigned a pretest probability of venous thrombosis using previously validated Wells’s model (Wells et al., 2003). A score of ≤0 indicates low pretest probability, a score of 1 or 2 indicates moderate pretest probability and a score ≥2 indicates high pretest probability. In patients with bilateral symptoms, the more symptomatic leg was used.

D-dimer assays were performed according to manufacturer’s instructions on the automated coagulometer (ACL 9000) using a commercial quantitative latex immunoturbidimetric D-dimer assay kit (Hemosil). The optimal discriminant value for the D-dimer assay was determined as per manufacturer’s instructions. A value of 250 ng mL⁻¹ was chosen as cut off (Villa et al., 2000). For the purposes of analysis, results were expressed as either negative (<250 ng mL⁻¹) or positive (≥250 ng mL⁻¹).

For the D-dimer assay the True Positives (TP), False Positives (FP), True Negatives (TN) and False Negatives (FN) were determined. In the primary analysis, the accuracy indices i.e., sensitivity (TP/(TP+FP)), specificity (TN/(TN+FP)), negative predictive value (TN/(TN+FN)) and positive predictive value (TP/(TP+FP)) of the D-dimer assay were calculated for all patients with suspected venous thrombosis. In the secondary analysis, the same indices were calculated for the following subgroups: Patients with suspected DVT with a high, moderate, or low pretest probability of disease. The data analysis was done by using statistical package S.P.S.S. software (version 10.0).
RESULTS

In the present study, the age of patients with suspected DVT ranged from 18-74 years (mean 43.46 years) with female to male ratio of 1.08:1. Majority of patients were in sixth decade of life. Deep vein thrombosis was diagnosed in 37 patients (female to male ratio: 1.06:1). D-dimer levels were raised in all DVT positive patients except for a 45 years old patient who had a D-dimer level of 145 ng mL⁻¹. There was no patient above 50 years of age with normal D-dimer levels. Weight of patients with suspected DVT ranged from 50-120 kg with mean of 64.6 kg. Majority of patients were in range of 61-70 kg.

Pain and swelling were the most consistent presenting symptoms in 93% of patients with suspected DVT and all patients with confirmed DVT on ultrasonography had pain and swelling as presenting symptoms. Twenty nine patients (58%) with suspected DVT had known risk factors for DVT, like history of previous DVT, malignancy, major surgery, pregnancy/puerperium, oral pills, obesity and trauma. Sixteen patients (43%), diagnosed with DVT confirmed on ultrasonography had no predisposing factor but all had raised D-dimer at presentation. Eighteen patients (46%) had single predisposing factor and three (8%) had 2 or more predisposing factors. All patients with DVT diagnosed on ultrasonography had raised D-dimer levels except one female on oral pills.

All the patients diagnosed with DVT had calf tenderness and Homans’s sign was positive. Ninety five percent patients had swelling of whole of limb while asymmetrical pitting edema was present in 62%. Forty six percent patients had a difference in calf circumference of more than 3 cm as compared to other limb.

Deep vein thrombosis was diagnosed sonographically in thirty seven out of fifty patients (74%). There were 13 sonographically negative suspected patients, 5 of which also demonstrated a negative D-dimer assay. There was a single false negative result (negative D-dimer with positive color Doppler ultrasonography). The patient was 45 years old female who was taking oral pills and had thrombus extending up to right iliac vein. None of the patients with negative color Doppler findings were found to be having thrombosis on repeat sonography at follow up. Correlation studies using color Doppler ultrasonography as the gold standard in the diagnosis of acute DVT showed the Latex D-dimer assay to have a sensitivity of 97.3% (CI 85.8-99.9%), a specificity of 38.5% (CI 13.9-68.4%), a positive predictive value of 81.8% (CI 67.3-91.8%) and a negative predictive value of 83.3% (CI 35.9-99.6%).

When pretest clinical probability was compared to the color Doppler ultrasonography results, this was statistically significant (p<0.001) indicating that diagnosis of DVT was more likely to be correct in patients with high compared to low clinical suspicion of DVT. High pre-test score was found in 60% patients, 20% had moderate score and 20% had low score.

The prevalence of venous thrombosis was 10, 80 and 93% in the low, moderate and high pretest probability populations, respectively. While the sensitivity and negative predictive value of this assay were high in patients with a low or moderate pretest probability of venous thrombosis, they were lower in those with a high pretest probability of venous thrombosis (Table 1).

When the low or moderate pretest probability categories are combined, the D-dimer has a sensitivity of 100% (CI 66.4-100%), a specificity of 36.4% (CI 10.9-69.2%), an negative predictive value of 100% (CI 39.8-100%) and a positive predictive value of 55.3% (CI 29.9-80.3%). Therefore, a D-dimer result less than 250 ng mL⁻¹ essentially excludes venous thrombosis in patients with a low or moderate pretest probability of venous thrombosis; these patients made up 40% of the study population who had a pretest probability assessment.
Table 1: Performance of the D-dimer test in patients suspected of DVT in different subgroups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Total</th>
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<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 30</td>
<td>n = 50</td>
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<tr>
<td>Female sex</td>
<td></td>
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<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>17</td>
<td>26</td>
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<tr>
<td>Mean age (years)</td>
<td>37.9</td>
<td>45.8</td>
<td>44.5</td>
<td>43.5</td>
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<tr>
<td>Venous Thrombosis</td>
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<tr>
<td></td>
<td>1</td>
<td>10.0</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>Sensitivity of D-dimer test</td>
<td>1/1</td>
<td>100.0</td>
<td>8/8</td>
<td>27/28</td>
</tr>
<tr>
<td>Specificity of D-dimer test</td>
<td>3/9</td>
<td>33.0</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Positive predictive value of D-dimer test</td>
<td>1/7</td>
<td>14.3</td>
<td>8/9</td>
<td>27/28</td>
</tr>
<tr>
<td>Negative predictive value of D-dimer test</td>
<td>3/3</td>
<td>100.0</td>
<td>1/1</td>
<td>1/2</td>
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<tr>
<td>Negative D-dimer test</td>
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**Treatment:** All patients with DVT received low molecular weight heparin for 7 days followed by oral anticoagulants for 6 months. The monitoring was done with prothrombin time and INR levels which was kept between 2-3.

**Follow up:** All patients with a negative color Doppler ultrasonography at presentation underwent a repeat ultrasound after one week. There was no fresh venous thrombosis found in repeat color Doppler ultrasonography in any of these patients.

**DISCUSSION**

The diagnosis of deep vein thrombosis remains a challenge to the clinicians. The present study was conducted to compare the latex D-dimer assay with color Doppler ultrasonography in diagnosis of DVT.

Mean age of the patients presenting with DVT in the literature varies from 56-61. The mean age of patients with confirmed DVT in the present study was 44 years. Patients in the present study were in younger age group mainly because of more of females who were pregnant or in puerperium. Moreover, there are regional differences in age related incidence of DVT (Hansson et al., 1997). Inactivity and reduced exercises associated with increasing age may lead to stasis of blood in veins leading to DVT (Sebastian and Sabiston, 1997). However, D-dimer test may not be of help in patients above the age of 70 years because the tests are usually positive even without deep vein thrombosis (Sebastian and Sabiston, 1997). Most studies have shown a higher number of female referrals for DVT testing. In the literature female to male ratio varies from 1:1 to 1.95:1. (Villa et al., 2000; Hansson et al., 1997; Sebastian and Sabiston, 1997; Schutgens et al., 2002; Killick et al., 1997; Diamond et al., 2005; Bradley et al., 2000; Sadouk et al., 2002). In the present study, female to male ratio was 1.08:1. Incidence of DVT in veins of left leg is more than in veins of right leg. The factors responsible are compression of left side veins by right iliac artery, an over distended bladder, gravid uterus and congenital webs within the veins.

Obesity is a risk factor for development of DVT. In the present study, mean weight of patients with diagnosed DVT was 66.4 kg. Lindmarker et al. (1994) had reported mean weight of patients with diagnosed DVT as 78.6 kg and Harenberg et al. (1990) had reported mean weight as 75.8 kg (Lindmarker et al., 1994; Harenberg et al., 1990). Mean weight in the present study was comparatively less due to more number of young females belonging to rural areas, having less body weight in comparison with urban population. Another possible reason may be because of geographical variation in body weight of patients.
The cardinal features of venous thrombosis of legs are pain and swelling. In study done by Diamond et al. (2005) presenting symptoms included leg pain in 42.6% of patients and leg swelling in 53.4% of patients with suspected DVT (Diamond et al., 2005). Villa et al. (2000) had reported pain and swelling as the most common presenting symptom in 86 and 72% patients, respectively (Villa et al., 2000). In the present study pain as well as swelling was present in 100% of patients.

In the present study 57% patients with suspected DVT had one or more predisposing factors. Eight percent patients had 2 or more risk factor. In study done by Diamond et al. (2005) 51% patients had known risk factors for DVT and 6% patients had 2 or more risk factors (Diamond et al., 2005).

In the present study, 43% patients having DVT were not associated with any predisposing factor. Diamond et al. (2005) reported 32% patients without any predisposing factor (Diamond et al., 2005) and Villa et al. (2000) had 29% patients without any comorbid conditions (Villa et al., 2000). Bick and Kaplan (1998) could not find a definable cause in 80-90% of patients of DVT (Haeger and Sjukhuset, 1969).

Calf tenderness, Homans’s sign and edema may be present but are not specific to the diagnosis of DVT (Green and Ouriel, 1999; Kontos, 1996). In the present study both calf tenderness and Homans’s sign were present in 100 percent of patients in whom DVT was confirmed on color Doppler. Pitting edema was present in 62% patients with DVT diagnosed on ultrasonography. Cranley et al. (1976) reported calf tenderness in 80% patients, limb edema in 90% and Homans’s sign in 80% patients (Cranley et al., 1976). Haeger and Sjukhuset (1969) reported calf tenderness and edema in 84 and 76% patients, respectively (Haeger and Sjukhuset, 1969).

In the present study, deep vein thrombosis was diagnosed sonographically in 74% patients of suspected DVT. Villa et al. (2000) diagnosed DVT in 72.1% patients (Villa et al., 2000). Diamond et al. (2005) reported DVT in 12.8% patients and Sadouk et al. (2000) diagnosed DVT in 20.3% patients (Hansson et al., 1997; Sebastian and Sabiston, 1997). The patients referred to our hospital usually have a pre-test clinical probability rated from moderate to high (80%). This is the reason of high prevalence of DVT. In our study 95% patients had thrombus extending into ileofemoral vessels. Diamond et al. (2005) reported proximal DVT in 73% patients whereas, Villa et al. (2000) reported proximal DVT in all patients with venous thrombosis (Diamond et al., 2005).

In 12% of patients with suspected DVT in the present study, D-Dimer test was negative. Villa et al. (2000) reported only 10% patients had negative D-dimer assay (Killick et al., 1997). Diamond et al. (2005) reported negative D-dimer in 43% patients and Sadouk et al. (2000) also found negative D-dimer in 50% patients (Diamond et al., 2005; Sadouk et al., 2000).

Diamond et al. (2005) did not report any false negative results of D-dimer test hence its sensitivity and negative predictive value was found to be 100% (Diamond et al., 2005). Sadouk et al. (2000) reported 83.3% sensitivity and the negative predictive value was 93.2% in his study (Sadouk et al., 2000). In study by Villa et al. (2000) sensitivity and negative predictive value were 98.4 and 88.9, respectively (Villa et al., 2000). In the present study, one false negative D-dimer test was reported and sensitivity remained high at 97.3% but NPV was at 83.3%. Thus when used with a discriminant value of 250 ng mL⁻¹, the Latex D-dimer assay had shown a high sensitivity in patients with suspected venous thrombosis. This sensitivity compares favorably with that of ascending venography, serial IPG and serial compression ultrasonography, the three widely accepted diagnostic strategies for DVT, all of which approximate 98-99% (Cogo et al., 1998; Hull et al., 1981, 1985; Huisman et al., 1986; Heijboer et al., 1993).
When comparing the clinical efficiency of the D-dimer assays reported in our study with the results of other studies, it is important to take into account that the prevalence of DVT is usually significantly lower in these later studies, being around 20% (Diamond et al., 2005; Sadouk et al., 2000). In the present study, the prevalence of DVT was close to 74%. However, the percentage of positive ultrasonography reported for patients with low, moderate and high pre-test clinical probability is 10, 80 and 98%, respectively which is consistent with the fact that patients in our study were rated with moderate and high pre-test clinical probability and yielded 3 out of 4 positive ultrasonographies. Villa et al. (2000) had also reported high prevalence of DVT and found the percentage of positive ultrasonography reported for patients with low, moderate and high pre-test clinical probability to be 3, 17 and 75%, respectively (Villa et al., 2000).

In patients with a low or moderate pretest probability of DVT, the negative predictive value of this assay is 100%. More than 40% of patients in our study had a low or moderate pretest probability of venous thrombosis and approximately 20% of these patients had a D-dimer result of less than 250 ng mL\(^{-1}\). The relatively low negative predictive value (50%) of this assay in patients with a high pretest probability of venous thrombosis is noteworthy. This observation is almost certainly the result of the high prevalence of venous thromboembolism in this subgroup (96.7%), as the sensitivity (96.5%) is not inconsistent with that in the other subgroups and negative predictive value is critically dependent on both sensitivity and prevalence. For example, in a population with a prevalence of venous thrombosis of 50%, a D-dimer assay with a sensitivity of 98% and a specificity of 50% would have a negative predictive value of 96%. Conversely, an assay like the qualitative D-dimer (SimpliRED) which has a sensitivity of approximately 90% and a specificity of approximately 75%, would be expected to have an NPV of only 75% in a patient subgroup with a prevalence of venous thromboembolism of 50%. Therefore, in patients with a high pretest probability of venous thromboembolism, we do not recommend obviating further testing in those with a negative D-dimer result.

A previous study has reported that the negative predictive value of a whole-blood agglutination D-dimer test is significantly lower in patients with cancer than in those without cancer, again almost certainly because the prevalence of venous thrombosis in patients with cancer is high (approximately 50%) (Green and Ouriel, 1999). This lends support to the findings of the present study. The results obtained should be generalizable to other patient populations because unselected patients were evaluated and the prevalence of venous thrombosis is consistent with that reported in other studies (Bernstein, 1984; Wells et al., 2003; Hull et al., 1981, 1985; Huisman et al., 1986; Heijboer et al., 1993; Lee et al., 1999; Wells et al., 1997).

The potential for bias was eliminated by having objective tests interpreted by clinicians unaware of the D-dimer results and assays performed by technologists unaware of the clinical status of the patients. Although the reference standard tests of venography was not performed in all patients with suspected DVT, the classification of patients in this study as venous thrombosis positive and negative was corroborated by clinical outcome on follow up. This approach has successfully been used to validate the use of serial impedance plethysmography in patients with suspected DVT (Hull et al., 1985; Huisman et al., 1986). However, it is still likely that a small proportion of patients who truly had calf DVT were misclassified as DVT negative. While the sensitivity and NPV of the latex D-dimer assay are similar to those of ELISA, the specificity of this latex assay seems somewhat higher. Based on these promising results, further clinical trials should be performed to determine the safety of withholding anticoagulant therapy in patients with a low or moderate pretest probability of deep venous thrombosis and a latex D-dimer result of less than 250 ng mL\(^{-1}\). If this approach is safe, it would reduce health care costs by allowing many patients who present with suspected DVT to be discharged without further expensive and invasive testing.
Thus it may be concluded that D-dimer assay would reduced the load on radiologist in busy institutions and prevent unnecessary anticoagulation in patients presenting at odd hours, however, further study is needed to evaluate its use in clinical practice, because there is a small risk of false negatives in patients having high pretest probability of deep vein thrombosis.

REFERENCES


