Elevated Fibrinogen in an Acute Phase Reaction Prolongs the Reptilase Time but Typically Not the Thrombin Time

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Key Words: Fibrinogen; Acute phase reaction; Reptilase time; Thrombin time; Dysfibrinogenemia

Abstract

The effects of elevated fibrinogen on thrombin and reptilase times have not been well documented. High fibrinogen levels are common (38% of specimens submitted to our coagulation laboratory). Among 102 patients in the present study, an endogenously elevated fibrinogen level was significantly associated, as follows, with prolonged reptilase times: 1 (4%) of 28 with normal fibrinogen levels, 6 (20%) of 30 with levels in the 400 to 700 mg/dL (4.0-7.0 g/L) range, 10 (34%) of 29 with levels in the 700 to 1,000 mg/dL (7.0-10.0 g/L) range, and 7 (47%) of 15 with fibrinogen levels greater than 1,000 mg/dL (10.0 g/L). This association was independent of patient age and fibrin degradation product titer. In contrast, thrombin time was not altered notably by elevated fibrinogen levels. In 4 patients studied further, the prolonged clotting times could be corrected or nearly corrected by adding calcium chloride or albumin, whereas no such corrections were demonstrable in samples from several hereditary dysfibrinogenemia control subjects. An elevated fibrinogen level is common and is associated with reptilase time prolongations. For patients with prolonged reptilase times, a fibrinogen assay is suggested before establishing a diagnosis of dysfibrinogenemia.

Dysfibrinogenemia is an uncommon hereditary condition characterized by dysfunctional fibrinogen. Many different mutations are known to cause hereditary dysfibrinogenemia.1 Dysfibrinogenemia mutations can cause bleeding, thrombosis, or both, or they may be clinically asymptomatic. If bleeding is present, it usually is mild, but severe bleeding has been reported. Dysfibrinogenemia has an estimated prevalence of 0.8% in patients with venous thrombosis.2 Arterial thrombosis is less frequent than venous thrombosis in these patients. Most patients with hereditary dysfibrinogenemia are heterozygous. Rare homozygous cases have been reported.

Acquired dysfibrinogenemia has been reported with liver disease or hepatoma.3 In addition, thrombin time and reptilase time prolongations have been observed commonly with amyloidosis owing to inhibition of fibrinogen conversion to fibrin.4 Rarely, heparin-like anticoagulants have been reported in patients with malignant neoplasms or other disorders, with prolonged thrombin times and normal reptilase times.5 High sialic acid content in fibrinogen was associated with prolonged thrombin and reptilase times in a case of renal cell carcinoma6 and with nephrotic syndrome.7 Newborns tend to have prolonged thrombin times, attributed to increased sialic acid and phosphorus content in newborn fibrinogen.8

The thrombin time and reptilase time, which measure the clotting time during the conversion of fibrinogen into fibrin, often are prolonged in dysfibrinogenemia because the fibrinogen is dysfunctional. Therefore, these assays can be used clinically to assess for dysfibrinogenemia.9 Table II. Thrombin cleaves fibrinopeptides A and B from fibrinogen, thereby converting fibrinogen into fibrin monomers, which then polymerize into fibrin clot. The thrombin time assay measures the clotting time of this last step in the coagulation
cascade and may be altered by impairment of fibrinopeptide release, fibrin polymerization, thrombin inhibitors, or any combination of these. It is prolonged with dysfibrinogenemia, decreased fibrinogen level, elevated levels of fibrin degradation products (FDPs), heparin, hirudin, argatroban, and other thrombin inhibitors. Reptilase is a thrombin-like protease from venom from the snake *Bothrops atrox*, and, like thrombin, it converts fibrinogen into fibrin clot. However, unlike thrombin, reptilase cleaves only fibrinopeptide A from fibrinogen. Also, the reptilase time is not prolonged by heparin, hirudin, or argatroban. As with the thrombin time, the reptilase time is prolonged by dysfibrinogenemia, a decreased fibrinogen level, or elevated levels of FDPs. In addition, prolonged plasma thrombin times in some patients were reported associated with elevated fibrinogen levels.9

The present study was designed to determine whether there is an association between elevated fibrinogen levels and prolonged reptilase or thrombin times and to determine the prevalence of such an association.

### Materials and Methods

We consecutively and prospectively obtained 102 citrated, unselected specimens (28 with a normal fibrinogen level, 30 with a fibrinogen level of 400 to 700 mg/dL [4.0-7.0 g/L], 29 with a fibrinogen level of 700 to 1,000 mg/dL [7.0-10.0 g/L], and 15 with a fibrinogen level of more than 1,000 mg/dL [>10.0 g/L]). Specimens had been submitted to the laboratory for various special coagulation assays, mostly hypercoagulation tests (91.2% submitted for hypercoagulation tests; 6.9% submitted for prolonged prothrombin time or partial thromboplastin time mixing studies and/or factor assays; 2.0% submitted for von Willebrand assays; with no significant differences among the 4 groups). In this study population, dysfibrinogenemia testing conceivably could be indicated as part of a hypercoagulation or bleeding evaluation.

Specimens were collected into citrate tubes, in a ratio of 1 part anticoagulant to 9 parts blood. The majority of collection tubes contained 3.2% citrate, but a minority of specimens received from outside laboratories had been collected in 3.8% citrate. Specimens were kept on ice during transportation to the laboratory. Specimens were centrifuged at 1,500g for 10 minutes, and fibrinogen assays were performed on receipt of the specimen by the laboratory. Specimens were then stored at –70°C until testing the thrombin and reptilase times within 2 weeks of collection. Specimen storage and assay conditions adhered to the National Committee for Clinical Laboratory Standards guidelines.10 Institutional review board approval was obtained for this study.

Fibrinogen was assayed using a Clauss-based, automated, optical detection method on an MDA-180 coagulation analyzer (Organon Teknika, Durham, NC) using MDA Fibriquik reagent (Organon Teknika).11

To perform the thrombin and reptilase times, specimens were thawed rapidly in a 37°C water bath. Thrombin and reptilase times then were performed immediately on an ST4 coagulation analyzer (Diagnostica Stago, Parsippany, NJ). To perform a thrombin time, 0.1 mL of patient plasma was incubated at 37°C in the instrument for 2 minutes, and then 0.1 mL of bovine thrombin reagent was added (Thromboquik, Organon Teknika). The final concentration of thrombin in the reaction was 1.5 U/mL. The time until clot formation after adding thrombin was detected mechanically by the instrument. To perform a reptilase time, 0.15 mL of patient plasma was incubated at 37°C in the instrument for 2 minutes, and then 0.05 mL of reptilase reagent was added (Atroxin, Sigma-Aldrich, St Louis, MO). The time until clot formation after adding reptilase was detected mechanically by the instrument. All testing was performed in duplicate, and the average of the 2 results was used for analysis. With each batch of testing, a thrombin time and a reptilase time were performed on pooled normal plasma (CryoCheck, Precision Biologicals, Dartmouth, Nova Scotia) for quality control purposes.

A small number of specimens were found to contain heparin (n = 9). Heparinized specimens were treated with Hepzyme (Dade Behring/IBEX Technologies, Newark, DE) before measuring the thrombin time in order to remove heparin. Specimens were not treated with Hepzyme for reptilase time
assays, because Hepzyme treatment can cause reptilase time prolongations (unpublished observations).

FDPs were measured in 20 µL of citrated plasma using a manual latex agglutination assay (FDP Plasma, Diagnostica Stago). The Student t test was used to determine statistical significance among the 4 fibrinogen groups.

**Results**

The reptilase times were significantly different among the 4 groups of fibrinogen levels [Table 2] and [Figure 1]. In contrast, no significant differences were found with the thrombin time among the different fibrinogen levels (Table 2) [Figure 2]. By using the reference range derived from the group with normal fibrinogen levels, reptilase times for 1 (4%) of 28 in the group with normal levels, 6 (20%) of 30 in the group with levels 400 to 700 mg/dL (4.0-7.0 g/L), 10 (34%) of 29 in the group with levels 700 to 1,000 mg/dL (7.0-10.0 g/L), and 7 (47%) of 15 in the group with levels above 1,000 mg/dL (10.0 g/L) would be considered prolonged.

In an additional analysis (data not shown), reptilase times were expressed as the difference (in seconds) from the corresponding control reptilase time. These control-adjusted reptilase times also were significantly prolonged with higher fibrinogen values, with P values essentially the same as those shown in Table 2. In contrast, control-adjusted thrombin times did not show any significant difference among the 4 fibrinogen groups.

Thrombin times were repeated on a fibrometer (BBL FibroSystem, Becton Dickinson, Franklin Lakes, NJ) for 6 randomly selected patients (2 patients each from the 3 groups with elevated fibrinogen levels) to determine whether the lack of effect of the fibrinogen level on the thrombin time was method-dependent. The thrombin time from the fibrometer was prolonged in only 1 of the 6 patients (17%). This patient had the lowest fibrinogen level of the 6 patients (450 mg/dL [4.5 g/L]). Only 5.6% of all patients with elevated fibrinogen levels (excluding the 2 specimens containing residual heparin) had a prolonged thrombin time according to the ST4 analyzer. These results suggest that the fibrometer might yield more thrombin time prolongations than the ST4 analyzer, but in both cases, the incidence is very low.

To determine whether the reptilase time prolongations were due to elevated FDPs, FDPs were measured in

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**Table 2**

Reptilase Time and Thrombin Time in Relation to Fibrinogen Level (mg/dL)*

<table>
<thead>
<tr>
<th>Fibrinogen Level</th>
<th>Normal (n = 28)</th>
<th>400-700 (n = 30)</th>
<th>700-1,000 (n = 29)</th>
<th>&gt;1,000 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reptilase time (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) fibrinogen level</td>
<td>273 (118-354)</td>
<td>522 (413-694)</td>
<td>833 (708-970)</td>
<td>1,094 (1,010-1,350)</td>
</tr>
<tr>
<td>Mean ± 2 SD</td>
<td>178</td>
<td>21.7</td>
<td>23.7</td>
<td>26.0</td>
</tr>
<tr>
<td>P†</td>
<td>—</td>
<td>&lt;.0005</td>
<td>&lt;.000001</td>
<td>&lt;.0000001</td>
</tr>
<tr>
<td>Thrombin time (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>175</td>
<td>175</td>
<td>16.8</td>
<td>18.6</td>
</tr>
<tr>
<td>Mean ± 2 SD</td>
<td>12.5-22.4</td>
<td>12.9-22.1</td>
<td>11.1-22.5</td>
<td>11.5-25.6</td>
</tr>
<tr>
<td>P†</td>
<td>—</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

* Values are given in conventional units; to determine values in Système International units (g/L), multiply by 0.01.
† Compared with the normal fibrinogen group.
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**Figure 2** Effect of fibrinogen on thrombin time. Open diamond, thrombin time prolonged; solid diamond, thrombin time normal; horizontal bar, mean thrombin time for specified fibrinogen group. Fibrinogen values are given in conventional units; to determine values in Système International units (g/L), multiply by 0.01.

In 2 additional patients, prospective follow-up was completed to measure their reptilase times once their fibrinogen levels returned to normal. In case 1, the initial fibrinogen level was 600 mg/dL (6.0 g/L), and the reptilase time was 34.2 seconds (with a negative FDP assay). These results were reproducible over a 1-month period. One year later, his fibrinogen level had returned to normal (394 mg/dL [3.9 g/L]), and his reptilase time had returned to normal at 23.5 seconds. The thrombin times (performed on the ST4 analyzer) were normal throughout. In case 2, the initial fibrinogen level was elevated at 425 mg/dL (4.2 g/L), and the reptilase time was prolonged at 25.7 seconds. Five weeks later, her fibrinogen level was within the reference range (347 mg/dL [3.5 g/L]), and her reptilase time was normal at 22.9 seconds. Her thrombin times were normal throughout, and her FDP assays were essentially normal. For a third patient (case 3), family studies were performed. Fibrinogen, reptilase times, and thrombin times were normal in all 3 of her adult children, consistent with an acquired cause for her reptilase time prolongation in association with her fibrinogen level of 811 mg/dL (8.1 g/L). In cases 1 and 3, thrombin times also were performed on a fibrometer during the acute phase reaction and were prolonged.

The prolonged plasma clotting times of hyperfibrinogenemic cases 1 and 3 were explored further. Studies of reptilase-induced clotting of isolated fibrinogen revealed delayed onset of clot formation and decreased clot opacity (results not shown). Further details were obtained in 1 of these 2 cases, in which clot opacity was 16% of normal when induced by reptilase and 54% when induced by thrombin. The fibrinogen profiles in sodium dodecyl sulfate polyacrylamide electrophoresis, of both unreduced and reduced samples and of plasmic digestion fragments, were normal. Normal amounts of fibrinopeptides A and B were released by thrombin, and the high-performance liquid chromatography profile of each peptide was normal, including normal amounts of phosphorylated A content. There were no detectable immune complexes containing fibrinogen or fibrin. Thus, in these 2 patients, the fibrinogen function was abnormal, and the defect did not seem to be hereditary, because in case 1, the clotting time prolongations returned to normal when the fibrinogen returned to normal, and in case 3 the patient’s 3 children had normal clotting times. Abnormal fibrinogen elevations of familial origin have not been reported, further suggesting an acquired defect (eg, possible increase in sialic acid content?) to account for the abnormality. Further evidence that the defect is acquired is the

randomly selected samples from each of the 4 fibrinogen groups (n = 39). Although there was a slight trend toward higher FDP levels with prolonged reptilase time **Figure 3** or with elevated fibrinogen (data not shown), there was no significant difference between the FDP level among individuals with normal fibrinogen levels and those with elevated fibrinogen levels (P = .17). Similarly, there was no significant difference between the FDP level among individuals with normal reptilase times and those with prolonged reptilase times (P = .8).

Patient age increased significantly with fibrinogen level. Mean (median) ages, in years, were 40 (48) for the group with normal levels, 56 (57) for the group with levels 400 to 700 mg/dL (4.0-7.0 g/L), 59 (62) for the group with levels 700 to 1,000 mg/dL (7.0-10.0 g/L), and 63 (72) for the group with levels above 1,000 mg/dL (10.0 g/L) (P < .005). To ensure that the difference in reptilase times was not due to increased age, an additional 16 consecutive, unselected specimens from patients older than 62 years (mean [median], 73 years [71 years]; range, 63-83 years) with normal fibrinogen levels were obtained, and their reptilase times were found to be normal. When considering the 16 older persons with normal fibrinogen levels together with patients in the group with normal fibrinogen levels, we found no significant difference in age between the combined normal fibrinogen group (n = 44) and any of the 3 groups with elevated fibrinogen levels (P = .1). In contrast, the reptilase times remained significantly prolonged in the 3 groups with elevated fibrinogen levels compared with the combined normal fibrinogen group (the P values were essentially unchanged compared with those shown in Table 2).
marked or nearly complete correction of the clotting times by adding calcium chloride (to a 3-mmol/L concentration) or albumin (to a 2.5% solution), since no such corrections were demonstrable with control samples from several hereditary dysfibrinogenemic propositi (results not shown).

Discussion

Reptilase times were significantly higher with higher fibrinogen levels. When using the reference range obtained from people with normal fibrinogen levels, 20% to 47% of patients with an elevated fibrinogen level could be classified as having an abnormal level, depending on the degree of fibrinogen elevation. In both cases for which follow-up was available, the reptilase time returned to normal when the fibrinogen level returned to normal. Therefore, an elevated fibrinogen level could lead to a misdiagnosis of hereditary dysfibrinogenemia. It is suggested to measure the fibrinogen level at the time of testing if the reptilase time is prolonged. If the fibrinogen level is elevated, the reptilase time assay should be repeated any time when the fibrinogen level returns to the normal range. Alternatively, fibrinogen-specific reference ranges could be applied to interpret the reptilase time result (for example, the mean ± 2 SD ranges in Table 2, but it still would seem appropriate to repeat the assay once the fibrinogen level has returned to normal).

An elevated fibrinogen level seemed to be a stronger influence on reptilase time prolongation than was the FDP level. The fibrinogen level was significantly associated with reptilase time, whereas the association between the FDP titer and reptilase time (or fibrinogen) was not significant. The FDP level tended to increase with the fibrinogen level, probably because patients with thrombosis were more likely to have elevated fibrinogen levels owing to an acute phase response to the thrombosis, and thrombosis generates FDPs. However, as mentioned, the association between FDP and fibrinogen levels did not reach statistical significance. Fibrinogen levels increased with age, probably because the prevalence and severity of illness and its associated inflammatory state increased with age. In general, reptilase time prolongations owing to FDPs are known to occur when the fibrinogen is decreased to less than 80 to 100 mg/dL (<0.8-1.0 g/L).

An endogenously elevated fibrinogen level, which occurs during acute phase reactions, is commonly associated with reptilase time prolongations. Thrombin time prolongations, along with reptilase time prolongations and elevated fibrinogen levels, were observed with nephrotic syndrome in a previous study. An endogenously elevated fibrinogen level, which occurs during acute phase reactions, is commonly associated with reptilase time prolongations. Thrombin time prolongations, along with reptilase time prolongations and elevated fibrinogen levels, were observed with nephrotic syndrome in a previous study.

The thrombin time was not significantly altered by elevated fibrinogen levels per se in the present study. The possibility that elevated fibrinogen levels rarely can be associated with thrombin time prolongations is not excluded by this study, particularly if using a fibrometer. However, the present results suggest that if elevated fibrinogen levels can cause thrombin time prolongations, it is much less common than reptilase time prolongations. Thrombin time prolongations, along with reptilase time prolongations and elevated fibrinogen levels, were observed with nephrotic syndrome in a previous study.

When a prolonged reptilase time or thrombin time is obtained in the setting of elevated fibrinogen, we found that adding calcium chloride (to a 3-mmol/L concentration) or albumin (to a 2.5% solution) corrects or nearly corrects the clotting time to normal, as first described by Jim. In contrast, calcium or albumin did not correct the prolongation in hereditary dysfibrinogenemia. Therefore, this technique could be useful for distinguishing acquired from hereditary causes for these prolongations.

An endogenously elevated fibrinogen level, which occurs during acute phase reactions, is commonly associated with reptilase time prolongations. To avoid misdiagnosis of hereditary dysfibrinogenemia, it is suggested to measure fibrinogen levels on specimens that demonstrate prolonged reptilase times. If the fibrinogen level is elevated, repeated testing at a later date would seem indicated. In addition, adding calcium chloride or albumin might help distinguish acquired from hereditary prolongations. As acute phase reactions are common, occurring with illness and injury, an elevated fibrinogen level is a common cause of reptilase time prolongations.
References


