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Comparative evaluation of whole blood D-Dimer test to plasma D - Dimer test for diagnosis of disseminated intravascular coagulation

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Three rapid D – dimer test methods were compared for the diagnosis of acute disseminated intravascular coagulation (DIC). These were (a) SimpliRED, an autologous red cell agglutination assay. (b) DIMERTEST latex agglutination assay, containing monoclonal antibody DD – 3B6 / 22[6], and (c) D – DI latex agglutination assay containing mouse anti–human D-dimer monoclonal antibodies. The D – DI latex method having higher sensitivity (100%) and specificity (81%) in clinically acute DIC was postulated as the gold standard and compared with the other two methods. The results suggest that D - DI latex agglutination assay containing mouse anti–human D–Dimer monoclonal antibodies are the better assay methods amongst all the three kits analyzed. It is advisable to look for the nature of the antibody used to coat the latex particles in plasma based kits. In emergency setting RBC kits may be of some use as rapid diagnosis is advantageous.

Keywords: D - Dimer test; Disseminated intravascular coagulation; Monoclonal antibody.

The D-Dimer tests used in most diagnostic laboratories to screen disseminated intravascular coagulation (DIC) are rapid latex agglutination slide tests using monoclonal antibodies for the qualitative and semi - quantitative determination of D-Dimer in plasma. The determination of cross–linked fibrin degradation products (XL-FDP) has proved specific for fibrin breakdown, and is replacing the fibrin/fibrinogen degradation products (FDP) assay. Rapid methods in detection of FDP have included agglutination inhibition assays, using either tanned red blood cells[1] or latex particles coated with fibrinogen[2]. Staphylococcal clumping was an early system utilizing the characteristic clumping of some strains of staphylococci in presence of fibrinogen[3]. Improved agglutination assays based upon antibody coated latex particles have produced a rapid diagnostic system[4-6]. The qualitative tests and bedside assays for D-Dimer have not demonstrated to have adequate sensitivity to rule out life-threatening condition such as pulmonary embolism. For this condition, recently described quantitative latex turbidimetric D–Dimer test or the ELISA D-Dimer test can be accurately used to diagnose this life threatening condition[7-8]. However, all these methods require processing of the blood samples to produce plasma or serum.

A new system for the detection of XL-FDP in whole blood has been developed[9-10]. The utilization of endogenous red blood cells removes the need for extensive sample preparation producing a unique test, which is supposed to be rapid and simple.

In this study, the accuracy of whole blood to plasma D–Dimer tests has been compared for the diagnosis of DIC.

Materials and Methods

The subjects included patients suffering from clinically acute DIC with bleeding manifestations from at least three sites, attending the Laboratory of Haematology Department of the Institute.

All blood samples were collected into 3.2% trisodium citrate vials. An aliquot of the whole blood was tested immediately using the SimpliRED method (according to manufacturer's specifications). SimpliRED (Agen Biomedical Limited, Acacia Ridge, Australia), is an autologous red cell agglutination assay, using a chemical conjugate of a monoclonal antibody specific to D-Dimer linked to a monoclonal antibody which binds to the red cell surface[11]. The conjugate will coat the red blood cell (RBC) but will not cause agglutination in samples with levels of XL-FDP's below 0.12 mg/l. XL-FDP's present in a blood sample at levels > 0.12 mg/l, will

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bind to the conjugate on the red blood cells causing cross–linking between conjugate groups of adjacent cells which results in visible RBC agglutination. In absence of D-Dimers the conjugate attached to the RBC does not cause agglutination.

The D-Dimer test was performed on the platelet poor plasma (PPP) using two different kits: Dimer test latex kit (Agen Biomedical Limited, Acacia Ridge, Australia) and D-DI latex kit (Diagnostica Stago, France). DIMERTEST latex test is a qualitative latex agglutination assay. It uses a highly specific D–Dimer monoclonal antibody DD–3B6/22[6], whereas, D-DI latex agglutination test uses mouse anti–human D–Dimer monoclonal antibodies. In Dimer test kit reactive fibrinolysis is demonstrated by latex agglutination at a plasma concentration of $\geq$ 0.20 mg/l of XL-FDP. It is specific for XL-FDP's, D-Dimer, D-Dimer E, and high molecular weight derivatives, which are all recognized by its monoclonal antibodies. A test is interpreted as positive when D-Dimer levels are $\geq$ 0.5 μg/ml in D-DI test kit. Test samples containing D-Dimers when mixed with the latex particle suspension make the particle agglutinate, producing macroscopic clumps. To eliminate bias all D-Dimer tests were performed without knowledge of the other test results or the patients' clinical outcome.

Thirty two patients clinically suffering from acute DIC were tested with each of the three D-Dimer test kits. Seven age and sex matched controls were also tested with all the three test kit. 21 age and sex matched controls were tested by D-DI test kit. Each subject was classified as positive for D-Dimer, only if macroscopic visible agglutination was present.

Results and Discussion

The specificity and sensitivity of D-Dimer test in acute DIC, when done by the D-DI test kit, containing mouse anti-human D-Dimer monoclonal antibody, was 81 and 100%, respectively making this test kit suitable for screening in acute DIC[12]. Keeping this in view, the D-DI test kit, containing mouse anti–human D-dimer monoclonal antibodies, was taken as the gold standard, and compared with the two other kits to determine their role in diagnosing clinically acute DIC (Table 1).

The kit with DD - 3B6 / 22[6] monoclonal antibody (DIMERTEST kit) had less sensitivity and specificity in comparison with the kit containing mouse anti–human D-Dimer monoclonal antibody (D–DI latex kit) for diagnosis of DIC (Table 2). Although, its positive predictive value is high, its role as screening test kit in clinically acute DIC appears limited, due to its low sensitivity.

SimpliRED test kit, containing a chemical conjugate of a monoclonal antibody specific to D-Dimer linked to a monoclonal antibody which binds to red cell surface, had higher specificity and sensitivity in comparison to kit containing DD-3B6/22[6] monoclonal antibody (DIMERTEST kit) for diagnosis of DIC (Table 3). It also had a higher positive predictive value in comparison to kit containing monoclonal antibody DD-3B6/22[6] (DIMERTEST kit). However, its sensitivity was same as that of the gold standard, but had lower specificity

Table 1—Results of D-Dimer test by different kits

	D – DI test	DIMERTEST	Simpli RED
No. of patient	32	32	32
Positive test	27 (84%)	19 (59%)	22 (69%)
Negative test	05 (16%)	13 (41%)	10 (31%)

Table 2— Comparison of D-DI test kit with DIMERTEST kit

	D – DI test (n = 32)	DIMERTEST (n = 32)
Positive by both	18	18
Negative by both	03	03
Other positive cases	09	02
Other negative cases	02	09
Sensitivity percentage (95% C.I.)	66.7%	(46 – 82.8%)
Specificity percentage (95% C.I.)	60.0%	(17 – 92.7%)
Predictive value positive (95% C.I.)	90.0%	(66.9 – 98.2)
Negative value positive (95% C.I.)	25.0%	(6.7 – 57.2%)

Table 3—Comparison of DI test kit with SimpliRED test kit

	D – DI Test (n = 32)	SimpliRED Test (n = 32)
Positive by both	22	22
Negative by both	04	04
Other positive cases	05	01
Other negative cases	01	05
Sensitivity percentage (95% C.I.)	81.5%	(61.3 – 93%)
Specificity percentage (95% C.I.)	80.0%	(29.9 – 98.9%)
Predictive value positive (95% C.I.)	95.7%	(76.0 – 99.8%)
Predictive value negative (95% C.I.)	44.4%	(15.3 – 77.3%)

Table 4— Comparison of results of whole blood plasma D-dimer test

	Whole blood	Plasma
No. of patients	32	64
Positive tests	22 (69%)	46 (71.9%)
Negative tests	10 (31%)	18 (28.1%)

than that of D-DI test kit (gold standard). This kit may not be appropriate as a screening test kit for detection of D-Dimer. On the other hand some advantages of whole blood assay include absence of requirement of centrifuge or specialized equipment and, can therefore be ideal for emergency room analysis (Table 4), where rapid diagnosis is advantageous[8].

It is thus concluded, that before selecting a kit for D-dimer it is advisable to look for the nature of antibody used to coat the latex particles in the plasma based kits. However, in an emergency setting with virtually no centrifuge available, RBC kits may be of some use.

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