Lupus anticoagulant as a cause of prolonged activated partial thromboplastin time (APTT) in preoperative patients

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ABSTRACT

Objective: This study aims at finding the prevalence of lupus anticoagulant (LA) in a selected group of patients going for elective surgery in whom the activated partial thromboplastin time (APTT) was undertaken as part of routine preoperative coagulation screening. Recent years have witnessed enormous expansion in our knowledge of the coagulation inhibitor, especially as a cause of the prolonged (APTT). **Materials and methods:** The study population consists of 900 consecutive preoperative patients admitted to the surgical wards at King Khalid University Hospital, Riyadh, over a period from December 1995 to March 1996. Four hundred and seventy patients were females and 430 were males; their ages ranging from 1 to 69 years. The coagulation tests undertaken included: APTT, platelet neutralization procedure (PNP), kaolin clotting time (KCT), prothrombin time, plasma fibrinogen and clotting factors VIII, IX, and XII. **Conclusion:** The prevalence of LA was found to be 5.3% among patients as compared to 2.9% among controls (healthy blood donors n=205). Among the 48 patients who were LA positive there was significant reduction in the clotting factor levels (<25%), most prominent with FXII (15/48) and FXI (10/48). The results of this study have established the fact that LA is a common cause of prolonged APTT in routine coagulation laboratory. These results emphasize the importance of establishing the assay procedures for LA in major general hospitals and also attract the attention of laboratory physicians to be on the look out for it.

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Prolongation of the activated partial thromboplastin time (APTT) is a frequent finding in routine hematology/coagulation laboratories.[1-5] Until recent years the most common identifiable causes of such prolongation of the APTT were anticoagulant (heparin) therapy and deficiencies of coagulation factors, both of which are easily identified and accurately diagnosed. However, many causes remain unexplained[2] and are ascribed to unidentified inhibitors. Recent years have witnessed enormous expansion in our knowledge and realization of importance of the inhibitor lupus anticoagulant (LA) especially its association with recurrent fetal loss, venous and arterial thrombosis and thrombocytopenia.[6] Like many routine laboratories, we faced the problem of unexplained prolonged APTT, however, we took special interest in the results of the coagulation screening tests that are performed routinely before patients undergo any surgical procedure. In our hospital such screening is mandatory, due to the fact that many patients usually fail to give accurate medical family history of bleeding tendencies. The aim of this report is to work out the prevalence of lupus anticoagulant as a cause of prolonged APTT in the preoperative coagulation screening performed in the Hematology Laboratory in King Khalid University Hospital, Riyadh Saudi Arabia.

Materials and methods. ***Patients.*** The study population consists of 900 preoperative patients admitted to King Khalid University Hospital, Riyadh, Saudi Arabia, for elective surgery over a period of 4 months (December 1995 to march 1996). There were 470 females and 430 males; ages ranging from 1 to 69 years. None of these patients had a history of thromboembolic phenomena, autoimmune disease or bleeding disorder. These patients did not include patients for ENT or eye surgery, which were performed at another hospital (King Abdul Aziz University Hospital). Patients who had circumcision are included in this study.

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Table 1 - Results of the mixing studies using APTT; duration of the incubation time of mixing procedure was: zero hours (without incubation) and 1 and 2 hours incubation at 37°C

Without mixing		Mixing 0 hour		Mixing 1 hour		Mixing 2 hours	
APTT X ± SD (RANGE)	APTT C X ± SD (RANGE)	APTT X ± SD (RANGE)	APTT C X ± SD (RANGE)	APTT X ± SD (RANGE)	APTT C X ± SD (RANGE)	APTT X ± SD (RANGE)	APTT C X ± SD (RANGE)
50.0 ± 11.4 (28.2-87.8)	32.4 ± 3.2 (28.8-49.5)	50.7 ± 12.6 (35.7-87.8)	31.9 ± 1.5 (29.2-35.4)	58.8 ± 15.6 (41.6-100)	34.5 ± 1.6 (31.3-38.1)	65.4 ± 16.6 44.6-109.4	36.7 ± 3.0 (32.4-50)

Statistical analysis:

Group	Zero hour	One hour	Two hours
Patients	50.7 ± 12.6*	58.8 ± 15.6*	65.4 ± 16.6*
Control plasma (pooled normal)	31.9 ± 1.5*	34.5 ± 1.6*	36.7 ± 3.0*

*Within each group all pairwise mean comparisons were statistically significant at 5% level of significance after adjustment for three pairwise mean comparisons.

Controls. Control subjects were 205 blood donors who fulfilled the standard requirements for acceptance as blood donors. They were selected as normal controls on the basis of their routine medical check-up that guarantees the freedom from diseases. Their ages ranged from 17-55 years. It proved difficult to get normal controls from children between 1-16 years of age.

Collection of blood samples. Blood samples for lupus anticoagulant screening as well as coagulation tests, were collected in tri-sodium citrate (0.11M) in a blood citrate ratio of 9:1. Platelet poor plasma (PPP) was separated in the cold (4-8°C) according to the recently recommended double centrifugation method of Triplett et al[6] as follows: citrated blood was centrifuged at 1500 g for 15 min. A plastic transfer pipette was then used to remove the supernatant PPP which was placed in a second plastic tube and centrifuged under identical conditions. The resulting PPP was tested immediately.

Coagulation tests. The following coagulation tests were performed in the Hematology Laboratory at King Khalid University Hospital, Riyadh, using a fully automated coagulation machine (Hemolab, Biomerieux, France): *Prothrombin time* (PT) and the *activated partial thromboplastin time* (APTT) were performed using commercial reagents (Isimat Hemolab and Silimat Hemolab, respectively, Biomerieux, France), according to the manufacturer's instructions.

Mixing studies: were performed on the samples that showed prolongation of the APTT that could not be corrected by mixing with 1:1 ratio of patient and normal pooled plasma. The APTT was performed on (i) immediate mixing (zero hour) and after (ii) 1 and (iii) 2 hours incubation at 37°C. The clotting time was recorded in duplicate and the test was taken as positive if the patient's APTT was more than 2 standard deviations (SD) above the upper limit of the normal range and the mixing studies on those samples which show prolongation of the APTT could not be corrected by mixing with 1:1 ratio of patient plasma and normal pooled plasma.

Plasma fibrinogen: was performed by an automated method (Hemolab) using the reagent Hemolab Fibrinomat (Biomerieux, France) and according to the manufacturer's intructions.

Coagulation factors VIII: C, IX, XI and XII assays: were also performed on the Hemolab automated machine using the reagents Cofac VIII: C, IX, XI and XII and according to manufacturer's instructions.

Other lupus anticoagulant screening tests. Platelet neutralization procedure (PNP) (8) - (Staclot PNP reagent, Stago Diagnostica, France) with the APTT test and according to manufacturer instructions using a coagulometer (ST4, Stago, France). The shortening is registered as significant if the shortening of the APTT is ≥ 8 seconds after the PNP.

Kaolin clotting time (KCT): as described by Exner et al.[7] An index of 10% or more was taken as positive for the presence of lupus anticoagulant.

Results. The total number of the coagulation screening tests performed in the routine Hematology Laboratory at the King Khalid University Hospital, during the four month period of the study was 22396 tests, (average/day = 186 tests). Of these, the pre-operative coagulation screening tests were carried out on 900 patients. Lupus anticoagulant was confirmed in 48 samples out of the 900 samples taken from pre-

Table 2 - Coagulation factor levels in patients with lupus anticoagulant

Clotting factors	X ± SD (RANGE)
FVIII:C	75.5 ± 37.1 (5.9-175)
FIX	70.6 ± 36.6 (2-195)
FXI	42.2 ± 25.8 (4-117)
FXII	40.7 ± 26.1 (10-109)
Fibrinogen	3.1 ± 1.2 (1.37-6.19)

operative patients. This is based on two or more of the following tests being positive: APTT, PNP, KCT. All 48 samples showed abnormal APTT; 45 and 44 samples had abnormal KCT and PNP, respectively. The mean age of patients diagnosed positive for LA was 29.3 yrs (range: 1-69.; SD = 20.9) and the male; female ratio was 19:29, with a clear predominance of females. Twelve patients were children. The prevalence of LA in the pre-operative patient population 48, out of 900 i.e. 5.3%, compared to that seen in healthy blood donars (2.9%).

Mixing studies: (Table 1) It is clear that when the incubation time of the 1:1 mixture of patient and normal plasma was extended to one and two hours before undertaking the APTT test, there was a simultaneous and progressive significant increase in the APTT; the APTT of control plasma was affected, but to a lesser degree by prolonging the incubation time.

Coagulation factor levels in patients with lupus anticoagulant: (Table 2) factors VIII: C, IX, XI and XII were all affected by the presence of LA. If an arbitrary level of 25% was taken to reflect the severity of reduction of levels, it was found that the prevalence of such severity was most prominent with FXII (15/48) and FXI (10/48) and least with FIX (3/48) and FVIII (2/48).. The low levels of intrinsic coagulation factors found is an artefact, due to the presence of lupus inhibitors in the test system.

Discussion. The APTT test if influenced by a number of variables, not least those associated with sample collection and storage. However, one major source of variation inherent to procedure itself is related to the phospholipid/activator combination. The source, concentration and composition of the phospholipid component have major influences on the sensitivity of the reagent to a variety of abnormalities. A high phospholipid concentration is often associated with a poor response to lupus inhibitor, whereas differing constituent phospholipids, affect sensitivity.[6,8,16] Therefore, a low phospholipid content reagent was used in the current study to heighten the sensitivity of the APTT. More reliable results might be obtained by investigating a mixture of test and normal plasma at least initially.[17] This approach was followed in this study to establish the presence of LA in preoperative patients. It is well known that postoperative deep vein thrombosis (DVT) is the most frequent complication of the postoperative period,[18] and that LA is a risk factor for arterial and venous thrombosis.[9] In the present study the prevalence of LA in blood donors is 2.9% is confirmed by two or more tests which have been used for the detection of the presence of LA. No such previous study has been carried out in Saudi Arabia as far as we know. It was previously reported in literature that LA is seen in apparently healthy individuals and various clinical situations such as infections, rheumatic and collagen vascular diseases, disorders of the nervous system and eyes, obstetrical disorders, thrombotic diseases with medication, autoimmune diseases, dermatological disorders and many other miscellaneous conditions.[9] In children, LA is most often seen in those patients with tonsillitis or inflamed adenoids which have not responded to recent treatment with penicillin or penicillin derivatives.[19] In this study LA is the second identifiable cause of prolonged APTT in our laboratory, after heparin. The prevalence of LA in these preoperative patients (5.3%) is the most common cause of a prolonged APTT in preoperative patients. This figure, although higher than in healthy blood donors (2.9%), did not attain statistical significance. Since the interaction of anti-phospholipid antibodies with their respective antigens increases with time, mixing studies were undertaken at zero hour, after one hour and after two hours incubation of 50:50 mixture of normal and patient plasmas at 37°C. The results of such mixing studies in patients' plasma, as compared to the control plasma indicates clearly that the inhibition of coagulation increases with the increase in the incubation time and this is more obvious in patients than control plasma. It has been reported that coagulation factor levels could be affected by the presence of LA.[21] The results obtained in this study showed a most significant reduction in the levels of factor XII and factor XI. Factor IX was affected to a lesser extent and the least affected being factor VIII:C. The low levels of intrinsic coagulation factors found is an artefact due to the effect of the inhibitor (LA) on the test system, non parallel curves in the assays confirmed this. In this study, VIIIC clotting activity is normal or slightly reduced or even increased in a few patients. This is probably due to the fact that factor VIII is an acute phase reactant which is elevated in many of these patients and is hardly affected by the simultaneous presence of LA. In conclusion, the present survey has established the prevalence of LA in a community not explored before. It also draws attention to the importance of establishing screening tests for LA in major hospitals and also to laboratory physicians in Saudi Arabia to be on the look out for LA. This study also

emphasizes the importance of the presence of LA as a positive high risk factor for thrombosis and thromboembolism in preoperative patients and to alert surgeons to take preventive measures to avoid such complications in those patients.

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