BLOOD COAGULATION ABNORMALITIES ASSOCIATED WITH ENVENOMING BY *TRIMERESURUS ALBOLABRIS* IN HONG KONG

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ABSTRACT

Snake bites in Hong Kong are most commonly due to Tr. albolabris (White-lipped pit viper, bamboo snake). We studied 21 cases of envenoming by Tr. albolabris prospectively in order to document the incidence and severity of associated coagulation abnormalities. Eighteen patients (86%) had increased blood concentrations of fibrin degradation products (FDP) ranging from 10-40 μg/l to greater than 200 μg/l (normal: less than 10 μg/l), the majority of whom also had detectable soluble fibrin monomers. Among these 21 patients, 10 had decreased blood concentrations of fibrinogen ranging from 0.3 kg/l to 1.9 g/l (normal: 2-4 gl/l). In 11 cases (52%), the euglobulin clot lysis time was shortened (less than 150 minutes) in association with elevated blood concentrations of FDP (n=10) and decreased circulating fibrinogen levels (n=8). Thrombocytopenia and/or prolongation of prothrombin time (PT), activated partial thromboplastin time (APTT) and/or thrombin time (TT) were present in 10 patients (28%). Increased blood concentrations of FDP and decreased circulating fibrinogen levels were present in most of these cases.

Envenoming by Tr. albolabris is therefore frequently associated with a coagulopathy compatible with increased fibrin/fibrinogenolysis. Measurement of blood concentrations of FDP is the most sensitive test for detecting the coagulopathy. There is, however, little correlation between the patterns of clinical manifestations and coagulation abnormalities although more severe clinical features were usually associated with high circulating FDP levels. Only one patient developed systemic bleeding but no fatality was observed. The coagulation abnormalities are usually correctable by replacement therapy. Further studies are required to study the mechanisms of this coagulopathy and its relationship with venom antigenaemia.

Keywords: *Trimeresurus albolabris, white-lipped pit viper, coagulopathy, fibrin/fibrinogenolysis, snake envenoming.*

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INTRODUCTION

Tr. albolabris is one of the eight venomous land snakes indigenous to Hong Kong[1]. In a previous survey of 242 cases of snake bites in Hong Kong, we found that *Tr. albolabris* was responsible for the great majority. Envenoming by this species was associated with features suggestive of an underlying coagulopathy which occasionally gave rise to significant clinical morbidity and rarely fatality[2]. Details were, however, not always well documented and exact information regarding the incidence and severity of coagulation disorders could not be obtained. A prospective study has now been conducted to document in more detail the clinical course and haematological sequelae which follow envenoming by *Tr. albolabris*.

MATERIALS AND METHODS

The study was carried out between October 1988 and May 1989 at the Prince of Wales Hospital, Shatin. This is a regional hospital situated in the north-eastern New Territories in Hong Kong, a large area of which is rural or semi-rural and where the majority of cases of snake bite occur.

A total of 21 consecutive patients with a definite history of bite by *Tr. albolabris* (Fig 1) were studied. Identification details were checked personally by either one of the authors (JC, CC) at or soon after admission. Positive identification

Fig 1 - A Photograph showing the *Tr. albolabris* (white-lipped pit viper) reproduced with permission by the Urban Council, Hong Kong Government. Taken from Romer JD, "Illustrated guide to the venomous snake of Hong Kong." [1]

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was based on the examination of the dead snake brought to the hospital in 7 patients and on the basis of the patients' descriptions in 14 cases.

The age, sex, site of bite, clinical features and treatment were recorded for each patient. Complete blood picture, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen, fibrin degradation products (FDP), soluble fibrin monomer, euglobulin clot lysis time, renal and liver function tests and electrocardiograms were performed daily for three days or until normality was restored.

Complete blood counts were performed with a Technicon H6000 cell counter and the blood smear checked manually after Field staining. PT, APTT and TT were measured on citrated plasma using standard methods[3]. Blood concentrations of fibrinogen were determined by the Clauss dilute thrombin time method and that of FDP, assayed using a thrombo-Wellcotest kit (Wellcome Diagnostics). This is a semi-quantitative procedure for the detection of fibrin/fibrinogen degradation products. A suspension of latex particles in glycine saline buffer is coated with antisera to highly purified preparations of human fibrinogen fragments D and E. The sensitivity of the latex reagent is adjusted so that in the presence of blood concentrations of FDP greater than 2 µg/ml, the latex particles clump together giving macroscopic agglutination. The serum is diluted up to 1/100 concentration which gives an estimated blood concentrations of FDP greater than 200 µg/l as the upper limit of detection. Euglobulin clot lysis time was done by published methods with a reference range of over 150 minutes[4]. Complete lysis in less than 15 minutes denotes acute fibrinolysis, whereas lysis time between 15 to 30 minutes is associated with clear fibrinolysis. Soluble fibrin monomer complex was determined by using glutaraldehyde treated human erythrocytes coated with purified fibrin monomer (FS test, Diagnostica Stago).

RESULTS

Table I summarises the clinical details and results of clotting studies of the 21 patients studied. There were 6 males and 15 females aged between 28 and 78. In 12 patients, the bites were sustained in the upper limb while in 9, in the lower limb.

Fifteen patients (71%) had clinical features of envenoming in the form of severe local oedema. In addition, 7 of these patients developed lymphangitis and regional lymph node enlargement. One patient had skin bruising while another had gastrointestinal haemorrhage and haematuria necessitating replacement therapy with platelet rich plasma and fresh frozen plasma. No patient required specific antivenom.

In 18 of the 21 patients (86%), there were increased blood concentrations of FDP ranging from 10-40 µg/l to greater than 200 µg/l (normal: less than 10 µg/l). The majority of these patients (n=17) also had detectable soluble fibrin monomers. Among these 21 patients, 10 had decreased blood concentrations of fibrinogen ranging from 0.3 g/l to 1.9 g/l (normal: 2-4 gl/l). In 11 cases (52%), the euglobulin clot lysis time was shortened (less than 150 minutes) in association with elevated blood concentrations of FDP (n=10) and decreased circulating fibrinogen levels (n=8). In addition, 10 patients (28%) had thrombocytopenia and /or prolonged PT, APTT and/or TT. In these patients, this was usually associated with increased blood concentrations of FDP, reduced circulating fibrinogen levels and shortened euglobulin clot lysis time.

While 6 patients did not exhibit any physical sign of envenoming which include local oedema, lymphadenitis and skin bruising and systemic bleeding, 4 of these had some degree of clotting abnormality. On the other hand, all patients who had clinical features of envenoming had increased blood concentrations of FDP and detectable soluble fibrin monomers to varying degrees. Patients with more than one physical sign tended to have high blood concentrations of FDP and a proportion of these patients also had thrombocytopenia and/or prolongation of PT, APTT and/or TT.

No abnormality was detected in serial renal and liver function tests or electrocardiograms in any of the 21 patients.

Table I - Summary of clinical details and coagulation results in all 21 cases of *Tr. albolabris* (white-lipped pit viper) bites.

Case no	PT	APTT	Fibrinogen	FDP	Soluble fibrin monomer	Euglobulin lysis time	TT	Platelet count	Clinical features
1	N	N	N	10-40	-	N	N	N	Nil
2	N	N	N	N	-	N	N	N	Nil
3	N	N	N	40-200	+	N	N	N	Oedema
4	N	N	0.7	>200	+	120	18 5	117	Oedema
5	N	N	1.1	>200	+	95	N	133	Oedema
6	N	N	N	>200	+	N	N	N	Nil
7	N	N	N	N	-	N	N	N	Oedema/ lymphadenitis
8	16 4	N	0 7	40-200	+	N	N	115	Oedema
9	N	N	1 1	>200	+	30	N	116	Oedema
10	N	N	0 9	>200	+	90	N	120	Oedema
11	N	N	1 5	>200	+	90	N	N	Oedema/ lymphadenitis
12	N	N	N	40-200	+	N	N	N	Oedema/ lymphadenitis
13	15.7	49.1	N	10-40	+	N	N	N	Nil
14	N	N	N	N	+	120	N	N	Nil
15	N	N	1.6	40-200	+	120	N	N	Oedema/skin bruising
16	N	N	1.7	10-40	+	N	N	N	Nil
17	N	45.2	N	>200	+	90	N	N	Oedema/ lymphadenitis
18	N	N	N	10-40	+	N	N	N	Oedema
19	15	41.6	N	40-200	+	120	N	N	Oedema/ lymphadenitis
20	18.5	N	0.3	40-200	+	130	21 5	N	Oedema/ lymphadenitis
21	N	N	1.9	40-200	+	120	N	85	Oedema/ lymphadenitis and systemic bleeding
Normal range	12-14 sec	25-40 sec	2 4 g/l	<10µg/l		>150 min	12-18 sec	150-400 x 10^9/l	

N = normal +/- = present/absent

DISCUSSION

Tr. albolabris belongs to the *Viperinae* family and species from this family have been shown to cause coagulopathy. This study documents the incidence and severity of haematological disturbances associated with *Tr. albolabris* bites in Hong Kong. *Tr. albolabris* is commonly found in the rural areas of Hong Kong and South China and is responsible for the majority of snake bites in these areas[1,2].

Our study indicates a high incidence of local oedema and subclinical haematological abnormalities following bites by *Tr. albolabris*. The increased blood concentrations of FDP and detection of soluble fibrin monomers suggest increased fibrin/fibrinogenolysis. There are several possible mechanisms underlying the coagulopathy. Increased fibrinogenolytic activities due to fibrinogenases (α- and β-) have been reported in the venom of *Tr. mucrosquamatus* which can cleave fibrinopeptides a and b from fibrinogen resulting in circulating soluble fibrin monomers. In the presence of the activity of β-fibrinogenase, no cross-linking of the FDP occur so that the blood concentrations of cross-linked FDP may remain normal in the presence of elevated circulating FDP[5]. We were, however, unable to measure the blood concentration of cross-linked FDP at the time of the study to clarify this aspect.

On the other hand, thrombin-like activity leading to defibrination has been reported in the venom of snakes belonging to the families of *Crotalidae* and *Viperinae*. This causes conversion of fibrinogen to a soluble fibrin polymer, in contrast to the formation of an insoluble fibrin clot due to the activity of normal thrombin. In typical cases of *Crotalidae*

envenoming, complete defibrination with undetectable fibrinogen and massively raised circulating FDP usually occur[6]. Hence while our findings are compatible with a thrombin-fibrinogen interaction, such thrombin-like activity is probably only weak in view of the relatively normal fibrinogen levels in most cases. This may also explain the normal PT, APTT and/or TT results in approximately 75% of the cases since the fibrinogen levels are still high enough for clot formation to occur. In this regard, all cases with prolonged PT and APTT also have high circulating levels of FDP, low concentrations of fibrinogen and detectable soluble fibrin monomers suggestive of severe envenoming.

Furthermore, the shortened euglobulin clot lysis time detected in some of the patients with elevated blood concentrations of FDP may indicate the release of endogenous plasminogen activator resulting in enhanced fibrinolysis. This activity has also been previously reported in the venom of *Crotalidae* and *Viperinae* families[6].

In *vitro* studies on the other members of the *Trimeresurus* family have also reported similar fibrinolytic properties in species from the families *Tr. gramineus* and *flavoviridus*[7-9]. Four haemorrhagic proteinases have been identified in *Tr. mucrosquamatus* venom, all of which have fibrinolytic activities[10]. In addition, phospholipase A_2 purified from *Tr. mucrosquamatus* and *Tr. gramineus* can induce thromboxane B_2 formation with platelet aggregation[11,12]. In this respect, five patients in the study had significant thrombocytopenia in addition to increased fibrin/fibrinogenolysis.

Over 70% of the patients had some forms of local oedema. Histamine release from mast cells has been shown to be important in the formation of oedema induced by *Tr. mucrosquamatus* snake bites[13]. Numerous proteins including three phospholipase A_2 enzymes have been identified from the venom of *Tr. flavoviridus* which can induce oedema in mouse footpads[14]. Other cytotoxic factors from *Tr. flavoviridus* have been purified but their significance is not yet clear[15].

Prolonged defibrination and venom antigenaemia with moderate thrombocytopenia detected five days after envenoming by a Green pit viper (also *Tr. albolabris* but with different markings) has previously been reported[16]. Death due to cerebral haemorrhage following a Green pit viper bite occurred in another patient with increased fibrinolytic activity, defibrination and thrombocytopenia[17]. A recent review of 11 cases of envenoming by the Green pit viper in Thailand showed that all patients had local swelling and 9 developed non-clotting blood which responded to a single dose of monospecific antivenom. However, no spontaneous bleeding was noted and coagulation studies indicated a pure defibrination syndrome with little effects on other factors of the clotting cascade or platelet function[18].

In the present study, there was a high incidence of local oedema and to lesser extent, lymphadenitis, skin bruising and systemic bleeding following *Tr. albolabris* envenoming. Subclinical coagulopathy was frequently present and elevation of blood concentrations of FDP was the most sensitive test to detect the clotting abnormalities. This was often accompanied by detectable soluble fibrin monomer, shortened euglobulin clot lysis time and decreased circulating fibrinogen levels. The association between the patterns of clinical manifestations and the severity of clotting abnormalities was, however, less well defined. While patients exhibiting more than one physical sign of envenoming tended to have high blood concentrations of FDP, others who appeared to be clinically well or developed minimal clinical features following envenoming often had abnormal blood levels of FDP. Thrombocytopenia and/or prolongation of APTT, PT and/or TT occurred less commonly. However, when present, these usually indicated significant envenoming as exhibited by the more severe clinical features (ie more than one physical sign) and other clotting abnormalities. No patient, however, required administration of antivenom and the clotting abnormalities were easily correctable by replacement therapy. Although no fatality occurred in this series of patients, we had previously reported a case of intracerebral haemorrhage following envenoming by *Tr. albolabris* in an elderly woman[2].

In conclusion, our study indicates a high incidence of subclinical coagulopathy compatible with increased fibrin/fibrinogenolysis following envenoming by *Tr. albolabris*. Measurement of FDP remains the most sensitive test for detection of the abnormality although correlation with the clinical features is less well defined. Severe systemic bleeding rarely occurs and the coagulation abnormalities are usually correctable by replacement therapy. Further studies including measurements of blood concentrations of cross-linked FDP are required to clarify the underlying mechanisms for the coagulopathy and its correlation with venom antigenaemia.

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