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Analysis of Disturbances in Secondary Haemostasis in Patients with Tick Bite

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Abstract

Tick bites are common in various regions of Africa and Europe and serve as the main mode of transmission for tick-borne pathogen. Tick's saliva is composed of variety of bioactive molecules which possess anti-haemostatic and anti-inflammatory properties. These features of the constituent bioactive molecules are believed to facilitate the transmission of tick-borne pathogens. Case reports of 419 patients diagnosed with tick-borne encephalitis were assessed to determine the disturbances in secondary coagulation caused after tick bites. In this retrospective case series research study, we present the changes in secondary haemostasis (Coagulation by Extrinsic & Intrinsic Pathways) in patients with tick bite.

Keywords: Tick bites, Ixodes ticks, Dermacentor ticks, Tick saliva, Secondary haemostasis

Introduction

Ticks are blood-feeding ectoparasites and vectors of human pathogens, including agents of Lyme disease and tick-borne encephalitis virus (TBEV). Ixodes Ricinus is a species of European tick in the Ixodidae (hard tick) family found in many parts of the world, mainly in Northern Africa and the Middle East [1].

Tick bites on host's skin and disrupts the capillaries and regional tissue. This tick bite is counteracted by host's defence processes which include hemostasis and immunological responses [2-3]. Ticks bite with their hypostome and two chelicerae, on tick bite its saliva is introduced within the host's body; tick's saliva is rich in variety of bioactive molecules which serve various functions; one among these bioactive molecules are the family of protease inhibitors which are a fraction of tick's salivary composition are also introduced in the host's body [4]. These Protease Inhibitors can be divided into inhibitors of serine proteases and inhibitors of cysteine proteases [5]. Serpins (serine protease inhibitors) have been identified in many tick species such as I. Ricinus [6], Haemaphysalis longicornis [7], *I.* scapularis [8] and Rhipicephalus Appendiculatus [9].

Tick serpins have been shown to suppress the activities of blood clotting factors, compliments and pro-inflammatory cytokines in vitro [10-11]. Ixodes Ricinus serpin-3 (Iripin-3) were found to inhibit the Extrinsic Blood Coagulation Pathway In Vitro; and Ixodes ricinus serpin-8 (Iripin-8) inhibited the Intrinsic Blood Coagulation Pathway In Vitro [10,11]. Apart from that, Iripins are also found to inhibit complement cascade [10-11].

Phenomenon of Haemostasis is extremely complex and comprises of several well-regulated steps. A complex relationship exists between circulating platelets, coagulation proteins and fibrinolytic system to maintain the state of homeostasis and provide assistance to prevent blood loss. It is broadly categorised into Primary Haemostasis (platelet adhesion and platelet aggregation) and Secondary Haemostasis (coagulation). Platelet adhesion with the site of injured vascular endothelium leads to the formation of primary hemostatic plug [12,13]. Secondary hemostasis (coagulation) can be started from activation of an extrinsic and/or intrinsic pathway. Activation of the coagulation cascade is driven by serine proteases (coagulation factors) that leads to the production of a stable fibrin clot. [12,13]

Extrinsic Pathway - The tissue factor is present in sub-endothelial tissue, which gets exposed on injury to the vessel or on disruption of endothelium. This exposed tissue factor binds to factor VII (Proconvertin) and activates it. The activated factor VII (factor VIIa) further activates factor X (Stuart-Power factor) and factor IX (Christmas factor) via proteolysis and leads to their activation. Activated factor IX (factor IXa) binds with its cofactor – activated factor VIII (factor VIIIa), which leads to the activation of factor X (factor Xa). Factor Xa binds to activated factor V (factor Va) and calcium and generates a prothrombinase complex that cleaves the prothrombin into thrombin [14].

Intrinsic Pathway - There occurs conversion of factor XII (Hageman factor) to activated factor XII (factor XIIa). This activated factor XII (factor XIIa) leads to conversion of factor XI to activated factor XI (factor XIa). Factor XIa with activated factor VII (factor VIIa) and tissue factor converts factor IX to activated factor IX (factor IXa). The activated factor IX combines with activated factor VIII (factor VIIIa) and activates factor X with thrombin production [14].

Common Pathway- Activated factor X (factor Xa) binds with activated factor V (factor Va) and converts prothrombin to thrombin. The common pathway terminates the coagulation process through the activation of thrombin (factor IIa) and the cleavage of fibrinogen to fibrin, the primary component of the clot [13,14]. The coagulation factors involved in secondary hemostasis and complement cascade are serine proteases [11]; tick's saliva is rich in serine protease inhibitors (serpins) which can make a significant impact on the activity of coagulation and complement cascade. In our study we exclusively focused only upon the effects on coagulation cascade.

Belarus is a landlocked country in North-Eastern Europe with a population around 9.4 million, more than 70% of which reside in urban areas. About 40% of the country is covered by forest and has a relatively higher incidence of tick bites. The most common tick species in Belarus are Ixodes ricinus and Dermacentor reticulatus [15]. Here we present the Prevalence of Disturbances in Secondary Hemostasis in patients with Tick Bite.

Materials & methods

We conducted a retrospective analysis of 419 medical cases of patients discharged from the Grodno Regional

Infectious Diseases Hospital with a definitive diagnosis of infectious lesion of the central nervous system caused by tick bite between years 2012 through 2020 to assess the Prevalence of Disturbances in Secondary Hemostasis in patients with tick bite.

Statistical analysis was carried out using the "Statistics" package, v.10 and the Excel program.

The authors calculated descriptive statistics, as well as complication profiles, of the study participants and a total of 419 cases were assessed and sorted according to the inclusion and exclusion criteria.

Inclusion criteria

1. Positive Enzyme-Linked Immunoassay (ELISA) test for Tick borne disease.
2. Age group 18 to 80.
3. No previous history of Idiopathic/ pathological bleeding disorders.

Exclusion criteria, the patient was not considered eligible if he/she had

1. Any diseases that cause disturbance in coagulation cascade (Haemophilia disorders (2 patients), Systemic Lupus Erythematosus (SLE) (1 patient)).
2. On medications that alter activated Partial Thromboplastin Time (aPTT) or Prothrombin Time (PT)(18 patients).
3. Serious comorbidities that can influence on coagulation cascade (Liver failure (7 patients), Renal failure (4 patients)).
4. Cases that lacked definite diagnostic results (0 patients).
5. Cases were excluded if more than one infectious aetiology was identified. (0 patients)

Results

After thoroughly analysing all the cases, we excluded 32 patients on aforementioned basis, thereby leaving us with 387 cases to include in this study. Tick identification was not done but based on epidemiological data it was found that ticks belonged either to the species Ixodes Ricinus (59.1%) or Dermacentor reticulatus (40.9%) [16].

The mean age of these 387 patients was 50.3 years (range 18 - 79). 258 patients (~67%) were male and 129 patients (~33%) were females.

Serological analysis

On ELISA 344 patients (89%) showed positive IgM, 13 patients (3.5%) showed both IgG & IgM and 2 patients (%0.5) showed IgG.

Tick borne encephalitis virus (TBEV) infection has a biphasic nature. 296 patients (76.5%) were found to have typical biphasic course of TBE illness. The first phase is characterized by nonspecific signs and symptoms, mostly flu-like symptoms followed by an asymptomatic interval of about one week. The second phase occurs with symptoms of CNS involvement (i.e., meningitis, encephalitis, meningoencephalitis, myelitis, radiculitis). Meningitis and encephalitis were the most frequent clinical forms of TBE. 205 patients (53%) were diagnosed with Meningitis. Meningitis typically manifests with high fever, headache, nausea and vomiting; many patients have photophobia and vertigo. Meningeal signs were present in most of patients. 73 patients (19%) were diagnosed with Encephalitis. Encephalitis can manifest itself as altered consciousness ranging from somnolence to stupor and, in rare cases even as coma. 21 patients (5.5%) were diagnosed with Meningoencephalitis. 8 patients (2%) were diagnosed with abortive form of TBE (a febrile illness with headache but no meningitis (i.e., the initial phase of TBE not followed by the second, meningoencephalitis phase of the disease)). No patients were found to have any

signs and symptoms of Cranial Neuritis. A Reporting Bias can be taken into account as development of a chronic progressive form of the disease or any long-term neurological sequelae were not reported or the follow up did not continue. 83 patients (21.5%) were asymptomatic or only had mild clinical signs and symptoms. No fatalities were reported during the period of hospitalisation.

Coagulation test

Table 1: PT Changes	
Mean	19.25416667
Standard Error	0.783433061
Median	18.05
Mode	18.6
Standard Deviation	5.427783464
Sample Variance	29.46083333
Kurtosis	-0.66751602
Skewness	0.26566343
Range	14.6
Minimum	15.2
Maximum	29.8
Count	267
Confidence Level (95.0%)	1.576064029
Upper CI (95%)	20.8302307
Lower CI (95%)	17.67810264

The patients that showed abnormal values of PT, a PTT or both were taken into account.

Extrinsic pathway (Table 1) - 267 patients (69%), had abnormal Prothrombin Time (PT). A prolongation in PT was noticed with a mean time of 19.25 seconds (95% CI: 17.678 - 20.830) was noted and a standard deviation of +/- 5.427 seconds (11-15 seconds normal range). Maximum time noted was 29.8 seconds and minimum time being 15.2 seconds.

Table 2: aPTT Changes	
Mean	46.012
Standard Error	0.55513722
Median	46.9
Mode	47
Standard Deviation	2.775686101
Sample Variance	7.704433333
Kurtosis	0.332565969
Skewness	-0.716836162
Range	13.1
Minimum	41
Maximum	54.1
Count	143
Confidence Level (95.0%)	1.14574691
UPPER CI (95%)	47.15774691
LOWER CI (95%)	44.86625309

Intrinsic pathway (Table 2) - 143 patients (37%), had abnormal activated Partial Thromboplastin Time (aPTT). A prolongation in aPTT was noticed with a mean time of 46.012 seconds (95% CI: 44.866 - 47.157) and a standard deviation of +/- 2.775 seconds (25-40 seconds normal range). Maximum time noted was 54.1 seconds and minimum time being 41 seconds.

Extrinsic and Intrinsic Pathway both - 138 patients (35%) had abnormal value of both PT and aPTT. No data about common pathway, TT (Thrombin Time) was available in the cases, therefore these findings were not included in this study.

Complete Blood Count (CBC) CBC showed mild to moderate Leucocytosis. Mean value of Leucocytosis was 11 × 10*9/L with a Standard deviation of +/- 1.4 × 10*9/L. Changes in ESR (Erythrocyte Sedimentation Rate) and concentration of CRP (C-reactive proteins) were insignificant in most patients.

Cerebrospinal Fluid (CSF) Only the patients with the diagnosis of Tick-borne encephalitis virus were included in this study. Other tick-borne infections (Tick borne relapsing fever (TBRF), Lyme borreliosis or any other tick-borne spirochaete infections) were not included. Elevated protein levels in CSF with pleocytosis was found in 294 patients (76%).

We did not find any cases with haemorrhage, petechial-purpuric rash, bruising and bleeding from mucous membranes; the findings that are consistent with other tick-borne spirochaete infections [17].

Discussion

Tick bites are common in Eastern European regions; these ticks are found mainly in forests and incidents of tick bites peak during spring and summer months when people frequently visit places inhabited by ticks. Due to inadequate covering of extremities tick bites are common. Ticks latch on the exposed areas of skin using their hypostome and two chelicerae and cause mechanical disruption of the natural skin barrier. Mechanical trauma caused to the skin by tick bite causes activation of various host's defence mechanisms. Starting with the haemostatic mechanisms of vasoconstriction, platelet adhesion, platelet aggregation and blood coagulation to prevent blood loss [18]. Innate immunity is activated as well, causing inflammation with edema, inflammatory cell infiltration, and itching at tick feeding sites. Long-term feeding and/or repeated exposures of the host to ticks also activate adaptive immunity [19]. Therefore, these defence mechanisms make tick survival and reproduction unfavourable [20]. Thus, to surpass these defence mechanisms - ticks secrete large variety of bioactive molecules at the site of trauma [21-23]. These tick defence mechanisms make conditions more viable and hospitable for survival, apart from that many infections which are either part of tick's life cycle or use the ticks as reservoirs are also transmitted to the host causing secondary diseases. From our study and in vitro findings [10-11], we can presume that tick salivary proteins and serpins can modulate host defences with anti-haemostatic, anti-platelet, anti-inflammatory, and/or immunomodulatory properties [10,24-26]. Tick saliva is a mixture of various salivary proteins, many of which might share the same function. Although the concentration of serpins like Iripin-3 & Iripin-8 might be lower at the site of an actual tick bite but the presence of other proteins in the tick saliva might be enough the create the desired anti-haemostatic effects in the host [10, 30]. The anti-haemostatic property of Iripin-3 can involve a complex mechanism by various other pathways - Iripin-3 formed covalent complexes, typical for the serpin "suicide" mechanism of inhibition [27], with kallikrein, matriptase, thrombin, and trypsin, as shown in [10]. Moreover, no SDS- and heat-stable complex were found to be formed between Iripin-3 and FVIIa in the absence or presence of tissue factor, suggesting Iripin-3 probably does not reduce the proteolytic activity of FVIIa through the classic serpin inhibitory mechanism [10]. Since these salivary molecules can target haemostasis and many other aspects of the immune response, they might be useful in the development of novel pharmaceuticals. These molecules can play a significant role for the treatment of immune-mediated inflammatory diseases, hypercoagulable states, diseases associated with excessive complement activation, or even cancer [18, 28-29].

Conclusion

To conclude, tick bites can cause disturbances in Secondary Homeostasis as shown in our study. There

were significant statistical evidences to support the hypothesis. Our findings were consistent with previously reported in-vitro anti-Hemostasic effects of tick salivary proteins [10,11]. The concentration of tick saliva at which anti-hemostatic effects are produced were not measured in our study and requires further investigation. In our study we exclusively focused only on the disturbances of coagulation cascade. Although our study was not focused on the anti-platelet, anti-inflammatory, and/or immunomodulatory properties of the tick's saliva due to lack of data and requires further investigation.

Data availability statement

All data are either contained within the manuscript and supporting information or available from the corresponding author on reasonable request.

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