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KININOGEN LEVELS AND THE INFLAMMATORY RESPONSE IN CASES OF DENGUE

Bruno Piccolo Santana
Universidade de Ribeirão Preto- UNAERP
Ribeirão Preto-SP
http://lattes.cnpq.br/9871996496247902

Pedro Osaki da Fonseca
Universidade de Ribeirão Preto- UNAERP
Ribeirão Preto-SP
http://lattes.cnpq.br/6554203805365925

Renata Dellalibera-Joviliano
Universidade de Ribeirão Preto- UNAERP
Ribeirão Preto-SP
http://lattes.cnpq.br/2840815150459820

Reinaldo Bulgarelli Bestetti
Universidade de Ribeirão Preto- UNAERP
Ribeirão Preto-SP
http://lattes.cnpq.br/2617500897651333

Abstract: It is known that dengue is a disease of viral etiology (DENV), transmitted by the mosquito of the genus Aedes, and that causes disturbances in the homeostasis of the circulatory system. The pathophysiology of the disease can be explained by the development of an inflammatory process in the body, with the release of several inflammatory mediators, such as kinins, and these are formed from precursor proteins, the high and low molecular weight kininogens (CAPM and CBPM, respectively). The study aimed to quantify the levels of kininogen in the blood of patients diagnosed with dengue and its possible relationship with the pathophysiology of the disease, as well as the importance of future approaches on the subject, for a better understanding and management of individuals affected by the infection.
Keywords: Arboviruses; Dengue; Kininogens; cinines

INTRODUCTION

Arboviruses are caused by Arboviruses (Arthropod-borne virus), which are so designated because part of their replication cycle occurs in insects, and can be transmitted to humans and other animals-which function as reservoirs and amplifiers of the disease- by the bite of hematophagous arthropods. Of the more than 545 known arbovirus species, about 150 cause disease in humans. The arboviruses that cause disease in humans and 6 other warm-blooded animals are members of five viral families: Bunyaviridae, Togaviridae, Flaviviridae, Reoviridae, and Rhabdoviridae. Arboviruses have become important and constant threats in tropical regions due to rapid climate change, deforestation, population migration, disorderly occupation of urban areas, precarious sanitary conditions that favor viral amplification and transmission. These viruses tend to have a restricted geographic and climatic distribution, as part of a special ecological subsystem represented by viruses, vectors, enhancing hosts and reservoirs. Most of Brazil has a tropical climate, being a suitable place for the existence of the vector and, therefore, for the occurrence of arboviruses (Lopes, Nozawa, Linhares, 2014). In the Brazilian epidemiological context, the most circulating arboviruses are DENV, CHIKV and ZIKV, although there are others with potential for dissemination in the country (Donalisio, Freitas, Von Zuben, 2017). However, DENV, which causes dengue, are the most important flaviviruses in Brazil, and the first evidence of DENV occurred at the end of the 18th century. At the moment, the main transmission cycle of DENV involves only humans and mosquitoes in large tropical urban centers. The main vector is A. aegypti, but A. albopictus and A. polynesiensis are included as secondary vectors. Dengue predominantly affects tropical regions of Asia, Oceania, Australia, Africa and the Americas. Subtropical and temperate areas are susceptible to the introduction and spread of the virus in the summer season (Lopes, Nozawa, Linhares, 2014).

Dengue virus belongs to the Flaviviridae family and the Flavivirus genus, and is represented by four serotypes, namely DENV-1 to DENV-4. The existence of more than one serotype, DENV-1 and DENV-2, occurred around 1940, and DENV-3 and DENV-4 were first isolated during epidemics in the Philippines in 1956. Clinical Manifestations of Arboviruses in Humans they can range from undifferentiated, moderate or severe febrile illness (FD), skin rashes and arthralgia (RA), to neurological syndrome (SN) and hemorrhagic syndrome (HS). FD usually presents with flu-like symptoms, such as fever, headache, retro-orbital pain, and myalgia. NS can manifest as myelitis, meningitis and/or encephalitis, paralysis, paresis, seizures

and coordination problems. RA manifests as maculopapular rash or rash, polyarthralgia and polyarthritis, while HS is evidenced by petechiae, hemorrhage and shock combined with an intense reduction of platelets. The first infected cells after viral inoculation by mosquito bites are probably skin dendritic cells. After initial replication and migration to lymph nodes, viruses appear in the bloodstream (viremia) during the acute febrile phase, usually for three to five days. The genesis of dengue symptoms is still unclear, but it is considered that the release of cytokines, as a result of the infection of dendritic cells, macrophages and the activation of TCD4+ and TCD8+ lymphocytes, plays an important role. In addition, the release of interferon by T lymphocytes may be closely related to the drop-in platelet count, by suppressing bone marrow activity, which generates symptoms such as petechiae spread throughout the body. From the bloodstream, viruses are disseminated to organs such as the liver, spleen, lymph nodes, bone marrow, and may reach the lungs, heart and gastrointestinal tract (Lopes, Nozawa, Linhares, 2014).

Inflammatory mediators are released by the body during arbovirus infection, one of the main mediators being kinins. Kinins are oligopeptides synthesized in plasma and/or interstitial fluid from high molecular weight proteins, and are involved in a series of biological events, including vasodilation, increased vascular permeability, pain modulation, smooth muscle contraction/relaxation and effects on cell proliferation (Ramalho, 2000). In addition, kinins influence the multiplication of the immune system (macrophages, dendritic cells and T and B lymphocytes), modulating their activation, proliferation and migration (Joviliano, Reis, Donadi, 2010). These peptides are part of the Kallikrein-Kinin System (KSK), which is composed of prokallikreins, kallikreins (tissue and plasma, which are serine proteases), kininogens, kinins, kininases and kinin-converting enzymes (Morais et al., 1999). Kininogens are multifunctional glycoproteins produced mainly by the liver, brain, kidneys, neutrophils and platelets, acting as intrinsic cofactors of the coagulation system. Plasma prekallikreins are mainly produced by the liver, being converted into kallikrein through negative charges on endothelial surfaces and by cysteine proteases present on the endothelial membrane. Tissue prokallikreins are synthesized in the salivary glands, kidneys, brain, pancreas and neutrophils, but the mechanism involved in the conversion of these zymogens is not yet known. Kininases I, known as carboxypeptidases M and N, remove the Arg residue from both kinins, forming Des-Arg-Bradykinin or Des-Arg-Lys-Bradykinin. Kininase II, also known as angiotensin-converting enzyme (ACE), inactivates Bradykinin and Lys-Bradykinin (Joviliano, Joviliano, Évora, 2010).

Plasma kallikrein specifically hydrolyzes high molecular weight kininogen, releasing Bradykinin, while tissue kallikrein releases Lys-Bradykinin from low molecular weight kininogen. Both Bradykinin and Lys-Bradykinin are agonists of the B2 receptor, which is expressed in several cell types and is responsible for mediating most of the physiological effects of kinins. Both peptides will be rapidly metabolized by kininase II, producing inactive peptides. However, by the action of kininase I, there is the formation of the metabolites Des-Arg-Bradykinin or Des-Arg-Lys-Bradykinia, which are specific agonists of the B1 receptor, which is expressed only in pathological situations in response to an intense stimulus. inflammation (Joviliano, Évora, 2010). During the response to the establishment of the infection, the inflammatory mediators initially produced would collaborate to activate cell populations

of the innate response, promoting antiviral and cytotoxic actions, for example. At first, this effector action would collaborate to resolve viremia and reestablish homeostasis. However, the exacerbation of the production of these inflammatory mediators can have an effect on the activation of the endothelium in a systemic way, culminating in plasma leakage, as observed in dengue (Srikiatkhachorn, Spiropoulou, 2014). The performance of cytokines in the immunopathogenesis of DENV is extremely important, since the response process starts from the high release of cytokines derived from the interaction of innate cells with activated T lymphocytes (Mesquita et al., 2010). Therefore, the participation of the kallikrein-kinin system as inflammatory mediators released in cases of arbovirosis and its participation in the pathological clinical picture of the disease is suggested.

This study will aim to understand the relationship between the SCC and the inflammatory response in dengue viral infection, as well as the participation of these inflammatory mediators in arbovirus infection. Furthermore, this study aims to measure the levels of kininogens (high and low molecular weight) in arbovirus infection, and to relate possible changes in these levels. The relevance of this research lies in the fact that the diseases caused by this family of viruses are of great importance in public health, due to a series of factors, ranging from the diversity of infectious agents involved and the plurality of clinical manifestations, to the lack of efficient laboratory support, the absence of immunoprophylactic measures for most current infections, and the difficulty in implementing and maintaining educational and sanitary measures. Allied to these difficulties and inherent to viral infections is the lack of specific therapy, relegating the treatment of arboviroses to the symptomatic control of clinical manifestations, which can unleash serious conditions, with neurological, articular, and hemorrhagic involvement.

METHODS AND PROCEDURES

In terms of materials and methods, the analysis of plasma samples collected with 3.8% sodium citrate was carried out in the following samples: control volunteers (n=4), without comorbidity, mean age of 42 years (G1); 4 patients with DENV, mean age 49 years (G2), confirmed by real-time RT-PCR for detection of viral RNA. The concentrations of the fractions that will be determined in plasma previously incubated (30 min) with kaolinite (1.5 mg/ml) for determination of low molecular weight kininogen, and with plasma and CSF without kaolinite treatment for total determination of kininogen. Treated and untreated samples will be subjected to acid denaturation and hydrolysis by trypsin. The immunoreactivity of the released kinin will be measured by the ELISA method using an antibody against bradykinin. The concentration of high molecular weight kininogen will be indirectly measured by the difference between the values of total kininogen and low molecular weight kininogen. The results will be expressed in units/mL of plasma. Nonparametric test was used for statistical evaluation ($p<0.001$).

RESULTS

The results were expressed as an average and the findings obtained were submitted to a non-parametric Spearman test. The average of the results of the analyzed groups (G1 and G2) for CAPM was 0.9 (G1) and 0.43 (G2). The average obtained for the CBPM was equivalent to: 1.2115 (G1) and 0.7875 (G2).

CONCLUSIONS

Considering that viral stimuli promote activation of the inflammatory response, our

data suggest that arboviruses, particularly the dengue virus, are capable of activating the kinin system, since there was consumption of the precursor proteins of this system. New approaches on the subject are important in order to seek diagnostic and therapeutic alternatives based on the pathophysiology of the Kallikrein-Kinin System and its repercussions in the body, offering a better prognosis for dengue virus infection.

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