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JUNGLE YELLOW FEVER: CLINICAL AND LABORATORIAL STUDIES EMPHASIZING VIREMIA ON A HUMAN CASE

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SUMMARY

The authors report the clinical, laboratorial and epidemiological aspects of a human case of jungle yellow fever. The patient suffered from fever, chills, sweating, headaches, backaches, myalgia, epigastric pains, nausea, vomiting, diarrhea and prostration. He was unvaccinated and had been working in areas where cases of jungle yellow fever had been confirmed. Investigations concerning the yellow fever virus were performed. Blood samples were collected on several days in the course of the illness. Three of these samples (those obtained on days 5, 7 and 10) were inoculated into suckling mice in attempt to isolate virus and to titrate the viremia level. Serological surveys were carried out by using the IgM Antibodies Capture Enzyme Linked Immunosorbent Assay (MAC-ELISA), Complement Fixation (CF), Hemagglutination Inhibition (HI) and Neutralization (N) tests. The yellow fever virus, recovered from the two first samples and the virus titration, showed high level of viremia. After that, specific antibodies appeared in all samples. The interval between the end of the viremia and the appearance of the antibodies was associated with the worsening of clinical symptoms, including bleeding of the mucous membrane. One must be aware of the risk of having a urban epidemics in areas where *Aedes aegypti* is found in high infestation indexes.

KEYWORDS: Arbovirus; Jungle yellow fever; Viremia; Clinical and laboratorial diagnosis.

INTRODUCTION

Yellow Fever (YF) is an arthropod-borne viral disease that is endemic in extensive areas of Brazilian territory. Outbreaks occur periodically in Northern, Central, Western and parts of Northeastern and Southeastern regions[3,10,16], mainly when human population enters into forested areas or when they live in dwellings inside these areas. Such situations usually expose these people to the attack of infected mosquitoes. It is important to emphasize that YF cases have also been detected in areas infested with *Aedes aegypti*, the vector of urban YF. Besides, the overlapping of endemic zones with areas infested with the urban vector increases the risk of outbreaks[14].

Clinical manifestations in human beings range from inapparent infections to mild, moderate, severe and malignant forms, causing disordered renal function, hepatic injury and high lethality. About 90% of the cases are constituted of mild forms, with high survival rate. However, most of the diagnosed cases in Brazil are those of severe pattern. Because of its typical clinical symptoms, the specific laboratorial surveys are directed towards the YF diagnosis confirmation. The mild forms of the disease are hardly ever diagnosed during nonepidemic periods. The YF virus is readily isolated from serum obtained during the first 4 days of illness[4], but it may be recovered from serum up to the 12th[1], 14 th[8] and 17 th[7] days. No studies on viremic period in natural infections have been reported. SPENCE[13] refers to a titer of $10^{2.9}$ LD_{50} from a single sample, obtained during the acute phase of disease.

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In addition to the classical techniques applied to the serological surveys of arboviruses, the MAC-ELISA have also been used successfully. This technique enables the early detection of specific antibodies and fast presumptive diagnosis of the disease.

Studies on epidemic periods or sporadic occurrence of YF cases are very important for epidemiological surveillance activities, specially in urban areas with *Aedes aegypti* high infestation indexes.

The purpose of this paper is to present a human case of jungle yellow fever, its clinical manifestations, laboratorial findings, emphasizing viremia. The prompt diagnosis is a decisive factor to lead off efficient and rapid measures of epidemiological vigilance.

CASE REPORT

On April, 3rd 1992, a 24-year-old white male was hospitalized at the Instituto de Infectologia Emílio Ribas, with YF as admitted diagnosis. He was unvaccinated against YF.

Two weeks before the onset of the symptoms he had been working in deforestation activities in Ribas do Rio Pardo, Mato Grosso do Sul State, prior to returning to Castilho, São Paulo State, where he lived. The occurrence of 4 cases of jungle YF had been reported[9] in the same period, in Ribas do Rio Pardo, where the infection of the patient had probably occurred.

The hospitalization was on 7th day after the onset of the disease and the patient complained of 39°C fever, headaches, chills, sweating and lumbosacral pains. He reported epigastric pains, nausea, vomiting, diarrhea and prostration on the 5th day of illness.

Physical examination revealed a regular condition, although presenting a certain degree of jaundice and painful hepatomegaly two fingers-width below the right coasted margin.

During the disease there was an aggravation of prostration, jaundice, vomiting and diarrhea. It was observed bleeding of gums from day 9 to 11 of illness.

The critical condition changed on the 13th day; the symptoms remitted gradually and the patient was discharged on April 22nd, 26 days after the onset of the illness.

LABORATORY RESULTS

Clinical and pathological exams brought about during the hospitalization showed the following normal values: creatinine 1.0 mg/dl; urea 25 mg/dl; glycemia 77 mg/dl; direct bilirubin 0.4 mg%; total bilirubin 0.9 mg%; alanine aminotransferase - ALT=SGPT - 3.036 u/l (5 - 35 u/l); aspartate aminotransferase-AST=SGTO - 3.600 u/l (8 - 40 u/l); creatinophosphoquinase 3.199 u/l (24 - 170 u/l); lactic dehydrogenase 4.688 u/l (150 - 360 u/l); blood: haemoglobin 15.3%; hematocrit 44%; platelets 212.000 mm^3. Blood indicated leucopenia 2.200 mm^3 (5.000 - 9.000 mm^3); bands strongely elevated 18% (2 - 5%); segmented slightly lowered 57% (58 - 66%) and lymphocytes 21% (24 - 30%); monocytes 4%; prothrombin time 24 s (12s) with 25% (100%) of activity which had been disturbed until 17th day of illness; active partial thromboplastin time 85 s (28 - 45 s); urine: normal values to pH and density; proteins: ++++ (absent); quantitative urinary sediment revealed desquamation of epithelials cells, erythrocytes and leukocytes with normal values.

VIROLOGICAL DIAGNOSIS

The blood samples collected on days 5, 7 and 10 of the illness were processed in order to isolate virus. The blood was diluted 1:10 with bovine albumin fraction V (0.75%) containing 100 units penicillin/ml and 100 ug streptomycin. This suspension was inoculated by intracerebral route into suckling mice, immediately after its arrival at the laboratory. The first sample (5th day) was sent by the Centro de Saúde de Castilho (SP), where the patient was first attended and the serum was inoculated in C6/36 cell culture. The other two samples were taken after his admission at the Instituto de Infectologia Emílio Ribas (IIER).

YF virus was isolated from the first two samples. Aliquots of serum and blood were kept in a mechanical freezer at -20°C and -70°C for ninety days, until they were submitted to virus titration, according to the technique described by SHOPE & SATHER [12].

Table 1 shows the virus titers during the viremia course and the difference between titers observed among samples which were stored at different temperatures. Significant differences were detected among specimens collected on days 5 and 7, about $10^{1.2}$ and $10^{1.4}$ LD_{50}, respectively higher in those aliquots stored at -70°C.

Definitive identification of the virus SPH-144990 isolated from the patient was performed using N tests in mice and immunofluorescence (IF) in C6/36 cells, using

TABLE 1
Results of neutralization tests in mice, with human acute and convalescent sera and blood against yellow fever antigen

Date of serum collection	Days after the beginning of the symptoms	Titer to YF*		
		serum		blood
		- 20°C	-70°C	- 70°C
04.01.92	5	3.9	5.1	4.6
04.03.92	7	1.3	nt	2.7
04.06.92	10	-	nt	nt

*log neutralization index
nt = not tested
- = <1.0

monoclonal antibodies. The results showed a close antigenic relationship between SPH-144990 and the reference strain of jungle yellow fever of Brazil: BeH-111; the serologic results also showed that the strongest cross-reaction occurred with Ilheus (ILH), St. Louis encephalitis (SLE) and Rocio (ROC) viruses which constitute a distinct serologic sub-group within the Flaviviruses.

TABLE 2

Results of serological tests of human sera according to the techniques and days after the onset of the symptoms, against YF antigen

Days after the onset of symptoms	MAC-ELISA	HI	CF	N
5	- *	-	-	-
7	10	-	-	-
10	2.600	80	-	-
13	5.120	80	8	2.26
14	10.240	80	8	2.31
26	20.480	320	256	4.41

*for MAC-ELISA < 10; HI < 20; CF< 8 and N < 2.

SEROLOGICAL DIAGNOSIS

Serological survey aiming at the detection of antibodies was performed by using sera samples obtained from the 5th, 7th, 10th, 13th, 14th and 26th days after the onset of the symptoms. Classical tests in arbovirology, i.e. HI[2,11], CF[5], N in suckling mice[12] and MAC-ELISA[6] were applied. The seroconversion is shown on Table 2. The rise in antibodies titers to the other flaviviruses is shown on Table 3.

One notices the persistence of the fever for two weeks, comprising the viremic and pos-viremic phases. The most critical period was observed from the end of the viremia until the appearance of the circulating antibodies. This period lasted from the 9th to the 11th day after the beginning of the symptoms. This was characterized by important haemorrhagic manisfestations and prolonged prothrombin times (from the 7th to 17th days).

THERAPY

The patient was treated in the Intensive Care Unit for three days (admitted on April 4th, 1992 - 8th day of illness), and then he was transfered to the infirmary where he stayed until his discharge on the 19th day after hospitalization.

Since there is no specific treatment for YF disease, general supportive and symptomatic treatment must be provided: analgesics for myalgia and headaches, intravenous fluids for dehydration and blood transfusion if there is haemorrhage, as well as nursing care. The patient was given intravenous fluids, fresh frozen plasma, vitamin K and cimetidin.

DISCUSSION

According to the classification recommended by WHO[17] for grade severity of YF clinical manifestations, the reported case should be classified as moderately severe. Most of the YF cases present virus particles in the circulating blood until the 4th day of illness. Thus, detecting viruses after this period may suggest an unfavorable prognostic. On 10th day of the disease the virus was absent. According to the results of the quantitative assays, one deduces that this had been the final period of the viremia.

TABLE 3

Results of hemagglutination inhibition (HI) and complement fixation (CF) tests of human acute and convalescent sera against Flaviviruses antigens.

Days after onset	HI titer to:						CF titer to:						
	IGP	ROC	SLE	ILH	DEN-1	DEN-2	IGP	ROC	SLE	ILH	DEN-1	DEN-2	DEN-4
5	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	8	8	-	-	-	-
14	-	-	-	40	-	-	-	8	8	-	-	-	-
26	160	20	40	160	20	40	-	32	32	16	32	16	32

- : for HI, <10
- : for CF, <8

CLINICAL-LABORATORIAL RELATIONSHIP

Figure 1 shows the time course of the main clinical features associated with the laboratorial findings.

The haemorrhagic manifestations took place in the borderline period between the end of the viremia phase

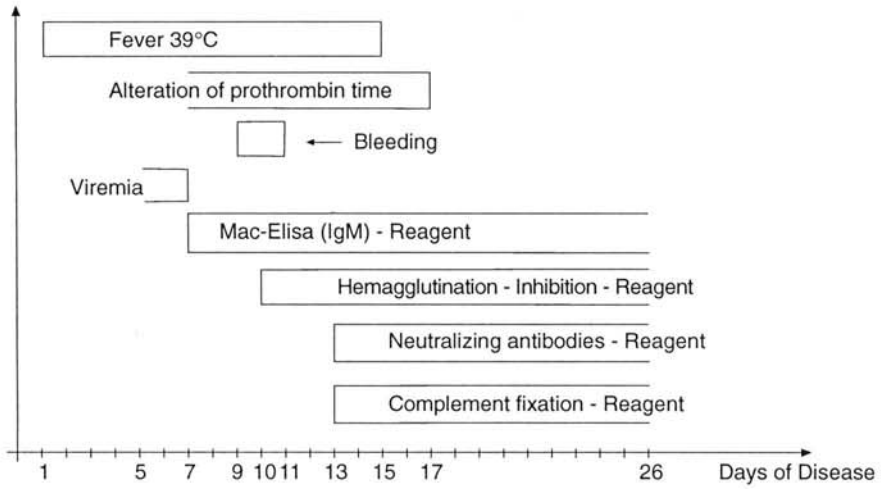

Fig. 1 - Distribution of clinical and laboratorial alteration in a human case of Yellow Fever according to the time of disease.

and the occurrence of measurable antibodies. It is suggested that the injury caused by the virus, followed by immune response, may have intensified the clinical symptoms. These symptoms included haemorrhagic manifestations, at the moment of the interaction antigen-antibody. It is also admitted that such haemorrhagic manifestation may be a corollary of the synthesis decrease of coagulation factors.

Probably due to the immediate medical care which provided the patient with all the therapeutic measures indicated for these cases, the evolution to a malignant form of the disease was avoided. The clinical and pathological data obtained from laboratorial tests were similar to those attributed to the cases of YF. The immunological profile of the patient was typical of primary infections, which showed seroconversion to the other Flaviviruses that only occurred on the 26th day.

In Brazil, YF virus has been frequently isolated from human blood during epidemics periods. Thus, viremia study improves the comprehension of the pathological disorders inherent to YF disease. High viral titer was observed on the 5th day of illness.

Noteworthy were the differences of $10^{1.2}$ and $10^{1.4}$ LD_{50} higher in the samples which had been kept at -70°C. It confirms the assertion that specimens taken for virus isolation must be processed as soon as possible by the laboratory. The storage must be done at low temperatures, in order to preserve the low-titered viral samples.

The seroconversion detected by using different techniques shows their efficacy and confirms the applicability of MAC-ELISA for fast detection of early antibodies due to its higher specificity and sensitivity.This test allows a presumptive diagnosis of a recent infection by using a single serum sample. It is worthy to note that in many cases, obtaining other samples can be very difficult. Besides, the MAC-ELISA can be performed in a short period of time.

Related to the epidemiological surveillance, this study shows clearly the risk that a single case can represent to the public health. Despite living in São Paulo State, the patient had been in Mato Grosso do Sul State, where cases of jungle YF are frequently notified. He could serve as an infection source for transmission cycles, starting therefore, an extensive epidemics, since *Aedes aegypti* is widespread in hundreds of counties, with high infestation indexes[15].

RESUMO

Febre amarela silvestre: estudo clínico e laboratorial, enfatizando a viremia, de um caso humano

Os autores estudaram um caso humano de febre amarela silvestre, sob os aspectos clínico, laboratorial e epidemiológico. O paciente apresentava febre (39°C), calafrios, sudorese, cefaléia, dor lombar, mialgia, dor abdominal em epigástrio, náuseas, vômitos, diarréia e prostração. Relatava permanência em área onde foram constatados casos de febre amarela silvestre e não havia histórico de vacinação anterior.

Frente às suspeitas que levaram à investigação do vírus da febre amarela, foram colhidas várias amostras de sangue no curso da doença. As amostras do 5º, 7º e 10º dias foram submetidas a provas de isolamento e quantificação do vírus, o que possibilitou o estudo da viremia. Empregando-se os testes de MAC-ELISA (detecção de IgM), Fixação de Complemento (FC), Inibição de Hemaglutinação (IH) e teste de Neutralização

(N), foi observada a resposta imune para anticorpos específicos nas amostras do 7º ao 26º dias.

Os resultados mostraram que no 5º e 7º dias havia persistência da fase virêmica, com títulos elevados. Ao término desta fase, com o aparecimento de anticorpos específicos, foi observado um agravamento do quadro clínico, com sangramento de mucosas.

Os autores alertam para a possibilidade de ocorrerem epidemias urbanas em áreas com alta infestação de *Aedes aegypti.*

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