

DETECTION OF INTRATHECAL IgM, IgG AND IgA IN A CUBAN WEST NILE VIRUS CONFIRMED CASE

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West Nile virus (WNV) is a flavivirus grouped in Japanese encephalitis virus serogroup due to serological antigenic characteristics. WNV is transmitted to humans through the bite of infected mosquitoes which acquire the virus after feeding on vertebrate amplifying hosts, mainly birds. WNV is endemic in great parts of Africa and it has been reported in Europe, the Middle East, Africa, India, parts of Asia, Australia (Kunjin virus, a subtype of WNV). In 1999, the virus was detected in the Western Hemisphere for the first time in New York City. After that, WNV has spread considerably throughout Latin America and the Caribbean^{1,2}.

Approximately 80% of WNV-infected patients are asymptomatic, 20% develop West Nile fever (WNF), and less than 1% develops West Nile neuroinvasive disease (WNND)³.

WNV central nervous system (CNS) disease occurs frequently in immunocompromised persons and the elderly¹. The specific mechanism by which WNV enters the CNS is unknown, but it is suggested from experimental WNV infection results high levels of inflammatory cytokines including tumour necrosis factor which can alter blood-brain barrier (BBB) permeability⁴.

CASE

We report the detection of intrathecal IgM, IgA and IgG synthesis in Cuban West Nile confirmed case to assess the possible dysfunction of blood cerebrospinal fluid (CSF) barrier using Reiber's formula.

The albumin and IgG concentrations were determined in serum and CSF by immunodiffusion method by NOR and LC Partigen immunoplates (Dade Behring, Marburg, Germany) in a Cuban confirmed case during WNV Surveillance in Cuba 2003. The albumin quotient ($Alb Q = \text{CSF albumin/serum albumin}$) was used to evaluate the integrity of the blood-CSF barrier using previously described protocols^{5,6}.

The presence of intrathecal synthesis of IgM, IgG and IgA was demonstrated by using Reiber's graph or reibergram⁵⁻⁷.

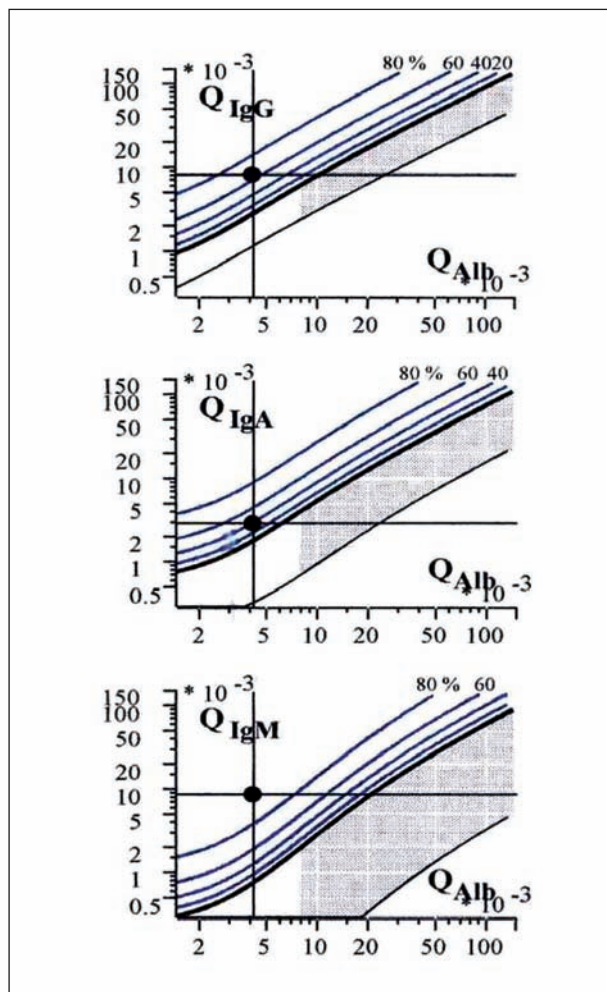


Figure. Reibergram from the WNV confirmed case. CSF/serum quotient diagrams for IgG, IgA, and IgM (Reibergrams). The upper hyperbolic curves (thick lines) represent the discrimination lines between brain-derived and blood-derived immunoglobulin fractions. Values above these upper discrimination lines represent intrathecal IgG, IgA, or IgM synthesis. The blue lines indicate the extent of intrathecal synthesis as intrathecal fractions (IgGIF, IgAIF, or IgMIF) with 20%, 40%, 60%, and 80% of the measured total immunoglobulin concentration in CSF, with reference to the discrimination line as 0% intrathecal synthesis.

DETECCIÓN INTRATECAL DE IgM, IgG E IgA EN UN CASO CONFIRMADO DE INFECCIÓN POR VIRUS DEL NILO OCCIDENTAL EN CUBA

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Received 18 September 2007, received in final form 6 June 2008. Accepted 11 June 2008.

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Table. WNV Antibody index.

Qspecific ^a	QAlbumin ^b	QIgG ^c	QLim ^d	WNV Antibody Index ^e
35.6 X 10 ⁻³	4.81 X 10 ⁻³	8 X 10 ⁻³	2.74 X 10 ⁻³	13.02

^aCSF WNV specific antibodies/Serum WNV specific antibodies quotient; ^bCSF Albumin/Serum Albumin quotient. Qalb is a marker of blood-CSF barrier; ^cCSF IgG/ CSF serum quotient; ^dQ IgG_{lim} = 0,93 √[(Q alb)² + 6 x 10⁻⁶] - 1,7 x 10⁻³; ^eIA=Qspec/QIgG.

WNV specific antibodies were calculated by using antibody index according to previous formulae^{7,8}.

The patient was identified as part of WNV Cuban surveillance in 2003 and exhibited febrile illness, intense and frontal cephalas, muscle weakness, encephalitis, myalgias, arthralgias and was hospitalized. This person had different jobs that required spending large amounts of time outdoors. This patient was localized in a community in central part of the Island⁹. The ethical approval of the study was granted according to the principles of the Declaration of Helsinki.

Both acute-phase and convalescent phase serum specimens from this patient was positive for flavivirus antibody by IgM and IgG ELISAs. CSF was also positive for IgM by WNV ELISA⁹.

The patient exhibited three class patterns of immunoglobulins intrathecal synthesis at the time of the lumbar puncture. It is important to point out that it was not the first diagnostic lumbar puncture performed on this patient. The sample was taken during the acute phase of the central nervous system infection.

At the Figure IgA, IgM and IgG intrathecal synthesis was observed by Reiber's graph or reibergramas. Notice that the plotted point are over the darkest hyperbolic line that indicates the limit between brain-derived fraction from the blood-derived fraction.

Table shows in details the figures of Qalb, QIgQ and Q lim and the Antibody index for WNV

DISCUSSION

WNV disease in the central nervous system occurs frequently in elderly and in immunocompromised individuals. The specific mechanism by which WNV enters the CNS is unknown, but experimental WNV infection results in elevated levels of inflammatory cytokines including tumor necrosis factor- α which can alter blood-brain barrier permeability^{3,4}.

Intrathecal Igs synthesis during a variety of CNS diseases, principally inflammatory diseases is originated by perivascular infiltrations from B lymphocytes locally matured which can be associated to CNS diseases. The relation among intrathecal fractions of one or more Ig classes or the predominance of one of them, have create a typical intrathecal synthesis pattern of some neurological diseases produced by bacteria, viruses and parasites among others^{8,10}.

Detection of WNV IgM in CSF is one of most impor-

tant criteria and is a diagnostic of neuroinvasive disease. In sera WNV IgM antibody persists for at least 6 months, and sometimes for 12–16 months in previously infected patients, however IgM antibody in CSF is detectable up to 7 months after illness¹¹. Other authors report the value of IgM and also IgA as indicator of CNS involvement following WNV infection¹².

The presence of intrathecal synthesis of three immunoglobulin classes is a clear evidence of an inflammatory process at CNS.

In our result is very interesting that the intrathecal fractions are present in decreasing order IgM>IgG>IgA.

There is a fact which clearly reveals that infectious process of CNS is due to WNV and it is that antibody index reaches a value greater than 1.5. That means that the causal agent of this infection is WNV.

Detection of intrathecal WNV IgM from CSF and sera has been detected by ELISA test. In some cases have been shown an unexpected onset of the intrathecal specific humoral immune response before serum immunoglobulins synthesis was recorded¹³.

Reiber's hyperbolic formula and Öhman's extended immunoglobulin indices are based on the demonstration of non-linear relationships between the Qalb and CSF-serum concentration quotients for IgG, IgA and IgM to determine whether there is a disruption of the blood-CSF barrier and intrathecal synthesis of immunoglobulins^{5,10,14-16}.

However Reiber's hyperbolic formula has been just used in a veterinarian studies which suggested a normal BBB integrity and increased intrathecal production of antibodies^{17,18}.

Our study is the first report of anti-WNV IgA and IgG antibodies intrathecal synthesis in a human being.

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