Arq Neuropsiquiatr 1994, 52(3): 445-446

POLYMERASE CHAIN REACTION IN THE DIAGNOSIS OF TUBERCULOUS MENINGITIS

PRELIMINARY REPORT

L.R. MACHADO, J.A. LIVRAMENTO*, S.P. BYDLOWSKI*, I. BENDIT*, L.M. BRAVO**, A. SPINA-FRANÇA****

SUMMARY - In this preliminary report the results of PCR for detection of DNA sequences (65 KDa antigen) of *Mycobacterium tuberculosis* in CSF samples from 20 patients are registered. In 10 patients there were clinical and laboratory findings suggesting the diagnosis of tuberculous meningitis (test group). In the other 10 patients, clinical and laboratory findings suggested meningitis or meningo-encephalitis from other etiologies (control group). In 7 patients from the test group antigenic DNA sequences of *Mycobacterium tuberculosis* were found in CSF by PCR; positive results were not registered in the control group.

KEY WORDS: PCR, CSF, tuberculous meningitis, meningoencephalitis.

PCR no diagnóstico da meningoencefalite tuberculosa

RESUMO - Neste relato preliminar são registrados os resultados da pesquisa de PCR para detecção de sequências de DNA (antígeno 65 KDa) do *Mycobacterium tuberculosis* no LCR. Foram estudadas amostras de LCR de 20 pacientes: em 10 havia suspeita clínica e laboratorial de neurotuberculose (grupo de teste); nos outros 10 havia suspeita diagnóstica de meningite ou meningoencefalite de outras etiologias (grupo controle). Em 7 dos 10 pacientes do primeiro grupo a pesquisa de sequências antigênicas de DNA do *Mycobacterium tuberculosis* por PCR foi positiva; em nenhum dos pacientes do grupo controle a pesquisa foi positiva.

PALAVRAS CHAVE: PCR, LCR, meningoencefalite tuberculosa, meningoencefalites.

Fast and accurate diagnosis is probably the most important factor in the prognosis of patients with tuberculous meningitis (TBM)[4,5]. The purpose of this communication is to evaluate the utility of polymerase chain reaction (PCR) for detection of DNA sequences (65 KDa antigen) of *Mycobacterium tuberculosis* in cerebrospinal fluid (CSF) samples in patients with TBM.

PATIENTS AND METHODS

A total of 20 patients were studied with infections of the central nervous system (CNS) divided in two groups. The first group consisted of 10 patients with clinical signs of meningo-encephalitis and CSF changes proper to subacute infectious syndrome (mild or discrete increase of cell number, with a variable neutrophils participation in the cytomorphologic profile; increased protein content; low glucose content; increased adenosine-

Centro de Investigações em Neurologia, Departamento de Neurologia, Faculdade de Medicina da Universidade de São Paulo. Laboratório de Genética Molecular e Biotecnologia, Fundação ProSangue Hemocentro, São Paulo/Laboratório de Neurodiagnóstico Spina-França, São Paulo/DiaGenix Biotecnologia e Diagnóstico, São Paulo. *Professor Assistente; **Bioquímico Pesquisador; ***Professor Emérito.Aceite: 20-junho-1994.

Dr. Luís dos Ramos Machado - Caixa Postal 5199 - 01061-970 São Paulo SP - Brasil. FAX (011) 289-8879.

deaminase activity) suggesting the diagnosis of TBM (test group). In the other 10 patients (control group), clinical and laboratory findings suggested meningitis or meningoencephalitis from other etiologies: viral meningitis (3); lymphoma associated meningoencephalitis (2); toxoplasmosis of the CNS (3); bacterial meningitis (1); cryptococcosis of the CNS (1). In the second group there were 3 patients with AIDS.

In all CSF samples classic tests for bacteria (Gram), fast-acid bacilli (Ziehl) and yeast (Moore and Weidman & Freeman) were performed, as well as cultures of sediment in appropriate media.

For PCR analysis, DNA was extracted from CSF sediment by alkaline hydrolysis. The 383 bp sequence of the 65 KDa antigen of *Mycobacterium tuberculosis* was amplified[2,5]. After agarose gel electrophoresis, amplification products were transferred to nylon membrane by Southern blotting technique and hybridized with a specific *M. tuberculosis* probe[1-3].

RESULTS

In the first group of patients, possibly with TBM, fast-acid bacilli were found in the CSF of 2 (20%). In these and in other 3 patients (50%),tuberculous bacilli developed in CSF Lowenstein cultures. In CSF samples from these 5 and from other 2 patients of the same group, in a total of 7 patients (70%), tuberculous bacilli antigen DNA sequences were detected in CSF samples by PCR.

In none of the control group patients specific antigen DNA sequences were detected by PCR.

COMMENTS

It was possible to confirm the hypothesis of TBM by PCR in 70% of the first group patients[3,4]. However, the true sensibility of PCR for tuberculosis in these samples may be higher, because the inclusion criterium (clinical and laboratory findings) was the probability one, not that of safety. There was no positive result in the second group of patients, suggesting a good specificity for this test[5].

In conclusion, in spite of the low number of cases analyzed in this study, the PCR, the for mycobacterial DNA sequences (65 kDa antigen) seems to be an important tool in the diagnosis of TBM, with a suitable sensibility/specificity engagement.

REFERENCES

1. Brisson-Noel A, Gicquel B, Lecossier D, Levy-Febault V. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet 1989, 1069-1071.
2. Hance AJ, Grandchamp B, Levy-Febault V. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Molec Microbiol 1989, 3: 843-849.
3. Kaneko K, Onodera O, Miyatake T, Tsuji S. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction (PCR). Neurology 1990, 40: 1617-1618.
4. Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM. Tuberculous meningitis: a 30-year review. Clin Infect Dis 1993, 17: 987-994.
5. Lee BW, Tan JAMA, Wong SC, Tan CB, Yap HK, Low PS, Chia JN, Tay JSH. DNA amplification by the polymerase chain reaction for the rapid diagnosis of tuberculous meningitis: comparison of protocols involving three mycobacterial DNA sequences, IS6110, 65 KDa antigen, and MPB64. J Neurol Sci 1994, 123: 173-179.