Neurol J Southeast Asia 1996; 1 : 39 – 42

T Lymphocyte subsets and impaired lymphoproliferative responses in apparently immunocompetent patients with cryptococcal meningitis during convalescence

Chai Beng TAN MBBS MMED, Bee Wah LEE MMED MD, *Chiang Chiang SEAH Dip Lab Med, HTL Tjia MBBS MMED

*Brain and Spine Centre, Tan Tock Seng Hospital, Singapore, *Department of Paediatric, National University Hospital, Singapore*

Abstract

Although cryptococcal meningitis (CM) usually affects the immunocompromised host, we have previously described CM in previously healthy subjects. In this study, we evaluated T lymphocyte subsets, lymphoproliferative responses and lymphocyte interferon-γ (IFN-γ) production of 9 such TM patients. T lymphocyte subsets, CD3, CD4, CD8, were evaluated by indirect immunofluorescence and flow cytometry. Lymphoproliferative responses to mitogens (PHA and ConA) were evaluated by tritiated thymidine uptake. IFN-γ production of PHA stimulated peripheral blood mononuclear cells was measured by radioimmunoassay. 12 healthy controls were similarly evaluated. Our results showed that there was no difference in the CD3, CD4 and CD8 subset expression between patients, in both acute and convalescent phases, and controls. However, lymphoproliferative responses to mitogens were impaired in the patients compared to controls. This impairment persisted into the convalescent period (PHA: $p < 0.01$, ConA: $p < 0.05$). IFN-γ production were also impaired compared to controls ($p < 0.03$). Taken together, our results indicate that despite normal T cell numbers, impairment of cell-mediated immune functions exists in these patients with CM.

Key words: cryptococcosis, immunocompetence.

INTRODUCTION

Cryptococcosis is a systemic fungal infection and meningeal involvement is the most serious complication. Susceptibility to crytococcal disease has been associated with impaired cellular immunity, and a large proportion of patients with this infection have underlying immunosuppressive disease such as the acquired immunodeficiency syndrome, malignancy, or are receiving immunosuppresive therapy [1-5]. In a proportion of cases, however, there does not appear to be any apparent factor predisposing them to the cryptococcal infection [6]. We have also previously described a group of apparently healthy patients who presented with cryptococcal meningitis (CM) [7]. In this study, we studied their cell-mediated immune status by evaluating T lymphocyte subsets, lymphoproliferative responses to mitogens, and *in-vitro* gamma interferon production of peripheral blood mononuclear cells following stimulation with phytohaemagglutinin.

MATERIALS AND METHODS

Patients and Controls

Nine patients with cryptococcal meningitis admitted to the Department of Neurology, Tan Tock Seng Hospital, between January 1990 and June 1993 were recruited into the study. These patients were apparently well and immunocompetent prior to illness. There was an absence of prior illness, immunosuppressive drug therapy, and HIV antibodies. The diagnosis of cryptococcal meningitis was based on the presence of pleocytosis and raised protein in the cerebrospinal fluid (CSF), and a positive CSF culture for cryptococcus. The patients were evaluated during the acute phase of the illness and 2 months to a year after completion of therapy. Twelve healthy volunteers were also evaluated. HIV antibody tests were carried out. Verbal consent from the patients and volunteers was obtained prior to study entry.

Lymphocyte subsets

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by Ficoll-Hypaque density gradient centrifugation [8]. Cell surface expression of T cell subsets was assessed by indiret immunofluoresence as previously described [9]. Briefly, PBMC were incubated in separate tubes with monoclonal antibodies to CD3, CD4 and CD8 (Becton Dickinson, USA), followed by one wash in staining buffer

Address for correspondence: Dr CB Tan, Department of Neurology, Tan Tock Seng Hospital, Moulmein Road, Singapore 308433.

(RPMI-1640 medium supplemented with 2.5% fetal calf serum, 0.01% sodium azide). Isothiocyanate-conjugated goat anti-mouse immunoglobulin antibody was used as the second antibody. The cells were then analysed by flow cytometry (FACScan, Becton Dickinson).

Lymphocyte proliferative response to mitogens

Lymphocyte proliferative responses to the mitogens, phytohaemagglutinin (0.5ug) and concanavalin A (10ug/ml) were assessed in microtiter plates containing 1 × 105 PMNC suspended in RPMI-1640 supplemented with 10% fetal calf serum, 2mM *L*-glutamine, and 100 U/ml penicillin and 50ug/ml streptomycin. After 72 hours the cells were pulsed with 1 uCi/well of tritiated thymidine was then counted using a beta scintillation counter (Beckman). Lymphocyte stimulation was assessed by the mean counts per minute (cpm) of triplicate stimulated cultures subtracted by mean cpm of unstimulated cultures.

PBMC interferon-γ production

Interferon-γ(IFN-γ)production by PHA-stimulated PBMC was made by incubating PBMC (2×10^6/ml) in culture medium supplemented with PHA (1.25 ug/ml) for 48 hours. The culture supernatants were then harvested and frozen until assayed for IFN-γ by radioimmunoassay (Centocor, Japan).

Statistical analysis was performed using the Student t, and Wilcoxon rank sum tests.

RESULTS

Patient parameters

The median age of patients and controls were 45 (range 24-61) and 37 (range 20-43) years, respectively. The male:female ratio in both groups were 2:1. There were no significant difference between the groups of subjects studied. All the patients were HIV antibody negative. None of the patients had any evidence of pulmonary cryptococcosis on chest radiography.

Laboratory results

The T lymphocyte subsets and lymphocyte proliferative responses to mitogens are shown in Fig 1 and 2. There was no significance difference in T cell subset percentages between patients in the acute and convalescent phases and controls. The median CD4/CD8 ratio were also similar between the groups (acute = 1.3, convalescent = 1.0, controls = 1.1). However, lymphoproliferative responses to mitogens were significantly depressed the patients at both acute and convalescent phases compared to normal controls (PHA: $p < 0.01$, Con A: $p < 0.05$). In addition, median gIFN production in the patients were also significantly lower compared to controls (acute:123 units/ml, convalescent: 224 units/ml, controls: 1026 units/ml)($p < 0.03$).

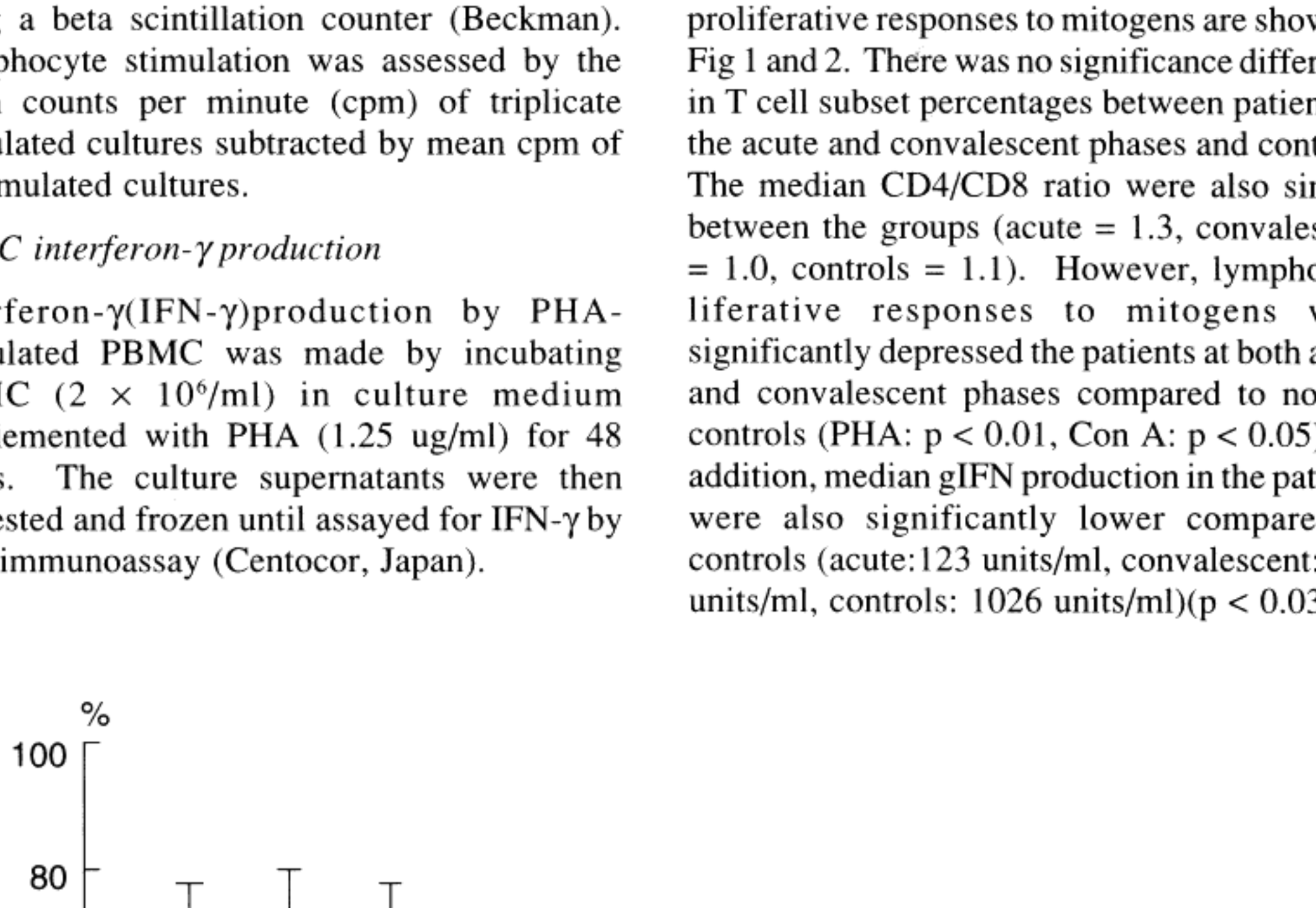

FIG 1: T lymphocyte subsets, CD3, CD4, CD8, in patients and controls.

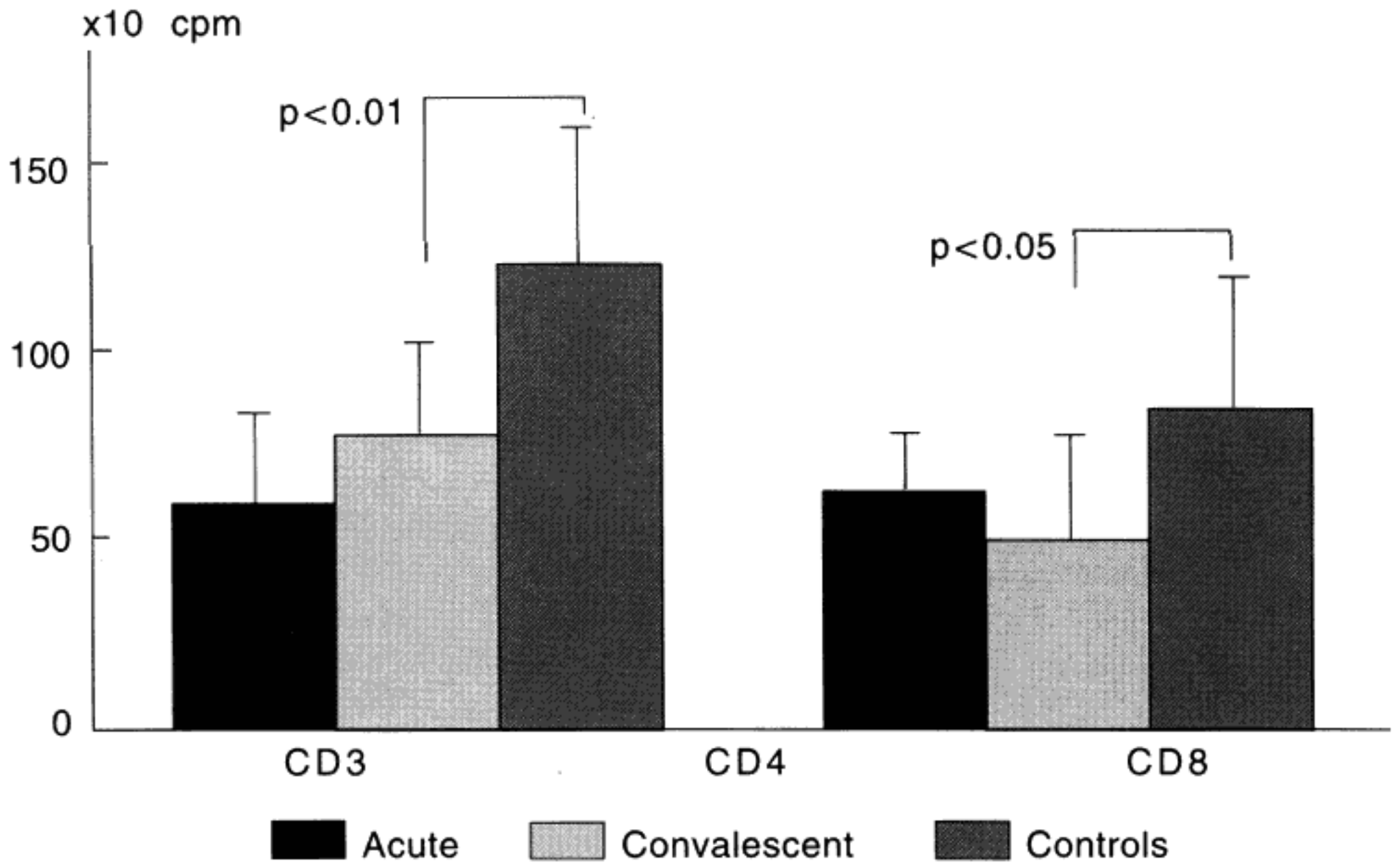

FIG 2: Lymphoproliferative responses to mitogens.

DISCUSSION

In this study we have demonstrated the presence of impaired cellular immune function in our subset of CM patients withiout prior history of major risk factors of immune suppression. This was evidenced by the presence of defective lymphoproliferative responses to mitogens and IFN-γ production of mitogen stimulated PBMC. Importantly, this impairment was present despite normal T cell subset numbers, hence excluding the more recently recognises forms of immunocompromise such as acquired immunodeficiency disease [5] and idiopathic CD4+ T-lymphocytopenia [10].

It has recently been established that a CD4+ cell-dependent acquired state of immunity protects the brain against cryptococcal neoformans infection. Experiments on normal and SCID mice indicate that yeast cells can establish small foci even in the brain of immunocompetent hosts, and protection against meningitis is achieved by competent CD4+ cells. This response was ablated by anti-CD4 monoclonal antibody treatment, and restored by infusion of CD4+ cells from primed donors [11]. Although the tests carried out in this study were not specific for immunity against Cryptococcus, the impaired IFN-γ suggests an abnormality in the T helper (CD4) subset in our patients.

It was impossible to study our patients prior to presentation. However, the persistence of impaired lymphoproliferative responses during convalescence suggests that this abnormality may be a longstanding one. Without prior history of apparent immunocompromise, it is possible that this defect is an acquired problem preceeding and causing the cryptococcal infection. The underlying reason for impaired responses in our patients is, however, unclear and deserves further investigation. Long term follow-up of our patients will be necessary to determine whether problems related to immunocompromise, such as recurrent opportunistic infections, would occur.

REFERENCES

1. Collins VP, Gellhorn A, Trmble JR. The coincidence of cryptococcus and disease of the reticuloendothelial and lymphatic system. Lancet 1951; 4: 833.
2. Zimmerman LE, Rappaport H. Occurrence of cryptococcosis in patients with a malignant disease of a malignant disease of the recticuloendothelial system. Am J Clin Pathol 1954; 24: 1050-72.
3. Goldstein E, Rambo ON. Crytococcal infection following steroid treatment. Ann Intern Med 1962; 56: 114-20.
4. Tay CH, Chew WLS, Lim LCY. Cryptococcal meningitis. Brain 1972; 5: 825-32.
5. Powderly WG. Cryptococcal meningitis and AIDS. Clin Infect Dis 1993; 17: 837-42.
6. Bennet JE. Cryptococcosis. In: Hoeprich PD, ed. Infectious diseases. New York: Harper & Row, 1974: 945-52.
7. Tjia TL, Yeow YK, Tan CB. Cryptococcal meningitis. J Neurol Neurosurg Psychiatry 1985; 48: 853-8.

8. Boyum A. Separation of leucocytes from blood and bone marrow. Scan J Clin Invest 1968; 21: 1-10.
9. Lee BW, Yap HK, Tan MA, Guan R, Quak SH, Choong L, Murugasu B, Woo KY, Jordan S. Cell-mediated immunity in patient on hemodialysis: relationship with hepatitis B carrier status. Am J Nephrol 1991; 11: 98-101.
10. Duncan RA, Von-Reyn CF, Alliegro GM, Toossi Z, Sugar AM, Levitz SM. Idiopathic CD4+ T lymphocytopenia - four patients with opportunistic infections and no evidence of HIV infection. N Engl J Med 1993; 328: 393-8.
11. Hill JO, Aguirre KM. CD4+ T cell-dependent acquired state of immunity that protects the brain against Cryptococcus neoformans. J Immunol 1994; 152: 2344-50.