To

My beloved husband

Dr.Abdul Ghani Abdul Jalil

&

My daughters

Siti Nurkhadijah & Siti Nuraishah,

&

My great parents

Hj.Muhammad Yahaya @ Ya

Hjh.Ruhani Syed Mohd Yusuff

&

My brother and sister

Abdul Halim Muhammmad

Siti Salmiah Muhammad

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Abstrak

Bahasa Malaysia

Tajuk: Pemeriksaan imbasan kepala untuk menentukan bahagian otak yang paling kerap terlibat bagi pesakit HIV/AIDS yang mempunyai ketidaknormalan sistem saraf otak dan kaitannya dengan jangkitan *T.gondii* and *Cryptococcus*.

Tujuan: Matlamat kajian ini adalah untuk ;

1. Mengenal pasti kawasan otak yang kerap terlibat.
2. Mengenal pasti hubung-kait di antara jangkitan *T.gondii* and *Cryptococcus* dengan kawasan otak yang terlibat
3. Mengenal pasti hubung-kait di antara hasil pemeriksaan skan CT dengan jangkitan *T.gondii* and *Cryptococcus*.
4. Mengenal pasti hubung-kait di antara kiraan CD4 dengan jangkitan *T.gondii* and *Cryptococcus*.

Kaedah : Kelulusan untuk menjalankan kajian di perolehi dari etika komiti dan pesakit yang terlibat. Kajian rentas telah dijalankan di HRPZ II dan Hospital USM selama 12 bulan bermula dari Januari 2005 hingga Disember 2005 ke atas 56 pesakit HIV/AIDS yang menjalani pemeriksaan imbasan CT bahagian kepala .

Keputusan : Terdapat 7/56 (12.5 %) pesakit peringkat pertengahan dan 49/56 (87.5%) pesakit peringkat akhir. Kebanyakan pesakit mendapat simptom non-focal iaitu sebanyak 52/56 (92.9%) kes dengan sakit kepala sebagai simptom yang paling kerap 17/52 (32.7%). Pesakit yang dijangkiti dengan *T.gondii* dan kulat *Cryptococcus* terdiri dari 70.7% (29/41) dan 23.3% (10/43) dari jumlah kesemua pesakit. Skan CT didapati normal dalam 13 (23.2%) and tidak normal dalam 43 (76.85) kes. Kebanyakan 'lesion' adalah lebih dari satu iaitu

melibatkan 24/38 (63.2%) kes. Secara amnya, kebanyakan 'lesion' adalah melibatkan bahagian otak 'cerebrum di mana melibatkan bahagian otak 'parietal' dalam 73.7% kes, diikuti oleh otak 'occipital' dalam 55.3% kes, otak 'frontal' 47.4% kes dan otak 'temporal' (36.8%). Kawasan 'basal ganglia' terlibat dalam 52.6% kes. Pesakit dengan jangkitan T.gondii didapati melibatkan bahagian otak 'frontal ' dalam 31% kes, 58.6% di bahagian otak 'parietal', 34.5% di bahagian otak 'occipital', 20.7% di bahagian otak 'temporal' dan 34.5% di kawasan 'basal ganglia'. Pada pesakit dengan jangkitan kulat 'cryptococcus', 40% melibatkan otak bahagian 'parietal', 30% di otak 'occipital', 10% di otak'temporal' dan 10% di kawasan 'basal ganglia'. Tiada 'lesion' didapati di bahagian otak 'frontal'. Tiada hubung kait didapati di antara kiraan CD4 atau pun hasil pemeriksaan CT scan dengan penyebab jangkitan iaitu *T.gondii* dan kulat *Cryptococcus* dan di antara *T.gondii* atau kulat *Cryptococcus* dengan kawasan otak yang terlibat di dapati dalam kajian ini..

Kesimpulan:

Dari hasil kajian ini, di dapati bahagian otak parietal adalah paling banyak terlibat bagi dua jenis jangkitan organism yang utama iaitu *T.gondii* dan kulat *Cryptococcus*. Tiada hubung kait didapati di antara kiraan CD4 atau pun hasil pemeriksaan CT scan dengan penyebab jangkitan iaitu *T.gondii* dan kulat *Cryptococcus* dan di antara *T.gondii* atau kulat *Cryptococcus* dengan kawasan otak yang terlibat. *T.gondii* adalah penyumbang utama kepada jangkitan kuman di bahagian otak pesakit HIV/AIDS yang terlibat. Penyakit tuberculosis dan jangkitan kulat '*Cryptococcus*' juga berpotensi besar untuk menyumbang kepada masalah jangkitan kuman di bahagian ini. Bahagian otak 'frontal' ,'temporal, dan kawasan 'basal ganglia' adalah kerap terlibat pada pesakit yang terkena jangkitan 'toxoplasma' dari pesakit yang terkena jangkitan kulat 'cryptococcus'.

English

Topic: CT brain study to determine the site of predilection of intracranial lesions in HIV/AIDS patient with neurological symptoms and its association with toxoplasma and cryptococcal infections.

Objective:

1. To determine the distribution of intracranial lesions.

2. To find out the association between site of intracranial lesions with toxoplasma and cryptococcal infections.

3. To find out the association between CT findings with toxoplasma and cryptococcal infections.

4. To determine the association between CD4 count with toxoplasma and cryptococcal infections.

Methods and Materials: Ethics committee approval and informed consent were obtained. This cross sectional study was carried out in Hospital USM, Kubang Kerian and Hospital Raja Perempuan Zainab II (HRPZ II) for 12 months from January 2005 until December 2005. Plain and contrast cranial CT scan were performed on a total of 56 HIV patients with neurological signs and symptoms.

Results: There were 7/56 (12.5 %) middle stage and 49/56 (87.5%) late stage patients. Majority of patient presented with non-focal symptoms 52/56 (92.9%) in which headache was the most frequent complaint 17/52 (32.7%). Patient with toxoplasmosis and cryptococcosis constituted 70.7% (29/41) and 23.3% (10/43) of total subject investigated. CT scans were normal in 13 (23.2%) and abnormal in 43 (76.85) cases. Majority of the lesions were multiple 24/38 (63.2%). In general, lesions were mostly found in supratentorial region within the parietal (73.7%) followed by occipital (55.3%), frontal (47.4%) and temporal

(36.8%) lobes. Basal ganglia region was affected in 52.6% of cases. Patients with Toxoplasmosis had lesions at frontal lobe in 31%, parietal lobe in 58.6%, occipital lobe in 34.5%, temporal lobe in 20.7% lobe and 34.5% at basal ganglia area. In patients with cryptococcosis, lesions were found 40% at parietal lobe, 30% at occipital lobe, 10% at temporal lobe and 10% at basal ganglia area. No lesion was found at frontal lobe in those patients with cryptococcosis in this study. No association was detected between CD4 counts or CT findings with etiology of the lesion (*Toxoplasma and Cryptococcus*) and between etiologies of lesion with site of intracranial lesions in this study

Conclusion:

We conclude that in general, parietal lobe is commonly involved in both opportunistic infections. No association was detected between CD4 counts or CT findings with etiologies of the lesion and between etiologies of lesion with site of intracranial lesions in this study. Toxoplasmosis however was a major opportunistic organism giving rise to various CNS manifestations in our HIV-infected patients with potential significant contribution of tuberculosis and cryptococcosis. Frontal lobe, temporal lobe and basal ganglia however, are more commonly involved in HIV patients with toxoplasmosis than in HIV patients with cryptococcosis.

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Abbreviations

CT	Computed tomography
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
CDC	Centers for Disease Control and Prevention (USA)
WHO	World Health Organization
UNAIDS	Joint United Nation Program on HIV and AIDS
MRI	Magnetic Resonance Imaging
GCS	Glasgow Coma Scale
ARC	AIDS Related Complex
ADC	AIDS Dementia Complex
IDUs	Injecting Drug Users
MOH	Ministry of Health
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
CNS	Central Nervous System

CM	Cryptococcal meningitis
PTB	Pulmonary tuberculosis
TM	Tuberculous meningitis
TE	Toxoplasma encephalitis
CSF	Cerebrospinal fluid
HUSM	Hospital Universiti Sains Malaysia
HRPZ II	Hospital Raja Perempuan Zainab II

SECTION ONE:

INTRODUCTION

1.0 Introduction

1.1 General

Acquired Immunodeficiency Syndrome (AIDS) a late-stage of HIV disease is one of the most destructive epidemics in recorded medical history. It is a devastating illness with progressive clinical course despite the advances in anti-viral treatments. Neurological manifestation is common either due to HIV itself or secondary to opportunistic infection. In addition, with improved treatment and prolonged survival, more HIV-infected patient reach an older age and are at risk for cerebrovascular diseases unrelated to HIV infection. Due to variability of intracranial causes in HIV-infected patients with neurological complaints, neuroimaging is essential to provide diagnostic and therapeutic information. The crucial role that computed tomography (CT) plays in establishing early diagnosis has been acknowledged.

1.2 Epidemiology

1.2.1 Global

AIDS has killed more than 25 million people since it was first recognized in 1981. In 2005, the epidemic has claimed 3.1 million (2.8 - 3.6 million) lives world-wide with 480,000 of them in South and South-East Asia (UNAIDS/WHO report, 2005). About 4.9 million (4.3- 6.6 million) people were newly infected with the virus in 2005. Across Asia, the epidemics are propelled by combinations of injecting drug use and unprotected sex.

1.2. 2 Malaysia

In Malaysia, the first, AIDS case was reported in December 1986. Since then, the cumulative HIV infection and AIDS cases continues to rise. In the year 2005, reported HIV infection was 70,559 cases and 10,663 AIDS cases with total number of 8,179 AIDS death (MOH, Malaysia, 2005). Males remain the majority (93.0%) of the reported HIV and AIDS cases (91%) within age group of 20 to 39 years. HIV transmission is mainly driven by infection through sharing needles among injecting drugs (IDUs) (75.1%), followed by heterosexual (13.6%) and homo/bisexual route (1.3%). Only 0.7% and 0.04% of the infection is attributed to vertical and blood transfusion respectively (MOH, Annual report, 2004). The intersection of drug injecting and HIV is most prominent in the east of the country. In Kelantan, estimated HIV prevalence among injectors was 41% in 2002, and in Johor and Terengganu it was 31% and 28% respectively (Ministry of Health Malaysia and WHO, 2004).

1.3 Biology of HIV

HIV belongs to a subgroup of retroviruses called lentiviruses. Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is the more virulent and easily transmitted, and is the source of the majority of HIV infections throughout the world where as HIV-2 is largely confined to west Africa. Both species originated in west and central Africa, jumping from primates to humans in a process known as zoonosis. HIV-1 has evolved from a simian immunodeficiency virus (SIVcpz) found in the chimpazee subspecies, *Pan troglodytes troglodytes*. HIV-2 crossed species from a different strain of SIV, found in sooty mangabey monkeys in Guinea-Bissau.

HIV is a retrovirus that primarily infects and destroys vital components of the human immune system such as CD4 + T cells, macrophages and dendritic cells. It also directly attacks organs, such as kidneys, heart and brain leading to acute renal failure, cardiomyopathy, dementia and encephalopathy.

Human immunodeficiency viruses are spherical, measuring 80-130 nm in diameter with three-layered structure (Figure 1). It composed of two copies of single-stranded RNA enclosed by a conical capsid, which is in turn surrounded by a plasma membrane that is formed from part of the host-cell membrane. Both types of HIV are quite similar, in which they have three major genes coding (gag, pol,env) for structural proteins with some subtype-specific genes.

1.4 Pathophysiology of HIV Infection

The clinical manifestations of HIV infection are a consequence of an ongoing, active viral infection. The process by which HIV infection causes progressive destruction of the human immune system is only partly understood. After the virus is acquired, it quickly invades cells of immune system which having surface molecule known as CD4 which serves as the virus receptor. Inside the infected CD4+ cells, the virus replicates itself producing numerous copies. This process leads to the death of the infected cell with release of the newly formed viruses. These virus progeny than invade another CD4+ cells and the cycle continues. The viral replication process is initially unchecked by host defense mechanism. But after weeks to few months, most HIV-infected persons developed immune response to the virus, which unfortunately partially effective in controlling the amount of viral replication that continues. Primary target cells are CD4+ cells T lymphocytes or sometimes referred as T helper cells. These cells play a crucial

role in coordinating the complex interaction that is required for a properly functioning immune system. The two parameters viral load and CD4 count are linked to each other since the rate at which CD4+lymphocytes are destroyed is directly proportional to the amount of ongoing viral replication as determined by plasma viral load measurement. Thus, patients with high viral load measurements have more rapid destruction of CD4+lymphocytes and usually lower CD4 count.

1.5 Immunopathogenesis of HIV-1 disease

Viruses enter the body through a variety portal of entry either as cell-free virions or in a cell-associated form. The virus binds to and infects $CD4^+$ cells and monocytes. The monocytes may also phagocytose virus. HIV infection can exist either as a latent or low level or chronic form. Upon activation of the infected cell, virus is produced resulting in cytopathic effect on T cells and too much less extent on monocytes. The monocytes serve as a reservoir for HIV, transporting the virus to various parts of the body, particularly the brain, thereby leading to neuropsychiatric abnormalities. In addition, monocytes can pass the virus to T4 cells. Cytopathicity follows upon activation of the T4 cells resulting in cell death and immunosuppression leading to development of opportunistic infections (Figure 2).

In the brain, additional means of virus passage from the brain parenchyma to the CSF (and reverse) involves endothelial cell infection and release of cells into the stroma of the choroids plexus. Here infection of stromal cells and macrophages may occur, followed by infection of choroids plexus epithelium. Transition of virus from brain parenchyma into the ventricular CSF and vice versa involves infection of ependymal cells and subependymal glia, although there is no experimental evidence for ependymal

cell infection. Damage to the nervous system as a result of HIV infection may occur via two general pathways;

i. First, it may involves direct virus infection of nervous system cell types that may result in either killing or alteration of cellular metabolic function(cytopathic effects). The retention of viral genome in neural cells in a persistent latent form may also lead to neuronal or glial dysfunction.

ii. A second general pathway centers on the cytotoxic action of HIV-specific products or aberrantly secreted cellular products that may interfere with nervous system function.

In addition, autoimmune mechanisms and such factors as virus strain may also play a role in nervous system dysfunction.

HIV-1 most likely enters the CNS in an infected macrophage. Infected microglial cells and astrocytes in the brain will release cytokines namely TNF-α, IL-1 and IL-6 which may amplify HIV-1 multiplication in macrophages. Microglia and macrophages release toxic viral components and quinolinic acid causing damage to the neurons. This cycle of immune activation within the brain parenchyma is most likely leads to an increased viral burden as well as to the broad spectrum of neurological disease caused by HIV-1infection in the brain (Figure 3).

1.6 Clinical course of HIV infection

Infection with HIV-1 is associated with a progressive loss of $CD4^+$ T-cells (lymphocytes). The rate of loss was measured to determine the stage of infection. The loss of $CD4^+$ T-cells is linked with an increase in viral load. The clinical course of HIV-infection generally includes three stages: primary infection, clinical latency and AIDS

(Figure 4). HIV plasma levels during all stages of infection range from just 50 to 11 million virions per ml.

1.6.1 Primary Infection

Primary or acute infection is associated with a rapid rise in plasma viremia which immediately follows the exposure to HIV. Most individuals (80 to 90%) develop an acute retroviral syndrome (ARS) characterized by flu-like symptoms of fever, malaise, lymphadenopathy, pharyngitis, headache, myalgia, and sometimes a rash. Onset occurs within a few weeks of infection and the syndrome generally resolves within two weeks. Following ARS, with average of three weeks, seroconversion occurs as antibody levels begin to rise and nearly all HIV-infected persons have detectable antibody 6 months after the infection.

1.6.2 Clinical latency

A strong immune defense reduces the number of viral particles in the blood stream, marking the start of the infection's clinical latency stage (Figure 6). Clinical latency can vary between two weeks and 20 years. During this early phase of infection, HIV is active within lymphoid organs, where large amounts of virus become trapped in the follicular dendritic cells (FDC) network. The surrounding tissues that are rich in CD4+ T-cells also become infected, and viral particles accumulate both in infected cells and as free virus. Individuals who have entered into this phase are still infectious.

1.6.3 AIDS

AIDS is the most severe manifestation of HIV infection where the cellular immunity has lost. At this stage, the CD4+ T cells are less than 200 per μl blood. At his stage , the infected individuals are prone for oppurtunistic infection and tumors typical for the immunocompromised host. In the absence of antiretroviral therapy, the median time of progression from HIV infection to AIDS is nine to ten years, and the median survival time after developing AIDS is only 9.2 months. However, the rate of clinical disease progression varies widely between individuals, from two weeks up to 20 years.

1.7 Toxoplasmosis

The protozoan *Toxoplasma gondii* is a coccidian, obligate, intracellular parasite responsible for zoonotic infection in man and other mammals. The cat is the definitive host. *T.gondii* exists in 3 forms of which are only present in the cat, are tachyzoites, bradyzoites, and sporozoites. Humans and other mammals are infected only by tachyzoites and bradyzoites. *T. gondii* causes broad spectrum of disease in various infected species, however, most are asymptomatic. Toxoplasmosis may be congenital or acquired.

1.7.1 Pathophysiology

The life-cycle of *T.gondii* consists of asexual and sexual stages. The asexual stages of *T.gondii* cause disease in humans and most animals. There are two asexual forms. The first form is tachyzoite, can invade almost all types of cells with the exception of non-nucleated erythrocytes, divides rapidly leading to cell death. The second form, called bradyzoite, divides slowly forming a cyst, present in multiple organs most prominently

in muscles and brain in latent form. Tachyzoite replication causes acute disease, while encysted bradyzoites are long-lived with slow turnover, and are responsible for maintaining the latent infection. Cysts in tissue is extremely resistant to the host's defenses, elicit no inflammation and presumably had little effect on surrounding cellular function until they breakdown and release bradyzoites, which can convert to tachyzoites and cause necrosis and inflammation. Reactivation of bradyzoites from cysts is responsible for most diseases in immuno-suppressed hosts. The sexual cycle takes place in superficial epithelium of the small intestine of both wild and domestic members of the cat family. Infected cat sheds noninfectious unsporulated oocysts in its feces. Sporulation occurs in at least 12 hours up to several days at room temperature after which they are infectious by mouth (sporozoite) for at least a year. In human, mode of transmission are either through fecal-oral or transplacental transmission.

1.7.2 Epidemiology

The prevalence of serum antibodies against toxoplasmosis varies throughout the world and depends on eating habits, hygiene, and climate. Toxoplasmosis appears to be more prevalent in hot humid climates.

1.7.3 Pathogenesis of infection

The primary route of infection is oral, with progression of the infection through the gastrointestinal tract to local lymphatic and spread to other organs was documented in mouse, but all of these steps have not been shown in humans (Schwartzman. J. D, 2001). Experimental animal studies involving the natural oral route of infection revealed importance of gut immunity in the acute stages of toxoplasmosis. Intraepithelial CD8+ lymphocytes isolated from infected mice shown to provide long-

term protection against infective challenge (Schwartzman. J.D, 2001). The key step in spreading the infection from the localized initial site is likely infection of circulating monocytes in the lamina propria; this cell subset has been shown to be permissive for *T.gondii* replication in both mice and humans, and therefore be responsible for transport of the parasite widely throughout tissues (Schwartzman. J.D, 2001). The initial pathological lesion is necrosis caused by death of parasitized cells, with a vigorous acute inflammatory reaction. As the disease progress, more lymphocytic infiltration develops but true granulomas are not formed if the host controls the tachyzoites replication, tissues are restored to anatomic integrity without scarring and cysts remain without sign of host reaction.

1.8 Cryptococcossis

Cryptococcus neoformans is a potentially pathogenic fungus that can be isolated from many common environmental sources. Infection with *Cryptococcus neoformans* is the most common fungal disease of the central nervous system. Rarely seen in otherwise healthy individuals, is more commonly occurs as an opportunistic infection in immunosuppressed patients, debilitated patient with cancer or diabetes, those treated with steroids or chemotherapy and most commonly AIDS patients.

1.8.1 Pathophysiology

Most initial infections occur through inhalation of the yeast from the environment. Cryptococci have large polysaccharide capsules that strongly resist phagocytosis. The inflammatory reaction to inhaled cryptococci produces a primary pulmonary–lymph node complex, which usually limits spread of the organism from this site. However

from lung and intra-thoracic lymph nodes, it circulates in the blood, especially in the immunocompromised host. Dissemination can occur during primary infection or during reactivation of infection years later. The most common site involved is the CNS. Hematogenous spread to CNS from pulmonary foci, which may be subclinical in which no pneumonitis is found in more than 85% of patients with cryptococcal CNS disease. Cryptococci also invade skin, bone, and genitourinary tract, but meninges appear to be the preferred site. The reasons are not clear, but several suggestions have been made in which cryptococcal capsule antigens may have limited ability in the cerebrospinal fluid (CSF) to induce an inflammatory response. Furthermore, the alternate pathway of complement is absent in the CSF. By contrast, CSF is a good growth medium for the organism in culture, possibly because of tropic properties of dopamine and other neurotransmitters in the CSF and absent cryptococcus-toxic proteins. Cryptococcal disease usually develops only when CD4 helper lymphocyte counts fall below 100 cells/mm^3 in which, at this stage, macrophage function also is impaired.

1.8.2 Epidemiology

C.neoformans is divided into two varieties: (i) *C.neoformans* var.*neoformans* (serotypes A and D), with world wide distribution and responsible for vast majority of HIV-associated infections: and (ii) *C.neoformans* var.*gatti* (serotypes B and C), confined to the tropics and subtropics, which often causes infection in immunocompetent individuals (Harrison. T. S, 2000).

In United States, the annual incidence of cryptococcosis is 2-7 cases per 1000 HIV-infected patients, up to 89% occurring as a CNS manifestation. It is the fourth most common cause of opportunistic infections after *Pneumocystis carinii,* cytomegalovirus (CMV), and mycobacteria, and CNS manifestations (66-89%) are by far more common

than those in other organs. CNS cryptococcosis is fatal unless treated with reported acute mortality rates of 6-14% in several studies. A minority of patients die within the first 6 weeks after diagnosis, despite treatment. Those who survive usually live for longer than 18 months but the rate of relapse after treatment is high (30-50%). CNS cryptococcosis is however rare in children with AIDS.

1.8.3 Pathogenesis of infection

Resistance to cryptococcosis depends primarily on cell-mediated immunity. Evidence from animal studies and the epidemiology of human infection clearly demonstrate that specific T-cell mediated immunity is critical in a protective immune response. This is supported by the pathology of cryptococcosis which is highly variable, reflecting the immune status of the host and organism factors, and is often notable for the paucity of inflammation, with large numbers of extra-cellular organisms forming cystic-like spaces in the tissue. The inflammation is granulomatous when present, with intra-cellular fungi. Macrophages are central to the immune response to *C.neoformans*, through antigen presentation and co-stimulation of T-cells, the chemokines and cytokines secretion and once activated, as final effector cells. However, the fungi are capable to survive and multiply within the macrophages, which under some circumstances may provide a protected niche. Despite the importance of T-cell-mediated immunity in defense against cryptococcosis, no cryptococcal t-cell antigens have been fully characterized to date. From animal studies, the subsequent T-cell-mediated responses involve both CD4 and CD8 cells. Immune protection also may relate to a THI pattern of cytokine release which is deficient in HIV disease. Pulmonary infection may be controlled without dissemination, but viable fungi remain in

interstitial and subpleural granulomata within macrophages and epitheloid cells (Harrison. T. S, 2000).

The typical pathologic findings of CNS cryptococcosis include meningitis, cryptococcomas, and dilated perivascular spaces from spread of the organism. Significant differences are reported in the inflammatory response to cryptococcal meningoencephalitis among patients with and those without HIV infection. Granulomatous inflammation is not common in patients with AIDS, whereas most patients without HIV have granulomas; this observation suggests a role for cell-mediated immunity in cryptococcal meningoencephalitis. An encephalitic component is common in HIV-associated patients, resulting in macroscopically or microscopically visible accumulations of organisms within the brain parenchyma. In cases not associated with HIV, cryptococcal meningoencephalitis is often confined to the subarachnoid space and perivascular Virchow-Robin spaces. In HIV-associated cases, large parenchymal cryptococcomas contain *C. neoformans* with cell wall pigmentation, suggestive of melanin. In patients with AIDS, altered immune functions have been suggested to allow *C. neoformans* to accumulate within the brain, predominantly extracellularly. Deficient macrophage/microglial effector function may be responsible for the altered pathology.

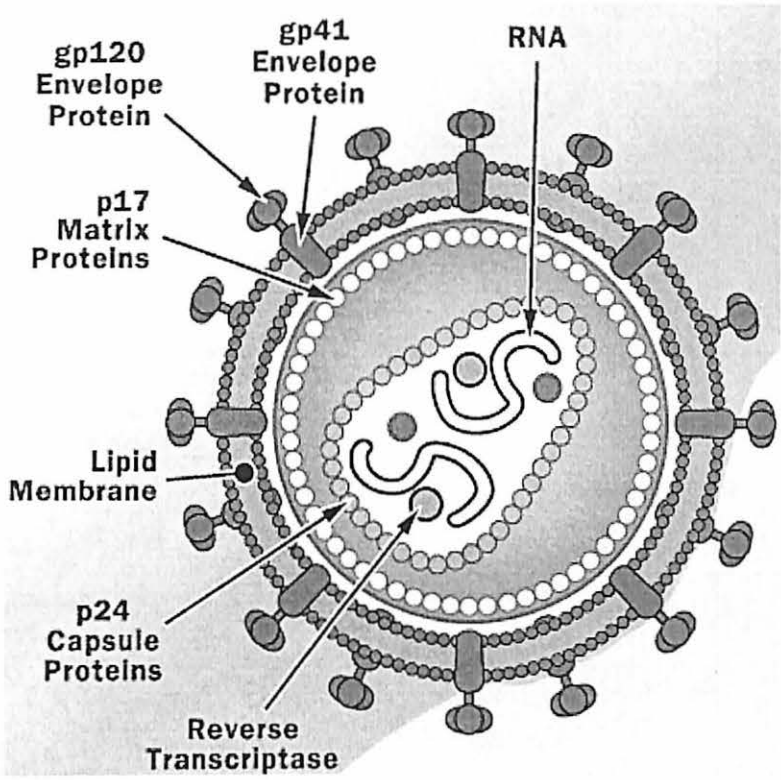

Figure 1: Diagram of HIV

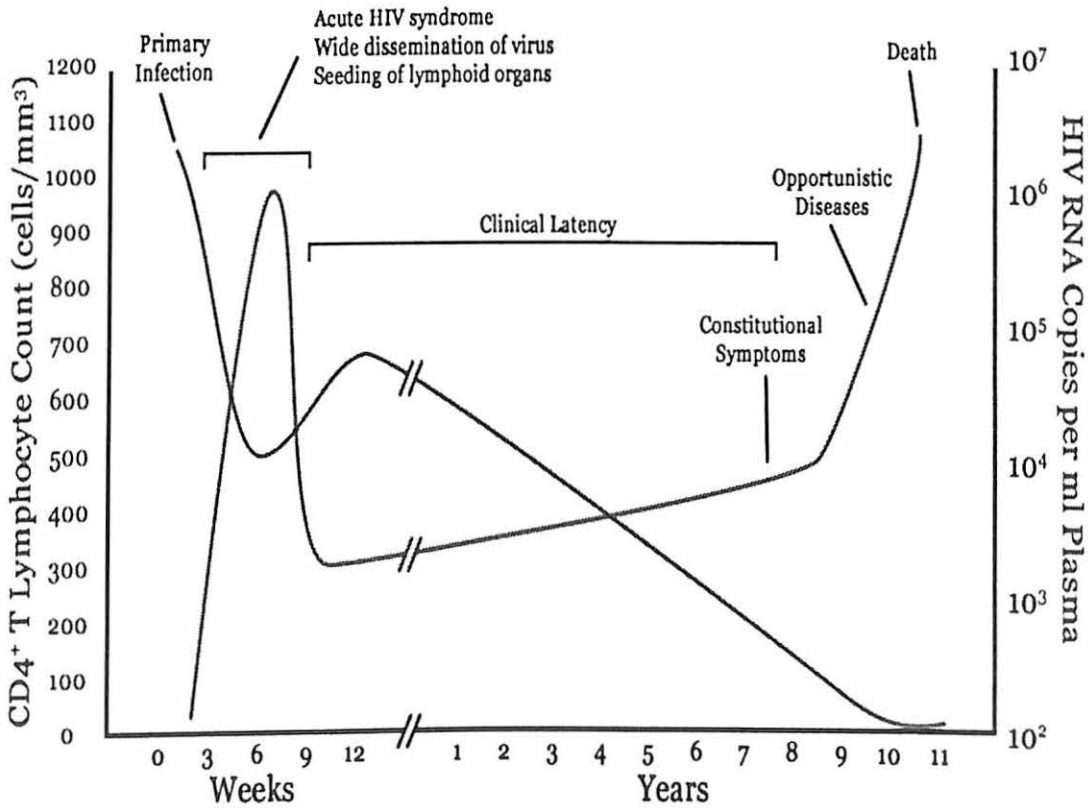

Figure 2:Graph showing HIV copies and CD4 counts over course of HIV infection

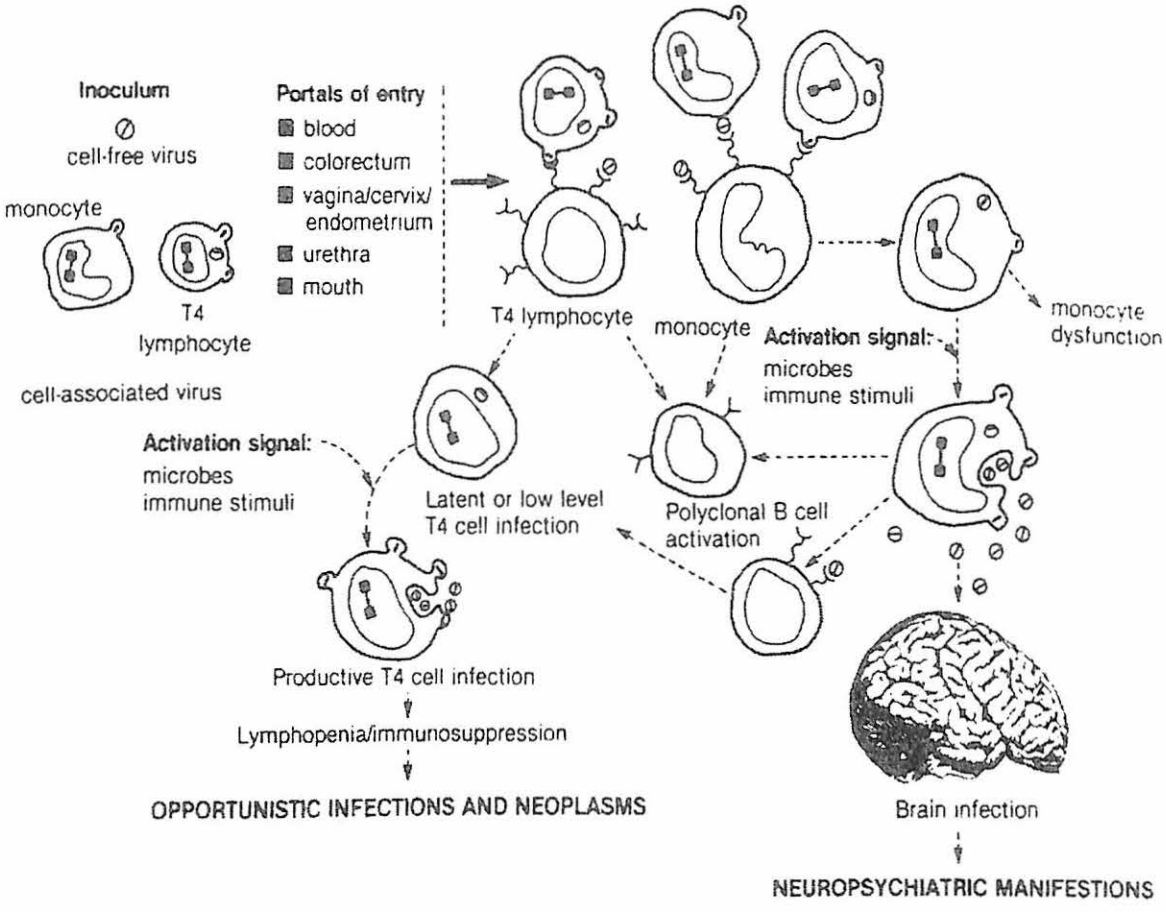

Figure 3: Potential mechanisms of pathogenesis of HIV infection

Virus enters the body through a variety of portals of entry either as cell-free virions or in a cell-associated form. The virus binds to and infects $CD4^+$ cells and monocytes. The monocytes may also phagocytize virus. HIV infection can exist either as a latent or low –level or chronic form. Upon activation of the infected cell, virus is produced resulting in cytopathic effect on T cells and to much less extent on monocytes. The monocytes serve as a reservoir for HIV, transporting the virus to various parts of the body, particularly the brain, thereby leading to neuropsychiatric abnormalities. In addition, monocytes can pass the virus to T4 cells. Cytopathicity follows upon activation of the T4 cells resulting in cell death and immunosuppression leading to development of opportunistic infections.

(Reprinted from Science. From Fauci, A.S., Science, 239, 617, 1988, Ackermann-W., Berthiaume, L., Tremblay, M., (2001) Viral Pathogenesis in Diagrams Textbook, CRC Press).

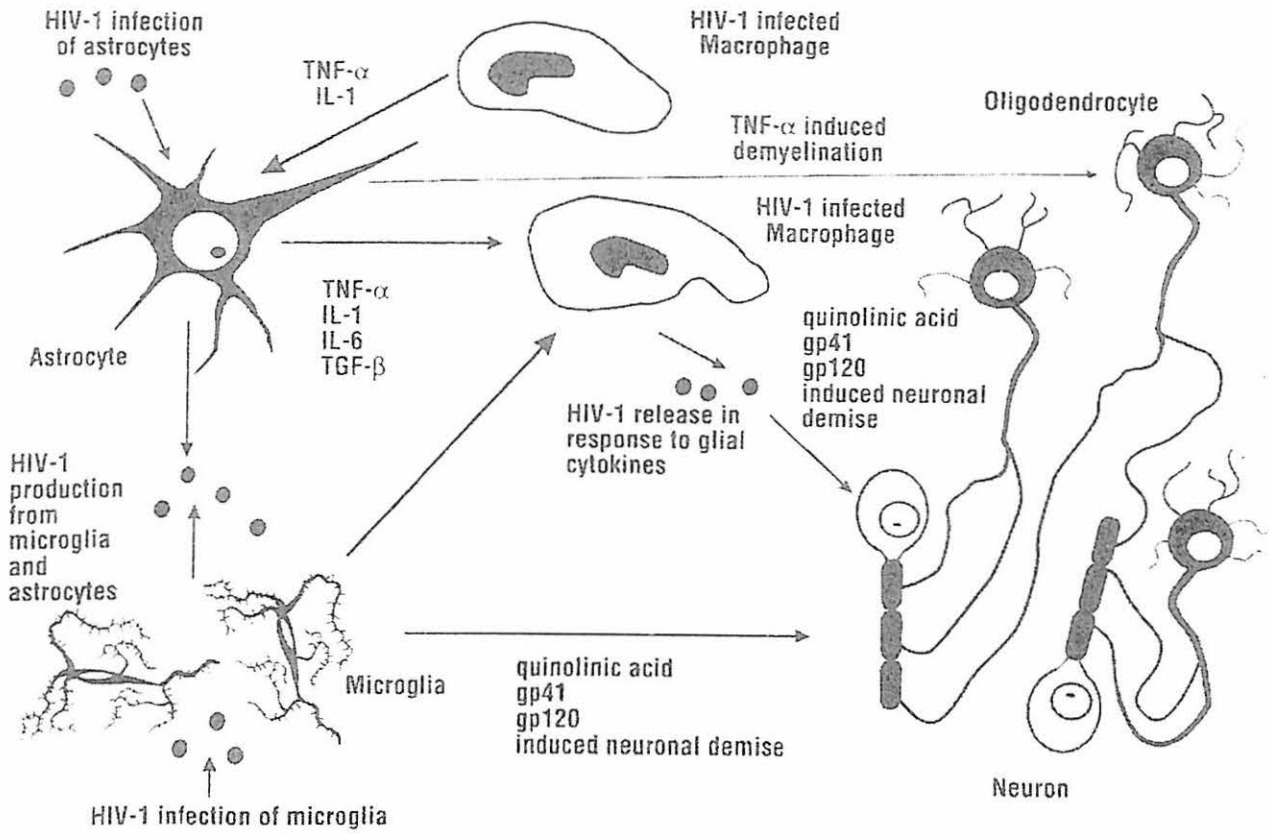

Figure 4: Proposed role of cytokines in the pathogenesis of HIV-1 infection of the nervous system

HIV-1 most likely enters the CNS in an infected macrophage. Infected microglial cells and astrocytes in the brain will release cytokines namely TNF-α, IL-1 and IL-6 which may amplify HIV-1 multiplication in macrophages. Microglia and macrophages release toxic viral components and quinolinic acid causing damage to the neurons. This cycle of immune activation within the brain parenchyma most likely leads to an increased viral burden as well as to the broad spectrum of neurological disease caused by HIV-1infection in the brain.

(From Atwood, W.J., Berger, J.R, Kaderman, R., Tornatore, C.S., and Major, E.o., Clinical Microbiol. Rev., 6, 339, 1993, Ackermann, H-W., Berthiaume, L., Tremblay, M., (2001) Viral Pathogenesis in Diagrams Textbook, CRC Press).

SECTION TWO:

LITERATURE REVIEW

2.0 Literature review

Malaysia is a country with a concentrated HIV epidemic, based on the current WHO/UNAIDS classification with HIV prevalence less than 1 percent among the general population and consistently higher than 5 percent among the IDU. As the number of persons with HIV and AIDS escalated in recent years, the management of these patients has become a significant clinical problem in many hospitals in the country. Among the many signs and symptoms that prompt these patients to the hospital, neurologic complaints are encountered routinely.

Because the highly neurotropic HIV easily crosses the blood-brain barrier, neurological complications of the virus occur in 10-30% of patients as an initial symptom and in up to 60-70% of AIDS patients during the course of the illness (CB Graham et al, 2001). Indeed, postmortem neuro-pathological series reveal that CNS abnormalities occur in up to 90% of patient with advanced AIDS (Connor et al, 2006). Neurological manifestations frequently develop when CD4 counts fall to < 200 cells/μl but it can occur during every disease stage. Certain complications and syndrome however, characteristically occur with greater frequency during certain stages of HIV disease. HIV/AIDS neurologic disorders can be classified as those caused by (1) primary HIV infection (HIV/AIDS encephalopathy), (2) secondarily by complications of immuno-suppression (infection, neoplasm and stroke), (3) complications of systemic HIV disease (metabolic and endocrinologic derangements associated with systemic HIV-related disorders and its therapies) and (4) toxic/metabolic complications of

antiretroviral and antimicrobial therapies. Neuropsychiatric problems in forms of psychosis or mood derangement can occur in advanced disease.

HIV is localized in macrophages, microglia and multineucleated giant cells found predominantly in the basal ganglia, subthalamic neucleus, substantia nigra, dentate nucleus and white matter (HIV is highly neurotropic but not neuronotropic). These cells express both CD4+ and β-chemokine receptors which permit HIV entry and mediate fusion with macrophage tropic strains. In adults, high level of HIV mRNA and expression of viral proteins in microglia and macrophages seem to correlate with the severity of clinical disease (Anita L. Belman, 2002).

HIV infection triggered neurologic autoimmune disease. Immune dysregulation can lead to "attack" on central or peripheral nervous systems results in neuronal injury and death. The most common sites are in the peripheral nervous system, i.e. nerves, nerve roots, muscle and the neuromuscular junction. HIV enter the brain early after primary infection carried into the CNS inside infected cells, macrophages and/or CD4+ T-lymphocytes or as cell-free viral particles. The movement of virus from the periphery into the brain is facilitated by immune dysfunction and structural blood brain barrier compromise. This occurs, in large measure, late in the course of disease and serves to speed the overall pathogenic process. Understanding the predilection for various sites of the neuraxis of infectious agent and tumors, layered on the HIV-induced disruption of the nervous system, will facilitate a logical approach for diagnosis. Protocols that have been proven useful in differentiating various opportunistic conditions is given in Table 2.

(Gary P. Wormser, Textbook of AIDS and Other Manifestations of HIV Infection, Fourth Edition, 2004).

The degree of immunosuppresion as defined by the CD4 cell count, determines to a large degree when individuals with HIV infection develop opportunistic infections or neoplasms. For example HIV dementia usually occur when CD4 count is below 200 cells/μl, toxoplasma encephalitis is common when CD4 is < 100-200 cells/μl, cryptococcal meningitis and CMV infection are common when CD4 < 50-100 cells/μl and progressive multifocal leucoencephalopathy (PML), primary lymphoma and herpes zoster are common when CD4 are < 50 cells/μl of blood. Bacterial, mycobacterial, syphilitic and non-AIDS related malignancy occurring at any CD4 count (Gary P.Wormser, Textbook of AIDS and Other Manifestations of HIV Infection, Fourth Edition 2004).

Clinically, the causes of focal central nervous system dysfunction such as tumor, toxoplasmosis, viral and bacterial infection cannot be distinguished from one another. At times even, stroke or hemorrhage can be atypical in AIDS patient. Elements from the patient's history (e.g. presence of headache, encephalopathy, focal or generalized seizures, rate of symptom progression), symptom complex to localize the site or sites of pathology, history of prior treatment, geographic and lifestyle background that would provided opportunities for acquisition of various organisms and stage of immunosuppression, help predict the diagnosis. However, features of common opportunistic infection and tumors are listed in Table 2 (Gary P. Wormser, Textbook of AIDS and Other Manifestations of HIV Infection, Fourth Edition, 2004).

Fungal, viral and mycobacterial meningoencephalitis are the most common cause of global cerebral dysfunction, and toxoplasma, lymphoma and progressive multifocal leucoencephalopathy account for the majority of focal presentation (Cohen .PT, The Aids Knowledge Based Textbook, Second Edition, Little Brown,1996). However, the causes of neurologic symptoms do not correlate closely with clinical findings. A patient's symptoms and signs may suggest focal process (focal motor or sensory signs), diffuse disease (confusion, decreased consciousness) or a meningeal component. The symptom complexes are often mixed or nonspecific. But, once a focal syndrome is suspected, even in a patient with a currently non-focal neurologic exam, radiologic evaluation using contrast enhancement is required. Radiological evaluation fall into two major categories: (1) structural: CT scan or Magnetic Resonance Imaging (MRI) and (2) functional: single photon emission computed tomography, positron emission tomography (PET scan), magnetic resonance spectroscopy and functional MRI.

The effectiveness of CT scan in detection of CNS disease in patients with AIDS has been well established in literature. The CT findings also have correlated well with the pathologic findings (M. Judith et al, 1986). Certain diseases especially of the white matter, however, have remained diagnostically elusive on CT scans in AIDS patients. In addition, meningeal diseases also often have not easily visualized on CT scans. Diffuse and infiltrative lesions such as CMV encephalitis and PML are difficult to diagnose by CT scan. Though CT scan or MRI studies lacked absolute specificity, CECT scan allowed better characterization of lesion with better spatial resolution, where as MR images demonstrated higher contrast resolution and were more sensitive

for use in detecting CNS lesions, thereby enabling earlier diagnosis. The greater sensitivity of MR imaging was especially apparent in detection of white matter lesions, lesion located at the periphery of corticomedullary junction, lesion at the base of skull and in demonstration of small lesions surrounded by edema (M. Judith et al, 1986). It was recommended that in centers with onsite MR units, MRI be used as the initial screening procedure in AIDS patient with neurologic symptoms. However, in Malaysia, many hospitals only have CT scanner unit, thus it is the most widely accepted as the primary radiographic diagnostic tool for AIDS patient with neurological manifestations.

De La Paz and Enzman summarized CT findings of nine different series that studied a total of 443 AIDS patient with neurologic complaints. CT was normal in 29% (range 10% – 40%), atrophy was present in 33% (range 22%-50%) and focal intracranial lesions were present in 38% (range 26%-53%). In patient with non focal symptoms such as headache, altered mental status or seizures, lesion appeared in 22% of scans (Elizabeth et al, 1993).

Although neurologic dysfunction definitely corresponds to the area of brain involved, the diagnosis may be difficult in patient with multiple lesions, simultaneous involvement of multiple areas of neuraxis such as brain and spinal cord or focal lesion superimposed on HIV dementia, neuropathy or metabolic encephalopathy. Additional problems interpreting neurologic signs emerge when patient's mental status is depressed by drug use, increased intracranial pressure or herniation, seizure activity or metabolic dysfunction. However, it is worth trying to localize systematically, especially when radiologic support is unavailable or clinical response will be used to follow

treatment efficacy. Cortical lesions cause dysfunction of higher cognitive process such as aphasia with difficulty producing speech if lesions are anteriorly located in the frontal lobe (Broca's area) or difficulty comprehending language if lesion is posteriorly located in the temporal lobe (Wernicke's area). Bilateral temporal lobe involvement leads to problem of storing or retrieving memory. If the occipital lobes are affected, a contra lateral loss of vision in the hemi field will be found. If the deeper areas of the hemisphere are involved, often in cases of toxoplasmosis or lymphoma, contra lateral weakness, tremor, rigidity or hemiballismus may occur. Cerebellar and brain stem involvement cause cranial nerve abnormalities such as dizziness or vertigo, diplopia, nystagmus, dysarthria and dysphagia, with intention tremor or in coordination and severe limb weakness or numbness opposite to the facial weakness (Gary P. Wormser, Textbook of AIDS and Other Manifestations of HIV Infection, Fourth Edition 2004).

The radiological picture is very useful in determining probable etiology. The organisms that have been reported in literature to cause encephalitis, brain abscess, or meningitis in AIDS patients include *Toxoplasma gondii*, CMV, *Papova* virus, *Candida albicans*, *Mycobacterium tuberculosis, Cryptococcus neoformans* and various pyogenic bacteria. The tumors include either primary lymphoma or Kaposi sarcoma. Opportunistic infections predominate, with toxoplasma encephalitis being the most frequent disease affecting the CNS. In Malaysia , a study conducted in Hospital Kuala Lumpur (HKL) in May 2001 found out that, the seroprevalence of toxoplasmosis was 51.2% (V. Nissapatorn et all, 2003). This seroprevalence even was much higher compared to other studies eg.15-37% in France, 21% in Malaysia, 22.45 in Thailand or 10-40% in USA (V. Nissapatorn et all, 2003).

CT picture may be quite variable because many different parts of the brain can be affected simultaneously by a single causative organism, or multiple infections of various etiologies can occur simultaneously in a background of brain changes as the result of the human immunodeficiency virus itself. Because the neuroradiologic findings are often confusing and non-specific, it is particularly important to correlate with serological findings for appropriate clinical diagnosis. Ring-enhancement is present in cases of lymphoma or other malignancy, toxoplasma or rarely in mycobacterial, bacterial or fungal abscesses. The latter infections may appear in the cortex or deeper, while lymphoma or toxoplasma encephalitis occur singly or in multiple locations often in basal ganglia, gray-white matter junction or deep white matter. Usually pronounced edema is seen, and the degree of enhancement is not different in tumor or infection. Viral infections cause enhancement only in cases of CMV which is located periventricularly. CMV however has become uncommon in the era of HAART. PML appear as single lesion in 10% of patients often in the posterior fossa (cerebellum or brainstem), and rarely enhances (Gary P. Wormser, Textbook of AIDS and Other Manifestations of HIV Infection, Fourth Edition, 2004).

The spectrum of CT appearances in Toxoplasmosis includes normal CT scans as well as nonspecific findings of atrophy and white matter area of low attenuation. Otherwise, toxoplasmosis characteristically appears as solitary or multiple iso- or hypodense lesions with ring- or nodular-enhancement pattern in contrasted scan. The ring enhancement is usually smooth but may be thick and irregular especially in larger lesion Porter et al found only 10% of lesions to be non-enhancing on a CT scan. Among

the rest, 82% were peripherally enhancing (Mathew MJ, Chandy MJ, 1999). The lack of contrast enhancement may be due to the paucity of inflammation or less vigorous peripheral vascular proliferation. The median number of lesions detected in CT scan was 2 and in about 27% of patients the lesion was solitary (Mathew MJ, Chandy MJ, 1999).

Weisberg and co-workers, 1988 also has described finding of a hypodense lesion with a central slightly hyperdense non-calcified region which showed homogenous nodular enhancement as quite characteristic of cerebral toxoplasmosis. Lesions usually located at corticomedullary junction and frequently affect the basal ganglia (75-88%). However, the subcortical lesions are most common (Wong.J, Quint.D.J, 1999). Weisberg and his colleagues (1988) also described that the lesions were more commonly located in the cerebral hemispheres and subcortical gray matter nuclear masses (thalamus, basal ganglia). Imaging characteristic suggestive of toxoplasmosis include a subcortical location of lesions, multiplicity of lesions, a uniform wall thickness less than 3 mm and marked vasogenic edema (Wong.J, Quint.D.J, 1999). Though multiple lesions are typical, a solitary lesion occurs in nearly one third of patients (Wong.J, Quint.D.J, 1999). The size of the lesion usually less than 1 cm to over 3 cm associated with surrounding mass effect and edema of variable degree (Scott W Atlas, 2002)..

Double dose delayed technique (using 200 ml of intravenous contrast by bolus or drip infusion with delayed scanning at 1 hour) has been found to be extremely effective in detection of these lesions as it permits maximal enhancement. The central portion of

ring lesions of toxoplasma may fill in on delayed scans. Dina, T.S, 1991 also found delayed double-dose contrast material-enhanced CT increases the detection rate and conspicuity of lesions.

On MRI, lesions are hypointense on T_1-weighted, hyperintense on T_2-weighted, occasionally hemorrhagic on T_1-weighted images with similar ring or nodular enhancement as seen on CT scan. 'Bull-eyes' lesions may be seen, representing successive expansion and contraction of necrotic focus with interruption of therapy (Schwartzman. J. D, 2001). Response to therapy observed by imaging studies is slower than the clinical response, taking up to 3 weeks to be evident. Complete resolution of lesions may take up to 6 months and small residua persist from large lesions may calcify (Schwartzman. J. D, 2001).

Because seropositivity for toxoplasmosis is so wide spread, a positive titer is non-diagnostic, only indicating past or recent exposure. A negative titer in a patient with a mass lesion of the CNS should arouse suspicion of other possible etiologies. But up to 22% of AIDS patient with toxoplasma encephalitis may not have detectable anti-toxoplasma IgG antibodies (Scott W Atlas, 2002).

Other pathology may mimic toxoplasmosis, such as CNS lymphoma, metastasis, cryptococcosis, tuberculoma and bacterial infection. Among all, CNS lymphoma especially primary form was the most difficult to be differentiated with toxoplasmosis based on imaging findings alone. CT appearance of primary CNS lymphoma in AIDS patients vary; some authors describe focal lymphoma mass lesions as being similar to