DETERMINATION OF JC VIRUS NON-CODING CONTROL REGION VARIANTS IN CEREBROLSPINAL FLUID AND URINE OF ADULT PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY PATIENTS AT THE UNIVERSITYTEACHING HOSPITAL

LUSAKA, ZAMBIA.

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DECLARATION

This work/dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma in this or any tertiary institution, and is not being submitted for a degree or diploma in any tertiary institution or for another degree or diploma at this institution. I declare that this Dissertation contains my own work except where specifically acknowledged. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Masters of Sciences in medical microbiology, University of Zambia guidelines.

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CERTIFICATE OF APPROVAL

The University of Zambia approves this Dissertation on "DETERMINATION OF JC VIRUS NON CODING CONTROL REGION VARIANTS IN CEREBROLSPINAL FLUID AND URINE OF ADULT PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATY PATIENTS AT THE UNIVERSITY TEACHING HOSPITAL".

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ABBREVIATIONS

Ag	:	Antigen
AIDS	:	Acquired Immune Deficiency Syndrome
CNS	:	Central Nervous System
CSF	:	Cerebrospinal Fluid
DNA	:	Deoxyribonucleic Acid
HFB	:	Human Fetal Brain
HIV	:	Human Immune Deficiency virus
JC virus	:	John Cunningham Virus
MS	:	Multiple Sclerosis
NCCR	:	Noncoding Control Region
PCR	:	Polymerase Chain Reaction
PML	:	Progressive Multifocal Leukoencephalopathy
UNZA	:	University of Zambia
UTH	:	University Teaching Hospital
VP1	:	Viral Protein 1

ABSTRACT

Background: John Cunningham virus (JC virus) is a polyomavirus that causes Progressive Multifocal Leukoencephalopathy (PML) a debilitating and often fatal disease of the central nervous system. JC virus is ubiquitous, approximately 50% of adults globally have antibodies against the virus and about 30% actively shed the virus in urine. PML develops in patients with immunological impairment such as those infected with Human Immunodeficiency Virus (HIV), patients with hematological malignancies and recipients of immunosuppressive drugs. JC virus has two variants archetype and rearranged which significantly play a critical role in the development of PML. This study was carried out to determine the JC virus variants present in cerebrospinal fluid (CSF) and urine of adult PML patients at the University Teaching Hospital.

Methodology: This was a cross-sectional study of adult patients at the University Teaching Hospital (UTH) who presented with symptoms of central nervous system infections requiring a lumbar puncture and met the inclusion criteria. A total of 326 patients who submitted urine and blood samples besides the CSF were enrolled. Polymerase chain reaction (PCR) to detect JC virus was done on all the CSF samples using the Corbett rotorgene thermal cycler. A second PCR on urine and CSF samples was done on all JC virus positive CSF using primers that detect JC virus archetype variant.

Results: Two hundred and seventy five (275) of the 326 were human immunodeficiency virus HIV antibody positive. Five (5) of the sampled CSF had detectable JC virus DNA. All the 5 JC virus from CSF had rearranged NCCR. Four out of the 5 (80%) had detectable JC virus DNA in urine 3 of which were archetype and 1 was rearranged NCCR. The median CD4 was 108.2 cells/ mm^3.

Conclusion: These findings suggest a much lower prevalence of JC virus compared to Europe and North America where seropositivity is about 50%. The study underlies and further implicates genetic rearrangements of the JC virus being responsible for the neuropathogenesis of JC virus in immunocompromised individuals especially those with HIV or AIDS disease.

Key words: *progressive multifocal leukoncephalopathy, JCV virus, archetype and rearranged*

1.0 NTRODUCTION

1.1 BACKGROUND

John Cunningham virus (JC virus) is a human polyomavirus that causes Progressive Multifocal Leukoencephalopathy (PML), a debilitating often fatal demyelinating disease which is characterized by progressive neurological dysfunction associated with multiple foci in the brain resulting from viral lysis of oligodendrocytes (Astrom *et al.,* 1958). PML is a rare disease which occurs following immunosuppression resulting from Human Immunodeficiency Virus (HIV) infection, hematological malignancies, organ transplant, and recipients of immunosuppressive and immunomodulatory therapy for treatment of inflammatory and autoimmune disease such as multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythromatous (SLE) and Crohns Disease (Ferenczy *et al.,* 2012). The incidence of PML differs depending on the underlying cause of immunosuppression. The incidence in HIV infected individuals is 3-4%, while that of patients on immunomodulatory therapy is approximately 1% (Major *et al*., 2010).

PML diagnosis is confirmed by detection of JC virus deoxyribonucleic acid (DNA) in cerebral spinal fluid (CSF) or by presence of demyelination, astrogiliosis, viral inclusions nuclei of oligodendrocytes, and lipid laden macrophages on brain biopsy.
(Berger *et al,* .2013). Although JC virus has been classically known to affect the white matter and cause PML, novel conditions have been described when it affects other areas of the brain such as cortical neurons causing JC virus encephalopathy and cerebellar granule cells causing JC virus granule cell neuronopathy (Gheuens *et al.,* 2013).

Although PML is rare, JC virus is ubiquitous .Approximately 50% of the population in the United States and Europe (west) have antibodies against JC virus with 30% actively shedding the virus in urine without any symptoms related to the disease (Boldorini *et* al., 2011).

Studies have not fully elucidated how the virus is transmitted however, the oral fecal transmission seems to be the major mode of transmission. The virus primarily infects stromal and immune cells of the upper respiratory tract cell by inhalation or ingestion, it is then trafficked to the bone marrow and kidney where it establishes latency. After immunosuppression the virus is trafficked to the brain hematogenously where it causes PML or other pathologies (Ferenczy *et al.*, 2012).

The JC virus genome is circular consisting of 5130 kilo nucleotide pairs, which consists of the conserved coding region which codes for the late and early proteins associated with viral genotypes and subtypes separated by a hypervariable noncoding control region (NCCR) also known as the regulatory region. The early protein coding region codes for various protein essential for viral transcription i.e. large T antigen (T Ag), small t (t Ag) antigen T' 135, T' 136, T'165 and agnoprotein whose function has not been fully elucidated. The late coding region codes for viral structural proteins; viral protein 3 (VP3), viral protein 2 (VP2) and viral protein 1 (VP1) which is the major structural protein and is used for genotyping the virus (Frisque *et al.,* 1984). The NCCR is hypervariable and contains the origin of replication it is thought to be the main determinant of cell type specificity and is composed of fairly well-conserved flanking regions that border the transcription start sites of the early and late coding regions, as well as a central region containing numerous transcription factor binding sites. It is thought to play a key role in determining viral neurotropism and neurovirulence. (Bellizi *et al.,*
2012).

JC virus variants based on NCCR are grouped into two groups, these are archetype CY and rearranged variants. Archetype NCCR is composed of a single copy of the 98-base pair repeat of a-c-e, with 23-base pair ("b") and 66-base pair ("d") sequence blocks between "a," "c," and "e" to yield an a-b-c-d-e structure. Deletions and duplications in the archetype NCCR result in

direct tandem repeats that give rise to the rearrangements seen in pathogenic variants The rearranged variants contains an enhancer element that exists as a 98-bp direct tandem repeat and therefore contains duplicate TATA boxes, (TATA box is a DNA sequence that indicates where genetic sequence can be read and decoded). Many studies have demonstrated that the TATA box(es) contained in the 98-base pair direct repeat structure is essential for transcription of early and late viral genes (Jensen *et al.,* 2001, Gosert *et al.,* 2010). While it is unclear in what tissues, or cells, rearrangement occurs, sufficient evidence suggests it occurs in lymphoid tissues and bone marrow (Houff *et al* 1988. Tan *et al* 2009). Rearranged JC virus variants have also a deletion of nucleotides in that'd' sequence section of the archetype NCCR (Jensen *et al,*
2001). Despite the non-coding control region been hypervariable, all JCV genotypes have a similar conserved T protein coding sequence. This is because the T protein coding sequence is essential for viral growth and its alteration results in nonviable viruses, hence the primers for detection of JC viruses are designed to target this region. (Ferenczy M.W. *et al,* 2012). The primers that detect archetype are designed to include the 'd' section nucleotide which are absent in the rearranged variants. The aim of the main aim of this study is to determine the JC virus NCCR variants in paired CSF and urine of PML patients.

1.2 STATEMENT OF THE PROBLEM

JC virus is ubiquitous with about 50% of the population in the west showing antibodies against the virus. JC virus causes PML one of the AIDS defining illness affecting about 3-5% of HIV infected patient and 1% of patients on immunomodulatory therapy (Major *et al,* 2010). The symptoms of PML are diverse, since they are related to the location and amount of damage in the brain, and may evolve over the course of several weeks to months. The most prominent symptoms are clumsiness; progressive weakness; and visual, speech, and sometimes personality changes. The mortality rate of PML is between 30-50% depending on the severity of the underlying cause of immunosuppression (Marzocchetti A.*et al,* 2009). Survivals of PML

are left with severe neurological disabilities. Currently there is no effective treatment for PML the best available therapy is reversal of the immune deficient state.

1.3 JUSTIFICATION OF THE STUDY

This was the first study to explore the circulating JC virus NCCR variants in CSF and urine of adult PML patients in Lusaka Zambia. This study provides new insights on JC virus NCCR variants in CSF and urine of PML patients in a population with a higher HIV prevalence 13% (ZHDS, 2014) compared to the west. The aim of the study was to determine the JC virus NCCR variants in CSF and urine of PML patients.

1.4 LITERATURE REVIEW

1.4.1 Global perspective

JC virus is among the most prevalent polyomaviruses affecting the human population with over 50% of adults in the west showing anti-JC virus antibody responses and up to 30% of the population displaying persistent viral shedding in the urine (Ferenczy *et al.,* 2012). Epidemiological data show that the virus establishes chronic latency infection in the kidneys of majority of the human population without any pathology. Studies have shown that factors that lead to immunosuppression can cause uncontrolled replication of the virus in carriers leading to PML and other newly discovered conditions such as JC virus granule cell neuronopathy and JC virus encephalopathy (Tan *et al.,* 2010).

Astrom *et al*., in 1958 were the first to describe PML as a complication of lymphoid malignancies. Studies done recently have also shown that there is a correlation between PML and immune suppression resulting from different factors among them hematological malignancies (Major *et al.,* 1999). Since the AIDS pandemic the number of PML cases drastically increased with about 4 of HIV patients developing PML (Major *et* al., 1999; Koralnik *et al.,* 2006). Use of immunosuppressive and immunomodulatory therapies for treating inflammatory and autoimmune diseases has also been associated with PML. Natalizumab, the immunomodulatory drug for treating multiple sclerosis, has been found to cause PML with increased dosage and exposure (Tan *et al.,* 2006).

Globally, at least 7 JC virus types have been identified based on the sequence analysis of the major capsid protein VP1. These types are numbered 1 to 8, with type 5 being found to be a minor member of type 3 (Ferenczy *et al,* 2012). In the West, type 1 and 4 are the most predominant, while type 2A occurs most commonly in the Asian and Native American population. In Africans and African-Americans, type 3 and 6 are frequently seen; 2D and 7C are isolated from Asians and South Asians, and in the Western Pacific population, types 2E,

8A and 8B are found (Agotsini *et al.,* 1998, Yanagihara, *et al., 2002*). Type 8A has only been found in Papua New Guinea (Jobe *et al.,* 2001).

In a study to determine JC virus genotypes in brain tissue from patients with PML and in urine from controls without PML, Frisque *et al* found that the brain tissues from patients with PML were infected with a significantly higher proportion of JC virus type 2 strains than were urine samples from the control group. Their study showed evidence indicating a biologic difference between JC virus genotypes and suggests a difference in their potential to cause PML (Frisque *et al,* 1999). Data from this studies indicate that there is variation in the distribution of JC virus genotypes and that this can affect the development of PML.

In Poland Kmieciak *et al* surveyed the occurrence rate and genotype distribution of JC virus variants in healthy Polish donors and found that the distribution of the virus in the polish population resembles that seen in other European countries, with the most abundant being genotype 1. However, genotype 4 which is the second most frequent genotype was not detected. Sequence analysis of the VP1 gene fragment revealed the following distribution of JC virus genotypes in the investigated groups: 1A, 1B, 2A, 2; and 2C. They further observed that the occurrence rate increased with age and was highest in the group above 60 years (Kmieciak *et al., 2001*).

In Northern Italy, Zanotta *et al* did a study to establish the molecular epidemiology of JC virus genotypes between immunocompromised and immunocompetent individuals. They found the overall prevalence of JC virus excretion at 32%. They detected JC virus DNA in approximately 40% of immunocompromised and 22% of immunocompetent individuals. They observed a difference in the distribution of the genotypes. Type 1 was the most prevalent genotype excreted in urine followed by genotype 2 and genotype 4. They observed a difference in the distribution of genotypes based on age and immune status (Zanotta *et al.,* 1999).

Despite JC virus having many genotypes based on the VP1 sequence, there are two types JC virus variants based on the NCCR region. These are archetype found predominantly in the urine of healthy individuals and rearranged variants seen predominantly in the brain and other tissues of PML patients (Major *et al.*, 2009). In a study of the JC virus NCCR arrangement in different biological specimens Pietropaola *et al*, results indicated the presence of different rearrangements among the analyzed biological fluids from the same patients. In the urine, they found NCCR that appeared very similar to that of the archetype, in the PBMCs and CSF, the NCCR sequences showed specific and characteristic rearrangements as compared to the archetype. They concluded that these different rearrangements could be correlated with the emerging of an NCCR organization more suitable for the development of PML Pietropaola *et al*,2009 *).*

In a study of distribution of JC virus variants in autopsy brain and extra neural organs of AIDS and non-immunocompromised individuals, Calderelli *et al* study showed that JC virus variants could be different in different body organs i.e. the brain, lung, liver, kidney, spleen and lymph nodes. They detected an almost unique variant with multiple rearrangements in the NCCR region and unusual base mutations in the VP1 region of the brain sample from another PML patient. Their finding indicated diffuse visceral involvement of JC virus was particularly frequent in AIDS patients with PML. Moreover, they concluded that the presence of rearrangements and mutations, involving different regions of the viral genome, observed in PML-affected brain tissues, could represent a risk factor for the development of PML in immunosuppressed individuals (Caldelleri *et al.*, 1999).

Pfister *et al,* did a study to determine the correlation between NCCR rearrangement in plasma and CSF to clinical outcomes in PML patient. They observed that patients with high proportion of NCCR rearrangements in plasma and CSF had poor clinical outcome. They also observed the predominance of archetype variants in patients who became survivors. They

showed an association between rearranged NCCR in plasma and CSF to poor clinical outcome (Pfisher *et al.,* 2001).

O'Neil et al, demonstrated the propagation of archetype and non-archetype variants in human brain fetal glial cells (HFB) invitro. They demonstrated interference activities of the JC virus NCCR variants when co-infected in HFB. Their study interestingly showed that archetype JC virus can be successfully propagated in HFB cells, although infection develops much more slowly than that caused by the PML JC virus prototypical variant. They concluded that the ability of archetypal and rearranged variant to enhance or retard each other's replication may have implications in vivo for the maintenance of JCV persistence and the growth of JCV variants (O'Neil F.J.*et al.*, 2009).

1.4.2 Regional perspective

Although JC virus is thought to be widely distributed globally there is paucity of data on its distribution in Africa, therefore it is not clear whether PML is less frequent in Africa than in the West. Stoner *et al,* reported and confirmed cases of PML in the central nervous system (CNS) of an AIDS patient in Uganda using immunocytochemistry and PCR. Stoners group reported type 3 JC virus by sequencing brain tissue of a Gambian patient who died 3 months after the onset of AIDS/ and PML in Germany. They observed that the non-coding control region had rearrangements that could be distinguished at several sites from East African and the African American JC virus strains. Notably their study showed that JCV type 3 is pathogenic in African AIDS patients. Furthermore, the finding of an African genotype in a patient who died in Germany months later suggests that this individual represented a latent JC virus infection acquired in Africa. (Stoner *et al,* 1998). In another study done in Ivory Coast the prevalence of PML in an autopsy series was 1.5% among HIV infected (Lucas *et al,* 1993).

In Zambia Siddiqi *et al*, confirmed the first cases of PML in Zambia. In the study of CNS opportunistic infections in HIV-infected Zambian adults at University Teaching Hospital, they detected JCV DNA in 20/331 (6%) of CSF samples of HIV-infected Zambians presenting with signs and symptoms consistent with CNS infection. In the Cohort Study of HIV-associated Seizures and Epilepsy in at the University Teaching Hospital in Zambia, Siddiqi et al detected JCV in 4/95 (4%) of HIV^{+} patients with new onset seizure had JCV DNA detected in the CSF (Siddiqi *et al*,2012).

As can be noted many studies done in the West have shown that the virus is ubiquitous but PML is a rare disease with survival highly depended on the variant present.

1.5 RESEARCH QUESTION

Which JC virus NCCR variants are present in the CSF and urine of adult PML patients in Zambia?

2.0 OBJECTIVES

2.1 GENERAL OBJECTIVE

- To determine the JC virus variants prevalent in the CSF and urine of PML patients at the University Teaching Hospital.

2.2 SPECIFIC OBJECTIVES

1. To determine the incidence of PML in adults HIV patients at UTH
2. To determine the JC virus NCCR variants in the CSF of PML patients at UTH
3. Determine JC virus NCCR variants in the urine of PML patients at UTH

3.0 MATERIALS AND METHODS

3.1 STUDY TYPE

This was a cross-sectional study.

3.2 STUDY SETTINGS

Patients were enrolled from the adult medical emergency unit and medical inpatient wards at the University Teaching Hospital (UTH). PCR studies were performed in the Department of Internal Medicine research labs at UTH.

3.3 STUDY POPULATION

Study subjects were black Zambians who were recruited from a larger study at University Teaching Hospital.

3.4 ELIGIBILITY CRITERIA

3.4.1 Inclusion criteria

Specific inclusion criteria were as follows: adults (≥ 18 y) presenting to UTH with meningismus, seizure, altered consciousness, headache, or focal neurological deficit who received a lumbar puncture for a suspected central nervous system infection.

3.4.2 Exclusion criteria

Exclusion criteria were: patients < 18 years or contraindications to lumbar puncture: space-occupying lesion of the posterior fossa, bleeding diathesis, vertebral deformities, and infection at the LP site.

3.5 SAMPLE SIZE

Three hundred and twenty six male and female who met the inclusion criteria where sampled.

3.6 SAMPLE PREPARATION

CSF samples from inpatient wards were sent to the UTH microbiology laboratory where CSF cell count, microscopy, cryptococcal antigen test and culture were done. Only patients whose CSF was cryptococcal antigen negative were considered for enrollment in the study. After consent was obtained the study nurse collected venous blood and urine from the patients. Blood was immediately sent to ZAMBART were CD4 enumeration was done which using BD facs count at Zambia AIDS Related Tuberculosis (ZAMBART) laboratory. The urine was stored at -20 degree Celsius awaiting DNA extraction. CSF was stored at 2-8 degrees Celsius in the microbiology laboratory and at -20 degrees Celsius after a day to two.

3.7 DNA EXTRACTION

DNA from CSF and urine was extracted in a fume hood with a strict adherence to general laboratory guidelines for safety and prevention of cross-contamination; by the Qiagen mini elute kit method adapted from Qiagen. The first stage was lysis which was achieved by adding 25 µl of Qiagen protease to 200ul of sample and 200 µl of lysing buffer called buffer AL in an eppendorf tube which was vortexed for 15 seconds and spun for 5 seconds. This was then incubated at 56 degrees Celsius for 15 minutes. The tube was then removed from the heating block and spun in a micro centrifuge for 5 seconds. 250 µl of 100 percent alcohol was added to the eppendorf tube and incubated for 5 minutes at room temperature after vortexing and spinning for 15 and 5 seconds respectively. The lysate was then transferred into the elution column and spun at 8000 RPM for 1 minute. The elution column was the put in a clean collection tube and 500 µl of wash buffer AW1 was added and incubated at room temperature for 1 minute and spun at 8000 RPM for 1 minute. The elution column was then put in a clean collection tube and the washing step repeated with wash buffer AW2. After the second wash 500 µl of pure alcohol was added and spin the column at 8000 RPM for a minute and spin again at 14000 RPM for 3 minutes.

200 µl of elution buffer was added to the column placed in a new clean labelled Eppendorf tube and spun at 14000 RPM for 1 minute. The collected DNA was then stored at ¯20 degrees Celsius.

3.8 DNA AMPLIFICATION

Real Time PCR amplification targeting a JCV T antigen coding sequence was performed using Rotor gene 6000, software 1.7 (R-040719, Corbett Research; Australia). The primer sets used were:-

JCT-1:5'-AGAGTGTTGGGATCCTGTGTTTT-3'

JCT-2:5'-GAGAAGTGGGATGAAGACCTGTTT-3'

JCT -probe5' CATCACTGGCAAACAT 3'.

The final mixture of 25µl comprised of a 12.5 µl of universal Master Mix consisting of Taq Polymerase, MgCl2, and dNTPs, 2.5 µl of primer probe mixing consisting of 32µl of forward primer, 32 µl of reverse primer and 2 µl of JCT probe in 134 µl of molecular grade water, and 10 µl of the template (sample) DNA. Amplification was carried out with the following conditions, an initial 50^0C hold for 2 min followed by a cycle at 95^0C for 10 min this will be followed by repeating 40 cycles of 15 seconds hold at 95^0C thereafter a 60 second hold at 60^0C acquiring of cycling A green and finally an extension cycle at 60^0C for 7 minutes. All samples were run in a duplicate manner and any disagreements between these duplicate runs were resolved using a third run that acted as a tie breaker. A positive control was included in all run. A second PCR was done on the CSF and urine of all JCT positive samples using the JRR primers with the following sequence.

JRR-1'GGAGCCCTGGCTGCAT-3',

JRR-2:5'TGTGATTAAGGACTATGGGAGG-3'

Probe 5' CTGGCAGTTATAGTGAAACC 3'

For detecting JCV archetype variant with the same cycling conditions.

3.9 DATA ANALYSIS

Descriptive statistics (mean) was used to analyze the data.

3.10 ETHICAL CONSIDERATION AND PERMISSIONS

Permission to conduct the study was sought from the UTH Senior Medical Superintendent and Permission to use equipment and facilities of Internal Medicine was sought from the head of the research group in the UTH was sought from the Head of the Department of Pathology, and Microbiology at UTH and permission to use the equipment and laboratory facilities at ZAMBART was sought from the laboratory manager

The lumbar puncture was a routine clinical procedure. Only when there was excessive CSF where patients followed for possible enrollment. Patient information and results were kept confidential and access to this information was restricted to the researcher and supervisors only. Enrolled patients were assigned a study number hence patients were identified by study numbers.

The study participants were provided with an information sheet and given a thorough explanation and rationale of the research after which the participants provided written informed consent without duress. All the above mentioned was done in private on a one to one basis to avoid undue influence that may have affected or substituted the patient's will for that of any other persons. The University of Zambia Biomedical Research Ethics Committee (UNZA-BREC) approval was (REF. No. 006-06-14).

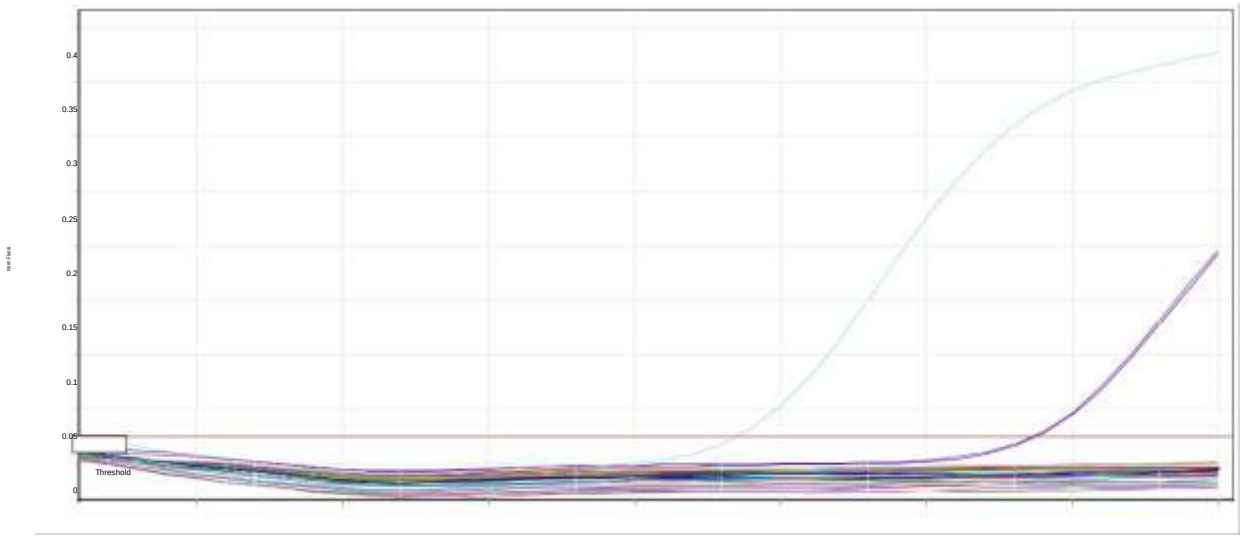
4.0 RESULTS

Three hundred and twenty six (326) CSF samples were analyzed for the presence of JC virus DNA. 169 were males and 157 were female. 275 (84.4 %) were reactive for HIV antibodies while 51 (15.6 %) were non-reactive. 5 (1.8 %) CSF samples where positive for JC virus. All the JC virus in CSF had rearranged NCCR none of the five JC virus DNA positive CSF was positive for archetype NCCR (amplification using JRR primers). Four of the 5 CSF JC virus DNA positive urines where positive for JC virus DNA using JCT primers. And three where positive for JC virus archetype (amplification using JRR primers). All patients that had JC virus DNA in the CSF were HIV-infected with a median CD4 count of 108 cells/ml^3. The mean age among PML patients was 39.

Figure 1: JC virus distribution by gender

GENDER	JC VIRUS DNA DETECTABLE
MALE	3
FEMALE	2

Figure 1: Representative Amplification Plot



5.0 DISCUSSION

PML occurs following immunosuppression which results from many factors, the incidence is highest in HIV-infected individuals compared to other causes of immunosuppression. Studies have shown that PML patients have CD4 count of less than 200cells/ml^3 (Gheuens *et al*, 2011). With a CD4 lower than 200 the ability to mount a formidable cellular immune response is compromised. The incidence of PML among HIV patients in this study was at 1.81%. Although the incidence is comparable to other studies done in different parts of Africa (Lucas *et al*, 1993), the incidence is less than the 6% previously reported in Zambia (Siddiqi *et al*, 2012). The reduction in the incidence of PML as seen by the reduction in the detectable JC virus DNA in CSF could be attributed to many factors which include improved use of highly active antiretroviral drugs which has been shown to have tremendous effect in halting HIV viral replication and improving host immunity thereby providing improved defence against these opportunistic infections (Engsig *et al*, 2009).

The incidence of PML at UTH in HIV infected adults (1.81%) is lower to that seen in the West which is approximately 4-5% in HIV infected individuals despite Zambia having a population with a very high incidence of HIV (13%) that increases in the urban population (ZDHS 2014). The reasons for low PML incidence despite a higher HIV burden compared to western population could be attributed to many factors which include JC virus genotypes present in the population. Studies have shown that the incidence of PML differs with the circulating JC virus genotype a population. Agotsin *et al*, in their study showed that genotype 2B was associated with a high PML incidence than genotype 4 (Agotsin *et al.,* 2001). Studies have shown that the genotypes present in Africa are distinct from the ones found in western populations. The other reason could be the difference in the HIV clades found in African population to that of the west. The viral interaction between the Tat in the HIV clade C found in Africa and JC virus could be less potent than the interaction with clade B found predominant in the western population there by halting

viral replication and resulting in reduced JC virus viral activity. Studies have shown that HIV proteins interact with JC virus to either enhance or reduce viral activity (Ferenczy *et al,* 2012). The low incidence could also be attributed to host genetic differences. In one study that looked at genetic factors and there association to PML, the prevalence was high among white males compared to African Americans. (Schutte *et al,* 2014).

The other reason could be a low JC virus prevalence in the general population of Zambia. Although JC virus DNA has been detected in CSF of Zambian PML patients there has been no studies done in Zambia to determine the seroepidemiology of the virus therefore it is not clear if JC virus is highly prevalent in Zambia than the west. The other reason in the reduction of the incidence could be the high burden of other opportunistic infections such us tuberculosis which causes death at higher CD4 counts before PML develops.

The JC virus variant detected in CSF was pathogenic/rearranged NCCR. Although studies have shown that the archetype variant can be propagated in human fetal brain cells and some have detected archetype DNA in the CSF and brain tissue of PML patients, the Rearranged variant has been predominantly detected in CSF(O'Neil *et al.,* 2009) (Newman *et al,* 1999).The NCCR contains an enhancer element that exists as a 98-bp direct tandem repeat and therefore contains duplicate TATA boxes, which can position mRNA start sites, as well as multiple transcription factor binding sites which are essential for transcription of early and late viral genes (Frisque *et a.,l*1983). The rearranged NCCR variants from PML patients confer increased early gene expression and higher replication rates compared to those of archetypal NCCR thereby increasing cytopathology (Rainer *et al.,* 2010). Archetype is rarely associated with PML and has been rarely detected in the CSF (Yogo *et al.,* (1990).The consistent isolation of re arranged NCCR in tissues obtained from PML patients strongly suggests the importance of this structure in viral pathogenesis (Frisque *et al.,* 1984) and (Major *et al.,* 1999).

Four out of the 5 PML patients had JC virus DNA in urine indicating viral shedding. This shows that most of the PML patients shed the virus in urine as observed by other studies. These are the ones that could possibly be transmitting the virus (Major *et al.*, 2010). Interestingly the variant in urine had 3 archetypal NCCR and one rearranged NCCR. Although few studies have indicated the presence of mixed variants (rearranged and archetypal NCCR) in urine. (Tom *et al*, 2013). Interestingly the possibility of transmitting the pathogenic NCCR it is not known, however there is need to explore the JC virus NCCR variants in healthy individuals to ascertain the prevalence of the rearranged JC virus NCCR in urine of immunocompetent individuals.

6.0 CONCLUSION

The main objective of this study was to determine the JC virus NCCR variants present in the CSF and urine of PML patients at UTH using PCR assay. Out of the 326 samples tested five (1.5%) had detectable JC virus DNA. Of the five patients with detectable JC virus DNA four (80%) had detectable JC virus DNA in urine. Three of the four JC virus detected in urine were archetypal and one had a rearranged NCCR. The study found a 1.81% prevalence of JC virus among HIV patients at UTH of which the JC virus in CSF had a rearranged NCCR and mixed variant in urine with the archetype variant been predominant. These findings suggest a much lower prevalence of JC virus compared to Europe and North Africa were the seropositivity to JC about 50%. The study underlies and further implicates genetic rearrangement in the NCCR region of the JC virus being responsible for the neuropathogenesis of the JC virus in immunocompromised individuals especially those with HIV and AIDS disease.

6.1 IMPLICATIONS AND RECCOMENDATIONS

Following the results of the study the following are the recommendation; there is need to establish the seroprevalence of JC virus in the population. There is need to genotype JC virus to ascertain circulating strains of the virus and compare them to strains from other regions. The determinants of neurotropism and neurovirulence needs to be established by genotyping the NCCR in order to fully elucidate how the virus causes pathology in the diseases. There is need for further studies to establish how HIV and JCV interact and lead to PML. There is also need for further studies to fully establish how the virus is transmitted to put up strategies on how to avoid getting infected with the virus especially for people who are at risk of developing PML.

Finally there is need for extensive studies to determine predictor of PML and identify people at risk of developing the disease.

6.2 LIMITATION / WEAKNESS

The positive control for JRR (archetype) was selected among samples there is need to use a commercially made control in order to pick low positives. This study looked at adults only and did not include children who are also known to have PML.

6.3 FUTURE DIRECTION

In future we intend to carry out a JCV prevalence survey in the general population that includes immunocompetent and immunocompromised individuals. We also intend to genotype the JCV variants to ascertain the prevalent genotypes.

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APPENDICES

Appendix I: Information Sheet

CSF Testing for TB and JCV in Zambia

- You have already had a test to remove spinal fluid from your back because your doctors want to test whether you have an infection of your brain.
- Dr. Omar Siddiqi and other doctors are performing a study on the fluid surrounding your brain to check for other infections that are not normally tested for by the hospital. These include tests for tuberculosis (TB) and infection called JC virus.
- If you agree to participate in this study we will test for TB and JC virus using spinal fluid that is left over from the regular testing.
- We would also like to take a sample of your blood and urine to see if you have signs of either of these infections in your body.
- There is no additional risk to you for participating.
- The testing will be performed in the future and may provide no extra benefit to you but the results of this study may improve the diagnosis of infections of the brain and spinal cord in the future.
- We may also review your medical record in the future for details related to your current presentation.
- Your personal information will not be shared with anyone outside of the research study. The information we collect from you will be stored on two password protected computers and in a locked file cabinet in the research study offices.
- To protect your privacy all of the samples we collect will be specially coded so that your name cannot be identified from the sample.
- You do not have to participate in this study.
- Your participation is voluntary and you will still receive medical care if you do not want to be a part of the study.
- Due to you your presentation, you may be offered HIV testing if you have not had this already. This is up to your treating doctors and is not a requirement for this study.

If you have any questions about this interview, you can contact any of the following people:

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Appendix II: Consent Form

"I voluntarily agree to participate in this study, to allow additional testing for infections on my leftover spinal fluid as well as providing a sample of blood and urine. I also agree to have my clinic records periodically reviewed by this research program"

……..	……..
Subject (print name)	Interview witness (print name)
……	……
Subject signature or thumb print	Interview Witness (signature)
…….	……..
Date signed	Date signed

Phone numbers:

.....

Please keep a copy of this form for your records

Appendix III: Data Collection Form

CSF:

Glucose_____ Protein _____ WBC_____ RBC _____

Gram stain_____ Diff _____

India Ink _____ CrAg _____ Culture _____

CSF JC virus _____ (Pos/Neg)

CSF JC virus variant _____

Urine JC virus variant _____