

Original Research Article

**SEROPREVALENCE OF HUMAN
CYTOMEGALOVIRUS AND RUBELLA VIRUS
ANTIBODIES AMONG ANTI-RETROVIRAL NAIVE HIV
PATIENTS IN LAGOS.**

ABSTRACT

BACKGROUND / AIMS: Opportunistic infections such as Cytomegalovirus and Rubella virus that pose no threat to healthy individuals can be life threatening in those with impaired immune systems. The aim of this study was to determine the sero-prevalence of human Cytomegalovirus (CMV) and Rubella Immunoglobulin M and G (IgM and IgG) antibodies among anti-retroviral naive patients in Lagos.

STUDY DESIGN: This is a cross sectional study.

PLACE AND DURATION OF STUDY: AIDS Prevention Initiative in Nigeria (APIN) clinic in Lagos University Teaching Hospital (LUTH) between April 2011 and May 2012.

METHODOLOGY: The study was investigated in 80 (28 males and 52 females) HIV infected adults attending the AIDS Prevention Initiative in Nigeria (APIN) clinic in Lagos University Teaching Hospital and the patients were aged between 18 and 60 years. IgG and IgM assay were performed using ELISA reagents (produced by Biotec laboratories, United Kingdom). Also, CD4+ cell counts were evaluated.

RESULTS: Seventy –four patients (92.5%) were positive for CMV IgM and fifty four patients (67.5%) were positive for CMV IgG. Also, 70 patients (87.5%) were positive for Rubella IgG and only one patient was positive for Rubella IgM. Majority of the patients (82.5%) had CD4+ counts that ranged between 1 and 400 cells/ μ l, 12 patients (15%) had CD4+ counts that ranged between 401 and 600 cells/ μ l while 2 patients (2.5%) had CD4+ counts greater than 600.

CONCLUSION: This study showed that the sero-prevalence of CMV and Rubella virus is high among anti-retroviral naive HIV patients in Lagos, Nigeria. This suggests a strong association between HIV, Cytomegalovirus and Rubella virus infections in this environment and should be a vital consideration in the initiation and choice of antiretroviral therapy.

Keywords: Cytomegalovirus; Rubella; Seroprevalence; Naive; Anti-retroviral; HIV; APIN

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). The virus attacks the cells of the immune system, particularly the CD4+ T lymphocytes depleting them from circulation thereby exposing the patient to a myriad of opportunistic infections [1]. In addition, infections that pose no threat to normal people can cause severe, even fatal disease in those with impaired immune systems [2].

Opportunistic Infections (OIs) have been recognized as common complications of HIV infection due to immune deficiency. OI is the main reason behind hospitalization and substantial morbidity in HIV infected patients [3]. It necessitates toxic and expensive therapies and reduces the expected life span of such patients. Virtually all HIV-related mortality is preceded by opportunistic infection [4].

49 Opportunistic Infections (OIs) encompass a wide variety of microorganisms that produce series of
50 infections in immunocompromised HIV seropositive patients. Viral pathogens causing OI evoke a
51 spectrum of illness ranging from asymptomatic to severe diseases in HIV infected individuals. Since
52 the onset of the acquired immunodeficiency syndrome (AIDS) epidemic in 1980; human herpes
53 viruses (HHV) have resulted in many of the secondary manifestations of human immunodeficiency
54 virus (HIV) infection such as painful rash caused by Herpes Zoster [5, 6].

55 Opportunistic Infections may be caused by bacteria, viruses, fungi and parasites that are normally
56 controlled by the immune system [7]. However the primary causes of death from HIV/AIDS are
57 opportunistic infections and cancer both of which are frequently as a result of the progressive failure
58 of the immune system [8, 9]

59 Human Cytomegalovirus (CMV) infection is typically unnoticed in healthy people, but can be life-
60 threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients,
61 or new born infants [10]. Cytomegalovirus is a common cause of blindness in AIDS patients and also
62 causes hydrops fetalis in infants and spreads readily in day care centers [2].

63 Rubella virus is typically a mild, often unrecognized disease that is difficult to diagnose but infection of
64 pregnant women can have tragic consequences [2]. HIV patients who are negative to Rubella IgG
65 antibodies face risk of infection which may be critical especially amongst women of child bearing age.
66 The primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face
67 which spreads to the trunk and limbs and usually fades after three days. Other symptoms include low
68 grade fever, swollen glands, joint pains, headache and conjunctivitis [11].

69 Considering the importance of human Cytomegalovirus and Rubella virus and their complications in
70 immunocompromised individuals, it seems necessary to investigate the seroprevalence of these
71 viruses among the anti-retroviral naive HIV patients. This will help medical personnel in making
72 informed decisions on the control or prevention of opportunistic infections that can arise from these
73 viruses and also in prescription of anti retro viral therapy to be used by the patients at the point of
74 care. Hence, this study was aimed at determining the seroprevalence of human Cytomegalovirus
75 (CMV) and Rubella virus antibodies in anti-retroviral naive adult HIV patients attending the AIDS
76 Prevention Initiative in Nigeria (APIN) clinic in Lagos.

77

78

79

80

81

82 **METHODOLOGY**

83 **STUDY DESIGN, POPULATION AND SETTING**

84 The research was carried out between April 2011 and May, 2012 at the AIDS Prevention Initiative in
85 Nigeria (APIN) clinic located at the Lagos University Teaching Hospital LUTH, Idi-araba, Lagos-state.
86 Apin is a non-governmental initiative jointly funded by Bill and Melinda Gates foundation and
87 Presidential Emergency Programme for Aids Reduction (PEPFAR) to cater for people living with HIV
88 by way of counselling, testing, care as well as anti-retroviral treatment. The enrolment of new patients
89 at this clinic is estimated to be 30 per week. The study group comprised of eighty patients, 28 males
90 and 52 females who had not started anti-retro viral therapy.

91 ELIGIBILITY CRITERIA

92 Patients between the ages of 18 and 60 years who had been previously screened at the clinic and
93 confirmed to be HIV positive and had not started receiving anti- retroviral therapy were recruited into
94 the study after their informed consent was sought. Standard questionnaires were also administered
95 on them.

96 SCREENING OF PATIENTS

97 Individuals that presented themselves for retroviral screening at the APIN clinic, upon counselling
98 were screened using the Alere Determine HIV 1 /2 test kit. Those that tested negative were given post
99 test counselling and discharged while those that tested positive were retested using western blot. This
100 testing was done by highly trained APIN staff.

101 LABORATORY METHODS

102 5ml of venous blood was drawn from each of the patients that had met the inclusion criteria into a
103 plain vacutainer bottle that was properly labeled. The samples were allowed to stand for an hour, after
104 which were spinned at medium speed (2500 revolution per minute) of the centrifuge. The resulting
105 sera were transferred into another sterile plain vacutainer bottles for viral assay and stored at -20°C till
106 they were assayed. A total of eighty sera samples were collected from 28 males and 52 females.

107 The reagent used for Rubella Immunoglobulin M and G (IgM and IgG) antibody detection in the
108 patients' sera was the rubella IgM and IgG fast EIA KIT manufactured by Biotec laboratories, United
109 Kingdom. While the kits used for Cytomegalovirus IgM and IgG antibody detection in the patients'
110 sera was the Cytomegalovirus IgM and IgG fast EIA KIT also manufactured by Biotec laboratories,
111 United Kingdom. The kits detect antibodies based on the ELISA principle (Nester *et al.*, 1998) which
112 has been shown to be a sensitive and reliable procedure for antibody detection.

113 Briefly, the patients' sera were diluted based on the manufacturer's instructions and added to 82
114 microwells (80 samples and 2 controls) previously coated with purified Rubella and CMV antigen. All
115 unbound materials were washed away using the washing buffer. The enzyme conjugate was then
116 added and the plate was incubated to allow the hydrolysis of the substrate by the enzyme. The
117 intensity of the colour generated is proportional to the amount of antibody in the sample. The negative
118 and positive control for each assay were run in duplicate and it was ensured that the mean O.D
119 values for the control falls within the range stipulated by the kit manufacturers. The patients CD4+ cell
120 counts were determined using flow cytometry.

121

122

123 STATISTICAL ANALYSIS

124 The statistical package for social sciences, SPSS statistics 17.0.1 (December, 2008) was used to
125 complete all data analysis. Sero-positivity rates were calculated and compared by gender and among
126 different 10-year interval age groups. Differences were evaluated using the chi-square test with Yates
127 correction. AP-value of <0.05 was considered statistically significant.

128

129 RESULTS

130 A total of 80 anti-retroviral naive HIV patients participated in this study, and out of which 28(35%)
131 were males and the remaining 52(65%) were females. Their ages ranged between 18 and 58 years.

132 Majority of the patients studied (51.3%) were in the 28-37 years age bracket while only (1.25%) were
133 above 57 years (table 1)

134 TABLE 1: Age and Gender Distribution of 80 HIV Patients attending APIN clinic in LUTH

S/N	AGE GROUP	NO (%)	MALE (%)	FEMALE (%)
1.	18-27	14 (17.5)	1(7.1)	13(32.9)
2.	28-37	41(51.3)	10(24.4)	31(75.6)
3.	38-47	18(22.5)	11(61.1)	7(38.9)
4.	48 -57	6(7.5)	6(100)	0(0)
5.	>57	1(1.25)	0(0)	1(100)
Total		80 (100)	28 (35)	52 (65)

135

136 Seventy- four patients (92.5%) were positive for CMV IgM. Fifty of them were female while 24 were
137 male. Fifty four patients (67.5%) comprising of 19 males and 35 females were tested positive for CMV
138 IgG, while only one male (1.25%) was tested positive for Rubella IgM. However, 70 patients (87.5%)
139 made up of 46 females and 24 males were positive for Rubella IgG (table 2)

140

141 Table 2: Prevalence of Cytomegalovirus and Rubella virus antibodies as it relates to gender among
142 80 HIV patients in LUTH

CYTOMEGALOVIRUS	RUBELLA
-----------------	---------

Gender	IgM+	IgM-	IgG+	IgG-	IgM+	IgM-	IgG+	IgG-
Male	24(32.4)	4(66.7)	19(35.2)	9(34.6)	1(100)	79(100)	24(34.3)	4(40)
Female	50(67.6)	2(33.3)	35(64.8)	17(65.4)	0(0)	0(0)	46(65.7)	6(60)
Total	74(92.5)	6(7.5)	54(67.5)	26(32.5)	1(1.3)	79(98.8)	70(87.5)	10(12.5)
	P VALUE	0.2128	P VALUE	0.8413	P VALUE	n/a	P VALUE	1.000

143

144 Among different age groups, patients aged between 18 and 27 years (comprising a total of 14
 145 patients) had 13 to be positive for CMV IgM , while 7 were CMV IgG positive; none was Rubella IgM
 146 positive and 11 were Rubella IgG Positive. For the second group, patients aged between 28 and 37
 147 years (comprising a total of 41 patients), 38 patients tested positive to CMV IgM while 32 patients
 148 were CMV IgG positive. No patient was Rubella IgM positive while 35 patients were Rubella IgG
 149 positive. In the third group, patients between 38 and 47 years of age(a total of 18 patients), 17 were
 150 CMV IgM positive and 8 were CMV IgG positive. None of the patients was positive to Rubella IgM
 151 while 17 were positive to Rubella IgG antibodies. In the fourth group, patients between 48 and 57
 152 years (comprising a total of 6 patients), 5 were CMV IgM positive while 6 were CMV IgG positive. Only
 153 1 of the patients was Rubella IgM positive and 5 were Rubella IgG positive. In the final group, patients
 154 with ages greater than 57 years , comprising of only one patient. The patient tested positive for CMV
 155 IgM and IgG but tested negative to Rubella IgM and positive to Rubella IgG antibodies (table 3)

156

157

158

159

160 Table 3: Prevalence of Cytomegalovirus and Rubella (IgG and IgM) for age groups in 80 HIV patients
 161 in LUTH

Cytomegalovirus	Rubella
-----------------	---------

S/N	Age	No	IgM+	IgM-	IgG+	IgG-	IgM+	IgM-	IgG+	IgG-
1.	18-27	14(17.5)	13(92.8)	1(7.1)	7(50)	7(50)	0	14(100)	11(78.57)	2(14.3)
2.	28-37	41(51.25)	38(92.6)	3(7.3)	32(78)	9(21.9)	0	41(100)	35(85.36)	6(14.6)
3.	38-47	18(22.5)	17(94.4)	1(5.6)	8(44.4)	10(55.5)	0	18(100)	17(94.4)	1(5.6)
4.	48-57	6(7.5)	5(83.3)	1(16.6)	6(100)	0	1(16.6)	5(83.3)	5(83.3)	1(16.6)
5.	>57	1(1.25)	1(100)	0	1(100)	0	0	1(100)	1(100)	0
Total		80	74(92.5)	6(7.5)	54(67.5)	26(32.5)	1	79(98.8)	70(87.5)	10(12.5)

162

163 The mean CD4+ count of patients positive for CMV IgM was 232 while that of patients negative for
 164 CMV IgM was 413.5. The mean CD4+ count of patients for CMV IgG was 237 and that of those
 165 negative was 262.4. For Rubella IgM, the mean CD4+ count of patients positive for Rubella IgM was
 166 514 while those negative was 242.4. Also, for Rubella IgG the mean CD4+ count of patients positive
 167 for Rubella IgG was 248 and that of patients negative was 230 (table 4)

168

169

170

171

172

173

174 Table 4: Seroprevalence of Cytomegalovirus, Rubella and mean CD4+ counts of positive and
 175 negative samples among participants (n=80)

Antibody	Number	Number	Number	MeanCD4+	Mean CD4+
	tested	positive(%)	negative (%)	counts of	counts of

			positive samples	negative samples	
<u>CMV antibodies</u>					
IgG	80	54(67.5%)	26 (32.5%)	237	262.4
IgM	80	74 (92.5%)	6 (7.5%)	232	413.5
<u>Rubella antibodies</u>					
IgG	80	70 (87.5%)	10 (12.5%)	248	230
IgM	80	1 (1.25%)	79 (98.75%)	514	242.4

176

177 Out of the 67 patients whose viral load counts were retrieved (the viral load counts of 13 out of 80
 178 patients recruited could not be retrieved from the clinic), none had viral load less than 50, 5(7.5%) had
 179 viral load between 50 and 500, 6 (9.0%) had viral load between 500 and 5000, 15 (22.4%) had viral
 180 load between 5000 and 50000, 24(35.8%) had viral load between 50000 and 500000 and 17(25.4%)
 181 had viral load greater than 500000 (table 5)

182

183

184

185

186

187

188

189

190

191 TABLE 5: Viral load counts among participants (n=67)

VIRAL LOAD	NO	MALE	FEMALE
	(%)		
<50	0	0	0

50-500	5 (7.5)	0	5
500-5000	6 (9.0)	3	3
5000-50000	15 (22.4)	8	7
50000-500000	24 (35.8)	8	16
>500000	17 (25.4)	6	11
TOTAL	67 (100)	25	42

DISCUSSION

Among the 80 patients recruited into this study, 74(92.5%) and 54 (67.5%) were positive for CMV IgM and IgG antibodies respectively. These values are similar to seroprevalence data from developing countries [12]. Also, 70 patients (87.5%) were tested positive to Rubella IgG antibodies while only one patient (1.3%) was positive for Rubella IgM. However, among the 80 patients investigated in this study, it was observed that 28 were males (35%) and 52 were females (65%), and it has been reported that incidence of HIV is more in females than in males [13]. In addition, Seroprevalence of CMV IgG antibody was more in the female patients (64.8%) than in males (35.2%), while CMV IgM antibody sero-status was 92.6% for female patients studied against 44.4% recorded by the male patients and this result is similar with the recent study in Benin, Nigeria [14]. Also, the sero prevalence of Rubella IgG was more in females (65.7%) than in males (34.3%), on the other hand, this study revealed that the Rubella IgM sero-status was recorded only in one male and there was none in female.

The patients were classified based on their ages into five classes or groups with a class width of 10. The modal class was the second class with ages ranging from 28-37 years. This further reiterates the seriousness of the HIV infection among this age group as it is obvious that this modal class represents the very active segment of the society. This was also reported by Nester *et al.*, (1998) which stated that AIDS is the leading cause of death among persons aged 25 to 44 years. Also in this study, highest CMV IgM and IgG seroprevalence (92.6% and 78% respectively) were recorded among patient with the age group of 28-37. However, highest Rubella IgG seroprevalence (85.36%) was recorded among the age group of 28-37 while one on male (16.6%) in the age group of 48-57 was positive for Rubella IgM.

The integrity of the immune system is a decisive factor in the development of CMV end-organ disease. In CMV viraemic AIDS patient stratified by CD4+ counts of <50, 50 – 100 and > 100

cells/mm³, the incidence of CMV end-organ disease over a period of 6 months was 25%, 5.5% and 1.3% respectively [15, 16]. In this study, the patients were stratified according to CD4 counts. Thirty-six patients (45%) had CD4 counts between 1 and 200. Since HIV attacks CD4 cells, the CD4 count of HIV patients is an important guide in determining the extent of progression of the disease and is often used by medical officers in determining the time to start anti-retro viral therapy (ART) in the patients. AIDS is defined as CD4 levels below 200 [17] and ART should start immediately CD4 counts fall below or with the occurrence of disease symptoms. The average CD4 counts of the patients that tested positive to CMV IgM antibody was 232 while the mean CD4 count of those that tested negative was 413.5. Thus it can be inferred that patients with lower CD4 counts are more susceptible to CMV infection.

For Rubella however, no significant correlation can be drawn as regards CD4 count as patients with relatively low CD4+ counts still tested negative to Rubella IgM antibodies (Mean CD4 count- 242.4). This may be due to the high immunity of the general population to Rubella virus infection. Viral load has important implication in taking decision on ART commencement [18]. United states department of health and human treatment services/ Henry J. Kaiser foundation recommend starting patients on ART when viral load is >10000 to 20000 copies per ml or when CD4+ cell count is less than 500 cells/mm³ [19]. However, International AIDS society – USA panel recommends starting patients on ART when viral load is more than 30000 copies per ml or when CD4+ cell count is less than 350 cells/mm³ and ART is deferred if viral load is less than 5000 copies/ ml [19].

Of the 67 patients whose viral load counts were retrieved only 17 patients (25.4%) had CD4+ counts below 10000. In resource limited settings like Nigeria where CD4+ count is used as a guide to start patients on ART, these 17 patients would have been eligible for ART but if viral load is considered then ART may be deferred (subject to physician's consideration) in these patients

In this study, the number of patients with CMV IgM antibodies (92.5%) indicating recent infection is worrisome, considering its effect in immunocompromised people and the scale of the CMV problem in developing countries (such as Nigeria) is still not known as most cases of CMV retinitis are never diagnosed. Yet it has been estimated that between 5% and 25% of all HIV infected patients in the developing world can be expected to develop CMV retinitis at some point during the course of their illness [20] leading to some to warn of a possible epidemic of blindness [21]. Consequently, treatment of opportunistic infection is an integral part of HIV management at the primary care level and treatment of CMV retinitis should be included [22].

CONCLUSION

The findings of our study indicated high prevalence of CMV and Rubella seropositivity among anti-retroviral naive HIV patients in Lagos. Hence screening of cytomegalovirus and Rubella virus would be a vital diagnostic step for HIV patients.

LIMITATION OF THE STUDY

The viral load counts of 13 out of 80 patients recruited for this study could not be retrieved from the clinic.

ACKNOWLEDGEMENT

We would like to acknowledge the support of the staff and management of the AIDS Prevention Initiative in Nigeria (APIN) Clinic. LUTH, Idi-Araba, Lagos, Nigeria

COMPETING INTEREST

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors AIA, OMI, ACI and AGO were involved in the concept design. Authors ASA, BBS and OMI were involved in drawing up of protocol, collection and analysis of specimen. Authors OOA, AIA and SAT were involved in literature research, statistical analysis and drafting of the manuscript. All authors read and approved the final manuscript.

CONSENT

All authors have declared that written informed consents were obtained from the patients for publication of this work.

ETHICAL APPROVAL

Ethical approval for this study was granted by the research ethics committee and review board, Lagos University Teaching Hospital (LUTH), Idi-araba, Lagos state, Nigeria.

REFERENCES

1. Sepkowitz KA . "AIDS – the first 20 years" N.Engl. J. Med. 2001; 344 (23): 1764-72. DOI: 10.1056/NEJM200106073442306. PMID 11396444
2. Nester EW, Anderson DG, Roberts CE Jr, Pearsall NN, Nester MT. Microbiology: A Human Perspective pp 400-790. Mc Graw Hill Publishers, New York. 1998. ISBN 0-697-28602-9
3. Brooks JT, Kaplan JE, Holmes K.K, Benson C, Pau A, Masur H: HIV Associated Opportunistic Infections- Going, Going, But not Gone: The Continued Need for Prevention and Treatment Guidelines. Clinical Infectious Diseases. 2009; 48(5): 609-611
4. Piscitelli Sc, Gallicano KD: Interaction among drugs for HIV and opportunistic infections. The New England Journal of Medicine. 2001; 344 (13):984
5. Gershon A.A: Varicella-zoster virus infections. Pediatrics in Review 2008; 29(1):5
6. Weaver BA: Herpes Zoster Overview: Natural History and Incidence. JAOJA: Journal of the American Osteopathic Association. 2009; 109 (6 Supplement 2):S2
7. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA. "Review of human immunodeficiency virus type 1-related opportunistic infections in sub-saharan Africa" Clin. Infect. Dis.2003; 36 (5): 656-662. Doi: 10.1086/367655. PMID 125946448.
8. Smith (edited by) Blaine T. Concepts in immunology and immunotherapeutics (4thed.). Bethesda. Md: American Society of Health-System Pharmacist. 2008. P.143. ISBN 978-1-58528-127-5

- 298 9. Cheung, MC; Pantanowitz, L; Dezube, BJ. "AIDS-related malignancies:emerging challenges in
299 the era of highly antiretroviral therapy". The oncologist.2005; 10(6): 412-26. DOI:
300 10.1634/theoncologist.10-6-421.PMID 15967835.
- 301 10. Ryan KJ, Ray CG (editors). Sherris Medical Microbiology (4th ed.). McGraw Hill. Pp.556; 566-
302 9.2004. ISBN 0-8385-8529-9
- 303 11. Edlich RF, Winters KL, Long WB, Gubler KD. "Rubella and congenital rubella (German measles)".
304 J Long Term Eff Med Implants. 2005; 15(3): 319-28. Doi: 10.1615/LongTermEff MedImplants.
305 v15.i3.80.PMID 16022642.
- 306 12. Urwijetaroon Y, Teawpatanataworn S, Kitjareontarm A. Prevalence of cytomegalovirus antibody
307 in Thai-northeastern blood donors. Southeast Asian J. Trop Med Public Health 24 (Suppl
308 1).1993;180-182.
- 309 13. Akinbami A.A., Akanmu A.S., Adeyemo T.A., Wright K.O., Dada M.O., Dosunmu A.O.
310 Cytomrgalovirus Antibodies amongst Immunocompromised (HIV) patients at Lagos University
311 Teaching Hospital (LUTH) Idi-Araba, Lagos. J. Med. 2010; 11: 151-154
- 312 14. Ojide C.K, Kalu E.I, Nwadike V.U, Ogbaini-Emovo E and Omoti C . Seroprevalence of
313 cytomegalovirus among HIV-infected Adult patients on HAART. International Journal of Tropical
314 Disease and Health.2013; 3(3):233-241
- 315 15. Gerard L, Leport C, Flandre P., Houhou N., Salmon-Ceron D., Pepin J.M et al., Cytomegalovirus
316 (CMV) viraemia and the CD4+ lymphocyte count as predictors of CMV disease in patients
317 infected with human immunodeficiency virus. Clinical infectious Diseases . 1997;24: 836-40.
- 318 16. Shinkai M, Bozzette J.A, Powderly W. et al. Utility of Urine and leukocyte cultures and plasma
319 DNA polymerase chain reaction for identification of AIDS patient at risk for developing human
320 CMV disease. Journal of infectious diseases. 1997; 175: 302-8
- 321 17. Mandell GL, Dolan R., eds. Mandell, Douglas and Bennett's Principles and practice of infectious
322 disease , 7th ed. Orlando, FL: Saunders Elsevier. 2009; Chap 118
- 323 18. Ingole N, Kukreja S. And Mehta P., "Role of HIV -1 Viral load in initiating Antiretroviral Therapy"
324 World Journal of AIDS, Vol 1 No4,2011; pp.149-154. Doi: 10.4236/wja.2011.14022.
- 325 19. Mylonakis E., Paliou and J.D Rich "Plasma Viral Load Testing in the management of HIV
326 infection" American Family Physician, Vol.63, No.3, 2001; pp 483-490
- 327 20. Kestelyn PG, Cunningham ET. HIV/AIDS and blindness Bull World Health Organisation.2001;
328 790: 208-213
- 329 21. Guex-Crosier Y. Telenti A. An epidemic of blindness: a consequence of improved HIV care? Bull
330 World Health Organ.2001; 79:181
- 331 22. Chua A, Wilson D, Ford N. HIV and Cytomegalovirus in Thailand. Lancet. Infect Dis, 2005; 5:328-
332 329

333

334

335

336

337

338

339

340

341

342

343

344

345

346