Characterization of Antibodies to Human Immunodeficiency Viral Proteins in the Sera of HIV Infected and Non HIV Infected HBsAg Seropositive Patients

Dr. Mathew Folaranmi OLANIYAN (Ph. D)
School of Medical Laboratory Technology, Baptist Medical Centre
P.M. B. 43, Saki – Oyo State, Nigeria
Tel: 234-805-224-8019 E-mail: olaniyanmat@yahoo.com

Abstract

This work was designed to characterize the HIV viral antibodies in HIV infected and non-HIV infected HBsAg seropositive patients. Fifty non HIV infected (male = 25; female = 25) and 50 HIV infected HBsAg seropositive patients (male -n = 25; female -n = 25) aged 6 - 64 years recruited from the medical outpatient department of Baptist Medical Centre, Saki – Ovo State – Nigeria were investigated as test subjects. Fifty apparently healthy HIV and HBsAg seronegative individuals (male = 25; female = 25) aged 4 - 72 years were recruited as control subjects. All subjects were counseled and were subjected to HBsAg and HIV immunoassays by Enzyme Linked Immunosorbent Assay and Western blot assay. All subjects were monitored for twelve months. The subjects were investigated on recruitment and 12months after recruitment. The result obtained indicated higher frequency of occurrence of each of the HIV antibodies to each of the viral proteins in HIV infected HBsAg seropositive patients than in non HIV infected HBsAg seropositive patients (94% vs 0% (gp 160,), 84% vs 0% (gp 120), 94% vs 4% (p66), 94% vs 4% (p51), 84% vs 0% (gp 41), 94% vs 0% (p31), 100% vs 24% (p24) and 84% vs 6% (p17) during the first bleeding. The result obtained after 12 months showed a slight difference with the expression of antibody to gp41 by 4% of the non HIV infected HBsAg seropositive patients in addition to antibody to p24 or p17 which confirms HIV infection. Some of the non HIV infected HBsAg seropositive patients expressed antibodies to the following proteins p66, p51, p24, p17 during the initial investigation and after 12 months. The frequency of occurrence of antibody to p24 obtained in all HIV - HBsAg and some of the non HIV infected HBsAg seropositive patients was higher compared antibodies to other HIV proteins. This recent work has therefore been used to suggest the possibilities of antibodies to HIV viral proteins (p66, p51, p24, p17) in HBsAg seropositive sera. It also confirms an encouraging degree of specificity of antibodies to HIV envelope glycoproteins (gp160, gp120, gp41) in the diagnosis of HIV infection.

Keywords: Hepatitis B surface antigen, Human immunodeficiency virus, Seropositive, Viral proteins, Antibodies

1. Introduction

Human immunodeficiency virus (HIV) is the aetiologic agent of Acquired Immunodeficiency Syndrome (AIDS). It is a retrovirus and the types include HIV1 and HIV2 with their subtypes. (Olusoji et al 2006). It primarily infects and destroys vital cells in the human immune system such as helper T. cells, macrophages and dendritic cells. (Joint United Nations Programme on HIV/AIDS, 2006). The virus has the following proteins which include the HIV1 viral Proteins that is: Envelope proteins (gp 160, gp 120, and gp 41); Gag protein (p24, p17, p6); polymerase protein (p66/p51 – reverse transcriptase, p12, p32) and HIV-2 Viral Proteins such as envelope protein (gp105, gp36), polymerase protein (p51,- reverse transcriptase, p14) and Gag protein (p26). (Olaleye, 2003).

Hepatitis B virus is a small double stranded DNA virus of hepadnaviridae, family. It consists of hepatitis B surface antigen (HBsAg) and an inner nucleocapsid consisting of hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg). The nucleocapsid encloses the viral DNA and DNA polymerase that has reverse transcriptase activity (Locarnini, 2004). The hepatitis B and HIV viral proteins (antigens) have their corresponding antibodies which could be characterized in the sera of infected patients. The HIV envelope encodes the envelope precussor, gp160, which is split into two smaller glycoproteins, gp120 and gp41, via cellular enzymes in Golgi apparatus (Levy, 1994). The gp120 forms the external surface envelope protein and contains the binding site for cellular entry receptors, as well as major immunodominant domains, while gp41

forms the transmembrane protein(Levy,1994). The envelope surrounds the core proteins that enclose the viral RNA genome and enzymes. The core protein encoded by gag gene, whose precussor,p55 gives rise to four smaller proteins(p24, p17,p9,and p6) by proteolytic cleavage. The core itself is made up of two proteins (p18, and p24). The pol precursor protein is cleaved into products consisting of the reverse transcriptase (p66/p51), the protease (p32),and the integrase (p12) proteins by protease enzyme. Detection of HIV-specific antibodies in the blood or other body fluids is the main method of testing for HIV and the standard procedure for diagnosis of HIV infection. The most commonly used serologic assay for diagnosing HIV infection is enzyme-linked immunosorbent assay (Hirsh and Curran 1990, Beerlaert et al., 2002; Rouet et al., 2004). In general, the assays used to detect specific HIV antibodies are classified into two tests: screening tests including ELIZA/EIA, rapid, and simple; and supplemental or confirmatory tests including Western blot, culture, antigen detection and immunofluorescence assays (Olusoji et al 2006)

Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are devastating disease agents that share common modes of transmission (Santiago-Munoz et al. 2005), therefore HIV positive individuals are at risk of co-infection with HBV and HCV infections.

In studies carried out in Northern Nigeria, the prevalence of HIV and HCV co-infection was reported as 6.2% in Kano, Abuja 12.5% (Emokpae et al.,2008) and Keffi, a neighbouring city to Abuja, 11.1%.(3) On the other hand, a higher prevalence of 20.6% was reported among persons with HIV/HBV co-infection in Keffi, North-Central Nigeria. (Forbi et al.,2007)

This recent work was designed to characterize antibodies to Human Immunodeficiency viral proteins in the sera of HBsAg seropositive patients and HIV – HBV patient. It is hoped that the result of this study will contribute immensely to the diagnosis and management of hepatitis B and AIDS/HIV infection.

This recent study is also significant because HIV-HBV coinfection has been less extensively studied than HIV-HCV coinfection (15th conference on retroviruses and opportunistic infections (CROI) on 26th of February, 2008 on line at www.hivandhepatitis.com).

2. Materials and method

2.1 Materials

- Subjects 50 non HIV infected (male = 25; female = 25) and 50 HIV infected HBsAg seropositive patients (male n = 25, female n = 25) aged 6 64 years were recruited from the medical outpatient department of Baptist Medical Centre, Saki Oyo State. Fifty apparently healthy HIV and HBsAg seronegative individuals aged 4 72 years (Male n = 25; Female n = 25) were recruited as control subjects.
- **Sample** five milliliter (5ml) of venous blood sample was collected from each of the subjects and preserved in a 5ml K_2 EDTA anticoagulant bottle. The plasma was extracted and subjected to immunoassays. The subjects were bled on recruitment and after 12 months of the initial bleeding.
- **Ethical Issue** ethical approval of Research and Ethical committee of Baptist Medical Centre, Saki was obtained before samples were collected.

2.2 Methods

- a. Counseling: Each of the subjects was pre and post test counseled to be able to meet their psychological needs.
- b. HBsAg test was carried out on all the subjects using a one step enzyme immunoassay technique of the sandwich types for the detection of HBsAg in human serum or plasma using the reagent kit (MONOLISA HBsAg Plus) of BIO-RAD Raymond Poin-care, Marnes La Coquette.
- c. i. HIV screening test was carried out on all the subjects with pre and post test

counseling by visually read qualitative immunoassay using the reagent kit of Abbot Laboratories Co. Ltd., Japan.

- ii. HIV confirmatory test was carried out on all the subjects by Western blot assay, using a reagent kit of Immunoetics, Inc., Avenue, Boston, U.S.A. (www.immunietics.com)
- d. The subjects were monitored for twelve months. At the twelveth month of the initial investigation, the subjects were bled for the second time and the sera were subjected to immunoassays using the same methods that were applied at the initial investigations.

3. Result

The result obtained indicated 94% vs 0% (Gp160), 84% vs 0% (Gp120), 94% vs 4% (P66), 94% vs 4% (P51), 84% vs 4% (Gp41), 94% vs 0% (P31), 100% vs 24% (P24), 84% vs 6% (P17) in HIV -HBsAg versus HBsAg

seropositive patients. None of the control subjects express antibody to any of the HIV viral proteins. The incidence of the antibody to each of the HIV viral proteins was higher in HIV-HBsAg seropositive patients than in non HIV infected HBsAg seropositive patients (Table 1).

The result obtained at the second bleeding therefore indicated a slight difference of 2(4%) (gp41) in the sera of non HIV infected HBsAg seropositive patients compared to 0 %(gp41) which was obtained at the initial bleeding/investigation (Table 2).

4. Discussion

The expression of antibodies to envelope proteins (gp 160, gp 120, and gp 41) by the HIV – HBsAg seropositive patients demonstrated by Western Blot assay is consistent with the previous reports of Olusoji et al; 2006, on HIV infected patients. This result also confirms a high degree of the specificity of the envelope proteins of HIV in the diagnosis of HIV infection as none of the non-HIV infected HBsAg seropositive patients was found to express any of the antibodies to the envelope proteins.

Polymerase proteins especially the reverse transcriptase – p66, p51 found in the sera of HIV infected HBsAg seropositive patients are used by HIV in reverse transcription leading to formation of DNA from RNA. Hepatitis B virus possesses DNA polymerase that has reverse transcriptase activity that helps the virus during replication to cause the reverse transcription of RNA protein to form DNA viral particles (Locarnini, 2004).

The presence of antibodies to p66 and p51 in the sera of non HIV infected HBsAg seropositive patients could therefore be associated with the above facts. Hepatitis B virus is the only non-retrovirus that uses reverse transcriptase in its life cycle. Presence of gag proteins (p24, p17) in the sera of non HIV infected HBsAg seropositive patients could be associated with the fact that they are antibodies to group of antigens which could be found in some viruses other than HIV. Cross reactivity of other antibodies with these proteins have been reported (Constantine et al., 1994; Olusoji et al., 2006). Consequently, expression of antibodies to p24 and p17 could not be solely used to determine HIV infection.

Presence of antibodies to HIV viral proteins (p66, p51, p24, p17) in the sera of some non HIV infected HBsAg seropositive patients could be attributed to the report of Janssens et al.,1997 that indeterminate Western blot reactions are common especially with African blood samples. Expression of the antibodies to p66, p51, p24, p17 in non HIV infected HBsAg seropositive patients without the expression of antibody to any of the envelope/glycoproteins could be interpreted as indeterminate result. This could be due to non viral antigens with epitopes similar to retroviral proteins and expression of endogenous retroviral sequences (Constantine et al., 1994).

Two of the non HIV infected HBsAg seropositive patients that expressed gp41 after 12 months in addition to antibody to p24 or p17could be associated with HIV infection which cannot be diagnosed on recruitment (Olusoji et al., 2006). The frequency of occurrence of antibody to p24 was higher than the antibodies to other HIV viral proteins. This protein is a gag protein and the antibody to this protein could be expressed by other related virus (Olusoji et al., 2006). The expression of specific antibodies to HIV viral proteins by HBsAg seropositive patients could be attributed to possible HIV- HBV coinfection because the two viruses share the same route of transmission (Ray and Ryan, 2004)

5. Conclusion

This recent work has therefore been used to suggest the possibilities of the presence of antibodies to the following HIV viral proteins such as p66, p51, p24 and p17 in the sera of some HBsAg seropositive patients. It also confirms an encouraging degree of specificity of antibodies to HIV envelope glycoproteins (gp160, gp120, gp41) in the diagnosis of HIV infection.

References

Beelaert G, Vercauteren G, Fransen K, et al. (2002). Comparative evaluation of eight commercial enzyme linked immunoorbent assays and 14 simple assays for detection of antibodies of HIV J Viral Methods, 105:197-206.

Constantine N.T, Jones Ks, Zhang X, Abdel – Hamid M. (1994). International Conference on AIDS. Page 7-12:10:133.

CROI. (2008). 15th conference on retroviruses and opportunistic infections. [Online] Available: www.hivandhepatitis.com.

Hirsch M, Curran J. (1990). Human Immunodeficiency viruses: biology and medical aspect. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B, eds. *Virology*. 2nd ed. New York Raven Press, 154-1570.

Emokpae MA, Nwokedi EE, Jegede EE. (2008). Impact of Hepatitis C Co-Infection on CD4 Cell Count in HIV Infected Subjects. *Online J Health Allied Scs.*,7(2):3.

Forbi JC, Gabadi S, Alabi R, Iperepolu HO, Pam CR, Entonu PE, Agwale SM. (2007). The role of triple infection with hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV) type 1 on CD4+lymphocytes levels in highly infected population of North Central, Nigeria. *Mem. Inst. Oswaldo Cruz*, 102(4):535-537.

Janssens W, Buve A. Nkengasory J. (1997). The Puzzle of HIV 1 Subtypes in Africa. AIDS 11:705-712. Joint United Nations Programe on HIV/AIDS. (2006).

Levy J. (1994). HIV and the pathogensis of AIDS. Washington, DC: ASM Press.

Locarnini S. (2004). Molecular Virology of Hepatitis B Virus. Semin. Liver Dis. 24 Suppl.: 3-10.

Menard D, Mavolomade E, Mandeng M, et al. (2003). Advantages of an alternative strategy based on consecutive HIV serological tests for detection of HIV antibodies in *Central African Republic J Virol Methods*,111:129-134.

Olaleye O. D. (2003). The Fingerprints of a Moster, Message from the dead to the Living. Printed by Ibadan University Press, U. I. Ventures Limited University of Ibadan ISBN 978-121-330-2. Inaugural Lecture. First Published, 24-40.

Olusoji A., Phyllis J. K., Oluwole D. and John A. I. (2006). AIDS in Nigeria; A Nation on the Threshold. Harvard Series on Population and International Health Publiched by Harvard Centre for Population and development Studies. U.S.A. [Online] Available: www.apin.harved.edu.

Rouet F, Ekouevi D, Inwoley A. et al. (2002). Field evaluation of a rapid human immunodeficiency virus (HIV) serial serologic testing algorithm for diagnosis and differentiation of HILV 2 infections in *West African pregnant women J Clin Microbiol*, 42:4147-4153.

Ryan K. J., Ray C. G. (editors). (2004). *Sherris Medical Microbiology*, 4th ed., Mc Graw Hill 247 -9: ISBN 0838585299.

Santiago-Munoz P Roberts S, Sheffield J, McElwee B, Wendel GD. (2005). Prevalence of hepatitis B and C in pregnant women who are infected with human immunodeficiency virus. *Am J Obstr Gyn*, 193 (Suppl.3):1270.

Table 1. Immunoblotting Profile of HIV Infected HBsAg Seropositive and Non-HIV Infected HBsAg Seropositive Patients

Antibodies to	HIV-HBsAg	HBsAg	Control
HIV Viral Proteins	Seropositive patients	Seropositive Patients	Subject
	(n=50)	(n=50)	(n=50)
Gp160	47(94%)	0%	0%
Gp120	42(84%)	0%	0%
P66	47(94%)	2(4%)	0%
P51	47(94%)	2(4%)	0%
Gp41	42(84%)	0%	0%
P31	47(94 %)	0%	0%
P24	50(100%)	12(24%)	0%
P17	42(84%)	3(6%)	0%

The above table shows the Immunoblotting Profile of HIV Infected HBsAg Seropositive and Non-HIV Infected HBsAg Seropositive Patients

Table 2. Immunoblotting Profile of HIV Infected HBsAg Seropositive and Non-HIV Infected HBsAg Seropositive Patients after 12Months of the Initial Investigation

Antibodies to	HIV-HBsAg	HBsAg	Control
HIV Viral Proteins	Seropositive Patients	Seropositive Patients	subjects
	(n=50)	(n = 50)	(n = 50)
Gp160	47(94%)	0%	0%
Gp120	42(84%)	0%	0%
P66	47(94%)	2(4%)	0%
P51	47(94%)	2(4%)	0%
Gp41	42(84%)	2(4%)	0%
P31	47(94%)	0%	0%
P24	50(100%)	12(24%)	0%
P17	42(84%)	3(6%)	0%

The above table shows the Immunoblotting Profile of HIV Infected HBsAg Seropositive and Non-HIV Infected HBsAg Seropositive Patients after 12Months of the Initial Investigation