Selected biochemical and hematological abnormalities in Nigerians with human immunodeficiency virus and hepatitis C virus coinfection

Olive Obienu
Sylvester Nwokediuko
Gastroenterology Unit, Department of Medicine, University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu, Nigeria

Background: Liver disease has emerged as a major cause of morbidity and mortality in patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection, now that antiretroviral therapy has become more effective and has prolonged life expectancy in HIV-infected patients. The main objectives of this study were to determine the prevalence of HIV/HCV coinfection and the pattern of hematological and biochemical abnormalities associated with such dual infection.

Methods: In this study, patients with HIV infection (cases) were tested for anti-HCV antibodies. There was a control group made up of apparently healthy individuals who came to hospital for medical examination for various reasons. They also had an anti-HCV antibody test. Those who tested positive for anti-HCV antibodies among the cases and control subjects were further evaluated for hemoglobin concentration, total white cell count, platelet count, and liver function.

Results: One hundred and eighty HIV-infected patients and 180 control subjects participated in the study. The seroprevalence of anti-HCV antibodies in the HIV-infected patients and control subjects were 6.7% and 4.4%, respectively ($P=0.57$). Serum total bilirubin, conjugated bilirubin, and alkaline phosphatase were significantly higher in the HIV/HCV coinfected patients compared with their HCV monoinfected counterparts ($P=0.0396$, $0.0001$, and $0.0016$, respectively). The mean hemoglobin, white cell count, platelet count, and CD4+ T lymphocyte count were significantly lower in the HIV/HCV coinfected patients than the HCV monoinfected control group ($P=0.0082$, $0.0133$, $0.0031$, and $0.0001$, respectively).

Conclusion: The seroprevalence of anti-HCV antibodies in HIV-infected Nigerian patients is 6.7%. Patients with HIV/HCV coinfection have lower blood counts, higher serum bilirubin, and higher serum alkaline phosphatase compared with patients having HCV monoinfection.

Keywords: human immunodeficiency virus, hepatitis C virus, coinfection, biochemical, hematological abnormalities

Introduction

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are global health problems. Due to shared routes of transmission, coinfection with the two viruses is common and represents an emerging area of interest both in clinical practice and research.

In the US, 15%–30% of HIV-infected individuals are coinfected with HCV. However, the proportion of HIV-infected patients with HCV varies substantially according to the HIV risk factor. Among patients who acquired HIV from blood product transfusion or intravenous drug use, even higher rates (60%–90%) of HCV
coinfection have been reported, whereas risk factors such as sexual transmission have lower rates.4

The mortality related to HIV has dropped considerably over the past 10–15 years with the introduction of highly active antiretroviral therapy.5 Following the introduction of highly active antiretroviral therapy, there has been a sharp increase in the number of deaths due to end-stage liver disease among the HIV-HCV coinfected population.6,7 This has led to increased research into the evaluation and management of coinfected patients.

Sexual transmission is the predominant source of the spread of the HIV/AIDS epidemic in sub-Saharan Africa.8 Consequently, the HIV/HCV coinfection rate is expected to be lower than that in developed countries. In Gambia it was reported as 0.6%.9 In Nigeria, prevalence rates ranging from 4.5% to 10.3% have been reported in various studies.10–13 However, there are no published studies regarding HIV/HCV coinfection in south east Nigeria.

Management of each of these viral infections requires a rigorous process of proper clinical evaluation and laboratory testing which are mandatory, not only at the initiation of treatment, but have to be repeated several times in the course of follow-up. The process becomes even more complex when there is coinfection. Both viruses affect the liver individually,14–17 and collectively.18–21 It follows that the clinician needs to have a good idea of certain parameters of liver function in HIV/HCV coinfected patients before, during, and after treatment. Therefore, this study was designed to determine the prevalence of HCV infection in HIV-infected patients and to evaluate selected biochemical and hematological indices.

**Methods and materials**

This was a cross-sectional study of consecutive treatment-naive HIV-infected patients seen at the University of Nigeria Teaching Hospital Enugu between May 2007 and April 2009. The study was approved by the University of Nigeria Teaching Hospital research ethics committee and informed consent was obtained from each participant before inclusion. There was a control arm made up of age-matched and gender-matched HIV-negative persons who came for pre-employment medical examination, a premarrige medical examination, or voluntary blood testing.

Venous blood (5 mL) was obtained from the cases and control subjects. Anti-HCV antibody was assayed in the two groups using a third-generation enzyme linked immunosorbent assay which utilizes recombinant HCV antigen manufactured by DRG International, Marburg, Germany. This test has a sensitivity of 95%, a specificity of 97.5%, and a coefficient of variation ±15%. Biochemical tests were also carried out on the sera of the HIV-infected patients (cases) and control subjects using the Bayer® Plus clinical chemistry analyzer. Hematological tests for the determination of hemoglobin, white cell count, and platelet count were carried out on the cases and control subjects using an automated hematology analyzer (Sysmex XT 2000i; Sysmex Corporation, Kobe, Japan). CD4+ T lymphocyte count was determined in HIV-infected patients using the CyFlow® Counter (Partec, Swedesboro, NJ).

**Statistical analysis**

The data were analyzed using SPSS version 15.0 (SSPS Inc, Chicago, IL). Continuous variables were summarized as means ± standard deviation, while categorical variables were presented as percentages. Differences between proportions were determined using the Chi-squared test, and P values ≤0.05 were considered statistically significant.

**Results**

There were 180 HIV-infected patients (80 males [44.4%] and 100 females [55.6%]). There were also 180 control subjects (91 males [50.6%] and 89 females [49.4]). The mean age of the HIV-infected patients was 36.4 ± 8.4 years while the mean age of the control subjects was 37.0 ± 7.9 years. Twelve HIV-infected patients (6.7%) and eight control subjects (4.4%) were anti-HCV positive. The difference between the proportions was not statistically significant (P = 0.57). Table 1 illustrates the anti-HCV serology status of the HIV-infected patients and control subjects.

Selected hematological indices in the cases and controls are depicted in Table 2. Mean hemoglobin concentration, mean leukocyte count, mean platelet count, and mean CD4+ T lymphocyte count were significantly lower in the HIV/HCV coinfected patients compared with the HCV monoinfected individuals (P = 0.0082, 0.0133, 0.0031, and 0.0001, respectively). Conversely, serum total and conjugated bilirubin were significantly higher in the HIV/HCV coinfected group compared with the HCV monoinfected group (P = 0.0396 and 0.0001, respectively). Serum alkaline phosphatase was

| Table 1 Anti-HCV in HIV-infected patients and control subjects |
|---------------------------------------------------------------|
| **Group**          | **Anti-HCV positive** | **Percentage** |
|-------------------|----------------------|----------------|
| HIV-infected patients (n = 180)        | 12            | 6.7            |
| Control subjects (n = 180)             | 8             | 4.4            |
| Total (n = 360)                        | 20            | 5.6            |

**Abbreviations:** HCV, hepatitis C virus; HIV, human immunodeficiency virus.
Albumin (g/L) 34.60
Total protein (g/L) 68.48
Alkaline phosphatase (iU/L) 102.3
μ conjugated bilirubin (mol/L) 13.55
Total bilirubin (Aspartate transaminase (iU/L) 7.9
Mean hemoglobin concentration (g/dL) 9.66
Total WBC (×10⁹ cells/μL) 4.04
Platelet count (×10⁹ cells/μL) 175.1
CD4+ T lymphocyte count (cells/μL) 220.5

Table 2 Selected hematological abnormalities in HIV/HCV coinfection and HCV mono-infection

| Parameter                           | HIV/HCV coinfection | HCV mono-infection | t value | P value |
|-------------------------------------|----------------------|--------------------|---------|---------|
| Mean hemoglobin concentration (g/dL)| 9.66 ± 1.80          | 12.47 ± 1.4        | 3.16    | 0.0082* |
| Total WBC (×10⁹ cells/μL)           | 4.04 ± 1.27          | 5.96 ± 0.89        | 2.95    | 0.0133* |
| Platelet count (×10⁹ cells/μL)      | 175.1 ± 80.85        | 333.6 ± 59.66      | 3.76    | 0.0031* |
| CD4+ T lymphocyte count (cells/μL)  | 220.5 ± 53.73        | 505.8 ± 90.4       | 5.81    | 0.0001* |

Note: *Statistically significant.
Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; WBC, white cell count.

significantly higher in the HIV/HCV coinfected patients compared with their HCV mono-infected counterparts (P = 0.0016). Table 3 shows selected biochemical abnormalities in the cases and controls.

Discussion
In this study, the seroprevalence of anti-HCV antibodies in the HIV-infected patients was 6.7% while in the control group it was 4.4%. The difference was not statistically significant. There is a possibility that some of the HIV-infected patients may have tested falsely negative for anti-HCV because of profound immunosuppression, as shown by the significantly lower mean CD4+ T lymphocyte count. This pattern has been reported by other investigators.22,23 In such situations, an HCV RNA assay would be the appropriate method for evaluating HCV infection. Unfortunately, that was not done in this study because of funding and technical constraints. Further studies including viral load estimation will shed more light on this.

Serum total and conjugated bilirubin were significantly higher in the HIV/HCV coinfected patients compared with the HCV monoinfected patients (P = 0.0396 and 0.0003, respectively). Cholestasis is a recognized histological feature of HIV/HCV coinfection.24 Whether the observed higher conjugated bilirubin level in the HIV/HCV coinfected group is related to this histological finding cannot be inferred because liver biopsy was not part of the study.

Furthermore, there is a special form of hepatitis known as fibrosing cholestatic hepatitis which has a strong association with immunosuppression. It is a rapidly progressive, sometimes fatal, form of liver injury originally reported in liver transplant recipients with recurrent hepatitis.25 It has now been recognized frequently in chronic hepatitis B patients and hepatitis C patients under immunosuppression.26 The histologic hallmarks in the liver include marked hepatocytic injury, severe cholestasis, and periportal and pericellular fibrosis.27,28 In contrast with the pathogenesis of chronic hepatitis in immunocompetent patients attributed to cellular immune-mediated hepatocytolysis, fibrosing cholestatic hepatitis has been postulated to result from unimpeded viral replication within hepatocytes, culminating in a direct cytopathic effect in the setting of immunosuppression.29 High-level expression of viral antigens (HbsAg and HbcAg) has been visualized directly by immunohistochemical staining in affected livers and measured by quantitative analysis, such as radioimmunoassay of tissue homogenates.30 The possibility that higher serum conjugated bilirubin levels in HIV/HCV coinfected patients represents an early stage in progression to fibrosing cholestatic hepatitis remains to be confirmed.

HIV infection modifies the natural history of HCV infection by accelerating the histological progression of HCV infection, leading to cirrhosis and end-stage liver disease in a shorter period of time.18,31–33 Another way to explain the higher level of serum conjugated bilirubin in the HIV/HCV coinfected group is to regard it as a manifestation of rapid histological progression to cirrhosis. The clinical importance of this observation is that such biochemical

Table 3 Selected biochemical abnormalities in HIV/HCV coinfection and HCV mono-infection

| Parameter                        | HIV/HCV coinfection | HCV mono-infection | t value | P value |
|----------------------------------|---------------------|--------------------|---------|---------|
| Alanine transaminase (IU/L)      | 7.9 ± 1.29          | 8.0 ± 1.96         | 0.1122  | 0.9127  |
| Aspartate transaminase (IU/L)    | 11.46 ± 1.73        | 11.16 ± 2.23       | 0.2755  | 0.7881  |
| Total bilirubin (μmol/L)         | 23.89 ± 8.72        | 14.1 ± 3.95        | 2.334   | 0.0396* |
| Conjugated bilirubin (μmol/L)    | 13.55 ± 2.27        | 6.92 ± 1.43        | 5.808   | 0.0001* |
| Alkaline phosphatase (IU/L)      | 102.3 ± 17.37       | 58.2 ± 20.46       | 4.165   | 0.0016* |
| Total protein (g/L)              | 68.48 ± 6.90        | 69.36 ± 1.35       | 0.279   | 0.7854  |
| Albumin (g/L)                    | 34.60 ± 3.096       | 36.58 ± 3.527      | 1.066   | 0.3094  |

Note: *Statistically significant.
Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; WBC, white cell count.
abnormalities may represent surrogate markers of liver disease progression. Larger prospective studies will be more elucidatory.

Hemoglobin concentration was significantly lower in the HIV/HCV coinfected patients compared with the HCV monoinfected control subjects. This may be due to an additive or synergistic effect of the two infections in the coinfected group. Anemia is a very common finding in patients with HIV infection, particularly in individuals with more advanced HIV disease. The causes include changes in cytokine production with subsequent effects on hematopoiesis, decreased erythropoietin concentration, opportunistic infectious agents, such as Mycobacterium avium complex and Parvovirus B19. Other less common mechanisms include vitamin B12 deficiency and autoimmune destruction of red blood cells. Similarly, in untreated HCV monoinfection, different types of immune-mediated cytopenias might be severe and clinically significant. However, hemolytic anemia and severe thrombocytopenia are the most frequently observed cytopenias. Pure red cell aplasia has also been reported in association with HCV infection. The mean platelet count was significantly lower in the HIV/HCV coinfected patients compared with the HCV monoinfected group. Again, this may be due to an additive or a synergistic effect of the two infections. Thrombocytopenia was first associated with the acquired immune deficiency syndrome before the discovery of HIV. The mechanisms involved include accelerated platelet clearance due to immune complex disease, antiplatelet glycoprotein antibodies, and/or anti-HIV antibodies that crossreact with platelet membrane glycoprotein (antigenic mimicry). Direct infection of megakaryocytes results in defective platelet production and megakaryocytic apoptosis. Similarly, thrombocytopenia is common in chronic HCV infection and the mechanisms include advanced liver fibrosis and manifest cirrhosis, lack of hepatic-derived erythropoietin, direct cytopathic involvement of platelets and megakaryocytes, and HCV-associated immunoglobulins which induce thrombocytopenia via an immunological mechanism similar to that operating in immune thrombocytopenic purpura.

Leukopenia is inherent in HIV infection. Apart from lymphocyte depletion, which is the critical event, granulocytopenia is a problem commonly encountered with HIV infection. The pathogenesis is multifactorial, including an autoimmune mechanism and impaired granulopoiesis.

In conclusion, the seroprevalence of anti-HCV antibody in HIV-infected Nigerian patients is 6.7%. Coinfected patients are more likely to have deranged hematological and biochemical parameters compared with HCV monoinfected patients. This may be due to an additive or a synergistic effect of the two infections.

Disclosure
The authors report no conflicts of interest in this work.

References
1. Thomas DL. HIV/HCV co-infection: comorbidity and clinical implications. Adv Stud Med. 2005;5:352–355.
2. Sherman KE, Rouster SD, Chung RT, Rajicic N. Hepatitis C virus prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis of the US Adult AIDS Clinical Trials Group. Clin Infect Dis. 2002;34:831–837.
3. Tedaldi EM, Hulisek KH, Malvestatto CD, et al. Prevalence and characteristics of hepatitis C virus co-infection in a human immunodeficiency virus clinical trials group: the Terry Beirn Community Programs for Clinical Research on AIDS. Clin Infect Dis. 2003;36:1313–1317.
4. Sulkowski MS, Thomas DL. Hepatitis C in the HIV-infected person. Ann Intern Med. 2003;138:197–207.
5. Palella FJ Jr, Delaney KM, Mooman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection: HIV Outpatient Study Investigators. N Engl J Med. 1998;338:853–860.
6. Darby SC, Ewart DW, Giagrande PL, et al. Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilic Centre Directors’ Organization. Lancet. 1997;350:1425–1431.
7. Bica I, McGovern B, Dhar R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis. 2001;32:492–497.
8. Schmid GP, Buve A, Mugyenyi P, et al. Transmission of HIV-1 infection in sub-Saharan Africa and effect of elimination unsafe injections. Lancet. 2004;363:482–488.
9. Mboto CI, Fielder M, Russel A, Jewel AP. The prevalence of HIV 1, HIV 2, hepatitis C and co-infection in The Gambia. West Afr J Med. 2009;28:306–309.
10. Agwale SM, Tanimoto L, Womack C, et al. Prevalence of HCV co-infection in HIV-infected individuals in Nigeria and characterization of HCV genotypes. J Clin Virol. 2004;31 (Suppl 1):S3–S6.
11. Inyama PU, Uneke JC, Anyanwu IG, Njoku MO, Idoko HJ, Idoko AJ. Prevalence of antibodies to hepatitis C virus among Nigerians patients with HIV infection. Online J Health Allied Sci. 2005;2:2.
12. Muktar HM, Alkali CN, Jones EM. Hepatitis B and C co-infection in HIV/AIDS patients attending ARV centre ABUTH, Zaria, Nigeria. Highland Med Res J. 2006;4:39–45.
13. Olokoba AB, Olokoba LB, Salawu FK, et al. Hepatitis C virus and human immunodeficiency virus co-infection in North-Eastern Nigeria. Res J Med Sci. 2008;2:217–219.
14. Albisetti M, Braegger CP, Stallmach T, Willi UV, Nadal D. Hepatic steatosis: a frequent non-specific finding in HIV-infected children. Eur J Paed. 1999;158:971–974.
15. Poles MA, Dieterich DT, Schwarz ED, et al. Liver biopsy findings in 501 patients with human immunodeficiency virus (HIV). J Acquir Immune Defic Syndr Hum Retrovirol. 1996;11:170–177.
16. Trojan A, Kreuzer KA, Flury R, Schmild M, Schneider J, Schroder S. Liver changes in AIDS. Retrospective analysis of 227 autopsies of HIV-positive patients. Pathol. 1998;19:194–200.
17. [No authors listed]. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic liver disease. MMWR Recomm Rep. 1998;47:1–39.
28. Rosenberg PM, Farrell JJ, Abraczinskas DR, Graeme-Cook FM, Furuta K, Takahashi T, Aso K, Hoshino H, Sato K, Kakita A. Fibrosing cholestatic hepatitis after renal transplantation for chronic hepatitis C virus infection among HIV/AIDS patients? NIGER J MED. 2007;16:231–234.

30. Adewole OO, Antey E, Ajuwon Z, et al. Hepatitis B and C virus co-infection in Nigerian patients with HIV infection. J Infect Dev Ctries. 2009;3:369–375.

32. Bierhoff E, Fischer HP, Willisch E, et al. Liver histopathology in patients with concurrent chronic hepatitis C and HIV infection. Tircarchs Arch. 1997;430:271–277.

34. Davies SE, Portmann BC, O'Grady JG, et al. Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. Hepatology. 1991;13:150–157.

36. Zylberberg H, Carnot F, Mamzer MF, et al. Hepatitis C virus-related fibrosing cholestatic hepatitis after renal transplantation. Transplantation. 1997;63:158–160.

38. Furuta K, Takahashi T, Aso K, Hoshino H, Sato K, Kakita A. Fibrosing cholestatic hepatitis in a liver transplant recipient with hepatitis C virus infection: a case report. Transplant Proc. 2003;35:389–391.

40. Rosenberger PM, Farrell JJ, Abraczinskas DR, Graeme-Cook FM, Dientag JL, Chung RT. Rapidly progressive fibrosing cholestatic hepatitis-hepatitis C virus in HIV co-infection. Am J Gastroenterol. 2002;97:478–483.

42. Toth CM, Pascual M, Chung RT, et al. Hepatitis C virus-related fibrosing cholestatic hepatitis after renal transplantation: response to interferon-alpha therapy. Transplantation. 1998;66:1254–1258.

44. Lau LJ, Bain VG, Davies SE, et al. High-level expression of hepatitis B viral antigens in fibrosing cholestatic hepatitis. Gastroenterology. 1992;102:956–962.

46. Jones R, Dunning J, Nelson M. HIV and hepatitis C co-infection. Int J Clin Pract. 2005;59:1082–1092.

48. Leen CLS. Hepatitis C and HIV co-infection. Int J STD AIDS. 2004;15:289–295.

50. Mohsen AH, Eastbrook PJ, Taylor C, et al. Impact of human immunodeficiency virus (HIV) on the progression of liver fibrosis in hepatitis C virus infected patients. Gut. 2003;52:1035–1040.

52. Zon Li, Groupman JE. Hematologic manifestations of the human immunodeficiency virus (HIV). Semin Hematol. 1988;25:208–218.

54. Zaulli G, Re MC, Visani G, et al. Evidence for a human immunodeficiency virus type-1 mediated suppression of infected hematopoietic (CD34+) cells in AIDS patients. J Infect Dis. 1992;166:710–716.

56. Furlini G, LaPlaca M. Tat protein stimulates production of transforming growth factor-B by bone marrow macrophages: a potential mechanism for human immunodeficiency virus-1 induced hemapoietic suppression. Blood. 1992;80:3036–3043.

58. Maciejewski JP, Weichold FF, Young NS. HIV-1 suppression in hematopoiesis in vitro mediated by envelope glycoprotein and TNF-alpha. J Immunol. 1994;153:4303–4310.

60. Spirak JL, Barnes DC, Fuchs E, Quinn TC. Serum immunoreactive crythropoietin in HIV-infected patients. JAMA. 1989;261:3104–3107.

62. Camacho J, Poveda F, Zamorano AF, Valencia ME, Vazquez JJ, Arnalich F. Serum erythropoietin levels in patients with advanced human immunodeficiency virus infection. Br J Haematol. 1992;82:608–614.
61. Zon LI, Groopman JE. Hematologic manifestations of the human immunodeficiency virus (HIV). Semin Hematol. 1988;25:208–218.
62. Murphy MF, Metcalfe P, Waters AH, et al. Incidence and mechanism of neutropenia and thrombocytopenia in patients with human immunodeficiency virus infection. Br J Haematol. 1987;66:337–340.
63. van der Lelie J, Lange JM, Voss JJ, van Dalen SA, von dem Borne AE. Autoimmunity against blood cells in human immunodeficiency virus (HIV) infection. Br J Haematol. 1987;67:109–114.

64. Stella CC, Ganser A, Hoelzer D. Defective in vitro growth of the hemopoietic progenitor cells in the acquired immunodeficiency syndrome. J Clin Invest. 1987;80:286–293.
65. Folks TM, Kessler SW, Orenstein JM, Justement JS, Jaffe ES, Fauci AS. Infection and replication of HIV 1 in purified progenitor cells of normal human bone marrow. Science. 1988;242:919–922.