Original Article

Possible Immunochemical Pattern of Anti-HCV, HBeAg, HBsAg HBeAb, and HIV-1 p24 in Newly Infected *Mycobacterium* Pulmonary Tuberculosis Patients

Mathew Folaranmi Olaniyan, Ruth Aderibigbe[1], Temitayo Afolabi[1]

Department of Medical Laboratory Science, Edo University, Iyamho, [1]Department of Medical Laboratory Science, Achievers University, Owo, Nigeria

Abstract

Background: Viral biomarkers in pulmonary tuberculosis (PTB) could be a primary or secondary viral infection to PTB which may depressed immunity or make the patient susceptible to the secondary infection. **Aim and Objective:** This work was, therefore, designed to determine frequency of anti-hepatitis C virus (HCV), hepatitis B envelope antigen (HBeAg), hepatitis B surface antigen (HBsAg), hepatitis B envelope antibody (HBeAb), and human immunodeficiency virus (HIV)-1 p24 in newly infected *Mycobacterium* PTB patients. **Materials and Methods:** Sixty newly infected *Mycobacterium tuberculosis* patients were recruited from the medical outpatient Department of Baptist Medical center, Saki-Nigeria. The patients were classified into females (30) and males (30) aged 38–79 years. *M. tuberculosis* was determined in the patients through fluorescence immunoassay, cultivation of sputum sample on Löwentein–Jensen medium, and radiological chest X-ray report. Anti-HCV, HBeAg, HBsAg HBeAb, and HIV-1 p24 were determined in the patients by immunochromatography and ELISA methods. **Results:** The viral immunochemical pattern obtained in the newly infected PTB patients showed a frequency of: 5% (3) (males - 3.3% [2] and females - 1.67% [1]) anti-HCV; 3.3% (2) (all males - 3.3% [2]) HIV-1 - p24; 15% (9) (males - 3 [5%] and females - 6 [10%]) HBsAg; 9 (15%) (males - 5% [3] and females - 10% [6]) HBeAg; 18.33% (11) (males - 6.7% [4] and females - 11.67% [7]) hepatitis B envelope (HBe) antibody; 3.33% (2) (males - 1.67%[1] and females - 1.67% [1]) HBeAg + HBsAg + HIV-1 - p24. **Conclusion:** This work revealed evidence of anti-HCV; HIV-1 - p24; HBsAg; HBeAg; and HBe antibody and HBeAg + HBsAg + HIV-1 - p24 in newly infected pulmonary *M. tuberculosis*-infected patients. Routine evaluation of viral biomarkers in PTB patients is necessary for effective management.

Keywords: Anti-hepatitis C virus, hepatitis B envelope antibody, hepatitis B envelope antigen, hepatitis B surface antigen, human immunodeficiency virus-1 p24, newly infected *Mycobacterium* pulmonary tuberculosis patients

Introduction

Pulmonary tuberculosis (PTB) is caused by the bacterium *Mycobacterium tuberculosis*. Tuberculosis (TB) is highly infectious that can spread through the air when patients with active TB cough, spit, speak, or sneeze.[1,2] This infection generally affects the lungs but can spread and affect other parts of the body. Most TB could be asymptomatic which is referred to as latent TB though, around 10% of latent infections progress to active disease if untreated.[1,2] PTB is an active TB that majorly involves the lungs (in about 90% of cases).[1] It most often affects lung higher lobes than the lower lobes. The symptoms of TB include a chronic cough with blood-stained sputum, fever, night sweats, and weight loss.[1,2] *Mycobacterium tuberculosis* has been characterized in human immunodeficiency virus (HIV) patients as an opportunistic infection. In co-infections, it comes in by chance as a result of low immunity.[2] Anti-hepatitis C virus (HCV) is a viral marker indication viral infection of HCV causing hepatitis C, whereas hepatitis B envelope antigen (HBeAg), hepatitis B surface antigen (HBsAg), and hepatitis B envelope antibody (HBeAb) are viral biomarkers of hepatitis B virus (HBV)[3] an infectious agent of hepatitis B.[4,5]

HIV, viral hepatitis of B or C could be a primary or secondary infection to PTB because any of the infectious agents could

Access this article online

Quick Response Code:

Website: www.jnsmonline.org

DOI: 10.4103/JNSM.JNSM_66_18

Address for correspondence: Prof. Mathew Folaranmi Olaniyan, Department of Medical Laboratory Science, Edo University, Iyamho, Nigeria. E-mail: ololaniyanmat@yahoo.com

How to cite this article: Olaniyan MF, Aderibigbe A, Afolabi T. Possible immunochemical pattern of anti-HCV, HBeAg, HBsAg HBeAb, and HIV-1 p24 in newly infected *Mycobacterium* pulmonary tuberculosis patients. J Nat Sci Med 2019;XX:XX-XX.

affect the immunity of the host in one way or the other leading to fatal complications. Innate host response is necessary for induction of adaptive immunity to *M. tuberculosis* leading to the production of antibody against the infectious agents which will involve the protective immune response of T-cell-mediated immunity[1] with CD4+ T cells regulated by pro- and anti-inflammatory cytokines.[2,6] HIV infects vital immune cells like CD4+ bearing T cells, macrophages, and dendritic cells.[2] HIV infection causes low levels of CD4+ T cells through pyroptosis of abortively infected CD4+ T cells, apoptosis of uninfected bystander cells, the direct viral killing of infected cells, and killing of infected CD4+ T cells by CD8+ cytotoxic lymphocytes that recognize infected cells.[2] Critical decrease in CD4+ T cells in HIV infection causes loss of cell-mediated immunity, and the body becomes progressively more susceptible to opportunistic infections such as TB which can eventually lead to Acquired Immunodeficiency Syndrome.[2]

Individuals who are seronegative to HBsAg, anti-HBc, and anti-hepatitis B surface (HBs) are those not infected with HBV but are susceptible to the infection. Individuals who are seronegative to HBsAg, but seropositive to anti-HBc and anti-HBs are said to be immune due to natural infection.[3-5] People who are seronegative to both HBsAg and anti-HBc, but seropositive to anti-HBs might have acquired the immunity to the virus due to hepatitis B vaccination.[3] Patients with acute hepatitis B are seropositive to HBsAg, anti-HBc, immunoglobulin M (IgM) anti-HBc, and seronegative to anti-HBs, whereas those with chronic hepatitis B are seropositive to HBsAg, anti-HBc, but seronegative to IgM anti-HBc and anti-HBs.[4,5] The presence of HBeAg is an indication that HBV is actively replicating[3,4] while its antibody indicates clearance of the envelope antigen and that the affected individual is a career of HBV. HBsAg may indicate acute or chronic hepatitis B.[5,6]

For the fact that viral infection could be primary or secondary to PTB due to the susceptibility of the patients with the primary infection to the secondary infection only anti-HIV has been widely characterized in PTB patients.[7,8] Active infection of *M. tuberculosis* occurs more often in those with HIV/AIDS.[9,10] The risk of developing active TB in HIV infection increases to nearly 10% per year.[10,11] The laboratory diagnosis of active TB includes chest X-rays, microscopic examination,[9] and culture of body fluids. Laboratory diagnosis of latent TB could be by tuberculin skin test or blood tests.[10,11]

Lagos, Kano, and Oyo have the highest TB prevalence rate. Other states experienced a drop in ases notified, resulting in a 4% overall decline in 2010.[12] Oyo increased by 46.5% from 2008 to 2010. The TB burden is compounded by a high.[12] The prevalence of HIV in the country which stands at about 4.1% in the general population.[12] The prevalence of HIV among TB patients increased from 2.2% in 1991-19.1% in 2001, and 25% in 2010. This indicates that the TB.

Situation in the country is HIV-driven.[12] The proportion of TB patients tested for HIV was 79% in 2010, with a 25% TB-HIV co-infection rate. About 59% of these patients were started on cotrimoxazole (CPT) prophylaxis and 1.8% provided with isoniazid (IPT) prophylaxis.[12] The proportion of TB/HIV co-infected patients on anti-retroviral (ARV) therapy was 33% in 2010.[12] The proportion of HIV-registered cases screened for TB was 57% in 2010.[12] The proportion of HIV cases that developed TB was 4% in 2010 and 3% in 2011. The age groups commonly affected by TB are the most productive age groups, with the 25–34 age group accounting for 33.6% (15,303) of the smear positive cases registered in 2010.[12]

This work was, therefore, designed to determine the possible immunochemical pattern of anti-HCV, HBeAg, HBsAg HBeAb, and HIV-1 p24 in newly infected *Mycobacterium* PTB patients.

Materials and Methods

Materials

Research design

This was observational-cross-sectional and case–control.

Area of study

Saki West is a Local Government Area in Oyo State, Nigeria. Its headquarters are in the town of Saki. It has an area of 2014 km² and a population of 278,002 at the 2006 census. Saki is located at the northern part of Oyo state in Nigeria. It has a Resettlement center of the 2nd Mechanized Division of Nigerian Army, The Oke-Ogun Polytechnic, and a Technical college. It is also one of the largest cities in Oyo State. The postal code of the area is 203.

Population of the study

The study population includes all 60 newly infected *M. tuberculosis* patients who attended the medical outpatient Department of Baptist Medical center, Saki-Nigeria between April and August 2018. The patients were classified into females (30) and males (30) aged 38–79 years.

Biological sample

Early morning sputum and venous blood sample were obtained from each of the patients for the determination of *M. tuberculosis* and HBeAg, HBeAb, HBsAg, anti-HCV, and HIV-1 p24, respectively.

Methods

Hepatitis B envelope antigen and hepatitis B envelope antibody assay using MyBioSource kit

Principle of the assay

This assay employs the qualitative enzyme immunoassay technique. The microtiter plate provided in this kit has been precoated with HBeAg for HBeAb assay or HBeAb for HBeAg assay**.** Samples were pipetted into the wells with HBeAb conjugated horseradish peroxidase (HRP). Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in opposite to the amount of human HBeAb bound in the initial step. The color

development is stopped, and the intensity of the color was measured at 450 nm.

Human immunodeficiency virus-1 p24 antigen ELISA using ZEPTROMETRIX retrotek kit

Principle

Microwells are coated with a monoclonal antibody specific for the p24 gag gene product of HIV-1. Viral antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured antigen is then reacted with a high tittered human anti-HIV-1 antibody conjugated with biotin. Following a subsequent incubation with Streptavidin-Peroxidase, color develops as the bound enzyme reacts with the substrate. Resultant optical density is proportional to the amount of HIV-1 p24 antigen present in the specimen.

Anti-hepatitis C virus ELISA assay

This was assayed using anti-HCV Core Antigen-antibody (ab50288) Abcam kit.

Hepatitis B surface antigen ELISA test

This was assayed using Diagnostic Automation/Cortez Diagnostics, INC kit by ELISA method

Principle

The HBsAg ELISA test kit employs an antibody sandwich ELISA technique where monoclonal antibodies unique to HBsAg, are precoated on polystyrene microwell strips. The plasma sample was added together with a second after incubation and washing, to eliminate unwanted serum proteins and unbound HRP-conjugate, chromogen solutions-containing tetramethyl-benzidine (TMB), and urea peroxide were added to the wells. The colorless chromogens were hydrolyzed by the bound HRP-conjugate to a blue-colored product. Sulfuric acid was added to stop the reaction, and the blue color then turns yellow. This color intensity is directly proportional to the amount of antigen in the samples. If the blue color remains colorless, it indicates HBsAg negative.

Identification of Mycobacterium tuberculosis in sputum using fluorescence microscopy (auramine-rhodamine staining)

Principle

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.[13]

Sputum culture for the identification of Mycobacterium tuberculosis

In addition to fluorescence microscopy, sputum was inoculated on Löwenstein–Jensen medium and incubated aerobically for 4 weeks. The following features were also used for the identification of the growth on the medium brown, granular colonies.[14]

Sampling procedure

The sampling procedure includes the consented patients with a history of prolong a cough, loss of appetite and weight in addition to positive X-ray report with respect to *M. tuberculosis* infection. Patients who were not on the anti-PTB drug were included in the study.

Ethical consideration

The proposal of this work was reviewed and approved by ethical and research committee of Baptist Medical center Saki-Nigeria before the commencement of this work. Informed consent was also obtained from each of the patient and controls.

Methods of data analysis

Data were collated, tallied, and analyzed with the aid of a Statistical Package for Social Sciences (IBM SPSS, version 18, American multinational information technology company, Armonk, New York) to determine the frequency, mean, and percentages.

Results

The viral immunochemical pattern obtained among the newly infected PTB patients showed a frequency of: 5% (3) (males - 3.3%[2] and females - 1.67% [1]) anti-HCV; 3.3% (2) (all males - 3.3% [2]) HIV-1 - p24; 15% (9) (males - 5% [3] and females - 10% [6]) HBsAg; 15% (9) (males - 5% [3] and females - 10% [6]) HBeAg; 18.33% (11) (males - 6.7% [4] and females - 11.67% [7]) hepatitis B envelope antibody; 3.33% (2) (males - 1.67% [1] and females - 1.67% [1]) HBeAg +HBsAg +HIV-1 - p24 [Table 1].

Discussion

Expression of viral biomarkers in newly infected PTB patients as indicated in the results could be associated with the following facts discussed in the following paragraphs.

M. tuberculosis co-infection has been reported. According to Verhagen *et al*.,[15] as HIV-infected infants and children are at increased risk of developing severe forms of PTB. The PTB diagnosis is complicated by diminished sensitivity and specificity of clinical features. Esteve *et al*.[16] also reported that immigrant patient presenting with a septic respiratory clinical picture in which the final diagnosis was miliary PTB and HIV co-infection. Kassu *et al*.[17] also reported that co-infection with HIV was very high in patients with PTB among adults at a teaching hospital, Northwest Ethiopia. Gao *et al*.,[18] also reported TB/HIV co-infection. These findings are consistent with the results obtained in this work.

According to Pawlowski *et al*.,[2] HIV infection is the most powerful known risk factor predisposing for *Mycobacterium* PTB infection and progression to active disease, which increases the risk of latent PTB reactivation.

Furthermore, Lomtadze *et al*.[19] reported that the high prevalence of HCV co-infection was found among patients

Table 1: Pattern of anti-hepatitis C virus, hepatitis B envelope antigen, hepatitis B surface antigen, hepatitis B envelope antibody, and human immunodeficiency virus-1 p24 in newly infected *Mycobacterium* pulmonary tuberculosis patients

	Total number of *Mycobacterium tuberculosis* patient screened	Positive patients	Negative patients	Male	Female
PTB patient with anti-HCV	60	5% (3)	95% (57)	3.3% (2)	1.67% (1)
PTB patient with HIV-1 - p24	60	3.3% (2)	96.67% (58)	3.3% (2)	-
PTB patient with HBsAg	60	15% (9)	85% (51)	5% (3)	10% (6)
PTB patient with HBeAg	60	15% (9)	85% (51)	5% (3)	10% (6)
PTB patient with HBeAb	60	18.33% (11)	81.67% (89)	6.7% (4)	11.67% (7)
PTB patient with HBeAg, HBsAg and HIV-1 - p24	60	3.33% (2)	96.67% (58)	1.67% (1)	1.67% (1)

PTB: Pulmonary tuberculosis, HCV: Hepatitis C virus, HBeAg: Hepatitis B envelope antigen, HBsAg: Hepatitis B surface antigen, HBeAb: Hepatitis B envelope antibody, HIV: Human immunodeficiency virus

with PTB in Georgia and that drug-induced hepatotoxicity was significantly associated with HCV co-infection. In addition, PTB and HBV infections are quite common in the developing world.[20]

In addition, Hussain *et al.*[21] also reported that the seroprevalence of HIV infection among PTB patients was 1.48% (18/1215) and that of HBsAg reactivity was found to be 2.96% (36/1215). During 2007–2010, the HIV-positivity varied between 1.5% and 1.45%, whereas HBV reactivity ranged between 2.4% and 3.63% and that substantial percentage of the PTB patients harbor HIV and HBV co-infections. This report can be associated with the findings of this work.

The findings of this study can also be linked with the report of Mengesha *et al.*[22] who found that PTB and hepatitis are the two common co-infections in patients infected with HIV. They also reported that the prevalence of hepatitis B and C coinfection was fairly high in HIV positive patients findings of this work are consistent with their reports.

The findings of this work could be associated with the effect of PTB on the immune status of the patients thereby making the affected individual susceptible to viral co-infection as evidenced in the report of Fenner *et al*.,[23] who analyzed data from 175,212 patients with possible low immunity who enrolled between 2000 and 2010 and identified 702 patients with incident CM (including 205 with a PTB history) and 487 with incident PCP (including 179 with a PTB history). They found that the incidence per 100 person-years over the 1st year of ART was 0.48 for Kaposi's sarcoma and 0.02 for nonHodgkin lymphoma. A history of PTB was associated with cryptococcal disease and *Pneumocystis jirovecii* pneumonia or Kaposi's.

Viral co-infection with *Mycobacterium* PTB could also be associated with the effects on immune system as described by van Crevel *et al*.,[24] that the different manifestations of infection with *Mycobacterium* PTB reflect the balance between the bacillus and host defense mechanisms. Conventionally, protective immunity to PTB has been ascribed to T-cell-mediated immunity, with CD4+ T cells playing a crucial role. Recent immunological and genetic studies support the long-standing notion that innate immunity is also relevant in PTB.[24] Furthermore, the subsequent inflammatory response is regulated by production of pro- and anti-inflammatory cytokines and chemokines. Different natural effector mechanisms for the killing of *M. tuberculosis* have now been identified. Finally, the innate host response is necessary for the induction of adaptive immunity to *Mycobacterium* PTB.[24]

Conclusion

This work revealed evidence of anti-HCV; HIV-1 - p24; HBsAg; HBeAg; HBe antibody, and HBeAg + HBsAg + HIV-1 - p24 in newly infected pulmonary *M. tuberculosis*-infected patients.

Recommendation

Routine evaluation of viral markers in PTB infection is recommended for effective management.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Glickman MS, Jacobs WR Jr. Microbial pathogenesis of *Mycobacterium tuberculosis*: Dawn of a discipline. Cell 2001;104:477-85.
2. Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. Tuberculosis and HIV co-infection. PLoS Pathog 2012;8:e1002464.
3. Duffy D, Mamdouh R, Laird M, Soneson C, Le Fouler L, El-Daly M, *et al.* The ABCs of viral hepatitis that define biomarker signatures of acute viral hepatitis. Hepatology 2014;59:1273-82.
4. Trépo C, Zoulim F, Alonso C, Petit MA, Pichoud C, Vitvitski L, *et al.* Diagnostic markers of viral hepatitis B and C. Gut 1993;34:S20-5.
5. Hadziyannis E, Laras A. Viral biomarkers in chronic HBeAg negative HBV infection. Genes (Basel) 2018;9. pii: E469.
6. Murray SA, Kendall M, Boyd K, Sheikh A. Illness trajectories and palliative care. BMJ 2005;330:1007-11.
7. Zumla A, Raviglione M, Hafner R, von Reyn CF. Tuberculosis. N Engl J Med 2013;368:745-55.
8. Blank J, Eggers L, Behrends J, Jacobs T, Schneider BE. One episode of self-resolving *Plasmodium yoelii* infection transiently exacerbates chronic *Mycobacterium tuberculosis* infection. Front Microbiol 2016;7:152.
9. Skolnik R. Global Health 101. 2nd ed. Sudbury, Massachusetts, Burlington, MA: Jones & Bartlett Learning; 2011. p. 253.
10. WHO. "Tuberculosis Fact sheet N 104" October, 2015; 23 August, 2012. Available from: https://www.who.int/tb/publications/factsheets/

en/. [Last retrieved on 2016 Feb 11].
11. Dolin R, Mandell GL, Bennett JE. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed., Ch. 250. Philadelphia, PA: Churchill Livingstone/Elsevier; 2010.
12. United States Embassy in Nigeria Economic Section, Nigeria Tuberculosis Fact Sheet; 2012. Available from: http://www.nigeria.usembassy.gov. [Last accessed on 2014 Feb 27].
13. Spring KR, Davidson MW. Introduction to Fluorescence Microscopy. Nikon Microscopy U; 2008. Available from: https://www.en.wikibooks.org/wiki/Chemical_Sciences:_A_Manual_for_CSIRUGC_National_Eligibility_Test_for_Lectureship_and_JRF/Fluorescence_microscope. [Last retrieved on 2008 Sep 28]. [Last accessed on 2011 Feb 19].
14. Cheesebrough M, editors. District Laboratory Practice in Tropical Countries. Part 2. Second edition; Published in the United States of America by Cambridge University Press, New York; 2006.
15. Verhagen LM, Warris A, van Soolingen D, de Groot R, Hermans PW. Human immunodeficiency virus and tuberculosis coinfection in children: Challenges in diagnosis and treatment. Pediatr Infect Dis J 2010;29:e63-70.
16. Esteve E, Supervía A, Pallàs O, Martínez MT, Montero MM, Del Baño F, *et al.* Miliary tuberculosis coinfection with human immunodeficiency virus. West J Emerg Med 2010;11:405-7.
17. Kassu A, Mengistu G, Ayele B, Diro E, Mekonnen F, Ketema D, *et al.* Coinfection and clinical manifestations of tuberculosis in human immunodeficiency virus-infected and -uninfected adults at a teaching hospital, Northwest Ethiopia. J Microbiol Immunol Infect 2007;40:116-22.
18. Gao J, Zheng P, Fu H. Prevalence of TB/HIV co-infection in countries except China: A systematic review and meta-analysis. PLoS One 2013;8:e64915.
19. Lomtadze N, Kupreishvili L, Salakaia A, Vashakidze S, Sharvadze L, Kempker RR, *et al.* Hepatitis C virus co-infection increases the risk of anti-tuberculosis drug-induced hepatotoxicity among patients with pulmonary tuberculosis. PLoS One 2013;8:e83892.
20. Patel ND, Singh SP. Antituberculosis therapy in patients with hepatitis B viral infection. Hept B Annu (serial online) 2012;9:16-48. Available from: http://www.hepatitisbannual.org/text.asp?2012/9/1/16/193287. [Last accessed on 2016 Nov 19].
21. Hussain A, Kulshreshthaa KK, Yadav VS, Katoch K. HIV and HBV co-infections among patients with active TB disease attending a primary health care centre in a rural area of North India. Egypt J Chest Dis Tuberc 2016;65:227-32.
22. Mengesha E, Airgecho T, Airgecho T, Negera E, Kebede M. Prevalence of triple viral infections of human immunodeficiency virus (HIV), hepatitis B and C among tuberculosis patients and associated risk factors: The case of West Arsi zone, Ethiopia. Afr J Microbiol Res 2015;9:1675-83.
23. Fenner L, Reid SE, Fox MP, Garone D, Wellington M, Prozesky H, *et al.* Tuberculosis and the risk of opportunistic infections and cancers in HIV-infected patients starting ART in Southern Africa. Trop Med Int Health 2013;18:194-8.
24. van Crevel R, Ottenhoff TH, van der Meer JW. Innate immunity to *Mycobacterium tuberculosis*. Adv Exp Med Biol 2003;531:241-7.