Expression of HBeAg, anti-HCV and HIVP24Ag-Ab in relationship with the evidence of anti-viral immune response in newly infected *Mycobacterium tuberculosis* patients

Abstract

Study Background: *Mycobacterium tuberculosis* coinfection with virus may generate active viral process that may bring about inflammation and liver damage due to anti-viral host immune response especially in hepatotropic viral infections. **Aim and Objective:** This work therefore sought to investigate the expression of HBeAg, anti-HCV and HIVP24Ag-Ab in relationship with the evidence of anti-viral immune response in newly infected *Mycobacterium tuberculosis*

patients**. Materials and Methods:** This work was carried out among forty one (41: Female – 17; Male-24) newly infected *M. tuberculosis* patients aged 46 – 64 years ; those who expressed viral immuno serological markers were studied as test subjects while those who were not infected with HIV, HCV and HBV were studied as Control. All subjects were negative to Giemsa thick blood film test for identification of *Plasmodium*. TNFα, HBeAg, anti-HCV and HIVP24Ag-Ab were determined by ELISA while ALT was determined by spectrophotometry. **Results:** The frequency of immune serological markers in *M. tuberculosis* patients include 17%(7**)**HBeAg; 9.8%(4)Anti-HCV; 2.4%(1)HIVP24Ag-Ab and 70.7%(29)*Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV. Plasma TNFα and ALT were significantly higher in patients with *Mycobacterium tuberculosis* + HBeAg and *Mycobacterium tuberculosis* + Anti-HCV compared with Mycobacterium tuberculosis patients not infected with HIV, HCV and HBV($p<0.05$). There was a significant increase in plasma TNFα in patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab compared with the *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV($p<0.05$).There was a significant increase in TNFα and ALT in patients with *M. tuberculosis* + HBeAg compared with the results obtained patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab($p<0.05$). There was a significant increase in plasma ALT in patients with *Mycobacterium tuberculosis* + HBeAg compared with patients *Mycobacterium tuberculosis* + Anti-HCV and patients with *Mycobacterium tuberculosis* patients + Anti-HCV compared *Mycobacterium tuberculosis* + HIVP24Ag-Ab($p<0.05$). **Conclusion:** This work revealed antiviral immune response in *Mycobacterium tuberculosis* patients as manifested by increase in plasma TNFα and ALT in relationship with the immune serological markers in *Mycobacterium tuberculosis* patients that include 17%(7**)**HBeAg; 9.8%(4)Anti-HCV; 2.4%(1)HIVP24Ag-Ab; and 70.7%(29)*Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV which can be prevented in *Mycobacterium tuberculosis* patients through adequate viral evaluation and vaccination against HBV and HCV.

Keywords: HBeAg, anti-HCV, HIVP24Ag-Ab, anti-viral immune response, ALT, TNFα, *Mycobacterium tuberculosis* patients

Introduction

Mycobacterium tuberculosis (MTB) infection can generate both innate and adaptive immune response. It is often thought as an opportunistic infection especially in HIV/AIDS. It is one of the infectious diseases that can be prevented through the use of vaccine(Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van *et al.,* 2010; Lawn and Zumla, 2011)

. Poverty, poor hygiene and lack of access to qualitative healthcare have been attributed to the scourge of the infection (Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van *et al.,* 2010; Lawn and Zumla, 2011). There is treatment for tuberculosis which has been free through foreign donors like Damien foundation. *Mycobacterium tuberculosis* infection in immunosuppression invade by chance. The risk factor include Diabetes, alcoholism, cigarrete smoking and malnutrition(Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van *et al.,* 2010; Lawn and Zumla, 2011)

Anti-viral immune response is manifested through the production of tumor necrosis factor (TNFα) produced by the Natural killer cells to induce inflammation, fever, production of antibodies, inhibit tumorigenesis and viral replication(Xiaoyan *et al.,*2017; Manisha *et al.,* 2018). Consequently, viral infections are accompanied by the expression of cytokines and chemokines that can be critical for the control of viral replication(Xiaoyan *et al.,*2017; Manisha *et al.,* 2018)

The Hepatitis e antigen (HBeAg) is an indication of acitive HBV, new acute infection; higher HBV DNA levels, increased level of hepatitis B Virus, active viral replication and that the person infected with Hepatitis B Virus can transmit the virus on to another (Gerald *et al.,* 2009)

It may disappear after six moth due to production of antibody to e antigen (HBeAb(Gerald *et al.,* 2009).Antibody to Hepatitis C Virus (Anti-HCV) is an indication of Hepatitis C \Virus(HCV) infection while HIVP24Ag-Ab is a seromarker for HIV infected psatients expressing both the antigen and antibody to HIV(Gerald *et al.,* 2009)

Expression of cytokines such as TNFα in response to infection/sepsis has a primary role of immune regulation(Brynskov *et al.,* 2002; Victor and Gottlieb, 2002). It is an endogenous pyrogen that can bring about fever, heat, swelling, redness, pain, loss of function apoptotic cell death, cachexia, inflammation, inhibit tumorigenesis and viral replication(Brynskov *et al.,* 2002; Victor and Gottlieb, 2002). Hepatotropic virus can induce liver damage as virus-specific cytotoxic T lymphocytes destroys the virus infected cells and production of antiviral cytokines(Brynskov *et al.,* 2002; Victor and Gottlieb, 2002). One of the manifestations of liver damage include elevated Alanine transaminase (ALT) (Ghouri *et al.,* 2010; Marshall, 2012). Alanine transaminase (ALT) or Serum Glutamate-Pyruvate Transaminase or Serum Glutamic-Pyruvic Transaminase (SGPT) is majorly in the liver which its plasma level rises in liver damage(Ghouri *et al.,* 2010; Marshall, 2012).

This work investigated expression of HBeAg, anti-HCV and P24Ag-Ab in relationship with the evidence of anti-viral immune response in newly infected *Mycobacterium tuberculosis* patients to provide useful information for preventive healthcare.

1-5 (Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van *et al.*, 2010; Lawn and Zumla, 2011)

Materials and Methods

Study Area

This work was carried out in Owo which is the headquarters of Owo local government area that hosts primary, secondary and tertiary health and educational institutions. It is a local government that constitutes Owo/Ose Federal constituency Ondo state in Nigeria.

Study Population

This work was carried out among forty one (41: Female – 17; Male-24) newly infected *Mycobacterium tuberculosis* patients aged 46 – 64 years in Owo local government area, Ondo state - Nigeria ; those who expressed viral immuno serological markers were studied as test subjects while those who were not infected with HIV, HCV and HBV were studied as Control. All subjects were negative to Giemsa thick blood film test for identification of *Plasmodium*

Research Design

Observational case control research design.

Alanine Transaminase

Alanine Transaminase activities was carried out by spectrophotometric method using Reagent Kit of Randox

TNF alpha ELISA

Plasma TNFα was measured in all the subjects by ELISA using Abcam's kit.

Laboratory Identification of Acid Fast Bacilli and *Plasmodium spp.*,

Laboratory detection of Acid Fast Bacilli and *Plasmodium spp.*, was determined by Microscopy using Ziehl Neelsen and Geimsha-Thick film methods as described by Cheesbrough, 20006).

Anti-HCV ELISA assay

This was detected in the subjects by Anti-Hepatitis C Virus Core Antigen antibody using Abcam kit.

HIV ELISA Test

HIVP24 Antigen and Antibody was evaluated in the subjects using Genscreen™ ULTRA HIV Ag-Ab Biorad Kit.

The Genscreen™ ULTRA HIV Ag-Ab is enzyme immunoassay technique based on the principle of sandwich technique for the detection of HIV antigen and antibodies specific to HIV-1 and/or HIV-2 virus in human plasma.

HBeAg ELISA Test

HBeAg ELISA was determined in the subjects using Bio-Rad kit .

Ethical Consideration

The proposal of this work was reviewed and approved by the Research and Ethical Committee of the Department of Medical Laboratory Science, Achievers University, Owo – Nigeria before the commencement of this work. Consent of the subjects was also obtained.

Data Analysis

The results obtained in this work was subjected to statistical analysis using IBM SPSS 20.0 to determine mean, standard deviation, student ‘t’ value and probability at 0.05 level of significance.

Results

The frequency of immune serological markers in *Mycobacterium tuberculosis* patients include 17%(7**)** HBeAg; 9.8%(4) Anti-HCV; 2.4%(1) HIVP24Ag-Ab; and 70.7%(29)*Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV (Table 1; Figure 1).

Plasma TNFα and ALT were significantly higher in patients with *Mycobacterium tuberculosis* + HBeAg and *Mycobacterium tuberculosis* + Anti-HCV compared with Mycobacterium tuberculosis patients not infected with HIV, HCV and HBV($p<0.05$; Table 1, 2; Figure 1).

There was a significant increase in plasma TNFα in patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab compared with the *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV($p<0.05$; Table 1, 2; Figure 1).

There was a significant increase in TNFα and ALT in patients with *Mycobacterium tuberculosis* + HBeAg compared with the results obtained patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab($p<0.05$; Table 1, 2; Figure 1).

There was a significant increase in plasma ALT in patients with *Mycobacterium tuberculosis* + HBeAg compared with patients *Mycobacterium tuberculosis* + Anti-HCV and patients with *Mycobacterium tuberculosis* patients + Anti-HCV compared *Mycobacterium tuberculosis* + HIVP24Ag-Ab($p<0.05$; Table 1, 2; Figure 1).

However there was no significant difference in patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab compared with the *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV Ab ($p>0.05$; Table 1, 2; Figure 1). There was also no significant difference in plasma TNFα in patients with *Mycobacterium tuberculosis* + HBeAg compared with those with *Mycobacterium tuberculosis* + Anti-HCV and patients with *Mycobacterium tuberculosis* + Anti-HCV compared with patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab ($p>0.05$; Table 1, 2; Figure 1).

Discussion

The frequency of immune serological markers in *Mycobacterium tuberculosis* patients include 17%(7**)** HBeAg; 9.8%(4) Anti-HCV; 2.4%(1) HIVP24Ag-Ab; and 70.7%(29)*Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV.

Regarding the above, it affirms *Mycobacterium tuberculosis* with HIV, HBV and HCV as earlier reported(Armin *et al.,* 2013; Francine *et al.,* 2018; Lubiao *et al.,* 2018). and that Tuberculosis therapy in *Mycobacterium tuberculosis* patients infected with hepatotropic virus may cause liver failure hence the need for liver function assessment (Lubiao *et al.,* 2018).

The prevalence of HCV infection among tuberculosis patients (9.8%)was higher than the overall prevalence of HCV infection in patients with TB was 7% reported by Meysam *et al*.,(2019) possibly due to socio-economic and environmental differences.

Plasma TNFα and ALT were significantly higher in patients with *Mycobacterium tuberculosis* + HBeAg and *Mycobacterium tuberculosis* + Anti-HCV compared with *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV.

Elevated Plasma TNFα and ALT in this study is a manifestation of anti-viral immune response because HBeAg indicates active HBV replication, increased infectiousness and viral load which can induce immune response leading to liver damage as HBV does not directly destroy hepatocytes[6][7][8] . Anti-HCV is also an indication of HCV infection which manifested anti-viral immune response which was reflected as elevated TNFα and ALT (Gerald *et al.,* 2009; Ghouri *et al.,* 2010; Marshall, 2012; Xiaoyan *et al.*,2017; Manisha *et al.,* 2018).

There was a significant increase in plasma TNFα in patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab compared with the *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV.

Presence of HIVP24Ag-Ab in a body system indicates HIV infection and increased TNFα is a reflection of anti-viral immune response to kill the virus and virally infected cells to induce , fever

inflammation, acute phase response and inhibit viral replication(Gerald *et al.*, 2009; Xiaoyan *et al.*,2017; Manisha *et al.,* 2018).

There was a significant increase in TNFα and ALT in patients with *Mycobacterium tuberculosis* + HBeAg compared with the results obtained patients with *Mycobacterium tuberculosis* + HIVP24Ag-Ab.

Again, HBeAg is an indication of active HBV, new acute infection; higher HBV DNA levels, increased level of hepatitis B Virus, active viral replication and that the person infected with Hepatitis B Virus can transmit the virus on to another while HIV can infect all organs [8]. Significant increase in TNFα and ALT in patients with *Mycobacterium tuberculosis* is as a result of anti-viral immune response against HBV infection in *Mycobacterium tuberculosis* patients (Gerald *et al.,* 2009; Ghouri *et al.,* 2010; Marshall, 2012; Xiaoyan *et al.*,2017; Manisha *et al.,* 2018).

There was a significant increase in plasma ALT in patients with *Mycobacterium tuberculosis* + HBeAg compared with patients *Mycobacterium tuberculosis* + Anti-HCV and patients with *Mycobacterium tuberculosis* patients + Anti-HCV compared *Mycobacterium tuberculosis* + HIVP24Ag-Ab.

Regarding Hepatitis B Virus envelope antigen, it indicates active viral replication and increased viral load which can generated corresponding degree of immune response thereby causing increased hepatocellular damage which was manifested in this work as elevated plasma ALT to signify a process of anti-viral immune response as HBV does not directly damage the hepatocytes but the immune response(Gerald *et al.,* 2009; Xiaoyan *et al.*,2017; Manisha *et al.,* 2018).

In respect of HCV infection compared with HIV infection the elevated plasma ALT in HCV patients than HIV patients is because though HIV can infect all organs but can induce immunosuppression thereby reducing the level of liver damage as the hepatocellular damage is always as a result of immune response. In addition HCV is an hepatotropic virus that can cause liver damage to leak ALT into the plasma to cause elevated plasma level (Gerald *et al.,* 2009; Ghouri *et al.,* 2010; Marshall, 2012; Xiaoyan *et al.*,2017; Manisha *et al.,* 2018).

Conclusion

This work revealed antiviral immune response in *Mycobacterium tuberculosis* patients as manifested by increase in plasma TNFα and ALT in relationship with the immune serological markers in *Mycobacterium tuberculosis* patients that include 17%(7**)** HBeAg; 9.8%(4) Anti-HCV; 2.4%(1) HIVP24Ag-Ab; and 70.7%(29)*Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV which can be prevented in *Mycobacterium tuberculosis* patients through adequate viral evaluation and vaccination against HBV and HCV..

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Table1: Frequency of HIVP24Ag-Ab, HBeAg, Anti-HCV, plasma values of TNFα, and ALT obtained in the subjects

	Frequency	**ALT(U/L)**	**TNFα(pg/ml)**	**Ziehl Neelsen AFB**	***Plasmodium***
Mycobacterium tuberculosis **patients + HBeAg**	17%(7)	41 ± 2.0	4.8 ± 2.0	Positive	Negative
Mycobacterium tuberculosis **patients + Anti-HCV**	9.8%(4)	28 ± 1.0	4.4 ± 1.0	Positive	Negative
Mycobacterium tuberculosis **patients + HIVP24Ag-Ab**	2.4%(1)	13 ± 2.0	4.0 ± 1.0	Positive	Negative
Mycobacterium tuberculosis **patients + two or more of HBeAg , anti-HCV, HIVP24Ag-Ab**	0%(0)	-	-	Positive	Negative
Mycobacterium tuberculosis **patients not infected with HIV, HCV and HBV Control(n=29)**	70.7%(29)	8.0 ± 1.0	3.3 ± 2.0	Positive	Negative

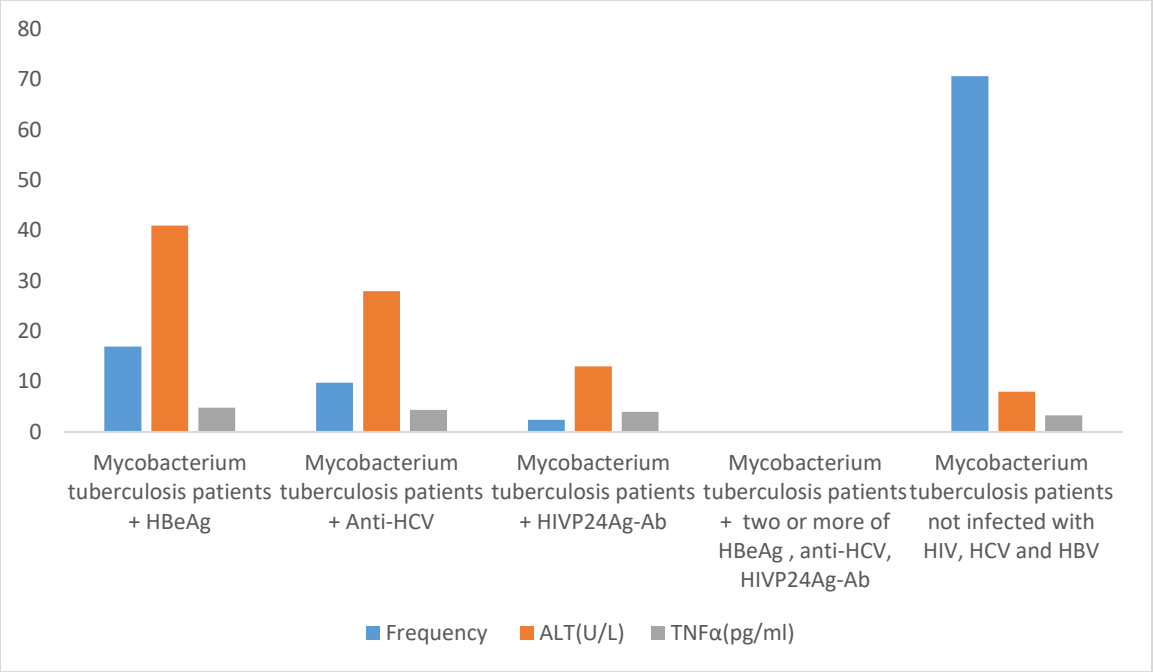

Figure1: Comparative description of the Frequency of HIVP24Ag-Ab, HBeAg, Anti-HCV, plasma values of TNFα, and ALT obtained in the subjects

Table2: Results of the Comparative analysis of the frequency of HIVP24Ag-Ab, HBeAg, Anti-HCV, plasma values of TNFα, and ALT obtained in the subjects

		***Mycobacterium tuberculosis* patients + HBeAg Vs *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV**	***Mycobacterium tuberculosis* patients + Anti-HCV Vs *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV**	***Mycobacterium tuberculosis* patients + HIVP24Ag-Ab Vs *Mycobacterium tuberculosis* patients not infected with HIV, HCV and HBV**	***Mycobacterium tuberculosis* patients + HBeAg** Vs ***Mycobacterium tuberculosis* patients + Anti-HCV**	***Mycobacterium tuberculosis* patients + HBeAg** Vs ***Mycobacterium tuberculosis* patients + HIVP24Ag-Ab**	***Mycobacterium tuberculosis* patients + Anti-HCV** Vs ***Mycobacterium tuberculosis* patients + HIVP24Ag-Ab**
TNFα(pg/ml)	**'t'value**	5.3033.	4.91935	3.1305	1.78885	3.57771	2.82843
	'p'value	0 .02*	0 .02*	0 .04*	0.1	0 .04*	0.05
ALT(U/L)	**'t'value**	14.75805	14.14214 .	2.23607	5.81378	9.8995	6.7082
	'p'value	0 .002**	0 .003**	0 .077	0 .014*	0 .005**	0 .01*

*Significant

**Highly Significant