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

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ABSTRACT

Mycobacterium tuberculosis (M. tuberculosis) co-infection 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. 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**.** This study was carried out among forty one (41) subjects (Female-17; Male-24) newly infected *M. tuberculosis* patients aged 46 – 64 years ; those who expressed viral immunoserological 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. The frequency of immune serological markers in *M. tuberculosis* patients include 17%(7)HBeAg; 9.8%(4)Anti-HCV; 2.4%(1)HIVP24Ag-Aband 70.7%(29)*M. tuberculosis patients* not infected with HIV, HCV and HBV. Plasma TNFα and ALT were significantly higher in patients with *M. tuberculosis* + HBeAg and *M. 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 *M. 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 *M. tuberculosis* + HIVP24Ag-Ab($p<0.05$). *There was a significant increase in plasma ALT in patients with M. tuberculosis* + HBeAg compared with patients with *M. tuberculosis* + Anti-HCV and also in patients with *M. tuberculosis* + Anti-HCV compared *M. tuberculosis* + HIVP24Ag-Ab($p<0.05$). This study revealed viral immunoserologic markers in *Mycobacterium tuberculosis* patients as 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 caused antiviral immune response in *M. tuberculosis* patients as indicated by increased plasma TNFα – pro-inflammatory cytokine and ALT - a liver enzyme and an index of liver damage due to host immune response to especially hepatotropic virus. Viral co-infection and the effect of anti-viral immune response may be prevented in *Mycobacterium tuberculosis* patients through adequate viral evaluation and vaccination against HBV and HCV.

Keywords: HBeAg, anti-HCV, HIVP24Ag-Ab, co-infection, immune-response,

INTRODUCTION

Mycobacterium tuberculosis (MTB) infection can generate both innate and adaptive immune response (Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van Zyl Smit et al. 2010; Lawn and Zumla, 2011). It is often thought as an opportunistic infection especially in HIV/AIDS (Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van Zyl Smit et al. 2010; Lawn and Zumla, 2011). It is one of the infectious diseases that can be prevented through the use of vaccine (van Zyl Smit 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 (Möller and Hoal, 2010; Van et al. 2010; Lawn and Zumla, 2011). *Mycobacterium tuberculosis* infection in immunosuppression occurs by chance. The risk factor include Diabetes, alcoholism, cigarrete smoking and malnutrition (Griffith and Kerr, 1996; Restrepo, 2007; Möller and Hoal, 2010; van Zyl Smit 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 (Li 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 (Li 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 (Mandell et al.2009). It may disappear after six moth due to production of antibody to e antigen (HBeAb) (Mandell 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 (Mandell 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, and 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 destroy the virus-infected cells and production of antiviral cytokines (Brynskov et al. 2002; Victor and Gottlieb, 2002). One of the manifestations of liver damage includes 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* patientsto provide useful information for preventive healthcare.

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.

Subjects

This observational case-control research work was carried out among forty one (41: Female – 17; Male-24) newly infected *Mycobacterium tuberculosis* patients aged 46 – 64 yearsin 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.*

Approval was granted by the Research and Ethical Committee of the Department of Medical Laboratory Science, Achievers University, Owo, Nigeria and informed consent was obtained from the subjects before the commencement of this work.

Inclusion Criteria: Only newly infected/diagnosed tuberculosis patients were recruited for the study.

Exclusion Criteria:

The excluded subjects include:

a). Individuals who were on hepatotoxic drugs or chemicals like alcohol were excluded
b). Individuals who were infected with *Plasmodium* were excluded from the study
c). Tuberculosis patients who have initiated treatment were excluded.

Analytical Methods:

Alanine Transaminase activities was carried out by spectrophotometric method using Randox® Reagent Kit whereas Plasma TNFα and Anti-Hepatitis C Virus were

measured by ELISA using Abcam® kit. HIVP24 Antigen and Antibody was evaluated in the subjects using Genscreen™ ULTRA HIV Ag-Ab Biorad Kit whereas HBeAg ELISA was determined in the subjects using Bio-Rad 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 respectively as described by Cheesbrough, 20006).

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. The results of the statistical analysis were presented in tables and figure.

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).

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**	70.7%(29)	8.0 ± 1.0	3.3 ± 2.0	Positive	Negative
Control(n=29)					

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).

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

		***M. tuberculosis* patients + HBeAg Vs*M. tuberculosis* patients not infected with HIV, HCV and HBV**	***M. tuberculosis* patients + Anti-HCV Vs *M. tuberculosis* patients not infected with HIV, HCV and HBV**	***M. tuberculosis* patients + HIVP24Ag-Ab Vs *M. tuberculosis* patients not infected with HIV, HCV and HBV**	***M. tuberculosis* patients + HBeAg Vs*M. tuberculosis* patients + Anti-HCV**	***M. tuberculosis* patients + HBeAg Vs*M. tuberculosis* patients + HIVP24Ag-Ab**	***M. tuberculosis* patients + Anti-HCV Vs*M. 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

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).

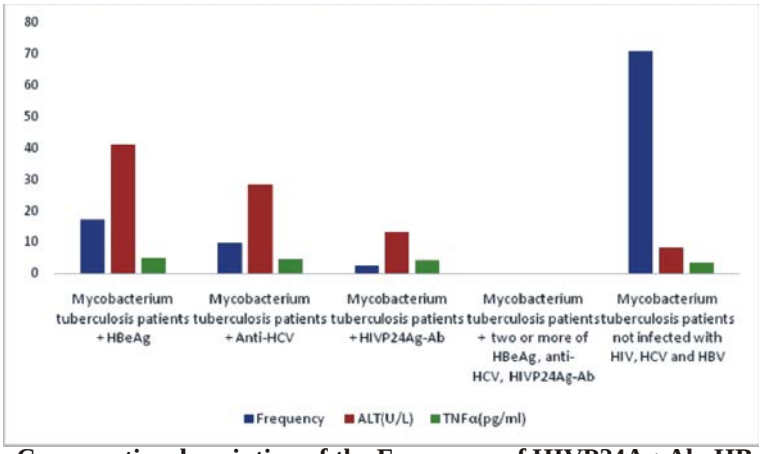

Figure1: Comparative description of the Frequency of HIVP24Ag-Ab, HBeAg, Anti-HCV, plasma values of TNFα, and ALT obtained in the subjects

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 HBVAb ($p>0.05$; 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* (Deffur 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* (Chen 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 Behzadifar 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 (Mandell et al. 2009; Li et al.2017; Manisha et al. 2018). Anti-HCV is also an indication of HCV infection which manifested anti-viral immune response which was reflected as elevated TNFα and ALT (Mandell et al. 2009; Ghouri et al. 2010; Marshall, 2012; Liet 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 (Mandell et al. 2009; Liet 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 (Mandell et al. 2009). *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* (Mandell et al. 2009; Ghouri et al.2010; Marshall, 2012; Li 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 (Mandell et al. 2009; Li 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 a hepatotropic virus that can cause liver damage to leak ALT into the plasma to cause elevated plasma level (Mandell et al. 2009; Ghouri et al. 2010; Marshall, 2012; Li et al. 2017; Manisha et al. 2018).

CONCLUSION

This study revealed viral immunoserologic markers in *Mycobacterium tuberculosis* patients as 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 caused antiviral immune response in *M. tuberculosis* patients as indicated by increased plasma TNFα – pro-inflammatory cytokine and ALT - a liver enzyme and an index of liver damage due to host immune response to especially hepatotropic virus. Viral coinfection and the effect of anti-viral immune response may be prevented in *Mycobacterium tuberculosis* patients through adequate viral evaluation and vaccination against HBV and HCV.

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