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Full Length Research Paper

Bladder tumour antigen seropositivity levels in human immunodeficiency virus seropositive subjects and HIV subjects co-infected with *mycobacterium tuberculosis* in Nnewi Nigeria

Manafa P. O.[1], Chukwuma G. O.[1], Akpuogwu U. A.[1], Chukwuma O. M.[1], Ibe N. C.[1], Nwene E. K.[2], Akulue J. C[1], Aneke J. C.[3] , Manafa V.I.[1], Onyenekwe C. C.[1]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus.
[2]Initiative for good health in Nigeria.
[3]Department of Haematology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

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Patients with chronic HIV infection may develop diseases such as tuberculosis and bladder cancer, associated with long-term exposure to several oncogenic risk factors. This study evaluated the levels of bladder tumour antigen seropositivity in HIV seropositive individuals and HIV seropositive subjects co-infected with tuberculosis. A total of 50 subjects were recruited. These include; 17 HIV seropositive subjects, 17 HIV seropositive subjects co-infected with tuberculosis, 16 apparently healthy individuals (control group). Bladder tumor antigen levels were estimated by the Enzyme Linked Immunosorbent Assay technique. The mean serum levels of bladder tumor antigen were significantly increased in HIV seropositive subjects and HIV seropositive subjects co-infected with tuberculosis compared with the control (P < 0.05). Similarly, there was a significant increase in mean serum levels of bladder tumour antigen in HIV seropositive males and HIV seropositive males co-infected with tuberculosis, compared with HIV seropositive females and HIV seropositive females co-infected with tuberculosis, respectively (P < 0.05). There existed no significant increase in the mean serum levels of bladder tumour antigen in HIV seropositive subjects co-infected with tuberculosis compared with HIV seropositive subjects without TB (p > 0.05). There is a need to explore further, the prognostic potentials of bladder tumour antigen in HIV and Tuberculosis infections.

Key words: HIV, bladder, tumour, antigen, tuberculosis.

INTRODUCTION

The human immunodeficiency virus is a lentivirus (International Committee on Taxonomy of Viruses, 2002), a subgroup of retrovirus that causes HIV infection. During HIV infection, the virus attacks and destroys the infection-fighting $CD4^+$ cells of the body's immune system, resulting to the progressive failure of the immune system and allowing life-threatening opportunistic infections and cancers to thrive (Douek et al., 2009). This virus is different in structure from other retroviruses. It is roughly spherically-shaped having a lipid bilayer envelope, with a diameter of about 120 nm (Fisher et al., 2007). It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid. There are two major viral glycoproteins in this lipid bilayer, gp120 and

*Corresponding author. E-mail: georgechuma@yahoo.com. Tel.:+2348034101608.

gp41. (Lu et al., 2011) HIV virus can infect a variety of immune cells such as $CD4^+$ T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and $CD4^+$ T cells is mediated through interaction of the virion envelope glycoproteins gp120 and gp 41 with the $CD4^+$ molecule on the target cells and also with chemokine co receptors. (Chan et al., 1997). It does so through the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell (Chan et al., 1997). Tuberculosis (TB) is an infectious disease usually caused by the bacterium *Mycobacterium tuberculosis* (WHO, 2010). Tuberculosis primarily affects the lungs, but can also affect other parts such as the bone, the central nervous system, and many other organ systems (Yates et al., 1993). The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats, and weight loss. *M. tuberculosis* is a small, aerobic, non-motile bacillus. The high lipid content of this pathogen accounts for many of its unique clinical characteristics (South wick, 2007). Mycobacteria have an outer membrane lipid bilayer (Niederweis et al., 2010). Because of the high lipid and mycolic acid content of its cell wall (Madison, 2001), the organism does not pick up stain when gram staining is performed on it. This organism primarily attacks the macrophages and replicates in them (Kumar et al., 2007). The most important risk factor of tuberculosis globally is HIV. About 13% of all people with *M. tuberculosis* are infected by the virus (WHO, 2011). Reduced production of IFN-γ or its cellular receptors lead to severe and fatal M. tuberculosis. During HIV infection, IFN-γ production is decreased dramatically which leads to an increased risk of developing, reactivation or reinfection by M. tuberculosis in these HIV/TB patients (Ottenhoff et al., 1998). A tumour can be seen as an abnormal growth of tissue resulting from uncontrolled, progressive multiplication of cells forming a mass which has no physiological function (Birbrair et al., 2014). A bladder tumour forms when abnormal cells in the bladder multiply without control, an event that causes a mass or lesion to form (WHO, 2014). Bladder tumour antigen is a human complement factor H related protein (hCFHrp) similar in composition, structure and function to human complement factor H (hCFH) with a molecular weight of 150KDa (Kinders et al., 1998). It is recognized by the monoclonal antibodies utilized in the BTA test, and is found in human plasma at concentrations of approximately 480 µg/mL (Bassi et al., 1998). This study will provide information on the levels of bladder tumour antigen in HIV seropositive subjects co-infected with mycobacterium tuberculosis. With increasing rates of bladder associated diseases among HIV infected individuals, this research is timely to possibly establish a link between between immuno suppressive diseases like HIV, TB and bladder tumours.

MATERIALS AND METHODS

Materials

i.) ELISA machine (Mindray MR-96A).
ii.) Bladder tumour antigen ELISA KIT by Perlong biotech, Beijing China.

Study site

The study was conducted in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria. It lasted from June to August, 2016.

Study population

This research is a case control study designed to evaluate the levels of bladder tumour antigen in HIV seropositive individuals co-infected with tuberculosis. A total of 50 subjects were recruited for the study. This includes 17 HIV seropositive subjects without tuberculosis, 17 HIV seropositive individuals co-infected with tuberculosis and 16 apparently healthy individuals (control group) randomly selected.

Inclusion criteria

i.) HIV seropositive subjects diagnosed with the infection for at least 5 year.
ii.) HIV subjects co-infected with Tuberculosis

Exclusion criteria

Individuals with history of chronic diseases such as Hepatitis, Herpes virus infection and diabetes.

Ethical consideration

The ethical approval for this research was obtained from Nnamdi Azikiwe University Teaching Hospital Ethical Committee (NAUTHEC).

Collection of sample

About 5mls of venous blood was collected aseptically from each of the subjects and dispensed into a plain container. The samples were allowed to clot and centrifuged at 5,000 rpm for 5 min. The serum was then separated into another plain container.

HIV testing

HIV detection was performed using the method as described by Manafa et al., (2003). The procedure makes use of Determine, uni-gold and Stat-pak test kits performed serially.

Principle of determine

This is an immunochromagraphic test for the qualitative detection of antibodies to HIV-1 and HIV-2. The sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window and no red line are formed at the patient window site.

Principle of UNI-GOLD

Uni-Gold HIV test is a rapid immunoassay based on the immunochromatographic sandwich principle. Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively, are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. During testing, two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash solution and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

Principle of HIV 1/2 STAT-PAK DIPSTICK

This assay employs a combination of antibody binding protein, which is conjugated to colloidal gold dye particles, and antigens to HIV1/2, which are bound to the membrane solid phase. The sample being tested and running buffer are applied to the sample pad. The running buffer facilitates the lateral flow of the specimen through the membrane and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST area producing a pink/purple line. In the absence of HIV 1/2 antibodies, there is no pink/purple line in the TEST area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimens and reagents have been applied properly and have migrated through the device.

Detection of *Mycobacterium tuberculosis*

GeneXpert procedure was adopted for identification of *M*. tubercule bacilli as described by Van Rie (2010).

Principle

The GeneXpert works on the principle of polymerase chain reaction .It detects DNA sequences specific for *M. tuberculosis* and rifampicin resistance by polymerase chain reaction(Van Rie et al., 2010).It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The GeneXpert MTB/RIF purifies and concentrates *M. tuberculosis bacilli* from sputum, gastric lavage, CSF and lymph node tissue, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies all the clinically relevant rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the *M. tuberculosis* genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate.

Procedure

This was according to the manufacturer`s protocol as follows:
The samples were processed directly from GeneXpert MTB/Rif test according to manufacturer's protocol. Sample reagent (SR) which contains NaOH and Isopropanol was added in 2:1 ratio to unprocessed sample in 15 ml falcon tube and the tube was manually agitated twice during a 15 minute incubation period at room temperature. Then 2 ml of the inactivated sample was transferred to the test cartridge using a sterile disposable pipette (provided with kits). Cartridge was loaded into the Genexpert instrument and an automatic process completed the remaining assay steps. Interpretation of data from MTB/RIF test was software based. The processing time was 90 min.

Interpretation of result

Detected: Mycobacteria have a high probability of resistance to RIF; should be confirmed by additional testing. If RIF resistance is confirmed, rapid molecular testing for drug resistance to both first-line and second-line drugs should be performed so that an effective treatment regimen can be selected.

Table 1. Comparison of the mean serum levels of Bladder Tumour Antigen in HIV seropositive subjects co-infected with TB, HIV seropositive subjects not infected with TB and HIV seronegative subjects.

Groups	N	Bladder tumour antigen (mmol/L)	Standard deviation	T-value	P-value
A. HIV / TB	17	77.84	± 13.82		
B.HIV positive	17	71.36	± 12.72	48.880	0.000**
C.HIV negative	16	37.28	± 8.94		
A VS B					0.195
A VS C					0.000**
B VS C					0.000**

Table 2. Comparison of mean serum levels of Bladder tumour antigen in HIV subjects co-infected with TB between different genders.

Gender	N	Bladder tumour antigen (mean) (ng/ml)	Standard Deviation	T-value
Male	10	83.86	± 9.92	
Female	6	62.80	± 10.49	3.282
		P= 0.004		

Not detected: Mycobacteria are probably susceptible to RIF; all tests that are positive for MTBC should have growth-based susceptibility testing to first-line TB drugs.

Indeterminate: the test could not accurately determine if the bacteria are resistant to RIF. Growth-based susceptibility testing to first-line TB drugs should be performed.

Evaluation of bladder tumour antigen

Bladder tumour antigen was evaluated using a method as described by the manufacturer (Perlong Biotech, Beijing China).

Principle

This immunometric assay is based on a double-antibody sandwich technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for human factor H (mouse anti-human factor H); this antibody will bind any Factor H introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of an HRP-labeled Factor H monoclonal antibody to detect the captured Factor H. The two antibody form a sandwich by binding to different locations on the Factor H molecule. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate TMB. After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow colour that can be measured at 450 nm.

Procedure

The desired number of coated wells (96) for the standard, test samples and control were secured in the holder and 50 µl, 5 µl, and 50 µl of standard, test samples and control were added in their appropriate wells respectively. Into the test wells, 45 µl of sample diluent was added. Enzyme conjugate (50 µl) was added to each well and thoroughly mixed for 30 s. The contents of the wells were incubated for 30 min at 37°C. After the incubation, the wells were washed 5 times and then stroked gently onto absorbent paper in other to remove water droplets. Substrate A (50µl) and Substrate B (50 µl) were dispensed into each well, gently mixed for 5 s and then incubated at 37°C for 15 min. After the incubation, the reaction was stopped by adding 50µl of stop solution to each well. It was gently mixed for 30 s, read at 450 nm.

Normal range

<88ng/ml in adult serum

Statistical analysis

The statistical analysis was performed using ANOVA (analysis of variance). The values were deemed significant if $P < 0.05$ (Tables 1, 2, 3 and 4).

Table 3. Comparison of mean serum levels of bladder tumour antigen in HIV seropositive subjects between different genders.

Gender	N	Bladder tumour antigen (mean) (ng/ml)	Standard deviation	T-value
Male	9	78.28	±11.42	
Female	8	60.98	±5.39	3.431
		P= 0.004		

Table 4. Comparison of mean serum levels of bladder tumour antigen of HIV seronegative subjects between different genders.

Gender	N	Bladder tumour antigen (mean) (ng/ml)	STD	t-value
Male	12	38.39	±9.42	0.960
Female	5	32.83	+5.84	
P=0.355				

RESULTS AND DISCUSSION

In this study, we observed a significant increase in the mean serum levels of bladder tumour antigen in HIV seropositive subjects not infected with TB and HIV seropositive subjects co infected with tuberculosis, compared with the control subjects, this is similar to that reported by Helleberg et al. (2014), they observed that the increase might be as a result of risk factors associated with the HIV seropositivity, such as illicit drug use, increased alcohol consumption and cigarette smoking. This could account for their increased rate of bladder tumour antigen levels. Tirelli et al. (2000), found that compared to HIV-negative patients with cancer, more HIV seropositive patients with cancer have a history of cigarette smoking and illicit drug use. Heicappell et al. (1999) reported that bladder tumour antigen is a human complement factor H-related protein (hCFHrp), produced *invitro* by several human bladder cancer cells but not other epithelial cell lines. Human complement factor H protein (hCFHp) is a soluble glycoprotein and complement regulator, essential for controlling the alternative pathway in blood and on cell surfaces (Esparza et al., 2004).

An increase was seen in the mean serum levels of bladder tumour antigen in HIV seropositive males and HIV seropositive males co-infected with tuberculosis, compared to their female counterparts ($p < 0.05$). Jemal et al. (2008), had earlier reported elevated plasma concentrations of bladder tumor antigen in HIV seropositive male subjects than in HIV sero positive female subjects. There are approximately 68,000 new cases of bladder cancer diagnosed each year in HIV seropositive individuals, with 75% of cases occurring in men (Elizabeth et al., 2009). Yoshiyuki and Kunio (1995), postulated that Population-attributable risk percent of occupational exposures is estimated as 35% in males and only l% in females. Occupational exposures are accordingly believed to be one of major contributes to male predominance in bladder cancer. Additionally, Pansy et al. (2009), reported patients with HIV-associated bladder cancer, most of whom received highly active antiretroviral therapy with median age of 58 years, and male to female ratio of 6:1.

Statistically, no significant difference existed in the mean serum level of bladder tumour antigen in HIV seronegative male subjects, compared with HIV seronegative female subjects ($P > 0.05$). Kinders and his colleagues in 1998 reported that normal human epithelial cell line were negative for production of a FH-like protein (CFHrp). This corresponds with the study done by Heicappell et al. (1999) that bladder tumour antigen can be produced *in vitro* by several human bladder cancer cells but not other epithelial cell lines In contrast to this report, Tevfik and Ebru (2012) showed that Hematologic malignancies are generally more common in males and this can be generalized to most other cancers. They suggested that the known differences in immunity may be responsible for this dichotomy. Other obvious differences include hormonal ones and the number of X chromosomes. Some of the differences may even originate from exposures during prenatal development.

However, no significant difference was observed in the mean serum level of bladder tumour antigen in HIV seropositive subjects co-infected with Tuberculosis compared with HIV seropositive subjects. Pansy et al.

(2009), worked on HIV/Tb co-infected individuals, and contrastingly reported that CXCR4 chemokine receptors are associated with advanced stages and recurrence of bladder cancer. In addition, CXCR4 are co-receptors in HIV disease and facilitate the entry of HIV into target cells. CXCR4-tropic HIV has been associated with faster T-cell depletion and AIDS progression (Rockstroh et al., 2009). CXCR4 expression is increased in active TB. TB up-regulates both CCR5 and CXCR4 receptors in monocytes in vitro. Hoshino et al. (2004) found that macrophages taken from bronchoalveolar lavage specimens from patients infected with TB preferentially expressed CXCR4 (chemokine receptor type 4). TB may have upregulated this molecule, leading to enhanced pathogenesis of bladder cancer (Hoshino *et al*, 2004). Saulius and colleague in 2007 discovered that in their series of 2218 operated patients, 46 (2.1%) cases of coexistence of lung cancer and tuberculosis were found. It is possible however, that tuberculosis of lung prepares conditions for the development of lung cancer. The contrast between this research work and previously done works relating to this topic, may be due to the difference in the duration of the Mycobacterium infection. Since the subjects used in this study have suffered from Mycobacterium infection for a shorter duration when compared to the subjects used in the previous research works.

Conclusion

HIV infection is known to render the immune system incompetent. In response to the efficacy of antiretroviral therapy (ART), there is increased life-expectancy for HIV-infected patients and exposure to several oncogenic risk factors, cancers now represent up to one third of all causes of deaths among HIV-infected patients. In this study, we have been able to show that bladder tumour antigen seropositivity levels were significantly increased in HIV seropositive subjects compared with the control group. Furthermore, there was a significant increase in mean serum level of bladder tumour antigen levels in HIV seropositive individuals co-infected with Tuberculosis compared with the control group. We have also shown that the levels of bladder tumour antigen seropositivity levels were significantly increased in HIV seropositive male subjects without TB and HIV seropositive male subjects co-infected with Tuberculosis, compared with HIV seropositive female subjects and HIV seropositive female subjects co-infected with tuberculosis. However, no significant difference was observed in the levels of bladder tumour antigen in HIV seropositive subjects compared with HIV seropositive subjects co-infected with Tuberculosis. There was also no significant difference in the mean serum level of HIV seronegative male individuals compared to HIV seronegative female group. The findings of this research suggest that individuals with HIV infection and HIV/ TB co-infection are at a higher risk of developing bladder cancer than HIV seronegative individuals. It also suggests the need for further study of the increased risk of bladder cancer among the HIV-infected patient population. Studies may be warranted to probe the possible association between HIV and *M. tuberculosis* co-infection, CXCR4 expression, and bladder tumour progression.

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