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## Microbial Assessment of Urine Samples of HIV Infected Patients on Antiretroviral Therapy

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### ABSTRACT

Patients on antiretroviral therapy (ART) usually suffer from side effects. As a result, treatment of HIV infection has become a complicated balancing act between the benefits of durable HIV suppression and the risks of drug toxicity. To investigate the urine of HIV infected patients on ART for possible side effects of ART with the aim of arming both the HIV positive community and health care providers with proper knowledge of possible outcomes and preventable measures. A total of 103 samples were screened at each phase - before ART and during ART, using the control tests as replicates. Three methods were used in the urine assessment: test strip (combi 10) method, microscopic method and cultural method. These were analyzed to investigate for the possible side effects of ART on HIV patients. Except for leucocyte, all other physicochemical parameters- protein, urine appearance,  $pH$ , nitrite, density/ specific gravity, glucose, bilirubin, urobilinogen, blood assessed using urine test strip showed increase in percentage ratio of positive tests during ART. However, microscopy of urine sediments during ART showed reduction in percentage ratios in cellular casts, white blood cell (WBC), and bacteria. Similarly, there was reduction in percentage ratio of bacteria growth during ART. The research showed significant negative effects in the urine parameters of HIV infected patients on antiretroviral therapy in terms of physicochemical parameters compared with the incidence rate before commencement of antiretroviral therapy. However, the reverse was the case in microscopy and cultural investigations. Results are useful in creating awareness in both the HIV positive community and among health care providers so that potentially preventable and treatable ailments resulting from administration of ART can be checked in time in the unsuspecting and asymptomatic individuals.

Keywords: HIV, ART, Patients, Health, Urine and Microbial assesement

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### INTRODUCTION

Antiretroviral therapies (ARTs) are tablets specially formulated to delay the progression of Human Immunodeficiency Virus (HIV) in both adults and children with HIV infection and those who have gone on to develop the symptoms of acquired immunodeficiency syndrome (AIDS) [1]. These drugs are formulated to

be taken orally, and to achieve their efficacy, they are usually taken in combination [1]. For example, nevirapine can be taken in combination with lamivudine and zidovudine. In some cases, monotherapy is recommended, especially lead-in period (period of beginning new ART). However, patients on

antiretroviral therapy (ART) usually suffer from side effects. As a result, treatment of HIV infection has become a complicated balancing act between the benefits of durable HIV suppression and the risks of drug toxicity. About 25% of patients stop therapy within the first year on ART because of side effects [2]. About the same number of patients does not take the recommended dosages of their medication due to concerns regarding the side effects [3]. Patients, who report significant side effects, are more often adherent to therapy [4].

The patient needs to be counseled in detail about potential side effects, in order to be able to recognize them and to consult his/her physician in time. This can save lives; for example, in the case of ART hypersensitivity reaction, or prevent irreversible damage such as polyneuropathy, and some costly side effects such as liver diseases, kidney diseases, neurons system disorders etc. Being prepared for the occurrence of possible problems and providing potential solutions improves both acceptance of treatment and the adherence. However, patients should not

be frightened by all these information. It may be difficult to distinguish between symptoms related to HIV infection and those caused by antiretroviral therapy. An accurate history, including any co-medication (not forgetting over-the-counter and “natural” products) is paramount. It is important to consider the intensity, variation and reproducibility of complaints, as other possible causes should be excluded before symptoms are judged as being side effects of treatment.

It must be stressed that majority of patients are able to tolerate ART well, even over years [5]. Nevertheless, the monitoring of treatment by an HIV clinician, is recommended in at least three-monthly interval, even in asymptomatic patients, and more often at the beginning of a new ART otherwise called lead-in period, when it should be weekly or on two-weeks intervals [1]. Standard evaluations include a thorough history- (allergies, other side effects), physical examination and measurement of vital signs and body weight. Routine investigations include of full blood count, liver, pancreas and renal function tests, and glucose levels.

### Materials and Methods

**Source of samples and sampling:** Urine samples used for this study were obtained from HIV patients attending Federal Medical Centre (FMC) and Ebonyi State University Teaching Hospital (EBSUTH), both located in Abakaliki, Ebonyi State, southeast, Nigeria. 58 urine samples were collected from HIV patients attending FMC, Abakaliki while 45 urine samples were collected from HIV patients

attending EBSUTH, Abakaliki. A total of 103 samples were used in the study. The samples were collected at random before patient's commencement of antiretroviral therapy (ART) and after 1month on commencement of ART. The samples were collected fresh in clean universal containers adopting standard procedures, and analyzed immediately after collection.

### Sample Analyses

**Physicochemical:** Urine test strips (UTS), precisely combi 10, were used to determine the physicochemical

parameters of samples. Each sample colour was observed and appearance recorded. A single test strip held in a

horizontal position then immersed in each sample in such a way that covered all the reagent areas. Excess urine on the test strips was removed by wiping edge of the strips on a clean absorbent paper. Throughout the incubation period, test strips were held in a horizontal position to prevent interaction with adjacent test areas. Under daylight, reagent areas on the test strips were compared with the corresponding reference standard colour chart on the test strip container 60 seconds (60-120 seconds for leucocytes) after immersion. Interpretation of results depended on how colour appearance on each test strip pad corresponded with the reference standard colour chart with respect to each parameter under investigation. Results were interpreted as normal or positive. Normal results in this research were adjudged negative results. After 1 month on commencement of ART, patients' urine samples were collected again and the above procedure repeated.

**Microbiological:** Test tubes containing each sample was loaded in bits in an electric centrifuge, and allowed to spin

for 5 minutes. The supernatant of each sample was decanted, and the natants taken with a pipette and 3drops of each sample made on a clean, sterile glass slide and covered with a clean, sterile cover slip making a wet preparation. The wet preparations were examined under the microscope using x40 magnification. White blood cells, cellular casts, and bacteria were observed. Each sample was further used to prepare culture. A drop of each sample was aseptically inoculated on a different culture media using sterilized wire loop flamed before and after each use. Streak method was used for the inoculation. The media [6] used was prepared and incubated according to the manufacturer's instruction. The numbers of viable bacteria that developed were counted, calculated and expressed as colonidophores forming units per gram (cfu/g). Isolation, characterization, and identification of bacteria were carried out using colonial, morphological and biochemical characteristics. The above procedure was repeated after 1 month on commencement of ART.

#### DATA ANALYSIS

The various data obtained were subjected to statistical analysis of mean, proportion, and Pearson correlation test.

Significance differences were determined at  $p < 0.05$ .

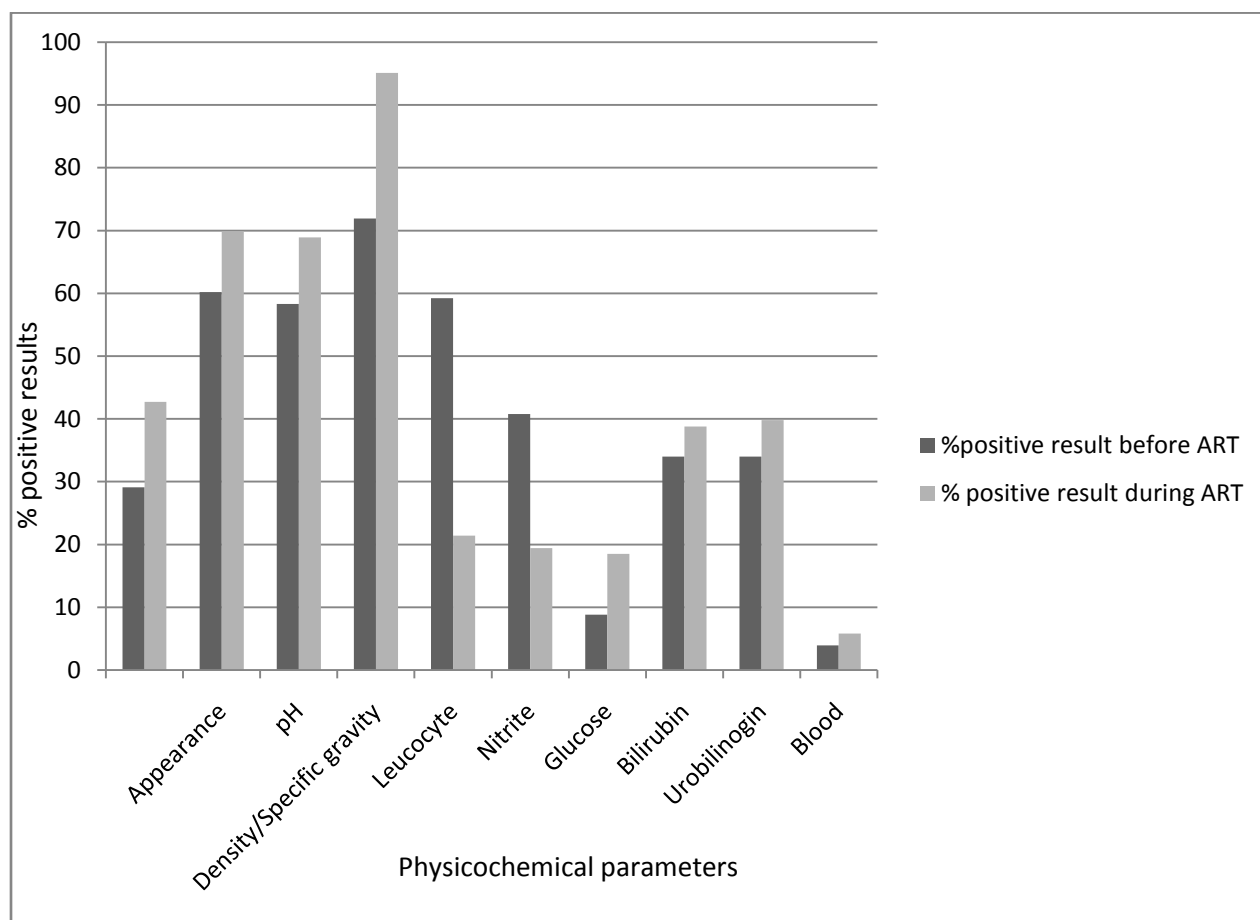
#### RESULTS

Results of the investigation of the urine samples of HIV infected patients attending two selected hospitals in

Abakaliki, Ebonyi State, Nigeria, before commencement of ART and while on ART are shown below:

**Table 1: Percentage distribution of positive test results of physiochemical parameters of urine samples of HIV infected patients before and during ART**

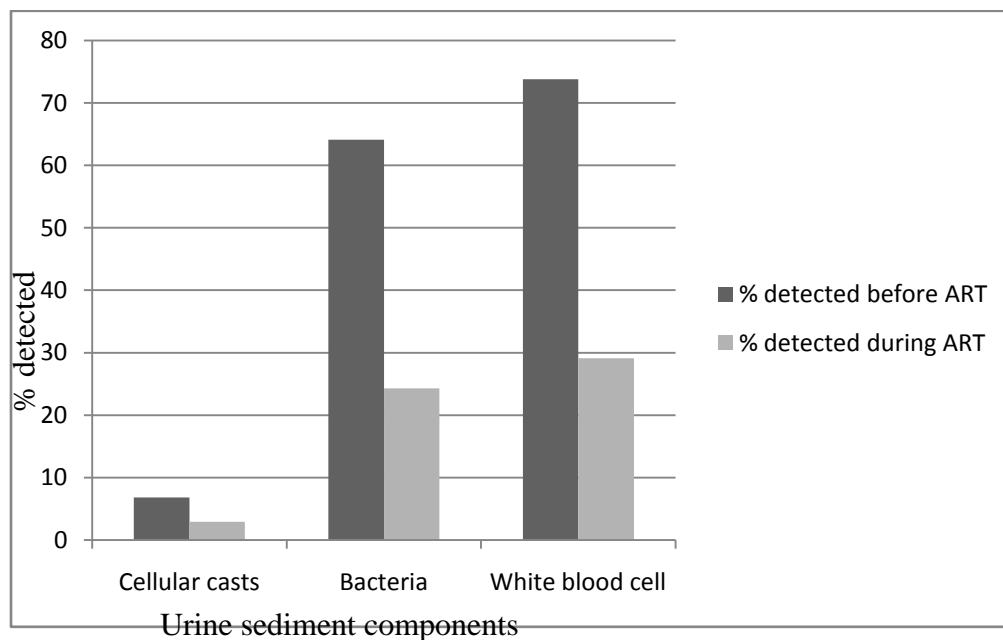
Physiochemical parameter	% positive test before ART	% positive test during ART
Protein	29.1	42.7
Appearance	60.2	69.9
pH	58.3	68.9
Density/Specific Gravity	71.9	95.1
Leucocyte	59.2	21.4
Nitrite	40.8	19.4
Glucose	8.8	18.5
Bilirubin	34.0	38.8
Urobilinogen	34.0	39.8
Blood	3.9	5.8

**Figure 1: Percentage distribution of positive test results of physiochemical parameters of urine samples of HIV infected patients before ART and during ART.**

**Table 2: Percentage detected of urine sediments in urine samples of HIV infected patients before and during ART under microscope.**

Urine sediment components	% detected before ART	% detected during ART
Cellular casts	6.8	2.9
Bacteria	64.1	24.3
White blood cell	73.8	29.1

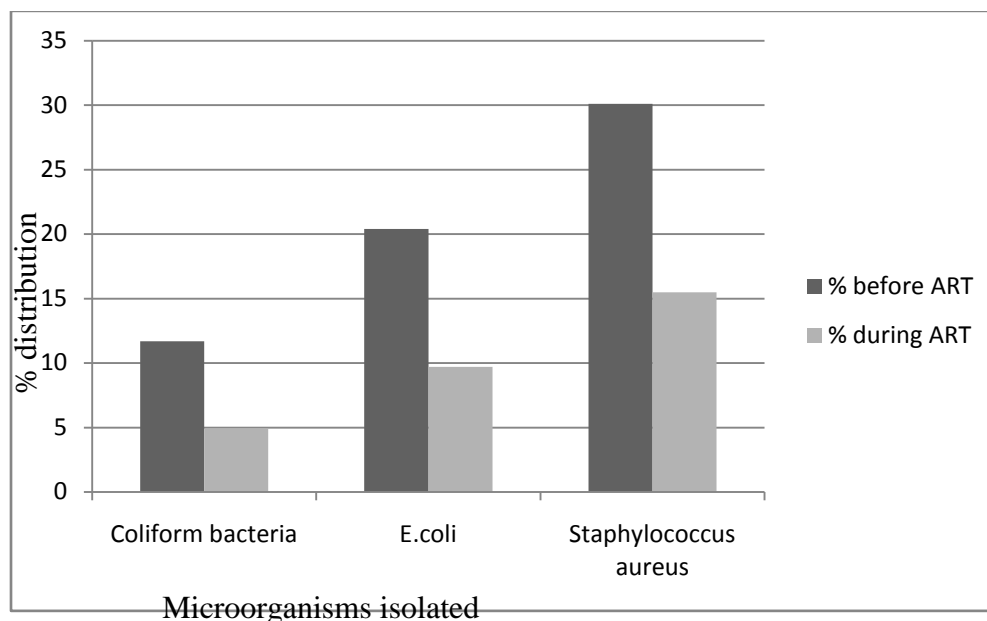
**Figure 2: Percentage detected of urine sediments in urine samples of HIV infected patients before and during ART under microscope.**



**Table 3: Percentage distribution of microorganisms isolated from urine samples of HIV infected patients before and during ART.**

Microorganism	% before ART	% during ART
Coliform bacteria	11.7	4.9
<i>E.coli</i>	20.4	9.7
<i>Staphylococcus aureus</i>	30.1	15.5

**Figure 3: Percentage distribution of microorganisms isolated from urine samples of HIV infected patients before and during ART.**



## DISCUSSION

The research showed a significant negative effect in the urine parameter of HIV infected patients on ART in terms of physiocochemical parameters compared to the incidence rate before they were placed on drug among patients that attend the FMC and EBSUTH, Abakaliki, Ebonyi State. The reverse was the case in microscopy of urine sediments and culture observations. A total of 103 individual urine samples were screened before and during ART. A total of 30 (29.1%) urine samples out of the total 103 urine samples tested positive to protein before commencement of ART, while 44 (42.7%) urine samples tested positive to protein during ART. The result analysis showed a 13.6% rise in protein content of the urine while on ART. It has been suggested that renal complications are

common among people living with HIV; in fact, up to 30% of them may have protein in their urine - a sign of kidney dysfunction; and the complication gets worsened during Highly Active Antiretroviral Therapy (HAART) [7]. Therefore, the presence of protein in their (HIV infected patients) urine before ART and the subsequent rise in protein in their urine during ART suggested that ART has a negative impact on the kidney of HIV patients.

The number of urine samples that showed abnormal colour (cloudy) instead of the normal amber (pale-yellow and clear) colour of urine were 62 (60.2%) before ART, and 72 (69.9%) during ART. This showed a 9.7% significant rise in cloudy urine during ART. The result suggested an

increase in the concentration of the urine as a result of toxicity effect produced by ART and increase in urine density.

Urine tests for pH, showed that 60 (58.3%) urine samples tested positive to acidic pH before ART, while 71 (68.9%) tested positive to acidic pH during ART. This showed a 10.6% rise in acidity of urine during ART. The result suggested that the persistent high pH values of the urine indicated urinary tract infections associated with bacteria origin. pH estimations are useful to evaluate the acidity or alkalinity of urine as it relates to numerous renal and metabolic disorder and in the monitoring of patients with certain diets.

There was also a 23.2% increase in urine density of HIV-patients during ART. The result suggested an increase in urine concentration as a result of kidney toxicity effect of ART.

It was interesting to note that before and during ART, the number of urine samples tested positive to leucocyte was 61 (59.2%) and 22 (21.4%) respectively; showing a (37.8%) sharp reduction of leucocyte in urine during ART. Leucocyte in urine indicates inflammatory diseases of the kidneys and urinary tract, and suggests the need for further investigation [8]. False positive results may be caused by contamination with vaginal secretion [1]. In this result, the reduction of leucocyte in urine by 37.8% during ART must not be unconnected with the increase in immunity induced by ART. During HIV infection, leucocyte increases in the urine due to the breakdown of white blood cells; but when on ART, it is controlled because the antigen (HIV) in the system has been controlled, thereby increasing the immunity.

Nitrite also reduced in the urine samples by 21.4% during ART. Nitrite identification is used in the diagnosis and treatment of urinary tract infections of bacterial origin. Since urinary tract infections are opportunistic infection associated with HIV infection, the reduction in nitrite suggested an immune boosting effect of ART.

Glucose test findings showed a 9.8% glucose rise in urine during ART. This suggested diabetic effects of ART. This result goes a long way to show that ART can render renal tubules insensitive to antidiuretic hormone (ADH).

Bilirubin increased from 34.0% to 38.8% before ART and during ART respectively. The increase of bilirubin in urine by 4.8% during ART may be as a result of bile products being released into the urine. Hence, the result suggested liver damage caused by ART. Urobilinogen tests also showed similar results with bilirubin tests.

Blood increased from 3.9% to 5.8% before and during ART respectively. The increase in blood suggested urological or kidney diseases. Hence, indicts ART on kidney dysfunction. Although, the increase in blood in urine may be as a result of blood from menstrual cycle in women patients. Normally, no blood should be seen in the urine.

Microscopy of urine sediments results showed a 3.9%, 39.8%, and 44.7% reduction in casts, bacteria, and white blood cells respectively during ART. The reduction showed that HIV patients prior to taking ART are more predisposed to opportunistic infections associated with HIV than patients on ART. This explains that ART suppresses opportunistic infection in HIV patients and as well boosts immunity.

Observation of bacteria growth on culture before ART was 64 (62.2%) culture plates compared with 31 (30.1%) culture plates during ART. The result showed a 32.1% reduction of bacteria such as coliforms, *E. coli*, and *Staphylococcus aureus* in the urine during ART. The reduction provides more credence to the antibiotic efficacy of ART. This result has shown that HIV patients before ART are more predisposed to bacteria implicated in urinary tract infection due to their compromised immunity. However, when patients were on ART, the immunity level increases, thereby, suppressing opportunistic infections. Therefore, ART confers some level of immunity to the body system of HIV patients making them less prone to some infections. Even the few number of patients that had the case of bacterial infection must have been at chronic stage. Chronic infections are those in which bacteria can be continuously detected, often at low levels; mild or no clinical symptoms may be evident [9].

A similar research work carried out by AIDS Society, June, 2007 on 187 patients who began highly active antiretroviral therapy (HAART) in a reference centre for HIV/AIDS located in Madrid corroborated my work. Examination of liver function was made at baseline and after 1 month of starting treatment. A significant increase in transaminases (more than twofold) were recognized in 26 (13.9%) patients, whereas bilirubin increase were seen in 7 (3.7%) individuals. 11 (5.9%) subjects needed to stop the medication because of either hepatic cytolysis (liver cell death). 4 (2.1%) individual developed clinical hepatic decomposition, and one

of them died. Their work concluded that hepatotoxicity is frequently seen in patients under HAART, and can force the withdrawal of antiviral treatment in a significant proportion of patients, occasionally resulting in fatal outcome.

According to the multimember AIDS Cohort study, HIV-infected men on ART has approximately a 5 fold increase in risk of diabetes compared with HIV-infected, untreated controls, and that ART conferred a 2-3 fold increase risk of hyperglycemia compared with HIV-infected, untreated controls. Their work further stated that HIV medication also may cause insulin resistance. For example, their study has shown that indinavir can cause insulin resistance, with this effect sometimes seen after administration of a single dose. The study also estimated that the risk of developing diabetes while on protease inhibitor based therapy ranges from 1% to 7%.

Framingham Offspring study, 1999 on 71 HIV-infected patients on ART detected diabetes in 7% and during ART were 5- 2%. This result differs slightly with the result of this study which was 9.8% rate among controls (before ART) and during ART.

According to Marques et al., 1989, 303 HIV-patients, 280 men and 23 women were studied during the average period of 6 months. All were screened with urinalysis and nitrite tests. Further investigation was done by urine culture. UTI was found in 19 (6.3%): 17 (6.1%) men and 2 (8.7%) women. In the study it was shown that HIV infection was not an isolated predisposing factor to UTI caused by non-opportunist bacteria.

## CONCLUSION

The knowledge that ART has some devastating side effect is solid, but my understanding of certain areas of renal

health is still rudimentary. Significant work remains to truly elucidate who is susceptible to kidney disease and why,



and how to treat certain kidney diseases that occur in HIV positive patients on ART.

Early screening for these side effects provides a greater likelihood of effective prevention and treatment. Screening is as simple as urine analysis for protein, bilirubin, leucocyte, pH and other assessment associated with urine and should be performed in every person living with HIV before and during ART. I, therefore, recommend that all HIV positive individuals on ART be screened for some possible side effects associated with ART at least once in every 3 months;

individuals at increased risk for side effects should be reassessed at least every month, and those with abnormal screening tests should receive further evaluation by their health care provider and see a specialist doctor.

Key to this recommendation is strict compliance with ART dosages and awareness of some possible side effects of ART. Without awareness in both the HIV positive community and among health care providers, potentially preventable and treatable ailments can progress unchecked in the unsuspecting and asymptomatic individuals.

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