Creatinine and Urea Levels in Patients on Tenofovir-Based Therapy at Harare Central Hospital, Zimbabwe

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Faith Chauke¹, Tawanda Jonathan Chisango², Danai Tavonga Zhou¹

University of Zimbabwe College of Health Sciences, Department of Medical Laboratory Sciences, P.O. Box AV 178, Avondale, Harare, Zimbabwe

Chinhoyi University of Technology, PO Box 7724, Chinhoyi, Zimbabwe

Emails: chauke.faith@yahoo.com, chisangotawanda@yahoo.com, danaizh@yahoo.co.uk

ABSTRACT

Antiretroviral therapy is the standard of care for HIV positive patients and its use has led to reduced mortality rate from HIV and HIV-associated diseases. Tenofovir is the first nucleotide reverse transcriptase inhibitor approved by the United States of America Food and Drug Administration (FDA) for use in combination with other antiretroviral drugs. However, nephrotoxicity of tenofovir has been reported in observational studies, case reports, animal models and cell culture as tenofovir accumulates in the proximal tubules causing dysfunction which can result in renal impairment. Our study aimed to determine kidney dysfunction in patients taking tenofovir by measuring serum creatinine and urea levels.

The study group comprised of 62 participants (45 females and 17 males) who had been on tenofovir-based therapy for more than 6 months. The patients attended Opportunistic Infections (OI) clinic at Harare Central Hospital, a referral hospital in Zimbabwe’s capital and samples were assayed at the University of Zimbabwe, Department of Medical Laboratory Sciences at Parirenyatwa Group of Hospitals.

In our study, the prevalence of impaired renal function in a sample of HIV positive patients attending Harare Central Hospital Opportunistic Infections (OI) Clinic was found to be 19.4%. The prevalence of renal impairment was measured as elevated creatinine and/urea levels. There was significant differences in creatinine levels by age and between males and females, p=0.0003 and p=0.0319 respectively.

One in every five patients had elevated serum creatinine/urea levels suggesting some evidence of renal dysfunction in our study of patients on tenofovir-based therapy. This suggests that there is need for continuous monitoring of patients on tenofovir-based antiretroviral therapy to enable clinicians to assess the risks posed by tenofovir on renal function in our setting. Routine monitoring will help patients as changes in creatinine and/or urea levels may indicate decline in kidney function even in patients who might be clinically and biochemically asymptomatic.
INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a worldwide disease, affecting millions of people across the globe and has become one of the leading causes of mortality among adults of reproductive age [1]. Human immunodeficiency virus (HIV) the retrovirus responsible for causing AIDS, is thought to have developed from a similar virus that affects chimpanzees and monkeys [2]. HIV is an enveloped spherical ribonucleic acid (RNA) virus measuring up to 120nm diameter, consisting of two antigenic variants: the more virulent HIV-1 found worldwide and HIV-2 found predominantly in West Africa [2][3]. HIV infects human host cells with CD4 antigens on their surfaces such as helper T cells, macrophages and macrophage lineage cells including monocytes, alveolar macrophages of lungs, dendritic cells of skin and microglial cells of the brain. Destruction of T-helper cells by HIV and re-infection of new cells results in suppression of the cell-mediated immunity [3][4].

HIV is transmitted through body fluids such as semen, blood, breast milk and vaginal fluids and hence spreads from an infected person to another through unprotected sexual contact, sharing contaminated sharp objects, blood transfusion, mother to child transmission via the placenta or breast milk [1][4]. HIV binds to CD4 receptors via the gp120 molecule on its envelope that interacts with either CXCR4 or CCR5 of host cells while gp41 facilitates fusion of virus and host CD4 cell. The virus loses its envelope in the process emptying its viral contents into the host cell after which the enzyme reverse transcriptase mediates transcription of RNA into proviral DNA. Proviral DNA then enters the host nucleus, fusing with host DNA and eventually manipulating host DNA to produce more viruses [1][4].

Many advances have been made in the prevention of HIV transmission and management of HIV/AIDS with antiretroviral therapy (ART) since the virus was discovered in the early 1980s [5]. ART-the standard of care for people with HIV infection prolongs life, reduces symptoms of disease, delays
disease progression to AIDS and has led to significant reductions in mortality from HIV infection [1] [6] [7]. Back in December 2014 the National AIDS Council of Zimbabwe reported that about 15% of the population were living with HIV, 76.9% of infected adults were on ART whilst 40.5% children were on antiretroviral treatment [8] [9]. Fortunately, HIV and AIDS statistics in Zimbabwe have shown a drop in the number of people who die from AIDS and AIDS related illness: from 170 000 in 2003 to about 60 000 in 2013 most likely due to more wide-spread availability of ART [10].

The available classes of antiretroviral (ARV) drugs are nucleotide/ nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and integrase inhibitors. NtRTIs include tenofovir, zidovudine, lamivudine, abacavir and stavudine; examples of NNRTIs are nevirapine and efavirenz.; PIs include atazanavir, lopinavir and ritonavir, whilst raltegravir is an example of an integrase inhibitor [9]. The preferred first line regimen for adults, adolescents and older children in Zimbabwe is tenofovir (NRTI), lamivudine (NRTI) and efavirenz (NNRTI); first-line regimens for children under 3 years of age include PIs, usually lopinavir/ritonavir [9].

Like many other drugs, ARV drugs may unfortunately have unique side effects; for example, stavudine was phased out by the World Health Organisation (WHO) on November 30 2009, on the eve of World AIDS day due to its serious toxicity which is also irreversible [1]. Tenofovir, an NRTI, approved by the United States of America Food and Drug Administration (FDA) became available in 2001 and is now the most prescribed ARV drug worldwide and is used in combination with NRTIs, PIs and NNRTIs [11]. Nephrotoxicity of tenofovir has, however, been reported in observational studies, case reports, animal models and cell culture due to accumulation in proximal tubules which causes renal dysfunction and renal impairment, whilst tenofovir-based therapy has been associated with abnormal creatinine and urea levels as a result of kidney impairment and may be elevated in some of these patients [12] [13].
The kidneys’ mainly function is to regulate fluid and electrolyte balance of the body by continually filtering blood and excreting waste substances [14]. Glomerulus filters substances from the blood and proximal convoluted tubule reabsorbs water, electrolytes and non-electrolytes, reabsorbing two thirds of the filtrate made up of glucose, amino acids, phosphate, potassium, urea, creatinine and other organic solutes [15] [16]. Renal impairment results in increased creatinine and urea levels in blood due to poor clearance of creatinine by the kidneys. The inability to eliminate waste material and to regulate the volume and composition of body fluid manifests as a rise in plasma urea or creatinine and as a reduction in measured glomerular filtration rate (GFR) [17]. Measuring urea together with creatinine is of clinical relevance because creatinine is a better measure of kidney function than urea alone. Urea levels may increase due to dehydration, high protein diet, steroid administration and protein catabolism. These factors do not affect creatinine levels, though serum creatinine levels are affected by gender, weight, age and lean body mass [14] [18]. We sought to establish the prevalence of kidney impairment in patients on tenofovir-based therapy at Harare Central Hospital Opportunistic Infections Clinic in Zimbabwe, using creatinine and urea levels as markers of renal dysfunction.

MATERIALS AND METHODS

STUDY DESIGN

A laboratory based cross-sectional study.

STUDY SITE

The samples used were obtained from patients attending Opportunistic Infections (OI) clinic at Harare Central Hospital, a referral hospital, then processed at the University Of Zimbabwe, College of Health Sciences, Department of Medical Laboratory Sciences at Parirenyatwa Group of Hospitals.
ETHICAL CONSIDERATIONS

Ethical clearance was granted by the Ethics Committee at Harare Central Hospital and the Joint Research Ethics Committee (JREC) at Parirenyatwa Group of Hospitals and University of Zimbabwe, College of Health Sciences. The samples were de-identified to ensure confidentiality and anonymity.

STUDY PARTICIPANTS

The participants were HIV positive patients, both male and female aged between 18 and 65 years, who had been on tenofovir-based therapy for at least 6 months.

BIOCHEMICAL TESTS

Samples were analysed using the Mindray BS 120 machine at the University of Zimbabwe, Department of Medical Laboratory Sciences. Frozen samples were thawed once prior to analysis and calibrators and controls were run first in order to ensure integrity of results. The picric acid method by Jaffe was used in the test for creatinine levels. This is a 2 point velocity method in which serum creatinine reacts with alkaline picrates resulting in the formation of a red complex whose intensity can be read at 505nm. Sodium lauryl sulphate/sodium dodecyl sulphate help prevent interference by proteins. The intensity of the colour formed is proportional to the creatinine concentration in the sample [19].

Creatinine + Picric acid $\xrightarrow{\text{OH}^-}$ Creatinine-picric acid complex

Urea was tested using the enzymatic method. The method uses the principle of urea hydrolysis in the presence of urease to carbon dioxide and ammonium ions. Ammonia ions formed react with α-ketoglutarate in a reaction catalysed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD$^+$ [19].

$$\text{Urea} + \text{H}_2\text{O} + 2\text{H}^+ \xrightarrow{\text{Urease}} (\text{NH}_4^+) \_2 + \text{CO}_2$$

$$\text{NH}_4^+ + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{H}_2\text{O} + \text{NAD}^+ + \text{L-Glutamate}$$
The decrease in concentration of NADH is proportional to urea concentration in the sample.

The serum creatinine normal reference ranges were as follows: Males 72-127μmol/L and females 58-96μmol/L whereas serum urea normal reference ranges was 2.8-7.2 mmol/L for both males and females [19].

RESULTS

The study group comprised of 62 patients, 45 of them female (73%), aged 18 to 62 years, who had been on tenofovir-based therapy for more than 6 months.

Table 1: Age and Sex Distribution of Study Participants

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males Frequency (%)</th>
<th>Males</th>
<th>Females Frequency (%)</th>
<th>Females</th>
<th>Combined Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4.4</td>
<td>2</td>
</tr>
<tr>
<td>20-29</td>
<td>1</td>
<td>5.9</td>
<td>8</td>
<td>17.8</td>
<td>9</td>
</tr>
<tr>
<td>30 – 39</td>
<td>3</td>
<td>17.6</td>
<td>13</td>
<td>28.9</td>
<td>16</td>
</tr>
<tr>
<td>40 – 49</td>
<td>8</td>
<td>47.1</td>
<td>15</td>
<td>33.3</td>
<td>23</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1</td>
<td>5.9</td>
<td>2</td>
<td>4.4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
<td>45</td>
<td>100</td>
<td>62</td>
</tr>
</tbody>
</table>
Age groups of study participants showed a normal distribution with the highest number of participants being aged between 40 and 49 years (Table 1). Median age for the whole study group was 43 years, mean age was 41 ± 12.19 years (Table 2), males were aged of 22 to 61 years and females were aged between 18 and 62 years.

Out of 62 samples, 12 (19.4%) had elevated creatinine and/urea which characterises impaired renal function. 9 out of 45 females (20%) had impaired renal function whilst 3 out of 17 males (17.6%) had impaired renal function (Fig 2).

Fig 2: Prevalence of Impaired Renal Function by Sex
Fig 3: Prevalence of Impaired Renal Function by Age (in years)

Fig 3 shows age-wise distribution of elevated creatinine and/urea as an indication of impaired renal function. The age group of 30-39 years had the highest prevalence of impaired renal function followed by 40-49 years. There were no recorded incidences of increased creatinine and/urea levels in those above 50 years.
Table 2: Serum Creatinine and Urea Levels in Patients on Tenofovir-based Therapy (values are expressed in mean ± SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females</th>
<th>Male</th>
<th>Combined</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39 ± 12.41</td>
<td>46 ± 10.05</td>
<td>41 ± 12.19</td>
<td>0.0319</td>
</tr>
<tr>
<td>Creatinine</td>
<td>82.49 ± 21.98</td>
<td>106.12 ± 20.86</td>
<td>88.97 ± 23.99</td>
<td>0.0003</td>
</tr>
<tr>
<td>Urea</td>
<td>3.57 ± 1.10</td>
<td>3.83 ± 1.55</td>
<td>3.64 ± 1.23</td>
<td>0.4615</td>
</tr>
</tbody>
</table>

The mean creatinine and urea levels in both males and females lay within normal reference ranges for both groups (Table 2). There was no significant difference in urea levels in males and females, p>0.05, but there was significant differences in creatinine levels and age between males and females, p=0.0003 and p=0.0319 respectively.

**DISCUSSION**

The available publications concerning renal safety of tenofovir are still very confusing most likely due to differences between what happens in real life and what is observed in clinical trials [20]. In some studies, kidney injuries due to tenofovir were found in 1-5% of patients and in such cases tenofovir was considered as a rare cause of renal impairment [21]. Other studies, however reported that long term use of tenofovir results in decreased renal function [22]. Mulenga L et al from Zambia, report that patients on tenofovir, more likely had baseline renal impairment than those on non-tenofovir therapy (16.7% against 12.4%) [23]. Adikwu Elias of Nigeria and others reviewed articles on tenofovir renal toxicity and noticed that the prevalence of tenofovir induced renal impairment falls between 0.7% and 17% [24].
The Surveillance Cohort Long-term Toxicity of Antiretrovirals (SCOLTA), a large observational study in Italy, followed up 754 HIV positive patients on tenofovir-based therapy over 19.5 months and found prevalence of elevated creatinine to be 2.5% in patients on tenofovir [25].

19.4% of the 62 samples had impaired renal function. 17.6% of males had and 20% of females had renal impairment and there was significant difference in creatinine levels and age between males and females. The 19.4% prevalence of renal impairment found in this study is higher than the values found from cohort studies carried out by Jones R et al in Chelsea and Westminster where 1058 study participants were on tenofovir [26]. Of the 1058 patients, 84 (8%) had elevated creatinine levels after exposure to tenofovir. However, they concluded that other drugs were associated more frequently with renal dysfunction. Their study participants were antiretroviral-naive patients, patients exposed to tenofovir and non-tenofovir containing regimens with no significant difference between the different groups (P < 0.001).

In this study, baseline creatinine values could not be used since the study was limited to a laboratory based cross-sectional one. In contrast, a retrospective cohort study was done in Toronto where baseline serum creatinine was measured and the patients were followed up in a period of 3 to 25 months [27]. Of the 172 patients, 15 (8.7%) had their serum creatinine levels increased more than 1.5 times the baseline values. Increases in baseline creatinine (P = 0.0005) and baseline creatinine clearance (P = 0.01) were significantly associated with ever having a 1.5 times increase in serum creatinine.

In a comparison study carried out by Gallant J. et al for patients on tenofovir and non-tenofovir regimens, they found out that serum creatinine levels increased by 0.15mg/dl (13.26µmol/l) as compared to 0.10mg/dl (8.84µmol/l) in non tenofovir group [28]. Risk factors in the non-tenofovir group included low CD4+ counts, diabetes and decreased renal function at baseline.
Mean age in the current study was 41 ± 12.19 years, mean creatinine level was 88.97 ± 23.99 years and mean urea level was 3.64 ± 1.23 years. Females had minimum creatinine value of 44µmol/l which is below normal and a maximum of 148.6 µmol/l which is above normal. Males had a minimum creatinine value of 69µmol/l and a maximum of 148 µmol/l, which were below and above normal ranges respectively. The seemingly large standard deviation was due to the difference between the minimum and maximum creatinine levels which were way below normal and way above normal (44µmol/l and 148.6 respectively). Low creatinine levels could have resulted from mass wasting or low CD4+ count (<50 cells/µl) in the patients. This then masks the effect of tenofovir on renal function as the average serum creatinine levels would remain low.

There were no recorded incidences of increased creatinine and/urea levels in those above 50. This could have been due to the limited number samples which result in missing out important observations. However, normal kidney function decreases with age.

**CONCLUSION**

In this study, the prevalence of elevated creatinine/ urea both markers of impaired renal function in HIV positive patients attending Harare Central Hospital Opportunistic Infections Clinic was found to be 19.4%. The prevalence can even be higher when the baseline creatinine levels are provided since some elevations in creatinine and urea can occur within the normal range. Literature review above also showed significant loss of kidney function over a small period of time, for example, 1.5 times increases in serum creatinine noted in 8.7% patients on tenofovir-containing regimens over a period of 3 to 25 months. This simply raises the question this question, “How much risk is then posed on lifetime taking of tenofovir?” Kidney function tests play an important role in monitoring HIV positive patients on antiretroviral therapy. The study of complications of antiretroviral therapy is necessary since the therapy is long term and can lead to the progression of chronic disorders over a long period of time.
The extent of adverse drug reactions in persons with normal baseline renal function should be assessed, since there are numerous complications associated with chronic renal impairment [30]. Moreover, HIV infected patients are at an increased risk of drug-induced renal toxicity hence the need for frequent monitoring of renal function in HIV patients receiving antiretroviral drugs [31].

LIMITATIONS

The sample size was reduced from the calculated minimum size due to limited resources and this could have reduced the chances of detecting the true results (type II error). Furthermore, the standard test for measuring renal function is calculated glomerular filtration rate (GFR), requiring urine samples and weight and this was not done. HIV-related renal impairment which can present as acute or chronic kidney disease can also be caused by HIV virus directly, hence the difficulty in making definite conclusions in this research [32]. HIV associated renal nephropathy has been found to be prominent in HIV positive patients of African origin either due to genetic predisposition or high viral load [24]. This study did not have measures of viral load as the test is not readily available to patients at public hospitals such as the one used in our study.

RECOMMENDATIONS

There is need for renal function tests on a larger study population to determine whether monitoring of patients on ART in Zimbabwe should be embraced as a health monitoring tool in ART patients on tenofovir-based therapy. Follow-up on patients over a long period of time may help determine if increases in urea and creatinine levels are of clinical relevance in our setting.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.
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19. Mindray® BS 120 Chemistry Analyzer Manual


