

Determination of Anthropometric and Immune Inflammatory Responses in Patients on Dolutegravir Based Antiretroviral Therapy at University of Nigeria Teaching Hospital Ituku-Ozalla

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Abstract

Dolutegravir, an integrase strand transfer inhibitor has been found to affect anthropometric indices. A total of 40 subjects comprising 19 male and 21 female were recruited for the study and apparently healthy sex and age matched individuals who tested sero-negative to HIV screening were used as controls. Standard weighing scale and measuring tape were used to determine body mass index (BMI) and Waist-to-Hip Ratio (WHR). Blood samples were collected and analyzed for pro inflammatory cytokines markers, specifically IL6 and TNF- α . The blood samples collected pre and six months post antiretroviral therapy were analyzed using the Anbio Fluorescence Immunoassay for IL6 detection and sandwich ELISA technique to determine the TNF- α concentration. The mean values of the BMI and WHR increased to $25.05 \pm 4.2 \text{ kg/m}^2$ and 0.93 ± 0.06 , respectively, after 24 weeks of treatment initiation. The mean pre-treatment values for IL6 and TNF- α were $33.44 \pm 11.37 \text{ pg/ml}$ and $122.52 \pm 57.75 \text{ pg/ml}$, respectively, compared to $425.50 \pm 111.17 \text{ pg/ml}$ and $162.51 \pm 77.26 \text{ pg/ml}$ after 24 weeks. Anthropometric indices and cytokine levels in treatment experienced patients were significantly higher compared to HIV Negative controls. The comparison of pre- and post-treatment values following Dolutegravir-based therapy revealed significant increases in BMI, WHR, and inflammatory cytokines (IL-6 and TNF- α). These findings suggest that the observed changes are likely due to increased adiposity, which triggers the production of pro-inflammatory immune mediators. This response is positively correlated with vascular and endothelial abnormalities, as well as oxidative stress.

Keywords: Anthropometry, Antiretroviral therapy, Highly Active Antiretroviral Therapy, Pro-inflammatory cytokines

Introduction

Chronic Inflammation is considered a risk factor in the development of certain non-communicable diseases including cardiovascular diseases, metabolic syndrome, diabetes, and certain types of cancer. Being overweight predisposes individuals to an inflammatory state by increasing the production of pro-inflammatory mediators such as IL-6 and TNF- α , while simultaneously reducing levels of the anti-inflammatory adipokine adiponectin. In overweight individuals, adipose tissue enlarges and becomes dysfunctional, leading to increased infiltration of immune cells and elevated levels of pro-inflammatory cytokines (IL-6, TNF- α), contributing to a cytokine storm and low-grade inflammation driven by hypoxia [1,2]. Excess macronutrient

intake activates adipose tissue, leading to the production of inflammatory mediators or pro-inflammatory cytokines, while simultaneously reducing the production of adiponectin, predisposing the individuals to a pro-inflammatory state, decreases nitric oxide availability, and increases reactive oxygen species, resulting in oxidative stress [1]. The elevated values of anthropometric indicators have predisposition to potential downstream metabolic effects leading to diabetic condition, hyperlipidemia, and cardiovascular disorders which ultimately result in a significant disease state or even death. Body Mass Index and Waist Hip Ratio/Waist circumference are good indicators/markers of adiposity in early and middle aged adults with the latter as measure of accumulated fat [3,4]. Several studies have reported significantly increased weight gain in people living with HIV (PLHIV) on Dolutegravir-

based regimens compared to those on other standard-of-care treatments that do not include integrase strand transfer inhibitors (INSTIs) [5-8]. Treatment with various HAART regimens, including Dolutegravir based ART could be associated with adverse and serious adverse events, such as metabolic syndrome and weight related complications, which can ultimately limit the overall benefits of the treatment. This study aimed to determine the anthropometric changes associated with the use of Dolutegravir-based ART six months after the initiation of treatment at our facility, University of Nigeria Teaching Hospital, Ituku-Ozalla. Additionally, it sought to examine the relationship between the anthropometric indices and pro-inflammatory cytokines levels in treatment experienced patients.

Materials and Methods

Newly diagnosed HIV positive patients were recruited for the study and the sample size was calculated using the formula below. Informed consent was obtained from the eligible subjects prior to sample collection and testing.

Sample size determination

Sample Size calculation

The Fischer's formula was used to calculate sample size [9]

$$n = Z^2Pq/d^2$$

Where n = Minimum sample size

Z = the abscissa of the normal curve that cuts off an area α at the tails (1.96)

P = Prevalence of HIV in Enugu based on NAHS survey in 2018 (2.1%) [10]

$$q = 1 - P$$

d = Desired level of precision (5%)

$$n = \frac{(0.96)^2(0.021)(0.979)}{(0.05)^2}$$

$$n = 31$$

Adding 20% attrition gives a sample size of 37

Therefore, the calculated sample size for the study was 37.

Data collated were analyzed using SPSS version 26.0 for windows. Descriptive statistics, including frequency and percentages, were used to summarize categorical variables, while means and standard deviations were computed for continuous variables. Means of continuous variables were compared using student's t test while the Chi square was used to compare proportions. A p-value <0.05 was considered statistically significant and results were presented in tables and charts.

Anthropometric assessment included measurements of the height, weight, and estimation of BMI and WHR

Height and weight were measured using a stadiometer and weighing balance recorded in meters (m) and kilograms (kg). WHR was measured using a standard measuring tape in centimeters (cm). A Healthy weight was defined as a BMI of 18.5-24.9 and a WHR of 0.85-0.9, as described by Casadei and Kiel [11].

Blood samples for the determination of IL-6 and TNF- α were collected via venipuncture and processed appropriately to obtain plasma or serum for analysis.

Plasma preparation

Blood was collected into EDTA specimen bottles and centrifuged at 3000 rpm for 20 minutes. The supernatant was carefully separated and collected as plasma.

IL-6 Assay

Principle of the assays: The Anbio IL 6 test kit is based on a sandwich immunodetection method. When the sample is added to the sample well of the test, a fluorescence-labelled detector IL-6 antibody binds to the IL-6 antigen present in the blood specimen. As the sample mixture migrates along the nitrocellulose matrix of the test strip by capillary action, the IL-6-antibody complexes are captured by anti-IL-6 antibodies that have been immobilized on the test strip. Thus, the higher the concentration of IL-6 antigen in the blood specimen, the more immune complexes accumulate on the test strip. The fluorescence signal intensity of the detector antibody reflects the amount of IL-6 captured. The Anbio Fluorescence Immunoassay meter then displays the IL-6 concentration present in the specimen.

Assay procedure: The determination of plasma IL6 levels was carried out using the Anbio fluorescence immunoassay kit for the quantitative measurement, with results is read using the Anbio fluorescence immunoassay meter, as described by Meisner [12]. The IL-6 test kit is a product of Anbio (Xiamen) Biotechnology Co. Ltd., Xiamen, Fujian, China.

Result: The Anbio FIA meter automatically calculated the test result and displayed the IL6 concentration of the test sample in pg/ml. The normal reference range is 0.2-7.8 pg/ml.

Tumor necrosis factor- α (TNF- α)

The principle of the sandwich enzyme-linked immunosorbent assay (ELISA) involves the use of a microELISA strip plate pre-coated with an antibody specific to TNF- α , was incubated after adding sample and standard antigens. Then Horseradish peroxidase (HRP)-conjugated antibody specific for TNF α was added to each well and incubated further. After incubation, unbound components were washed away, and the Tetra

Methyl Benzidine (TMB) substrate solution was added to each well. Wells containing Tumor Necrosis Factor-alpha (TNF-α) bound to the HRP-conjugated antibody produced a blue color through a chromogenic reaction. Upon addition of the stop solution, the color changed to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value was directly proportional to the concentration of TNF-α in the sample.

The determination of plasma Tumor Necrosis factor alpha (TNF α) was carried out using a sandwich-type ELISA technique, with measurements taken using an Enzyme-Linked Immunosorbent Assay (ELISA) reader, as described by Hayrapetyan et al. [13]. The TNF-α ELISA test kit was manufactured by SUNLONG Biotech Co. Ltd., Hangzhou City, Zhejiang Province, China. The normal range of TNF-α was <24.47 pg/ml.

Method of data analysis

The collected data were analyzed using SPSS version 26.0 for windows. Descriptive statistics, including frequency and percentages were used to summarize categorical variables, while means and standard deviations were calculated for continuous variables. Means of continuous variables were compared using Student's t-test, and the Chi-square test was used to compare proportions. A p-value of <0.05 was considered statistically significant. Results are presented in tables and charts.

Results

BMI and WHR of the patients significantly increased following initiation of the Dolutegravir-based ART regimen ($p < 0.05$), with BMI rising to $25.05 \pm 4.42 \text{ kg/m}^2$ ($p < 0.001$) and WHR increasing to 0.93 ± 0.06 , as shown in **Table 1**.

IL-6 and TNF-α levels were significantly increased six months after initiation of Dolutegravir-based ART ($p=0.029$ and $p=0.02$, respectively), indicating a notable elevation in inflammatory response post-treatment (**Table 2**).

Discussion

The Dolutegravir-based regimen interferes with the melanocortin system by inhibiting Proopiomelanocortin (POMC)/Cocaine- and Amphetamine-Regulated Transcript (CART) neurons, which express POMC genes responsible for producing α-MSH. This disruption prevents α-MSH from binding to the MC4 receptor on second-order neurons in the hypothalamus, thereby inactivating the anorexigenic pathway [14]. Moreover, the Dolutegravir-based regimen promotes weight gain by stimulating the release of Neuropeptide Y (NPY) and activating its receptors, thereby enhancing orexigenic activity that increases appetite and food intake. Additionally, Dolutegravir suppresses leptin—an anorexigenic hormone that normally inhibits Agouti-related protein (AgRP)/NPY neurons—leading to increased secretion of AgRP and NPY, further contributing to weight gain. The Dolutegravir-based regimen inhibits the binding of α-MSH to MC4R and MC3R, thereby causing abnormal orexigenic function, resulting in weight gain [15]. Additionally, Dolutegravir downregulates adipokines such as leptin and adiponectin, a consequence of increased adiposity. This downregulation promotes adipogenesis, lipogenesis, and oxidative stress, all of which are associated with obesity-related complications [16]. Consequently, the observed increase in BMI among patients on Dolutegravir-based ART is linked to a heightened risk of developing non communicable diseases such as cardiovascular diseases, diabetes, cancers, and other metabolic co-morbidities. The findings of this study are consistent with those of previous studies conducted by Sax *et al.*, Achhra *et al.*, Venter *et al.*, Shah *et al.*, and Kumar *et al.* [17-21]. According to Fegal *et al.* [20], BMI and WHR are stronger indicators of obesity as they help differentiate between lean body mass and fat mass. Systemic toxicity resulting from excessive weight gain—caused by glucose accumulation, unoxidized long-chain fatty acids, mitochondrial dysfunction, and increased production of reactive oxygen species (ROS)—can be induced by Dolutegravir-based regimen treatment, thereby activating the synthesis of pro-inflammatory adipokines [23]. Excessive weight gain leads to increased adipocyte infiltration and

Table 1. Comparison of mean anthropometry before and after Dolutegravir based ART regimen.

	Pre Mean ± SD	Post Mean ± SD	t	P value
Body Mass Index (BMI)	23.48 ± 4.09	25.05 ± 4.42	4.250	<0.001
Waist-to-Hip Ratio (WHR)	0.91 ± 0.06	0.91 ± 0.06	2.820	0.008

Table 2. Comparison of mean values of Pro-inflammatory cytokines evaluation pre and post 24 weeks of Dolutegravir-based ART regimen commencement.

	Pre Mean ± SD (pg/ml)	Post Mean ± SD (pg/ml)	t	P value
Interleukin 6 (IL6)	33.44 ± 11.37	425.50 ± 111.17	2.270	0.029
Tumor Necrosis Factor α (TNF-α)	122.52 ± 57.75	162.51 ± 77.26	3.373	0.002

alters the functional properties of white adipose tissue (WAT) through the recruitment of immune cells that secrete pro-inflammatory cytokines. This shift in WAT phenotype promotes a chronic low-grade inflammatory state, thereby predisposing individuals to cardio-metabolic risks.

The outcome of this study also indicated significantly increased levels of pro inflammatory cytokines, IL6 and TNF- α , following treatment with the Dolutegravir-based ART regimen. This regimen appears to stimulate the release of IL-6 and TNF- α by adipocytes, thereby enhancing systemic inflammation associated with increased weight gain [24]. Mitochondrial dysfunction induced by Dolutegravir-based ART may contribute to immune system impairment by promoting the generation of reactive oxygen species (ROS), inflammation, and subsequent cytokine release. Additionally, the secretion of pro-apoptotic factors such as cytochrome c during mitochondrial impairment further amplifies the inflammatory response through enhanced release of pro-inflammatory cytokines. This finding is in agreement with the study by Amoani *et al.* [25], who reported that IL6 and TNF- α were significantly higher in patients on ART when compared to ART-naïve HIV-positive subjects.

Conclusion

The Dolutegravir-based ART regimen resulted in significant weight gain and was associated with elevated levels of pro-inflammatory cytokines when compared to non-Integrase Strand Transfer Inhibitor (INSTI) regimens. These changes may contribute to an increased risk of metabolic disorders, cardiovascular complications, and other non-communicable diseases. Therefore, routine monitoring of anthropometric parameters, blood glucose levels, and lipid profiles is recommended to mitigate the potential long-term adverse effects of Dolutegravir-based therapy. Further research is warranted to investigate the monitoring of adipokines and the inhibition of pro-inflammatory cytokine signaling pathways as potential therapeutic targets. These approaches may hold promise for drug intervention and precision medicine strategies in the management of overweight and obesity.

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