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Assessment of oxidative toxicity and folate status in HIV patients on dolutegravir-based antiretroviral therapy

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Abstract

Dolutegravir (DTG), a key component of antiretroviral therapy (ART), has demonstrated potent virologic suppression and superior efficacy compared to standard regimens in HIV management. However, concerns about its long-term safety persist, with emerging evidence suggesting potential adverse effects. Notably, studies have reported an increased risk of neural tube defects in infants born to women exposed to DTG during pregnancy, as well as associations with neuropsychiatric effects and sideroblastic anemia. This cross-sectional study investigated plasma folate and malondialdehyde (MDA) levels—markers of antioxidant status and oxidative stress, respectively—in HIV-positive patients receiving DTG-based ART at the University of Nigeria Teaching Hospital, Enugu. A total of 120 participants were recruited, comprising 40 treatment-naïve patients initiating DTG-based ART, 40 patients on DTG-based ART for 6 months, and 40 HIV-negative controls. Plasma folate was measured using chemiluminescence immunoassay, while MDA levels were determined spectrophotometrically. Results showed significantly elevated MDA levels in both treatment-naïve (5.72 ± 3.61 μmol/L) and 6-month DTG-treated patients (8.94 ± 5.03 μmol/L) compared to controls (1.19 ± 0.18 μmol/L). Conversely, folate concentrations were markedly lower in the DTG groups (2.23 ± 1.52 and 1.89 ± 0.54 ng/mL, respectively) than in controls (11.11 ± 1.31 ng/mL). These findings suggest that DTG-based ART may elevate oxidative stress while reducing antioxidant levels, underscoring the need for careful monitoring of its biochemical effects in HIV-positive individuals.

Keywords: Dolutegravir; Oxidative toxicity; Folate status; Antiretroviral therapy; HIV

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1. Introduction

Dolutegravir (DTG), an integrase strand transfer inhibitor, has become a cornerstone of modern antiretroviral therapy (ART) owing to its potent virologic suppression, high genetic barrier to resistance, and favorable safety and efficacy profile across diverse populations. Its widespread adoption as part of first-line ART regimens has contributed significantly to global progress toward HIV epidemic control. Despite these clinical successes, concerns regarding the long-term safety of DTG have emerged, particularly in relation to neuropsychiatric adverse events (NPAEs), hematological alterations, teratogenicity through interference with folate metabolism, and potential contributions

to oxidative stress and metabolic dysregulation. These safety signals have sparked renewed scrutiny, especially in resource-limited settings where DTG is being rapidly scaled up as part of universal treatment strategies.

Real-world evidence has increasingly highlighted the neuropsychiatric burden associated with DTG. A cross-sectional study in Uganda reported that 41.7% of adult patients on DTG experienced at least one NPAE, with 9.1% experiencing severe or life-threatening outcomes requiring clinical intervention.[1] Comparable findings were reported in a multicenter study in Ethiopia, underscoring the importance of clinical vigilance in settings with limited access to psychiatric support.[2] Further evidence from the randomized DOBINeuro trial showed that patients switched from DTG/abacavir/lamivudine to bictegravir-based regimens experienced improvements in sleep disturbances, although other neuropsychiatric symptoms persisted, suggesting that DTG-associated tolerability challenges are nuanced and may not be completely reversible.[3]

In parallel, a growing body of experimental and clinical research has examined the relationship between DTG and folate metabolism. Preclinical evidence demonstrates that DTG disrupts the expression and function of critical folate transporters—including reduced folate carrier, proton-coupled folate transporter, and folate receptor-α—in human placental models and pregnant mice, thereby impairing cellular folate uptake under folate-deficient conditions.[4,5] In mice maintained on low-folate diets, DTG exposure induced neural tube defects (NTDs), such as exencephaly and cleft palates, whereas folic acid supplementation mitigated these abnormalities.[6]

Human evidence aligns with these findings: the ADVANCE trial in South Africa reported divergent folate responses depending on the ART regimen, with women on tenofovir alafenamide/emtricitabine (FTC) + DTG showing increased serum folate over 12 weeks, whereas those on tenofovir disoproxil fumarate (TDF)/FTC + DTG or TDF/FTC/Efavirenz experienced declines.[7] This variability suggests that DTG's impact on folate may depend on background regimen composition, baseline nutritional status, or host-related factors. The importance of adequate folate intake in mitigating DTG-associated teratogenic risks has been emphasized by several investigators. A 2024 review highlighted that supplementation or dietary fortification protects NTDs in pregnancies exposed to DTG, particularly in populations with poor baseline folate status.[8] Initial surveillance in Botswana suggested an elevated risk of NTDs in infants conceived on DTG,[9] and subsequent analyses reinforced this association in the context of low dietary folate intake.[10]

Mechanistic work further supports this biological interaction. Cabrera *et al.*[4] demonstrated that DTG acts as a non-competitive antagonist of folate receptor 1, inhibiting folate uptake despite adequate serum levels. Animal and cell-based studies confirmed that DTG-induced neurodevelopmental toxicity—including dopaminergic neuronal loss, reduced viability of stem cell-derived brain organoids, altered neurogenic gene expression such as *ngn1*, and impaired locomotor activity in zebrafish embryos—was largely attenuated by folic acid supplementation.[6,11-13]

Although the initial NTD safety signal has been attenuated by subsequent large-scale monitoring—with risk estimates declining as surveillance expanded[9] and pharmacovigilance studies have not confirmed a definitive link between DTG and NTDs,[14] clinical attention has shifted toward other emerging safety concerns, including weight gain, metabolic complications, and oxidative imbalance.[15]

A growing body of work suggests that DTG may induce oxidative stress and mitochondrial dysfunction, leading to systemic metabolic dysregulation. Elevated reactive oxygen species (ROS) production and perturbations in mitochondrial pathways have been observed, contributing to lipid accumulation, insulin resistance, and metabolic dysfunction in adipocytes. These effects appear to be mediated through impairment of fatty acid oxidation, dysregulation of lipoprotein lipase, and increased expression of pro-inflammatory cytokines such as tumor necrosis factor α and interleukin-6, mechanisms that mirror established pathways of oxidative stress-related metabolic disorders.[16,17] Such findings raise important concerns regarding long-term cardiometabolic outcomes in individuals on lifelong DTG therapy.

The central nervous system effects of DTG further compound its safety profile. Its penetrance across the blood-brain barrier has been associated with symptoms such as insomnia, depression, and anxiety, which in some cases necessitate treatment discontinuation. Systematic reviews estimate that up to 11.8% of patients discontinue DTG due to NPAEs, with older age, female sex, and ART-naïve status conferring increased risk.[14] Pharmacogenetic data suggest that individual susceptibility may be modified by genetic variation, with polymorphisms such as NR1I2 c.–22-7659C>T shown to reduce NPAE risk.[18] Preclinical evidence adds further mechanistic plausibility, indicating that DTG may exert neurotoxic effects through N-methyl-D-aspartate receptor activation, glutamate-mediated ROS production, and eryptosis, with recent work implicating endoplasmic reticulum stress at the blood-brain barrier as an additional pathway of DTG-induced central nervous system toxicity.[19]

Taken together, accumulating evidence underscores that, while DTG remains a highly efficacious antiretroviral agent with substantial public health benefits, its long-term safety profile requires continued vigilance. Concerns spanning neuropsychiatric events, folate metabolism, oxidative stress, and metabolic outcomes highlight the need for integrated pharmacovigilance, mechanistic research, and context-specific clinical guidance, particularly in regions with limited dietary folate intake and rising ART scale-up.

2. Materials and methods

2.1. Materials

2.1.1. Study design and setting

This was a quasi-experimental, prospective cohort study conducted at the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu State, Nigeria. The study was designed to evaluate the effects of DTG-based ART on hematological indices, micronutrient status, oxidative stress markers, and toxicity biomarkers among people living with HIV (PLWH).

2.1.2. Study population and group distribution

A total of 120 participants were recruited through purposive sampling and categorized into three groups of equal size (n = 40 per group):

(i) Group 1 - Treatment-naïve HIV-positive group: Newly diagnosed, ART-naïve HIV-positive individuals
(ii) Group 2 - DTG-experienced HIV-positive group: HIV-positive individuals who had received DTG-based ART for a minimum of 24 weeks
(iii) Group 3 - HIV-negative control group: Age- and sex-matched HIV-negative individuals without chronic illness or known hematological disorders.

Recruitment was conducted at the UNTH HIV clinic and the general outpatient department. HIV diagnosis was confirmed using the national HIV testing algorithm, while HIV-negative status was verified through serological screening.

2.2. Methods

2.2.1. Sample size determination

The minimum sample size was estimated using Fisher's formula for cross-sectional studies, based on the national HIV prevalence rate in Nigeria of 2.1%.[20] A minimum of 102 participants was required to achieve adequate statistical power at a 95% confidence level and a 5% margin of error. To compensate for possible attrition, 120 participants were enrolled, distributed equally across the three study groups (40 per group).

2.2.2. Eligibility criteria

Inclusion criteria were as follows:

(i) Aged 18–55 years
(ii) Confirmed HIV serostatus (positive or negative)
(iii) Written informed consent
(iv) For the DTG-experienced group: ≥24 weeks of continuous treatment with DTG-based ART.

Exclusion criteria included:

- Pregnancy
- Concurrent opportunistic infections (e.g., tuberculosis)
- Known metabolic, hematological, renal, or hepatic disorders
- Use of antioxidant supplements, cytotoxic drugs, or medications known to interfere with oxidative stress or folate metabolism.

2.2.3. Ethical considerations

Ethical approval for the study was obtained from the Health Research Ethics Committee of the UNTH (NHREC/05/01/2008B-FWA00002458-1RB00002323). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment. Confidentiality and data protection were maintained through data anonymization and restricted access to personal identifiers.

2.2.4. Sample collection and processing

Venous blood (5 mL) was collected aseptically from each participant into two tubes:

(i) Ethylenediaminetetraacetic acid tubes: For hematological profiling and plasma separation
(ii) Plain vacutainer tubes: For serum separation.

Samples in plain tubes were allowed to clot, then centrifuged at 3,000 rpm for 10 min, and serum aliquots were stored at −20°C until biochemical and immunological analyses were performed. Plasma and serum samples were thawed only once to minimize degradation of analytes.

2.2.5. Laboratory procedures

a. Determination of plasma folate levels

Plasma folate concentrations were quantified using the Maglumi 600 fully automated chemiluminescence immunoassay analyzer (Snibe Co., Ltd., China). The assay was based on a competitive chemiluminescent immunoassay in which folic acid was labeled with N-(4-aminobutyl)-N-ethylisoluminol and immobilized onto magnetic microbeads through folate-binding protein antibodies.

Test samples, calibrators, or controls were incubated with the labeled antigen, and the resulting complexes were magnetically separated from unbound fractions. Following a wash step, chemiluminescent starter reagents were added to initiate light emission. The relative light units generated were inversely proportional to the folate concentration in the test sample.[21]

b. Determination of malondialdehyde (MDA)

MDA concentrations were measured using the thiobarbituric acid reactive substances (TBARS) assay with a semi-automated chemistry analyzer (EMP Semi-Autochemistry Analyzer, Model: 168, Manufactured in China), following the method of Gutteridge and Wilkins.[22]

The assay is based on the reaction of MDA, a byproduct of lipid peroxidation, with thiobarbituric acid under acidic and high-temperature conditions to form a stable pink chromogen. Absorbance was measured spectrophotometrically at 532 nm. The concentration of MDA was calculated using the Beer-Lambert law:

$$\left[MDA\right](\mu M) = \frac{(A_{532} - A_{blank)} \times 10^6}{\varepsilon \times l} \quad \text{(I)}$$

Where ε is the molar extinction coefficient and l is the path length.

Alternatively, concentration was expressed as:[23]

$$[MDA](nmol/mL) = (A_{532}\text{-}A_{blank}) \times 6.41 \quad \text{(II)}$$

2.3. Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., USA). Descriptive statistics (mean ± standard deviation for continuous variables and frequencies/percentages for categorical variables) were computed.

(i) Comparisons of means across the three groups were performed using independent sample *t*-tests and one-way analysis of variance, where appropriate
(ii) Chi-square tests were used for categorical variables
(iii) Effect sizes were calculated using Cohen's *d*, allowing interpretation of the magnitude of observed differences beyond statistical significance
(iv) A $p<0.05$ was considered statistically significant
(v) Results are presented in tables and figures to enhance clarity.

2.4. Confidentiality and data management

All participants' personal identifiers—including names, age, and contact details—were anonymized and coded before analysis. Laboratory samples were labeled with numeric codes rather than participant information. Data were stored on a password-protected computer with access limited to the research team. Individual laboratory results were communicated privately to each participant during counseling sessions at the HIV clinic.

3. Results

3.1. Baseline characteristics of the study population

Table 1 summarizes the sociodemographic characteristics of the study population. The distribution of participants by sex was fairly equitable, with males comprising 48–51% and females 49–52% across groups, showing no significant difference (p=0.816). The mean age of participants was 37.32 ± 8.63 years, spanning 19–53 years, representing mostly young- and middle-aged adults in their physiologically active stages.

However, age distribution differed significantly between groups (p<0.001). While the majority of HIV-negative

Table 1. Sociodemographic characteristics of study participants across treatment groups

Baseline characteristic	Subject	Control	x^2	*p*-value
Age group				
19–28	6 (16.2)	24 (64.9)	20.254	<0.001
29–38	13 (35.1)	8 (21.6)		
39–48	14 (37.8)	5 (13.5)		
49–58	4 (10.8)	0 (0.00)		
Sex				
Male	18 (48.6)	19 (51.4)	0.054	0.816
Female	19 (51.4)	18 (48.6)		
Educational status				
Primary	10 (27.0)	10 (27.0)	0.670	0.715
Secondary	16 (43.2)	13 (35.1)		
Tertiary	11 (29.7)	14 (37.8)		
Socioeconomic status				
Low	27 (73.0)	26 (70.3)	1.019	0.601
Middle	10 (27.0)	10 (27.0)		
High	0 (0.00)	1 (2.7)		
Occupation				
Artisan	7 (18.9)	0 (0.00)	20.567	0.001
Business/trader	21 (56.8)	14 (37.8)		
Civil servant	7 (18.9)	17 (45.9)		
Farmer	0 (0.00)	3 (8.1)		
Student	0 (0.00)	3 (8.1)		
Unemployed	2 (5.4)	0 (0.00)		
Marital status				
Single	14 (37.8)	18 (48.6)	0.881	0.348
Married	23 (62.2)	19 (51.4)		

Note: Data are expressed as *n* (%).

controls was younger adults aged 19–28 years (64.9%), the largest proportion of PLWH on DTG-based ART were in the 39–48 age category (37.8%), suggesting that HIV-positive individuals tended to be older.

Educational attainment was comparable between groups (p=0.715), with secondary education being the most frequent, followed by tertiary and primary education. Socioeconomic status was also similar (p=0.601), with over 70% of participants in both groups classified as low-income status, and only a minority reporting middle- or high-income status. In contrast, occupational status differed significantly (p=0.001): a majority of HIV-positive participants were traders (56.8%), while nearly half of the control groups were civil servants (45.9%). Marital status distribution showed no significant variation between groups (p=0.348), with both groups having a predominance of married individuals.

These findings suggest that, while educational and socioeconomic backgrounds were largely balanced across groups, the HIV-positive cohort was slightly older and more commonly engaged in trading, whereas controls were more likely to be in formal employment.

3.2. Biochemical outcomes

A significant increase in MDA levels was observed after 24 weeks of DTG-based ART (Table 2). Mean plasma MDA concentrations rose from 5.72 ± 3.61 μmol/L at baseline to 8.94 ± 5.03 μmol/L post-treatment (t = 2.767, p=0.009), reflecting a pronounced elevation in oxidative stress after therapy.

Comparison with HIV-negative controls further underscored the burden of oxidative imbalance. Mean MDA levels in the control group were markedly lower (1.19 ± 0.18 μmol/L), and both pre- and post-treatment values in the DTG group were significantly elevated relative to controls (p<0.001).

Folate levels did not show a statistically significant within-group change over 24 weeks of therapy (p=0.753). Mean plasma folate decreased from 2.23 ± 1.52 ng/mL at baseline to 1.86 ± 0.54 ng/mL post-treatment, suggesting only a minimal short-term effect of DTG initiation on folate status.

However, when compared with HIV-negative controls, who had substantially higher folate levels (11.11 ± 1.31 ng/mL), both pre- and post-treatment HIV-positive participants exhibited severe folate depletion (p<0.001). This pattern highlights that folate deficiency is a persistent feature of HIV infection, independent of immediate DTG exposure.

The effect size plot with dashed lines marking thresholds for small, medium, and large effects. It illustrates that folate shows an extremely large decrease, while MDA shows moderate-to-very large increases depending on the comparison.

To complement significance testing, Cohen's d effect sizes were calculated (Table 2 and Figure 1).

Within the groups (pre vs. post), the increase in MDA from baseline to post-treatment corresponded to a medium-to-large effect (d = 0.74). In contrast, the decline in folate corresponded to a small-to-medium effect (d = 0.32).

Between the groups (HIV vs. controls), very large to extremely large effects were observed for MDA, with values of d = 1.78 for pre versus control and d = 2.18 for post versus control. For folate, the differences were extremely large and negative, reflecting markedly lower levels in HIV patients compared to controls; specifically, d = −6.25 for pre versus control and d = −9.25 for post versus control.

These findings provide additional insight into the magnitude of observed biochemical alterations. The within-group effect of DTG on oxidative stress was moderate-to-large, suggesting that treatment contributed to further increases in lipid peroxidation beyond baseline HIV-associated levels. Conversely, the within-group decline in folate was small and not statistically significant, indicating that folate depletion is more strongly attributable to HIV infection itself rather than short-term DTG use. However, the between-group comparisons revealed extremely large differences in folate status, demonstrating the severity of micronutrient deficiency among PLWH relative to HIV-negative individuals.

Table 2. Mean values of malondialdehyde and folate among treatment-naïve, treatment-experienced, and control participants with Cohen's *d* effect sizes

Parameter	Control	Pre-DTG	Post-DTG	*p* (Pre vs. post)	*p* (Pre vs. control)	*p* (Post vs. control)	Cohen's *d* (pre vs. post)	Cohen's *d* (pre vs. control)	Cohen's *d* (post vs. control)
Malondialdehyde (μmol/L)	1.19±0.18	5.72±3.61	8.94±5.03	0.009	<0.001	<0.001	0.72 (moderate-large ↑)	1.66 (very large ↑)	1.94 (very large ↑)
Folate (ng/mL)	11.11±1.31	2.23±1.52	1.86±0.54	0.753	<0.001	<0.001	0.29 (small ↓)	6.8 (huge ↓)	8.2 (huge ↓)

Note: Data are expressed as means±standard deviations.
Abbreviation: DTG: Dolutegravir.

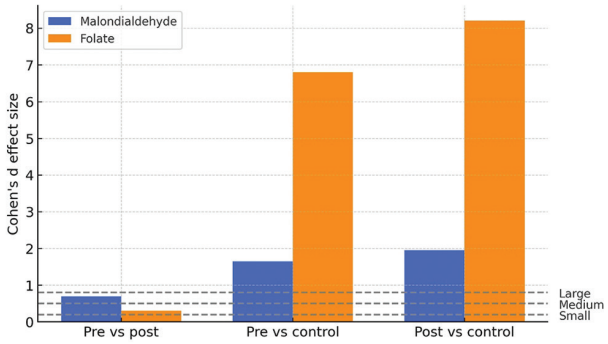

Figure 1. Effects of dolutegravir treatment on malondialdehyde and folate

4. Discussion

This study demonstrates that DTG-based ART is associated with significant biochemical alterations, most notably elevated oxidative stress and reduced folate availability. Taken together, these findings reveal two distinct but interconnected biochemical patterns.

First, DTG therapy was associated with a statistically significant and clinically meaningful increase in MDA, with effect size analysis confirming a moderate-to-large impact (d = 0.74). Both pre- and post-therapy MDA levels were substantially higher than in HIV-negative controls, indicating that oxidative imbalance is both a consequence of HIV infection and further exacerbated by ART. The moderate-to-large increase in MDA concentrations post-treatment is consistent with elevated lipid peroxidation and oxidative imbalance. Oxidative stress, reflected by biomarkers such as MDA, is a well-established driver of mitochondrial dysfunction, cellular senescence, and inflammatory activation in PLWH.[24,25] These mechanisms have been implicated in neurocognitive decline, cardiovascular disease, and accelerated aging, even among virally suppressed patients.[26] The persistence of high oxidative stress despite ART initiation suggests that integrase inhibitor therapy may not fully attenuate, and may even exacerbate, oxidative injury through ROS generation and impaired antioxidant defenses.

Second, folate levels remained markedly and consistently lower in HIV-positive participants compared with controls. The within-group change after DTG therapy was small and not statistically significant (Cohen's d = −0.33), but the between-group effect sizes were extremely large, with values such as d = −1.49 for pre versus control and d = −2.61 for post versus control. This suggests that while DTG initiation does not cause an acute decline, HIV infection itself is strongly associated with severe folate depletion. Folate is indispensable for one-carbon metabolism, DNA synthesis, and hematopoiesis, and its depletion predisposes patients to megaloblastic anemia and impaired immune recovery.[27] More importantly, maternal folate deficiency has been consistently linked to NTDs, raising major public health concerns for women of reproductive age receiving DTG-based ART. Our findings align with prior reports showing reduced folate levels among ART-experienced populations,[28,29] though they contrast with recent clinical trial data from Barlow-Mosha *et al.*,[30] which demonstrated more pronounced folate changes. Such discrepancies may reflect differences in baseline nutritional status, treatment duration, or population characteristics.

The oxidative and nutritional disturbances observed in this study are consistent with previous reports. Elevated MDA has been documented in both ART-naïve and ART-treated HIV populations, reflecting heightened lipid peroxidation and cytokine-driven inflammatory pathways.[31-33] Mechanistically, cytokine-mediated activation of lipoxygenase pathways may further potentiate ROS generation.[34] Similarly, folate depletion in HIV has been attributed to anorexia, increased metabolic turnover, and viral replication-driven nucleotide demand.[35,36] Given the estimated daily production of up to 10 billion virions in untreated HIV, such demands are likely to accelerate micronutrient depletion, compounding nutritional deficiencies.

Folate deficiency has dual consequences. It impairs DNA synthesis and causes defective S-phase progression, both of which are reversible upon repletion. In addition, it compromises immune function through reduced T-cell proliferation and blunted mitogen responses.[37] In the context of oxidative stress, these disturbances may act synergistically. ROS-mediated injury depletes antioxidant reserves, further lowering folate bioavailability, while folate deficiency compromises DNA repair capacity, thereby amplifying oxidative damage.[38] This bidirectional interplay could underlie the neuropsychiatric symptoms, hematologic toxicity, and heightened teratogenic risk reported in DTG-treated populations.[4] An additional layer of complexity may arise from genetic polymorphisms in folate metabolism (e.g., MTHFR variants), which could modulate individual susceptibility to drug-nutrient interactions and oxidative injury, further reinforcing the need for personalized monitoring strategies.

Clinically, these findings highlight that patients on DTG-based ART face a dual burden. They experience worsening oxidative stress after treatment initiation, coupled with profound pre-existing folate deficiency that remains uncorrected by therapy. This underscores the need for integrated management approaches that extend beyond viral suppression. Routine monitoring of oxidative

stress markers and serum folate should be considered, alongside adjunctive strategies such as targeted antioxidant support and folate supplementation. Such interventions may mitigate metabolic toxicity, improve hematological outcomes, and reduce neurodevelopmental risks in exposed infants. Although concerns about potential teratogenicity with DTG have been reported mainly from observational studies of exposure at conception, our study did not include pregnant women. Therefore, our findings do not provide direct evidence on this risk but rather suggest the need for further validation in pregnant populations. Further longitudinal and interventional studies are warranted to establish causality and evaluate the therapeutic efficacy of these strategies.

4.1. Perspectives on oxidative stress in HIV

The elevated oxidative stress observed in this study must be contextualized within the broader landscape of HIV management. Chronic HIV infection induces a pro-oxidant state, and while ART effectively reduces viral load, residual immune activation continues to sustain ROS generation. Mitochondria, as key regulators of cellular metabolism and apoptosis, are particularly vulnerable to oxidative injury. Persistent mitochondrial dysfunction has been linked to ART-related toxicities, including neuropathy, lipoatrophy, and lactic acidosis.[39] Although DTG is considered safer than thymidine analogues in this regard, the rise in MDA suggests that integrase inhibitors may not be metabolically inert.

Importantly, oxidative stress acts as a bridge between HIV and its non-AIDS comorbidities. Cardiovascular disease, osteoporosis, frailty, and neurocognitive impairment are all more prevalent in PLWH compared with the general population, even among those on suppressive ART. Our findings strengthen the hypothesis that oxidative stress contributes to this excess burden and may serve as a therapeutic target.

4.2. Comparative insights across ART classes

Previous studies have shown differential effects of ART classes on oxidative stress. Protease inhibitors, for instance, increase oxidative stress by inducing mitochondrial ROS production and promoting dyslipidemia. Nucleoside reverse transcriptase inhibitors, particularly stavudine and zidovudine, cause direct mitochondrial DNA depletion through inhibition of polymerase-γ.[40] By contrast, integrase inhibitors were initially thought to spare mitochondria, yet our findings suggest that the benefits may be relative rather than absolute. Understanding these nuances is critical as DTG becomes entrenched as the global first-line regimen.

4.3. Folate depletion: Clinical and public health significance

The extreme folate deficiency in our cohort highlights a major nutritional challenge. Folate insufficiency predisposes PLWH to anemia, impaired immune recovery, and teratogenic risk. While ART has transformed HIV into a chronic condition, quality of life and comorbidity prevention remain pressing concerns. Nutritional supplementation represents a low-cost intervention with the potential to improve outcomes.

Globally, the intersection between HIV and folate deficiency is particularly concerning in sub-Saharan Africa, where both HIV prevalence and baseline micronutrient deficiencies are high. Women of reproductive age are a critical population, as folate deficiency is directly linked to NTDs. The Botswana findings of increased NTDs among infants exposed to DTG at conception underscore the urgency of integrating micronutrient monitoring into HIV programs.[9]

4.4. Systematic interplay: Folate, one-carbon metabolism, and redox balance

Folate deficiency compromises one-carbon metabolism, leading to impaired DNA synthesis, methylation defects, and reduced production of nicotinamide adenine dinucleotide phosphate, which is essential for regenerating glutathione. Thus, folate deficiency and oxidative stress reinforce each other in a vicious cycle. This mechanistic insight provides a strong rationale for dual-target interventions: antioxidant therapy to counteract ROS and folate supplementation to restore one-carbon metabolism.

4.5. Clinical implications and future directions

The clinical implications of our findings are multifaceted. Monitoring oxidative stress markers (e.g., MDA, F2-isoprostanes) and serum folate in routine HIV care may help identify high-risk patients early. Nutritional counseling and supplementation should be prioritized, especially for women of childbearing age. Although antioxidant interventions are not yet standardized, they warrant further study in the context of DTG-based therapy.

Future research should move beyond observational findings to include randomized controlled trials evaluating the efficacy of folate and antioxidant supplementation in improving hematological, neurocognitive, and pregnancy outcomes. Moreover, integrating genetic screening for folate metabolism polymorphisms may allow for more personalized care.

5. Conclusion

This study provides preliminary evidence that DTG-based ART is associated with alterations in oxidative stress and

folate status among PLWH. While the findings are clinically significant, they must be interpreted with caution, given methodological limitations, including reliance on plasma folate alone, the use of the TBARS assay for MDA, and unmeasured confounding factors such as diet, lifestyle, and concomitant medications. Importantly, the observed changes likely reflect the combined influence of DTG, companion drugs (TDF/3TC), and underlying HIV-related inflammation rather than DTG alone. Nevertheless, the results highlight the need for routine monitoring of folate and oxidative stress biomarkers in patients on ART and suggest a potential role for targeted supplementation strategies. Future research incorporating longitudinal designs, functional folate markers, more specific assays, and cost-effectiveness analyses will be essential to validate and extend these findings, particularly in vulnerable groups such as pregnant women.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: All authors
Investigation: Onwuka Kalu Chima
Methodology: All authors
Writing–original draft: Onwuka Kalu Chima
Writing–review & editing: All authors

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital (NHREC/05/01/2008B-FWA00002458-1RB00002323). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment. Confidentiality and data protection were maintained through data anonymization and restricted access to personal identifiers.

Consent for publication

Specific consent for publication of participant data was not obtained because all data were anonymized and analyzed in aggregate. No identifiable information was included; therefore, additional consent was not required under the approved study protocol.

Availability of data

Data supporting the findings of this study are available from the corresponding author on reasonable request.

References

1. Mwebaza J, Meya D, Musiime V, Birungi C. Prevalence of neuropsychiatric adverse events and associated factors among adult patients on dolutegravir attending Mulago ISS clinic. *HIV Med.* 2023;24(4):491-501.

   doi: 10.1111/hiv.13428

2. Tesfaye E, Demelash K. Burden of care among caregivers of patients with mental illness in Ethiopia: A systematic review and meta-analysis. *BMC Public Health.* 2025;25:2414.

   doi: 10.1186/s12889-025-23461-1

3. Rossetti B, Ferrara M, Taramasso L, *et al.* Evolution of self-reported neuropsychiatric symptoms after switching from dolutegravir/abacavir/lamivudine to bictegravir/emtricitabine/tenofovir alafenamide: Results from the randomized DOBINeuro trial. *Infect Dis Ther.* 2025;14(1):293-304.

   doi: 10.1007/s40121-024-01083-1

4. Cabrera RM, Souder JP, Steele JW, *et al.* The antagonism of folate receptor by dolutegravir: Developmental toxicity reduction by supplemental folic acid. *AIDS.* 2019;33(11):1789-1798.

5. Gilmore JC, Hoque MT, Dai W, *et al.* Interaction between dolutegravir and folate transporters and receptor in human and rodent placenta. *EBioMedicine.* 2022;75:103771.

   doi: 10.1016/j.ebiom.2021.103771

6. Tukeman GL, Wei H, Lin YL, Wlodarczyk BJ, Finnell RH, Cabrera RM. Dolutegravir-induced neural tube defects in mice are folate responsive. *AIDS.* 2024;38(4):439-446.

   doi: 10.1097/QAD.0000000000003639

7. Chandiwana NC, Chersich M, Venter WDF, *et al.* Unexpected interactions between dolutegravir and folate: Randomized trial evidence from South Africa. *AIDS.* 2021;35(2):205-211.

   doi: 10.1097/QAD.0000000000002741

8. Zhou Q, Dong G, Wang Q, *et al.* Preconception folic acid supplementation for the prevention of birth defects: A prospective, population-based cohort study in mainland China. *BMC Pregnancy Childbirth.* 2024;24(1):114.

   doi: 10.1186/s12884-024-04319-9

9. Zash R, Holmes L, Diseko M, *et al.* Neural-tube defects and antiretroviral treatment regimens in Botswana. *N Engl J Med.* 2019;381(9):827-840.

doi: 10.1056/NEJMoa1805230

10. Assefa N, Abdullahi YY, Abraham A, *et al.* Consumption of dietary folate estimates and its implication for reproductive outcome among women of reproductive age in Kersa: Cross-sectional survey. *BMC Nutr.* 2021;7(1):69.

doi: 10.1186/s40795-021-00482-w

11. Jakimiuk A, Piechal A, Wiercińska-Drapało A, Nowaczyk A, Mirowska-Guzel D. Dolutegravir induces FOLR1 expression during brain organoid development. *Front Mol Neurosci.* 2024;17:1463159.

doi: 10.3389/fnmol.2024.1463159

12. Alcorn K. *Adequate Levels of Folate Protect Against Neural Tube Defects When Taking Dolutegravir*. *NAM aidsmap*; 2024. Available from: https://www.aidsmap.com [Last accessed on 2025 Sep 25].

13. Quirós Roldán M, Bresciani A, Savoldi A. Dolutegravir and folic acid interaction during neural system development: Zebrafish embryo model. *Int J Mol Sci.* 2024;25(9):4640.

doi: 10.3390/ijms25094640

14. Saint-Lary O, Martin C, Leclerc T. Post-marketing safety concerns with dolutegravir: A comprehensive pharmacovigilance review. *Front Pharmacol.* 2025;16:1399821.

doi: 10.3389/fphar.2025.1399821

15. Masenga SK, Kabwe LS, Chakulya M, Kirabo A. Mechanisms of oxidative stress in metabolic syndrome. *Int J Mol Sci.* 2023;24(9):7898.

doi: 10.3390/ijms24097898

16. Jain RG, Furfine ES, Pedneault L, White AJ, Lenhard JM. Metabolic complications associated with antiretroviral therapy. *Antiviral Res.* 2001;51:151-177.

doi: 10.1016/S0166-3542(01)00147-7

17. Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. *J Biomed Biotechnol.* 2012;2012:936486.

doi: 10.1155/2012/936486

18. De Greef J, Yombi JC, Vincent A, *et al*. Dolutegravir and risk of neuropsychiatric adverse events: A pharmacogenetic study. *J Infect Dis.* 2025;231(6):1425-1429.

doi: 10.1093/infdis/jiaf098

19. Jakimiuk A, Piechal A, Wiercińska-Drapało A, Nowaczyk A, Mirowska-Guzel D. Central nervous system disorders after use of dolutegravir: Evidence from preclinical and clinical studies. *Pharmacol Rep.* 2023;75(6):1138-1151.

doi: 10.1007/s43440-023-00515-y

20. National Agency for the Control of AIDS (NACA). *National HIV/AIDS Indicator and Impact Survey (NAIIS) 2021: Technical Report*. Abuja, Nigeria: NACA; 2021.

21. Azim M, Hasan M, Ansari IH, Nasreen F. Chemiluminescence immunoassay: Basic mechanism and application. *Bangladesh J Nuclear Med.* 2018;18(2):171.

doi: 10.3329/bjnm.v18i2.35240

22. Gutteridge JM, Wilkins SC. Copper dependent hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. *FEBS Lett.* 1982;137:327-330.

doi: 10.1016/0014-5793(82)81330-8

23. Buege JA, Aust SD. Microsomal lipid peroxidation. In: Fleischer S, Packer L, editors. *Methods in Enzymology.* Vol. 52. United States: Academic Press; 1978. p. 302-310.

24. Piantadosi CA, Suliman HB. Mitochondrial dysfunction in oxidative stress. *Free Radic Biol Med.* 2017;122:134-146.

doi: 10.1016/j.freeradbiomed.2018.01.003

25. Shytaj IL, Norelli S, Chirullo B, Palamara AT. Oxidative stress and HIV infection: Role in pathogenesis and therapeutic strategies. *Expert Rev Anti Infect Ther.* 2020;18(5):447-460.

doi: 10.1080/14787210.2020.1762438

26. Reust CE. Common adverse effects of antiretroviral therapy for HIV disease. *Am Fam Physician.* 2011;83(12):1443-1451.

27. Bailey LB, Gregory JF 3rd. Folate metabolism and requirements. *J Nutr.* 2015;145(3):593-596.

doi: 10.3945/jn.114.204009

28. Flax VL, Adair LS, Allen LH, *et al*. Plasma micronutrient concentrations are altered by antiretroviral therapy and lipid-based nutrient supplements in lactating HIV-infected Malawian women. *J Nutr.* 2015;145(8):1950-1957.

doi: 10.3945/jn.115.220109

29. Alani A, Vincent O, Adewumi A, *et al*. Plasma folate studies in HIV-positive patients at the Lagos University Teaching Hospital, Nigeria. *Indian J Sex Transm Dis AIDS.* 2010;31(2):99-103.

doi: 10.4103/0253-7184.74995

30. Barlow-Mosha LN, Ahimbisibwe GM, Chappell E, *et al*. Effect of dolutegravir on folate, vitamin B12 and mean corpuscular volume levels among children and adolescents with HIV: A sub-study of the ODYSSEY randomized controlled trial. *J Int AIDS Soc.* 2023;26(9):e26174.

doi: 10.1002/jia2.26174

31. Korencak M, Byrne M, Richter E, *et al.* Effect of HIV infection and antiretroviral therapy on immune cellular functions. *JCI Insight.* 2019;4(12):e126675.

doi: 10.1172/jci.insight.126675

32. Oyeyipo IP, Skosana BT, Everson FP, Strijdom H, du Plessis SS. Highly active antiretroviral therapy alters sperm parameters and testicular antioxidant status in diet-induced obese rats. *Toxicol Res.* 2018;34(1):41-48.

doi: 10.1039/c7tx00205h

33. Dhonju K, Gautam A, Dahal A, *et al.* Dolutegravir-induced acquired sideroblastic anemia in an HIV-positive patient: A challenging hematologic complication. *Clin Case Rep.* 2023;11(12):e8301.

doi: 10.1002/ccr3.8301

34. Kovacic P, Somanathan R. Broad overview of oxidative stress and its complications in human health. *Open J Prev Med.* 2013;3(1):32-41.

doi: 10.4236/ojpm.2013.31005

35. Rothenberg SP. Increasing the dietary intake of folate: Pros and cons. *Semin Hematol.* 1999;36(1):65-74.

36. Babafemi T. *General HIV Medicine*. Vol 1. Hyderabad: TFC Press; 2004. p. 10-11.

37. Dhur A, Galan P, Herberg S. Folate status and the immune system. *Prog Food Nutr Sci.* 1991;15(1-2):43-60.

38. Molloy AM, Scott JM. Folates and prevention of disease. *Public Health Nutr.* 2001;4(2B):601-609.

doi: 10.1079/PHN2000128

39. Lewis W, Dalakas M, Biesecker GM. Mitochondrial toxicity of antiviral drugs. *Nat Med.* 2003;6(3):249-255.

doi: 10.1038/nm0303-249

40. Savinelli S, Newman E, Mallon PWG. Metabolic complications associated with use of integrase strand transfer inhibitors (INSTI) for the treatment of HIV-1 infection: Focus on weight changes, lipids, glucose and bone metabolism. *Curr HIV/AIDS Rep.* 2024;21:567-580.

doi: 10.1007/s11904-024-00683-5