Effect of Probiotic Yogurt Supplementation on Intestinal Inflammation and Oxidative Stress in HIV-Positive Patients on Antiretroviral Therapy: Randomized Controlled Trials in 24 and 48 Weeks

Kasamba Ilunga Eric1, Balaka Ekwalanga Michel2, Mulumba Kadiebwe David3, Kalumba Kambote Athy4, Ilunga Nikulu Julian5, Lungu Anzwal6, Kalenga Mwenze Kayamba Prosper7, Malangu Mposhy Emmanuel Prosper8

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Department of Biomedical Sciences, Faculty of Medicine, University of Lubumbashi, R Congo

3Department of Veterinary Medicine, University of Lubumbashi, DR Congo

4Department of Internal Medicine, Faculty of Medicine, University of Lubumbashi, DR Congo

5Center of Excellence, Faculty of Medicine, University of Lubumbashi, R D Congo

Abstract: Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART) have been associated with high oxidative stress in HIV patients. The disparity in antioxidant levels and secondary inflammation due to intestinal bacterial translocation in people living with HIV is the leading cause of morbidity and mortality associated with HIV infection. Using a simple 1: 1 randomization, Efavirenz (EFV) and Lopinavir (LPV) -treated antiretroviral (ARV) -treated patients were additionally supplemented with probiotic yogurt and then followed for 48 weeks and evaluated at inclusion, at mid- and at the end of the course, by the determination of markers: immunological (CD4), virological (viral load), inflammatory (soluble Hs CRP, CD14 and CD163) and oxidative stress [Superoxide dismutase (SOD), glutathione peroxidase (GPx ) and Zinc]. The results confirm that HIV infection induces inflammation by a very significant increase in SCD14, sCD163 and HsCRP; and a collapse of the antioxidant protective system characterized by decreased levels of SOD, GPxs and Zinc. At weeks 24 and 48, a significant reversal of markers of inflammation and oxidative stress was observed under the ART + Probiotic Yogurt arm. Only the value of Zinc remained at a subnormal rate. All patients in the same arm had their undetected viral load since week 24. Although probiotic yogurt supplementation with ART has a significant impact on immunologic, virologic, inflammatory and oxidative stress markers compared to ARV only, it should be associated with Zinc for an optimal evolution of seropositive patients.

Keywords: HIV, Inflammation, Oxidative Stress Probiotic

1. Introduction

HIV-1 induces oxidative stress by deregulation of oxidative stress pathways with increased ROS production and by inducing mitochondrial dysfunction [1, 2]. Although antiretroviral therapy can eliminate viremia and improve the immunological status of HIV-infected individuals for a prolonged period of time, both nucleoside and non-nucleoside inhibitors of Retro-Transcriptase (RT), as well as inhibitors of viral protease, trigger mass production of ROS in various cell types [3,4] also playing an important role in the development of a broad spectrum of virus-associated pathologies and is one of the factors contributing to the emergence and / or the perpetuation of chronic inflammatory syndromes. There is damage and dysfunction of the digestive system. This enteropathy due to HIV is characterized by a pronounced loss of CD4 + lymphocytes, increased intestinal permeability and microbial translocation that promotes systemic immune activation involved in the progression of the disease (5) The capitalization of these clinical observations suggests that the translocation microbial could affect the progression of HIV disease, the response to treatment, and non-AIDS comorbidities. Given its adverse effect on overall immunity, several interventions aimed at preventing or blocking microbial translocation are currently being investigated as new therapeutic agents for HIV / AIDS (6).

Thus, in the search for new therapeutic approaches to restore antioxidant defense systems and inhibit microbial translocation and / or attenuate chronic immune activation in HIV-infected individuals, in order to complement treatments aimed at the direct suppression of viral replication. We conducted a study to evaluate the effect of probiotic yoghurt supplementation on intestinal wall inflammation and oxidative stress on disease progression

2. Methods

A simple 1: 1 randomization, selecting 229 HIV-positive patients on ART, of whom only 178 were elected and included in the study because of 141 under Bras Efavirenz (68 under ART and 73 under ART + probiotic yogurt) and 37 under LPV / r ( 18 on ART and 19 HAART + Probiotic Yogurt) combined with Tenofovir (TDF) and Lamuvidine (3TC) according to the following criteria: written informed consent, age ≥ 18 years, patient on antiretroviral therapy for more than 6 months, meet criteria following, negative pregnancy test at the selection visit (♀ of childbearing age) and the non-inclusion criteria are defined as follows:

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presence of Grade 4 laboratory abnormalities at the selection visit, ALT or AST > 3X limit Sup of normal, hemoglobin < 8.5 g% (♀), < 9.0 g% (♂), estimated creatinine clearance < 50 ml/min, according to the Cockcroft-Gault formula suspicion of TBC at X-ray thorax. And followed for 48 weeks.

The parameters of interest were as follows: age, sex; therapeutic regimen, CD4 count (s) count, sCD14 (Enzo life science), sCD163 (Avisera Biosciences) (Hypersensitive CRP (Enzo life science), Glutathione peroxidase (Randox), sulfoxide dismuthase (Randox), Zinc (Randox)), the viral load (Roche CAPCTM) The data collected at inclusion, in weeks 24 and 48 were analyzed using the software Epi Info version 7.2.2.2, and Excel 2010.

3. Results and Discussion

Table 1: Randomization: Patient Status at Inclusion and Week 24 and 48ko

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
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</thead>
<tbody>
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<tr>
<td>Eligible patients</td>
<td>178</td>
</tr>
<tr>
<td>Ineligible patients</td>
<td>51</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>19</td>
</tr>
<tr>
<td>Tuberculosis (TBC)</td>
<td>23</td>
</tr>
<tr>
<td>Refusal consent</td>
<td>9</td>
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77.7% of eligible patients were included in our study, tuberculosis (10.0%) and pregnancy (8.29%) were the main causes of non-eligibility with a refusal of consent of 3.93% or 9 patients of the selected group. After 24 and 48 weeks of follow-up, 7 deaths in week 24 and 2 in week 48, ie 3.92% of cases, the lost were respectively divided again: 11 in week 24 set 9 in week 48 or 20 cases in total (11.23% ); 4 cases of tuberculosis occurring during our cohort, ie 2.31% of patients included. Most of our patients are female, ie 71.9% with an average age of 30 (28-45).

In their Cameroon series Dimala CA et al (7) found that the mean age was 40.2 ± 8.0 years and 70% of them were women, which is close to our observation; In addition, Woldemedhin B et al., Studying the reason for the change of regimen in HIV / AIDS patients who initiated highly active first-line antiretroviral therapy in southern Ethiopia found that the majority of patients (69.29%) were women (8), same observation made by Abo Y et al (9) in West Africa where their series consisted of 67% women, with a median age of 35 years. As for the mortality rate, it is lower compared to the estimates of Farahani M and all (10) which report a mortality rate in sub-Saharan countries of 18.5% (95% confidence interval). 13.8 to 23.7). But, our rate is similar to that found in Kenya by Onyango, DO et al (11) where it is estimated at 6% with a tuberculosis incidence of 10% as in our study.

1) Immunological marker monitoring

The CD4 follow-up results during randomization show that a significant difference in variation between the two arms, ART + Yogurt significantly improved CD4 count, an increase of 92.7% and 396.6% respectively under supplementation, in probiotic yogurt and EFV (p = 0.00206) and LPV (p = 0.00278) versus 43.6% and 46.5% respectively under EFV and LPV only. The results under ART only approximate those of Roul H et al who showed a cumulative incidence of CD4 recovery after 6 years of virological control was 69.7% with CD4 recovery as the main factor. their rate at the beginning of treatment (12); and Kroezes S et al who observed CD4 CD4 incidence rates were 12.5 times higher for AIDS (13); But the results of the CD4 level obtained with ART alone are far lower than those obtained with ART + Yogurt.
obtained with ART and probiotic yogurt supplementation, which is confirmed by Irvine SL et al who found in their study that the introduction of probiotic yogurt, made by local women in a low-income community in Tanzania, was significantly associated with an increase in CD4 count among consumers living with HIV (14), the same observation was made by Anukam KC (15) in his study on Yogurt containing Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 probiotics that helped treat moderate diarrhea and increase CD4 counts in HIV / AIDS patients.

2) Tracking markers of inflammation

Generally, within 48 weeks, high levels of macrophage activation markers, such as sCD163, sCD14 in the blood, as well as inflammatory biomarkers, such as Hs-CRP, were observed under ARV treatment, and decreases non-significantly under ART only. And a very significant decrease was found under the ARV + Probiotic Yogurt arm and this regardless of the ARV base.

The figure above shows a high level of soluble CD14 for all patients on ART at the inclusion of randomization under EFV [3560 (2300-5980)] and under LPV [3500 (2210-5840)], which translates persistent inflammation despite ART. This persistence of high sCD14 levels was also observed by Macatangay BJ et al (16) who noted among study participants who started antiretroviral therapy in the first six months of HIV infection, high levels of sCD14 and CRP and remained similar to levels seen prior to antiretroviral therapy, suggesting that immune lesions occurring during the early stages of infection persist despite short-term virological suppression; Sereti I et al (17) also observed that sCD14 levels decreased during antiretroviral treatment, but remained high compared to non-HIV infected participants. Negi N et al also noted in their results that elevated levels of sCD14, Endo Cab and IgM LPS in HIV-1 infected individuals are potent predictors of disease progression and could be considered Candidate biomarkers for disease surveillance (18). Sereti at al (17) suggest that additional interventions to reduce inflammation may be necessary to optimize clinical outcomes in HIV-infected individuals. Thus, under probiotic yogurt and HAART, our results show a significant decrease in the level of sCD14. Indeed, Pei R (19) et al claim that yogurt consumption after nine weeks showed that ΔAUC ratios of YO and YN LY / sCD14 ratios were less than half those of control groups (P = 0, 0093). Similarly, Kong LC et al (20) in their study of dietary habits associated with inflammation and gut microbiota in overweight and obese subjects, found that the group with the healthiest eating behaviors (lower consumption of sweets and sugary drinks, and higher fruit consumption, but also yogurts and soups) had the lowest inflammatory markers (sCD14).
In the analysis of Figure 3, we note that the level of sCD163 mono-macrophage activation marker remains high in patients on antiretroviral therapy, which is consistent with the statement of Castley A et al (16), who report that HIV status was associated with a significant increase in the expression of CD64, CD143 and CD163 on CD16 + monocytes regardless of the virologic response to HIV treatment. This finding is also that of Burdo TH (21) who observed that the sCD163 rate was high in the plasma of people suffering from chronic HIV infection (> 1 year in duration), compared to HIV-seronegative. And under effective antiretroviral therapy, the sCD163 level decreased in parallel with the level of RNA-HIV viral load without equaling the level of seronegative subjects, suggesting the presence of residual monocyte/macrophage activation, even with viral loads, plasma levels below the limit of detection. And recently, Generoso M et al (22) confirmed in a child study that high plasma levels of sCD163 in HIV-infected children correlate with disease progression and T-cell activation and that initiation of antiretroviral therapy normalizes sCD163 levels and can reduce HIV-related morbidity and improve long-term outcomes. Ticona E et al (23) evaluating the biomarker of inflammation during antiretroviral suppressive therapy, found that prior to antiretroviral therapy, soluble CD163 concentrations were higher and remained higher after 24 months of suppressive therapy. Knudsen TB et al (24) associate rate increase sCD163 was an independent marker of all-cause mortality in a cohort of HIV-infected individuals, suggesting that activation of monocytes/macrophages may play a role in pathogenesis of HIV and be a target for intervention. While in our study, the rate of sCD163 decreased significantly in patients receiving antiretroviral therapy combined with probiotic yogurt supplementation.

Figure 4 shows the evolution of the hypersensitive CRP level; which in patients with inclusion have high values as found by Borato DC et al (25) who has significantly elevated levels of Hs-CRP were observed only in the TARV group which indicates a predictor of events cardiovascular in HIV positive patients, same finding made by Muswe R et al (26), Kozić Dokmanović S et al (27), Hattab S et al (28) investigating the impact of various antiretroviral regimens on markers of immune activation and inflammation, found that levels of hs-CRP and sCD14 remained stable and no difference was found between LPV/r and EFV as in our study. Naomi Trupper (29) measured in HIV-positive post-
ART patients and extra-virgin olive oil HsCRP at lower levels due to the effect of hydroxytyrosol concentration, a potent antioxidant found in olives with antiviral and anti-inflammatory properties and acts as a microbicide and reduces HIV transmission.

3) Tracking markers of Oxidative Stress

Inflammation during HIV infection contributes to the development of cancer, mainly by causing oxidative stress and DNA damage. And thus disrupting the system of enzymatic and non-enzymatic antioxidant defenses, in our study, we observe a decrease in the activity and Superoxide dismutase and glutathione peroxidase as well as a decrease in the Zinc rate. This situation showed no significant change under ART and very significant under ART with probiotic yogurt supplementation.

![Figure 5: Profile of Superoxide dismutase](image)

The SOD level is significantly decreased on ART at baseline and the rest on ART only at week 48, this observation is also that of Makinde O et al (30) who after vitamin A supplementation in HIV-positive people on ART, the SOD rate in its series has remained low. Similarly, Suresh et al (31) evaluating total antioxidant capacity and a new biochemical early marker of oxidative stress in HIV-infected individuals, found a significant decrease in vitamin E, vitamin C and SOD levels. What Quaye O et al (32) also note in Ghana among HIV-infected patients was that SOD activity was significantly reduced in antiretroviral-treatment-naive patients compared to those on antiretroviral therapy and the control group. In addition, our results demonstrate that antiretroviral therapy combined with probiotic yogurt supplementation improves SOD activity and restores oxidative stress defense activity.

![Figure 6: Glutathione peroxidase profile](image)

Figure 6 high, shows a low activity of Glutathione peroxidase under ART that significantly improves with ART and probiotic yogurt. What Look MP1 et al (33) have also noted in evaluating the various antioxidant markers in people living with HIV that stages I to III of HIV disease are characterized by significant alterations in the antioxidant defenses provided by selenium, GSH-Px, SH groups and GSH. Ogunro PS et al (34), who studies the correlation...
between the concentration of selenium and glutathione peroxidase, have also confirmed that selenium and glutathione peroxidase are reduced to scavenging antioxidants, and that their concentration decreases significantly with evolution of the disease. This is exactly what Wanatabe et al (35) state that HIV infection was associated with increased oxidative stress and appears to affect the protective activity of Glutathione Peroxidase. While Stephensen CB et al (36) insist on the relationship between Glutathione peroxidase activity and good nutrition because in their conclusion, they specify that the GPX activity seems to have been induced by the oxidative stress associated with the infection. HIV and antiretroviral therapy. Thus, young and well-nourished subjects can develop a compensatory antioxidant response to HIV infection.

The Zinc rate remains low in our series throughout 48 weeks of treatment with ART or ART supplemented with Probiotic Yogurt. Shivakoti R et al (37), in their studies, found that despite increasing micronutrient levels, the prevalence of individual deficiencies remained virtually unchanged after 48 weeks of antiretroviral therapy and noted that antiretroviral therapy alone is not sufficient to improve micronutrient deficiency. In addition, low levels of zinc and chronic inflammation are known to be common in persons infected with the human immunodeficiency virus (HIV) (38) and zinc deficiency may promote systemic inflammation. (39), which is confirmed by Mburu AS et al (40) in a study in Kenyan adults living with HIV showing that plasma zinc levels were lower in those with inflammation. Similarly Osuna-Padilla I et al (41) found frequent deficiencies in serum zinc concentration in HIV-positive individuals; in Iran, Khalili H et al (42) also noted that serum zinc and selenium levels in individuals infected with human immunodeficiency virus were significantly lower compared to healthy individuals.

The results of all these high publications have confirmed our study which demonstrates that HIV infection induces both inflammation and oxidative stress, the result of which is the degradation of the quality of life of the infected person and status remains despite effective antiretroviral therapy. Probiotic yogurt supplementation provides both ingredients that reduce inflammation because fermented foods may also have improved nutritional and functional properties due to the transformation of substrates and the formation of bioactive or bio-available end products (43) that have significant anti-inflammatory effects (44) that lower the indirect markers of bacterial translocation and T-cell activation, and induce an improvement in thymic production and improvement of soluble CD14 inflammatory biomarkers and C-reactive protein with high sensitivity (45). This is particularly the case of the Lin PP (46) team demonstrated by the administration of probiotics orally fermented in rats and observed a suppression of the inflammatory pathway related to TLR-4 which is the same pathway. Bacterial cell wall LPS fixation via soluble and membrane-bound CD14 and MD2 factors, and improves the antioxidant level of patients by the proteolytic activity of probiotics that release bioactive peptides, amine acids, antioxidant enzymes and other compounds (47) and the protein fraction contains antioxidant activity, in particular casein, antioxidant enzymes; lactoferrin; conjugated linoleic acid; Coenzyme Q10; vitamins C, E, A and D3; equol; Uric acid; carotenoids; and mineral activators of antioxidant enzymes (48). In addition, fermentation leads to an increase in the capacity and bioavailability of antioxidants (49) (50) (51); also, the survival rate of ferments during digestion would play a role in bioavailability of antioxidants (52). And the highest antioxidant activity shows that Lactobacillus acidophilus has an antioxidant capacity measured at 54.86% neutralization of free radicals and that of Streptococcus thermophilus at 45.17% (53). And these bacteria are those isolated from the probiotic yogurts used in our series.

4. Conclusion

Inflammation and oxidative stress induced by HIV infection are two major problems that prevent optimal progression of patients on antiretroviral therapy. They maintain a low level of CD4, very remarkable increase in biomarkers of inflammation (sCD14, sCD163 and HsCRP) and a permanent oxidative stress by collapse of the main antioxidant system defenses enzymes (superoxide dismutase and glutathione peroxidase) anis Zinc cofactor, the deficiency of which also has systemic inflammation.
Probiof yoghurt supplementation with Lactobacillus acidophilus and streptococcus bulgaricus resulted in improved levels of CD4 and enzymes in the antioxidant system, significantly reducing biomarkers of inflammation. Only the Zinc concentration remained at sub-normal values regardless of the intervention.

References


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