Zinc-enriched cookies improve immunity and decrease opportunistic diseases in AIDS patients

Biscoitos enriquecidos com zindo melhoram a imunidade e diminuem as doenças oportunistas em pacientes com AIDS

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RESUMO

Introdução: a síndrome da imunodeficiência adquirida (AIDS) é uma doença do sistema imunológico humano decorrente da infecção pelo vírus da imunodeficiência humana (HIV). Métodos: o estudo foi realizado entre 2015 e 2016 com pacientes atendidos em um Centro Especializado em Doenças Infecciosas Parasitárias do Paraná. A pesquisa foi dividida em duas etapas, onde foram verificados os efeitos da suplementação sobre as células TCD4+, carga viral (CV) e número de doenças oportunistas. 20mg/dia/zinco foram adicionados aos biscoitos por 3 meses. Resultados: a análise dos dados não mostrou diferença estatística na CV, no entanto a suplementação mostra que é capaz de promover um aumento na contagem de TCD4+. Os pacientes relatam uma melhora em seu bem-estar e uma diminuição da incidência de doenças oportunistas. Conclusão: assim, observou-se que a suplementação de zinco foi altamente eficaz para aumentar a contagem de células TCD4+, melhorando positivamente a resposta imunológica e a qualidade de vida. Além disso, a suplementação em forma de biscoito se mostrou inovadora e eficaz.

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ABSTRACT

Introduction: Acquired Immunodeficiency Syndrome (AIDS) is a disease of the human immune system resulting from infection by the Human Immunodeficiency Virus (HIV) virus. Methods: The study was carried out between 2015 and 2016 with patients who attended a Specialized Center for Parasitic Infectious Diseases in Paraná. The research was divided into two stages, where the effects of supplementation on TC4 + cells, viral load (LV) and number of opportunistic diseases were verified. 20mg / day of zinc was added to the cookies for 3 months. Results: Data analysis does not show statistical differences in VL and Zinc supplementation; however, supplementation shows that it is able to promote an increase in the TCD4 + count. Patients reported an improvement in their well-being and a decrease in the incidence of opportunistic diseases. Conclusion: Thus, it was observed that zinc supplementation was highly effective in increasing TCD4 + positively improving the immune response and quality of life. In addition, the supplementation in the form of biscuits proved to be innovative and very effective.

Palavras-chave: minerals; food supplement; biscuits; HIV; immune system.

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is the name given to the set of opportunistic diseases resulting from the weakening of the immune system mediated by the Human Immunodeficiency Virus (HIV) (HEMELLAR et al., 2019; LU, et al., 2018). Epidemiologically, AIDS translates as a global epidemic and is no longer restricted to only subpopulations of risky behavior (HAMELLAR et al., 2019; SOARES, ARMINDO, ROCHA, 2014; BELOUKAS et al., 2016). It is estimated that there are already more than 35 million individuals infected with HIV (MOREIRA, CHIARELLO, 2008; WHO, 2017).

It mainly infects helper T cells (helper T cells) of the specific group number 4 or TCD4 +, which are responsible for modulating the immune response and its surface phenotypic marker, the CD4 + receptor, which has an affinity for the gp120 protein. of the HIV viral envelope (PARHAM, 2011; ROBBINS et al., 2001), responsible for triggering the mechanism of lymphocytic invasion by the virus.

A mandatory intracellular parasite, HIV requires cellular machinery for replication, which includes cellular enzymes, structures and elements that are absent from the virus (SOARES, ARMINDO, ROCHA, 2014; PARHAM, 2011; BRITO, CASTILHO, SZWARCWALD, 2001). Through the reverse transcriptase enzyme, HIV promotes the transformation of its own RNA into DNA, a mechanism by which the virus assumes cellular machinery in favor of viral replication. Thus, when viral replication begins, depletion of TCD4 + lymphocytes and cell apoptosis occurs, compromising the
host's immune response to various etiological agents (LU et al., 2018; SOARES, ARMINDO, ROCHA, 2014; PARHAM, 2011; BRITO, CASTILHO, SZWARCWALD, 2001).

The body's immune response becomes more deficient as HIV advances through the immune system. Additionally, immunocompetence is impaired if there is nutritional deficiency, which implies a deficient functioning of cellular structures, and the formation of proteins, enzymes and cells. Among the most common complications in infected patients are changes in taste and appetite, weight loss, alopecia, diarrhea, chronic weakness and deleterious psychological consequences. The loss of micronutrients is vitally important for the proper functioning of the organism (KAISER et al., 2014; ASDAMONGKOL, PHANACHET, SUNGKANUPARPH, 2013; BAUM et al., 2010).

In this sense, Zinc (Zn) is a micronutrient involved in the activity of more than 300 enzymes, which also participates in the synthesis and degradation of carbohydrates, lipids and proteins, in the maintenance of normal growth and development, in the functioning of the antioxidant defense and sensorineural function, in the transcription and translation of polynucleotides, and in the correct functioning of the immune system (READ et al., 2019; KAISER et al., 2014; ASDAMONGKOL, PHANACHET, SUNGKANUPARPH, 2013; BAUM et al., 2010).

In addition, zinc can affect the phagocytic process of macrophages and neutrophils, interfere with cell lysis mediated by killer cells and the cytolytic action of T cells, where it is part of enzymes such as ecto-5'-nucleotidase (5'NT), present in the cell membrane, or in the zinc finger nucleases (READ et al., 2019; PARHAM, 2011; KAISER et al., 2014; ASDAMONGKOL, PHANACHET, SUNGKANUPARPH, 2013; DIRAJLAL-FARGO et al., 2019; GNATIENKO et al., 2018).

Zinc deficiency is an aggravating factor for the progression of AIDS, since its deficiency can contribute to an increase in viral replication (KAISER et al., 2014; ASDAMONGKOL, PHANACHET, SUNGKANUPARPH, 2013; DIRAJLAL-FARGO et al., 2019; GNATIENKO et al., 2018). Therefore, supplementing patients' diets in practical ways such as cookies is highly recommended, aiming not only at the adequate amount of vitamins and calories, but also micronutrients.

In AIDS patients, zinc deficiency impairs the immune system's already weakened response. The use of this mineral could be considered positive and a complementary alternative for the treatment of AIDS. Therefore, this study aimed to
assess the effects of supplementation with zinc-enriched biscuits on general well-being, symptoms, TCD4+ cell count and viral load (VL) of patients diagnosed with AIDS at the Specialized Center in Parasitic Infectious Diseases (CEDIP) in Cascavel, Paraná, Brazil.

METHODS

Selection of participants

The present study is a longitudinal cohort. After approval by the ethics committee under number 33713113.0.0000.5564 the inclusion criteria were: individuals of both sexes (n=37) aged between 21 and 50 years diagnosed with AIDS; CD4+ cell count below 300 cells/mm³. Patients who met the inclusion criteria were listed, and then randomly selected for the study. The selected patients who wished to participate in the study signed a Free and Informed Consent Form (ICF) and were assessed at the beginning of the research (Step 1) and 3 months after the first assessment (Step 2).

Formulation of the biscuit enriched with zinc

The study of Teixeira et al. (2018) was used as a reference for the preparation of biscuits enriched with zinc. Chelated zinc was added to a biscuit-based formula consisting of eggs, refined sugar, baking powder, cocoa, butter, wheat flour, rice flour and bean flour, in order to obtain an approximate amount of 3.3 mg of zinc per biscuit.

Data collection from research participants

The patients attended CEDIP from February 2015 to December 2016. In step 1 of the study, the personal, sociodemographic and health status data of the patients were assessed through a targeted questionnaire. The count of VL and TCD4+ cells (CD4+) for each participant was obtained from the archived medical records. Plasma zinc levels of the participants, before and after supplementation, were measured. Participants underwent nutritional status assessment using body weight and height measurements to classify their body mass index (BMI). At the end of step 1, participants were instructed to consume six cookies enriched with zinc (approximately 20 mg of Zn/day) during the day, for a period of 3 months.

In step 2, after three months of consumption of the cookies, a new questionnaire was applied to re-assess the health status and verify the cookie consumption; the
assessment of nutritional status was repeated and the data of VL and TCD4+ cell count updated, when possible.

**Blood collection techniques and instrument**

Blood samples were collected from the participants to determine plasma zinc levels. For this procedure, surgical gloves, tourniquet, 70% alcohol, cotton, dressings, EDTA tubes and 10 mL syringes and disposable needles were used.

**Determination of blood zinc levels**

To determine zinc levels, the samples were analyzed after previous digestion of approximately 500 mg of blood with 6 mL of bidistilled nitric acid (Merck) in open vessels, by conventional heating. After cooling, the digestions were diluted with ultrapure water to 50 mL in a polypropylene container.

To detect zinc values, an inductively coupled plasma optical emission spectrometer (ICP-OES) with axial vision configuration (SpectroCiros CCD, Spectro Analytical Instruments, Germany) was used.

**Data analysis**

The data were tabulated in the Microsoft Excel 2010 program and statistical analyses were performed using the Prism 7.01 software. To compare the parameters before and after supplementation with the biscuit, the paired t test was used, considering a statistically significant difference if \( p < 0.05 \). In addition, possible correlations between VL and CD4+ cells, the duration of the disease and the number of opportunistic diseases were verified using the multiple linear regression test.

**RESULTS**

In step 1 of the study, the participation of 51 volunteers who attended CEDIP, a specialized center in Cascavel-PR, from 2015 to 2016 was recorded. The general data of participants are shown in Chart 1.

**Chart 1** General data of participants in step 1 of the study.

| Age (years) | 30 – 54 |
| Number of daily meals | 2 – 5 |
The descriptive data demonstrate a median age of 42 years and 36 months of disease diagnosis, mostly by routine blood tests (70.60%). Regarding eating habits, most patients rated their appetite as good, with adequate water ingestion, and an average of 4.00 daily meals and 1.50 liters water/day.

In step 2, 18 of the initial 51 participants answered the questionnaire and performed the assessment of nutritional status. Almost 100% of patients who ate the zinc-enriched biscuits relished the biscuit received, and only 1 (5.56%) patient reported gastrointestinal discomfort after consumption. Regarding the time stipulated for biscuit consumption, 1 (5.56%) patient did not consume all of the biscuits and 3 (16.67%) reported consumption in less than 3 months.

Although there is no statistical correlation between VL and CD4+ cell counts, and between the duration of disease and the number of opportunistic diseases, in the period in which the supplementation occurred, the number of opportunistic diseases observed by patients was significantly lower (Table 1). When asked about changes in their clinical condition, 11 patients (61.1%) observed improvements such as greater disposition, stronger hair and nails, and improved immunity.
Table 1 Changes observed in blood, nutritional and clinical parameters of AIDS patients after the consumption of cookies supplemented with zinc.

| Parameter                                | Before supplementation (Min - max) | After supplementation (Min - max) | p      |
|------------------------------------------|-----------------------------------|-----------------------------------|--------|
| Viral Load (cells/mm³) (n=24)            | 47.00 - 1636.00                    | 43.00 - 1345.00                   | 0.4609 |
| TCD4+ count (copies/mL) (n=24)           | 65.00 - 533.00                     | 134.00 - 543.00                   | 0.0003*|
| IMC (kg/m²) (n=17)                       | 18.13 – 41.12                     | 19.61 – 42.64                    | 0.3060 |
| Number of opportunistic diseases (n=18)  | 6 ± 12                            | 1 ± 17                            | 0.0027*|
| Plasmatic zinc (mg/L) (n=33)             | 0.82 - 2.74                       | 0.80 - 2.67                       | 0.8207 |

*The p value was considered as statistic significant if p<0.05.

DISCUSSION

The results of the present study revealed that Zn supplementation was not able to decrease viral load, considering the supplementation provided and the proposed treatment time, a result similar to the study by Read et al. (2019).

Zinc participates in the synthesis and degradation processes, in the transcription and translation of polynucleotides and in the correct functioning of immune system proteins (DIRAJLAL-FARGO et al., 2019; OLENDER et al., 2012; SNEIJ et al., 2016), that is, zinc may be involved in all aspects of the viral life cycle, inhibiting retroviruses like HIV. On the other hand, in vivo, HIV stimulates the influx of zinc to macrophages, preventing the death of these cells, and it can remain latent and viable until the end of antiretroviral treatment. The lack of responsiveness of the immune system determines the increase in opportunistic diseases (DIJARAJLAL-FARGO et al., 2019; GNATIENKO et al., 2018; TEIXEIRA, et al., 2018; XU et al., 2008; SWINGLES et al., 2007; OLENDER et al., 2012; SNEIJ et al., 2019; VISSER et al., 2017).

A fact of great relevance in this study was in relation to the number of opportunistic diseases experienced by patients; it was found that after supplementing cookies containing 20mg of Zn / day, there was a significant decrease in these diseases, a fact that was also observed in other studies with zinc supplementation (READ et al.,...
In addition, the decrease in these comorbidities improves the well-being of most patients, which is related to an improvement in general health, including a greater willingness to undertake daily activities, and stronger hair and nails. These data are corroborated by the study of Sneij et al. (2016), who demonstrated a correlation between zinc levels and the emergence of opportunistic diseases. The depletion of immune cells is related to cellular processes that support responses to invaders, causing more or less opportunistic diseases (Read et al., 2019; Di Rajjal-Fargo et al., 2019; Caza et al., 2016). A study by Caza et al., (2016) demonstrates the failure of the immune system against opportunistic diseases, such as fungal meningoencephalitis. With an adequate offer of food and the improvement of well-being, not only do levels of zinc improve, but the calorie intake also enables constitutional elements to form, such as zinc finger nucleases (Kaiser et al., 2006; Asdamongkol et al., 2013). A study published by Olender et al. (2012) demonstrated the role of zinc finger nucleases on the multiplication of TCD4+ lymphocytes, retarding HIV progression and contributing to a better quality of life. The supplementation of 20 mg/day proposed by this study was shown to be capable of promoting a significant increase in TCD4+ cell counts, in agreement with other authors (Read et al., 2019; Di Rajjal-Fargo et al., 2019; Gnatienko et al., 2018; Sneij et al., 2016).

The clinical improvement reported by most patients in this study can be correlated with the reduction of some biological markers. Dijaeajlal-Fargo et al. (2019) evaluated inflammation markers in the serum of HIV-positive patients before and after zinc supplementation. Even though they administered a zinc concentration which was lower than that applied in this study, the decrease in inflammation marker levels was statistically relevant. Gnatienko et al. (2018) demonstrated an improvement in mortality biomarkers after zinc supplementation, via a reduction in coronary heart disease risk and the slowing of HIV progression, due to an increase in TCD4+ lymphocytes and other components of the immune system.

**CONCLUSION**

In addition, zinc supplementation in biscuits showed a positive response, especially for TCD4 + lymphocytes and the number of opportunistic diseases. The
The number of TCD4 + lymphocytes increased, which contributed to the reduction in the number of opportunistic diseases in the analyzed period. The general improvement in health status reported by study participants shows that zinc supplementation can be very promising. Supplementation seems to be a viable alternative for the population affected by HIV, and aims to innovate public policies aimed at young and elderly, groups in which this syndrome gains an epidemic.

The present study has some limitations, such as the zinc analysis. The number of replicates analyzed was probably not sufficient to presume a more certain level in the plasma. Inflammatory parameters, if analyzed together, could provide important information relative to zinc levels offered to patients and parameters to determine whether the disease is accelerating or not. Also, the number of participants included was lower than initially considered due to a lack of volunteers and a loss of data by the patients.

REFERENCES

ASDAMONGKOL, N.; PHANACHET, P.; SUNGKANUPARPH, S. Low plasma zinc levels and immunological responses to zinc supplementation in HIV-infected patients with immunological discordance after antiretroviral therapy. Jpn J Infect Dis. 2013.

BAUM, M. K.; LAI, S.; SALES, S.; PAGE, J. B.; CAMPA, A. Randomized, Controlled Clinical Trial of Zinc Supplementation to Prevent Immunological Failure in HIV-Infected Adults. Clin Infect Dis. 2010.

BELOUKAS, A.; PSARRIS, A.; GIANNELOU, P.; KOSTAKI, E.; HATZAKIS, A.; PARASKEVIS, D. Molecular epidemiology of HIV-1 infection in Europe: An overview. Infect Genet Evol. 2016.

BRITO, A. M. de; CASTILHO, E. A. de; SZWARCwald, C. L. AIDS e infecção pelo HIV no Brasil: uma epidemia multifacetada. Rev Soc Bras Med Trop. 2001.

CAZA M.; HU, G.; PRICE, M.; PERFECT, J. R.; KRONSTAD, J. W. The Zinc Finger Protein Mig1 Regulates Mitochondrial Function and Azole Drug Susceptibility in the Pathogenic Fungus Cryptococcus neoformans. mSphere. 2016.

DIRAJLAL-FARGO, S.; YU, J.; KULKARNI, M.; SATTAR, A.; FUNDERBUG, N.; BARKOUKIS, H. et al. Brief Report: Zinc Supplementation and Inflammation in Treated HIV. J Acquir Immune Defic Syndr. 2019.

GNATIENKO, N.; FREIBERG, M. S.; BLOKHINA, E.; YAROSLAVTSEVA, T.;
BRIDDEN, C.; CHENG, D. M. et al. Design of a randomized controlled trial of zinc supplementation to improve markers of mortality and HIV disease progression in HIV-positive drinkers in St. Petersburg, Russia. HIV Clin Trials. 2018.

HEMELAAR, J., et al. Global and regional molecular epidemiology of HIV-1, 190 2015: a systematic review, global survey, and trend analysis. Lancet Infect Dis. 2019.

KAISER, J. D.; CAMPA, A. M.; ONDERCIN, J. P.; LOUUNG, G. S.; PLESS, R. F.; BAUM, M. K. Micronutrient supplementation increases CD4 count in HIV-infected individuals on highly active antiretroviral therapy: A prospective, double-blinded, placebo-controlled trial. J Acquir Immune Defic Syndr. 2006.

LAZZAROTTO, A. R.; DERESZ, L. F.; SPRINZ, E. HIV/AIDS e Treinamento Concorrente: a Revisão Sistemática. Rev Bras Med do Esporte. Sociedade Brasileira de Medicina do Exercício e do Esporte; 2010 Apr [cited 2020 Jun 18];16(2):149–54.

LU, D-Y.; WU, H-Y.; YARLA, N. S.; XU, B.; DING, J.; LU T. R. HAART in HIV/AIDS Treatments: Future Trends. Infect Disord - Drug Targets. 2018 Mar 2018(1):15–22.

MOREIRA, E. A. M.; CHIARELLO, P. G. Nutrição e Metabolismo - Atenção Nutricional - Abordagem Dietoterápica em Adultos. Guanabara Koogan; 2008.

OLENDER, S.; WILKIN, T. J.; TAYLOR, B. S.; HAMMER, S. M. Advances in antiretroviral therapy. Top Antivir Med. 2012.

PARHAM, P. O sistema imune. 3ª. Artmed; 2011.

RAYMOND, A. D.; GEKONGE, B.; GIRI, M. S.; HANCOCK, A.; PAPASAVVAS, E.; CHEHIMI, J. et al. Increased metallothionein gene expression, zinc, and zinc-dependent resistance to apoptosis in circulating monocytes during HIV viremia. J Leukoc Biol. 2010.

READ, S.A.; OBEID, S.; AHLENSTIEL, C.; AHLENSTIEL, G. The Role of Zinc in Antiviral Immunity. Adv. Nutr. 2019.

ROBBINS, S. L.; COTRAN, R.S.; KUMAR, V.; COLLINS, T. Fundamentos de Robbins: patologia estrutural e funcional. Fundam Robbins Patol estrutural e Func. 2001.

SNEIJ, A; CAMPA, A. Lower Plasma Zinc Levels in Hyperglycemic People Living with HIV in the MASH cohort. J AIDS Clin Res. 2016.

SOARES, R.; ARMINDO, R. D.; ROCHA, G. A imunodeficiência e o sistema imunitário. O comportamento em portadores de HIV. Arq Med. 2014.

SWINGLES, S.; MANN, A. M.; ZHOU, J.; SWINGLER, C.; STEVENSON, M. Apoptotic killing of HIV-1-infected macrophages is subverted by the viral envelope glycoprotein. PLoS Pathog. 2007.
TANG, A. M.; GRAHAM, N. M. H.; HIRBY, A. J.; MDCALL, D. L.; WALTER, C.; WILLET, W. C.; SAAH, A. J. Ingestão de micronutrientes na dieta e risco de progressão para a síndrome da imunodeficiência adquirida (AIDS) em homens homossexuais infectados pelo vírus da imunodeficiência humana tipo 1 (HIV-1). American Journal of Epidemiology, Volume 138, Issue 11, 1 de dezembro de 1993, Pages 937-951.

TEIXEIRA, A. T.; MACHADO, C.; RIOS, T. S.; LUCCA, L.; GONÇALVES, T. L.; WEBER, J.; BENVENÚ, D. M. Suplementação com biscoito enriquecido com zinco em um indivíduo com deficiência do mineral: um estudo de caso. Biosaúde (Londrina), v. 20, p. 55-68, 2018.

VISser, M. E.; DURAO, S.; SINCLAIR, D.; IrlAM, J. H.; SIEGFRIED, N. Micronutrient supplementation in adults with HIV infection. Cochrane Database Syst. Rev. 2017.

WHO. HIV/AIDS Fact sheet. Media Cent. 2017.

XU, Y.; ZHU, H.; WILCOX, C. K.; VANT’ WOUT, A.; ANDRUS, T.; LLEWELLYN, N. et al. Blood Monocytes Harbor HIV Type 1 Strains with Diversified Phenotypes Including Macrophage-Specific CCR5 Virus. J Infect Dis. 2008.

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