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Cytogenetic changes in oral mucosal cells of human immunodeficiency virus-infected children and adolescents undergoing antiretroviral treatment

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate possible cytogenetic changes in children and adolescents with human immunodeficiency virus on antiretroviral therapy, through the micronucleus test in oral mucosa.

METHODS: This was a prospective study consisted of 40 individuals, of whom 21 comprised the human immunodeficiency virus group and 19 comprised the control group. Children and adolescents with human immunodeficiency virus were enrolled. The inclusion criteria were <18 years old and consent in participating in the study. The exclusion criteria were the presence of numerous systemic comorbidities, oral lesions, the habit of smoking, alcohol consumption, and X-rays or CT scans taken within 15 days prior to sample collection. A gentle scraping was performed on the inner portion of the jugal mucosa on both sides. A total of 2,000 cells per slide were analyzed for the determination of mutagenicity parameters as follows: micronuclei, binucleation, and nuclear buds. For measuring cytotoxicity, the following metanuclear changes were evaluated: pyknosis, karyolysis, and karyorrhexis, in a double-blind manner. The repair index was also evaluated in this setting.

RESULTS: The human immunodeficiency virus group showed high frequencies of micronuclei (p=0.05), binucleated cells (p=0.001), and nuclear buds (p=0.03). In the cytotoxicity parameters, represented by the cell death phases, there was an increase with statistical difference (p≤0.05) in the karyorrhexis frequency (p=0.05). Additionally, repair index was decreased in the human immunodeficiency virus group.

CONCLUSION: These results indicate that human immunodeficiency virus -infected individuals undergoing antiretroviral therapy have cytogenetic changes in oral mucosal cells.

KEYWORDS: Child. DNA damage. HIV. Micronucleus tests. Mouth mucosa.

INTRODUCTION

Currently, 79 million people have been infected worldwide with the human immunodeficiency virus (HIV), whose predilection for immune system cells, which induces acquired immunodeficiency syndrome (AIDS), has been responsible for the death of 39 million people since the beginning of the epidemic[1]. Still, it is responsible for 88% of contaminations of children up to 13 years. From this age, the main route of contamination is sexual. During the past 10 years, there has been an increase of 64.9% in young males aged 15–19 years, while there has been a significant decrease among women in this age group[2].

Antiretroviral therapy (ART), started in the late 1980s, has been constantly evolving, acting especially on the replication of the virus in several stages and combining different classes of antiretrovirals. Antiretroviral drugs are classified according to their mechanism of action. There are more than 25 drugs divided into six types: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, integrase inhibitors, and entry inhibitors. In fact, ART has shown a reduction in mortality and a significant increase in survival. It also shows an improvement in the quality of life and suppression of viral load in many cases, which decreases the chance of transmission, preventing vertical transmission[3,4]. In 2004, a study reported that only 26% of children achieved complete viral suppression with 72 weeks of ART[5], in contrast to the percentage observed by Gulick et al.[6] whose remission in adults was 89% after 48 weeks of ART treatment. In the same study, Aboulker et al.[7] demonstrated 30% of selection of HIV resistance mutations in children, leading to virological failure, and emphasized the

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importance of early initiation of ART in children. In Europe and the United States, the infant mortality rate fell between 80 and 90% after the introduction of ART, and although it is considered a chronic disease at present, it is estimated that the survival of these children is 30 times lower than a healthy child[8]. However, metabolic, neurological, cardiovascular, renal, and cancer disorders are the complications not related to AIDS, but related to the set of aging conditions mediated by HIV, or even by the persistent inflammatory immune process, even if under treatment[9,10].

Micronuclei (MN) are small fragments of nuclei (DNA), present in the cytoplasm of nucleated cells, and are used as a sensitive biomarker of chromosomal damage, genome instability, and mutagenicity[11,12]. MN originate during cell division, from a damage that exceeds the repair capacity, which can lead to the loss of an entire chromosome (aneugenic event), or a chromosome fragment (clastogenic event), and that in the final phase of cell division, telophase, receive a nuclear envelope, giving a similar appearance to the nucleus with a smaller size in relation to the main nucleus[13]. In the 1980s, Stich and Rosin proposed a modification capable of identifying such MN in exfoliated cells of the oral mucosa[14], with the advantage of being a less invasive test and easy to perform in relation to other genetic tests. This methodology has since proved to be an important biomarker of effect, versatile, and low cost, which focuses on prevention, predicting an interaction between a chemical, physical, or biological substance, with biological receptors, ideally when there is no disease. It should be noted that these are not diagnostic tests[15], but internationally recognized tests, which are considered robust, simple, and amenable to automation, with established regulatory guidelines[16]. Based on the information presented, the objective of this study was to evaluate possible cytogenetic changes in children and adolescents with HIV on ART, through the micronucleus test in oral mucosa.

METHODS

Casuistics

The study was approved by the Institutional Human Ethics Committee from Federal University of São Paulo, under the protocol 0485/2019. All the legal guardians of participants signed the informed consent form and participants signed the assent form.

This prospective study consisted of 40 individuals; of whom 21 comprised the HIV group and 19 comprised the control group. All participants received detailed information about the project and the consent form was delivered to children and adolescents (<18 years), after being signed by their respective guardians. Children and adolescents (aged from 0 to 18 years), with HIV were enrolled, who were regularly monitored at the Specialized Child Care Service (SAE infantil) in the city of Santos – SP, Brazil, and whose total was 21 people from August to December, 2019. The inclusion criteria were <18 years old and consent in participating in the study. The exclusion criteria were the presence of systemic comorbidities, oral lesions, the habit of smoking, alcohol consumption, and X-rays or CT scans taken within 15 days prior to sample collection. A single examiner, a dentist, collected, stained, and examined the unidentified samples. The control group was enrolled by direct approach randomly in public places in the city of Santos – SP. Exclusion criteria were similar to that of HIV group.

Micronucleus test in oral cells

The buccal mucosal MN test followed the protocol described by Belien et al.[17] With the aid of a wooden spatula previously moistened in saline solution, a gentle scraping was performed on the inner portion of the jugal mucosa on both sides, for approximately 10 times each side. The material was deposited on a clean and dry histological slide. The slides were stained by Feulgen-Fast-Green technique. A total of 2,000 cells per slide were analyzed, at 1,000´ magnification, for the determination of MN, binucleation (BN), and nuclear buds (NB), and cytotoxicity parameters such as pyknosis (PK), karyolysis (KL), and karyorrhexis (KR) in a double-blind manner. The correct identification of such parameters was established by Bolognesi et al.[18]. For this purpose, the following criteria were established for the correct identification of cytogenetic changes of MN: (1) intact main nucleus and cytoplasm; (2) one-third diameter of the main nucleus; (3) similar stain and texture of the main nucleus; and (4) MN in the same focus as that in the main nucleus; KR: the nucleus may also exhibit extensive fragmentation indicative of advanced nuclear fragmentation; BN: two main nuclei within a single cell and the nuclei are of similar size and staining intensity; NB: the main nucleus has a sharp constriction forming a bud of nuclear material being attached to the main nucleus by a narrow or wide nucleoplasmic bridge; PK: the nucleus is small and shrunken with a diameter that is approximately one-third of that in a fully differentiated cell being uniformly and intensely stained; and KL: they do not have a DNA-containing nucleus or other structures that stain with Feulgen.

The repair index (RI), proposed by Ramirez and Saldanha[15], represented by the formula RI=(KL+KR)/(MN+NB), was also evaluated in this setting.

Statistical analysis

All data were submitted to the normalization using Kolmogorov-Smirnov test. After that, non-parametric data were confirmed by all data collected in this setting. The test used to evaluate the metanuclear alterations and DNA RI between the control group and HIV was the non-parametric Mann-Whitney U test. The statistical significance level was set at 5%. The statistical analysis was conducted by the Bio Stat software (version 5.0, Maringá-Brazil).

RESULTS

In the HIV group, there was an exclusion of only one transsexual teenager, who was a smoker and also used other drugs such as LSD ((lysergic acid diethylamide) and ecstasy (3,4-methylenedioxy-methamphetamine). The distribution was 9 boys and 11 girls, all of whom reported good eating habits including fruits, except the 4-month-old baby who used milk formula. Only one adolescent reported occasional use of marijuana and eight reported the use of mouthwash. Only 4 cases were in treatment between 1 and 3 years, and the other 16 cases were in treatment from the beginning of life. Regarding the control group, all participants were included and the distribution was 8 boys and 11 girls, of whom 6 reported using mouthwash and none were using any medication. The groups are represented in Table 1.

The HIV group presented an increase in statistical difference ($p \leq 0.05$) in relation to the control group in the parameters of mutagenicity, the frequency of MN ($p=0.05$), BN ($p=0.001$), and NB ($p=0.03$), as shown in Table 2.

In the cytotoxicity parameters, there was an increase in statistical difference in the frequency of KR ($p=0.05$). KL and PK showed no significant increase ($p>0.05$), as shown in Table 2. Finally, the RI shown in Table 2 suggests a higher repair capacity in the control group when compared to the HIV group.

DISCUSSION

Our results demonstrated that children infected with HIV undergoing ART therapy possess cytogenetic damage in the oral mucosa as depicted by increasing mutagenicity, cytotoxicity, and low DNA repair capacity. It is important to highlight that the success of ART is undeniable in relation to the higher life expectancy and quality of life, giving chronicity status to the infection caused by HIV. However, the side effects of ART are well documented and others are still being studied. There are 12 types of drugs used in this HIV group, in different conjugations, always using a cocktail of at least 3 drugs per individual, according to the guidelines commonly used for the management of HIV infection. Nucleosides reverse transcriptase inhibitor drugs are essential constituents that make up ART, and patients in general receive two drugs of this class in combination with a third active drug of another class[19]. This protocol may change according to the body response, from the viral load and clinical response, and which can be influenced by both virus mutations and individual interruptions or adverse reactions in treatment. In a study with mice, alterations such as hepatocellular adenomas, carcinomas, and pulmonary alveolar/bronchiolar adenomas were reported with the use of tenofovir and

Table 1. Demographic characteristics of study participants.

Parameters	Control group (n=19)	HIV group (n=21)
Mean age	6.9 (4.9)	13.1 (4.72)
Gender	8/11 M/F	9/11 M/F
Time of therapy (years)	–	9.3+6.1
Time of infection (years)	–	9.4+6
Educational level		
Primary level	19	21
Secondary level	0	0
Ethnicity		
Black	0	0
White	14	11
Mixed	5	10
Use of mouthwash	6	8
Use of illicit drugs	0	1

Table 2. Total number [Median (Min–Max)] of oral cells presenting genotoxicity, cytotoxicity, and DNA repair index in children infected with human immunodeficiency virus undergoing antiretroviral therapy

Groups	Normal	Karyolysis (KL)	Karyorrhexis (KR)	Pyknosis (PK)	Micronucleus (MN)	Binucleation (BN)	Nuclear bud (NB)	(KL+KR)/ (MN+NB)
Control (n=19)	1,633 (1,556–1,748)	199 (50–328)	21 (6–34)	138 (64–285)	0 (0–1)	0 (0–1)	0 (0–1)	227 (31–309)
HIV (n=20)	1,618 (1,450–1,704)	220.5 (175–266)	43.5 (34–53)*	122.5 (63–182)	1.5 (1–2)*	1.5 (1–2)*	0 (0–0)	91 (33–309)*

*$p \leq 0.05$ when compared to control.

efavirenz[20], but this risk with tenofovir and entecavir in humans was not evident in the recent review published by Tseng et al.[21] Nephrotoxic effects, liver disease, and bone hypomineralization have been described in nucleoside reverse transcriptase inhibitor drugs[19]. In this study, the HIV group presented statistical difference in all mutagenicity parameters (MN, NB, and BN), in relation to the control group of healthy children and adolescents submitted to ART.

Regarding cytotoxicity, parameters were also altered, specifically in KR, which may suggest that the organism is constantly being challenged, by the mechanisms of cell cycle control and tissue death. We can still observe that even with an increase in the rate of cell death, which could mask the presence of mutagenic alterations, MN, NB, and BNs were increased in the HIV group, which suggests a high mutagenicity detectable by the proposed methodology. These results were confirmed by the decrease in the efficiency of the repair system in the experimental group. Despite few studies involving children, in 2007, Witt et al.[22] evaluated the chromosomal damage of infants exposed to ART by transplacental route and observed increased MN in erythrocytes in these infants born to seropositive mothers, who used zidovudine during pregnancy, and were also prophylactically medicated for 6 weeks after birth. The genotoxicity and mutagenicity of this drug had already been described in other studies in vitro and in animal models[23]. In 2013, Olivero et al.[24] observed that the use of ART, with reverse transcriptase inhibitor drugs (zidovudine, lamivudine, abacavir, and nevirapine) in the offspring of pregnant monkeys not carrying HIV, which were medicated during pregnancy and after birth, revealed genomic instability and mutagenic effects that persisted for 3 years (final year of research). Moraes Filho et al.[25] evaluated that cytotoxicity and genotoxicity of different concentrations of ART composed of tenofovir, lamivudine, and zidovudine, lamivudine, and efavirenz, in mice, through the comet assay and bone marrow micronucleus test. The increase of MN in buccal mucosal cells was observed by Lima et al.[26], in adults with HIV using ART, with low viral load. Our results are in line with the studies identified above that evaluated the effects on rodents and adult individuals.

This study has some limitations. First, it was not possible to evaluate to what extent ART only is able to induce cytogenetic damage in oral cells. Second, the lack of previous research studies on the topic compromise an in-depth discussion regarding the data. Taken together, our results indicate that children infected with HIV and submitted to ART demonstrate genomic damage and cytotoxicity in buccal cells. However, further studies are necessary to elucidate the issue.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The project was approved by the Research Ethics Committee of UNIFESP (Federal University of São Paulo) under protocol number #3.461.911.

AVAILABILITY OF DATA AND MATERIALS

Data sharing are available upon request.

AUTHORS' CONTRIBUTIONS

MESA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **MMC:** Conceptualization, Data curation, Supervision, Writing – original draft. **DVS:** Methodology, Software, Validation, Visualization. **DAR:** Conceptualization, Formal Analysis, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **CMCBM:** Data curation, Investigation, Writing – original draft.

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