Original Article

Quantification of micronuclei in exfoliated cells of human immunodeficiency virus/AIDS-infected female patients

Snehal Shah[1], Sasidhar Singaraju[2], Einstein T Bertin[3], Medhini Singaraju[2], Ashish Sharma[2]

[1]Consultant Oral Pathologist, Central Lab, Indore, [2]Department of Oral and Maxillofacial Pathology, Rishiraj College of Dental Sciences and Research Center, Bhopal, Madhya Pradesh, [3]Maitri Dental College and Research Center, Durg, Chhattisgarh, India

Abstract

Background: Micronucleus (MN) is a biomarker for cytotoxicity, which is formed during cell division. Increased MN scoring has been successfully used to recognize population groups at risk for cancers of oral cavity, cervix, urinary bladder and esophagus. Incorporating MN score along with cytological smear testing gives a better and cost-effective screening for high-risk patients.

Objective: This study evaluated the effectiveness of using MN score assessed from Papanicolaou (PAP) smears, as a biomarker for chromosomal damage in human immunodeficiency virus (HIV) patients.

Materials and Methods: Oral smears of 25 female HIV/AIDS patients, without habits such as chewing or smoking tobacco, and taking antiretroviral therapy (ART) at ART center, were recruited for the study. After careful oral examination and oral rinsing with normal saline, smears were prepared on slides by scraping the buccal mucosa with a wooden spatula. All the slides were fixed in 95% ethyl alcohol and stained with PAP stain, and 1000 cells were counted per patient. Based on Tolbert *et al*.'s criteria, MNs were identified, and quantitative scoring of MN was done on the basis of morphological assay.

Results: Mean ± standard deviation values of frequency of MNs in HIV-infected females were 73.40 ± 19.70 and in normal females were 38.08 ± 8.56.

Conclusion: MN scoring on the epithelial cells of buccal mucosa can be used as a biomarker in screening procedures for HIV patients.

Keywords: Biomarker, human immunodeficiency virus/AIDS, micronucleus, Papanicolaou smear

Address for correspondence: Dr. Sasidhar Singaraju, Department of Oral and Maxillofacial Pathology, Rishiraj College of Dental Sciences and Research Center, Airport Road, Gandhi Nagar, Bhopal, Madhya Pradesh, India.
E-mail: ssingaraju_64@yahoo.com

Received: 29.11.2017, **Accepted:** 03.04.2019

INTRODUCTION

Human immunodeficiency virus (HIV)/AIDS is a growing epidemic in developing countries. During the past two decades, more articles have been written on HIV and its related disease states than any other infectious process.[1] Oral lesions in this disease are a basic component of entire disease course and considered as a marker of disease progression and immunosuppression.[2,3] Oral lesions strongly associated with HIV/AIDS include oral candidiasis, oral hairy leukoplakia, periodontal diseases, oral warts, Kaposi's sarcoma and recurrent oral ulcers.[2,4] Oral health is the most neglected part of the treatment in HIV/AIDS patients.[2]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_251_17

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Shah S, Singaraju S, Bertin ET, Singaraju M, Sharma A. Quantification of micronuclei in exfoliated cells of human immunodeficiency virus/AIDS-infected female patients. J Oral Maxillofac Pathol 2019;23:301.

Micronuclei (MNs) are good prognostic indicators and biomarkers of cytotoxicity. It is formed during the metaphase/anaphase. Scoring of MNs can be performed easily on different cell types relevant for human biomonitoring such as lymphocytes, fibroblasts and exfoliated epithelial cells. MN observed in exfoliated cells is induced when the cells are at the basal layer. MN scoring is a sensitive as well as relatively easy method of scoring when compared to other molecular methods used to detect genotoxicity. Increased MN scoring recognizes chromosomal damage in infective and inflammatory diseases, cancers of oral cavity, cervix, urinary bladder and esophagus.[5]

So monitoring the frequency of MN could reflect the amount of DNA damage and chromosomal breakdown in chronic infective diseases. Oral exfoliative cytology is valuable for mass screening purposes. It is a nonaggressive technique, well accepted by the patients and important for prognostication of lesions.[6]

With this view in mind, the present study was carried out to assess the levels of MN in oral exfoliative cytology of healthy control female participants and HIV-positive female patients. Aim and objectives of this study were to evaluate and compare the frequency of MNs in Papanicolaou (PAP)-stained smears of oral exfoliated cells from healthy control female participants with HIV-positive female patients and also to evaluate the effectiveness of using MN score assessed from PAP smears, as a biomarker of chromosomal damage in HIV patients.

MATERIALS AND METHODS

Study groups

Twenty-five participants (female) with HIV infection and without any tobacco-related habit and receiving antiretroviral therapy (ART) were selected as the study group, and 25 healthy participants (female) having no obvious oral lesions or habits of consumption of tobacco, other tobacco-related substances or any other such substances that could compromise the oral hygiene status were selected as control group. Participants (both infected and healthy) with any grade of gingival inflammation, periodontitis, other viral or fungal infections and pregnant and lactating females were excluded from the study.

Collection of exfoliated cells

Participants were asked to rinse their mouth gently with water. Oral mucosal scrapings were obtained from buccal mucosa of Group I and Group II participants using a slightly moistened wooden spatula. The cells were immediately smeared on coded, precleaned microscopic slides and fixed in 95% ethyl alcohol.

Cytological staining and evaluation

The cytosmears were separately stained with PAP stain. The slides were mounted with cover glass using DPX mountant. All the slides were observed under light microscope using low magnification (×400) for screening and high magnification (×1000) for counting of MN.

Scoring criteria

The most commonly used method, that is, the zigzag method, was followed for screening of slides. One thousand cells with intact nuclei and cell boundaries were counted on each slide. For the purpose of designating an extranuclear body as an MN, the following criteria given by Tolbert *et al*.[7,8] were considered as follows:

- Rounded smooth perimeter suggestive of a membrane
- Less than a third the diameter of the associated nucleus but large enough to discern shape and color
- Staining intensity similar to that of the nucleus
- Texture similar to that of nucleus
- Same focal plane as nucleus
- Absence of overlap with, or bridge to, the nucleus.

Only those structures fulfilling the above-mentioned criteria were recorded as MN [Figure 1]. The same person scored 1000 intact cells blindly in each case to determine the MN percentage. The data obtained were statistically analyzed. Mean, standard deviation (SD), median and minimum and maximum values of frequency of MNs in HIV-infected females and normal females were calculated. The Shapiro–Wilk test showed that the frequency of MNs in HIV-infected female and normal female groups followed normal distribution. Hence, parametric test, namely unpaired *t*-test, was used for comparison of the frequency of MNs between HIV-infected females and

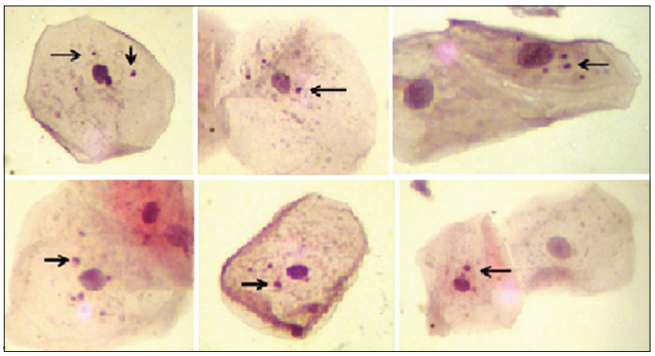

Figure 1: Photomicrograph showing micronuclei (arrow) in oral exfoliated epithelial cells of human immunodeficiency virus-infected females, stained with Papanicolaou stain (×100)

normal females. $P < 0.001$ was considered statistically highly significant.

RESULTS

The present study comprised a total of 50 participants divided as 25 cases of HIV-infected females and 25 healthy control participants without any habits of consumption of tobacco, other tobacco-related substances or other such substances.

Mean ± SD values of frequency of MNs in HIV-infected females were 73.40 ± 19.70 and in normal females were 38.08 ± 8.56. Median values of frequency of MNs in HIV-infected females were 69.00 and in normal females were 37.00. Minimum and maximum values of frequency of MNs in HIV-infected females were 37–126 and in normal females were 23–53.

Mean difference of frequency of MNs between HIV-infected females and normal female was 35.32. Unpaired *t*-test showed highly significant difference for the frequency of MNs in HIV-infected female and normal female groups ($t = 8.222$ and $P < 0.001$). Frequency of MNs in HIV-infected females was significantly higher than normal females.

DISCUSSION

MNs are extranuclear cytoplasmic bodies. They are induced in cells by numerous genotoxic agents that damage the chromosomes.[9] MNs are found in intermediate and superficial squamous cells. It has to be differentiated from apoptotic bodies found in superficial squamous cells, bacteria, Candida spores and artifacts such as stain particles and granules.[5]

The damaged chromosomes, in the form of acentric chromatids or chromosome fragments, lag behind in anaphase when centric elements move toward the spindle poles. After telophase, the undamaged chromosomes, as well as the centric fragments, give rise to regular daughter nuclei. The lagging elements are included in the daughter cells, too, but a considerable proportion is transformed into one or several secondary nuclei, which are, as a rule, much smaller than the principal nucleus and are therefore called MNs[6] [Figure 1].[8] Bigger MNs result from exclusion of whole chromosome following damage to the spindle apparatus of the cell (aneugenic effect), whereas smaller MNs result from structural aberrations, causing chromosomal fragments (clastogenic effect).[9]

Increased frequency of MNs in chronic infective/inflammatory diseases reflects the amount of DNA damage. Gradual increase in MNs has been considered a hallmark of aneuploidy and is used for assessing mutagenicity of the test compounds.[10]

In this study, MN score in buccal smears from HIV-positive female patients was compared with MN score of healthy patients. We noted highly significant difference of MN score in the two groups as higher in HIV-positive patients. There are limited studies of MN scoring on PAP-stained smears, and literature search did not yield much data focused on HIV-positive smears.

Two hypothesized mechanisms that contribute toward the formation of MN in buccal smears of HIV-positive patients are viral protein R (VPR) gene and ART therapy. VPR is an accessory gene of HIV Type 1 (HIV-1) encoding a virion-associated nuclear protein.[10,11] VPR of HIV-1 induces cell cycle abnormality causing cell accumulation at G2/M phase leading to increased ploidy and results in high frequency of MN formation. The incidence of MN in VPR-expressing cells was shown to be more than 50 fold higher than in control cells. Studies suggest that VPR plays an important role in regulating nuclear import of the HIV-1 preintegration complex and is required for virus replication in nondividing cells. VPR also induces cell cycle arrest in proliferating cells, stimulates virus transcription and regulates activation and apoptosis of infected cells, thus playing a critical role in tumor development and has an important role in the development of AIDS-related tumors.[10]

This study revealed 2–3-fold increase in the presence of MN in HIV-positive participants. This confirms the data of Chan *et al*.,[12] who concluded the presence of MN in the VPR-expressing cells. Shimura *et al*.[10] found 50-fold increase in VPR-expressing cells, which are quite higher than as compared to our study.

ART is the most common treatment provided to HIV patients. Mostly, it consists of two nucleoside reverse transcriptase inhibitors (NRTIs) along with one non-NRTI or one protease inhibitor.[13] NRTIs are known for causing hematologic disorders, myopathy, cardiotoxic effects, peripheral neuropathies and hepatotoxic effects.[14] These heterogeneous adverse effects of NRTIs are related to defective mitochondrial DNA replication secondary to the NRTI-induced deleterious inhibition of the mitochondrial DNA polymerase gamma.[15] These effects can also lead to the toxicity of the genome, which in turn can cause change in cellular functions, cancer and cell death.[16] Genotoxicity effect may lead to cellular malignant transformation.[17]

Witt KL *et al*.,[18] Lourenco *et al*.[19] and Kaushik *et al*.[20] ssessed MN frequency in patients who are on ART, in reticulocyte, mononucleated and binucleated cells, and peripheral blood respectively, found elevated MN frequency. Herd *et al*.[21] also reported that radiation-induced MNs are more in ART patients as compared to without ART patients. Qadir *et al*.[2] evaluated oral mucosal changes in patients of HIV/AIDS taking ART and found increase in MNs up to 51.4%. In this study, all the patients were on ART for a period ranging from 2 to 7 years.

Wide variation (37–126) in the MN scores was observed in the HIV-positive smears studied. This variation among different individuals in the same group may be attributable to environmental exposure to genotoxic agents, lifestyle factors, micronutrient deficiency, genetic makeup, baseline MN frequencies, ethnicity and other factors associated with carcinogenesis and chromosomal damage. This also suggests that MN formation is a multifactorial event.[5,22]

CONCLUSION

MN scoring on the epithelial cells of oral cavity could be used as a biomarker in cancer screening. This is an easy, simple, reliable and reproducible, which can be done on routinely stained PAP smears to pick up premalignant lesions in HIV patients. Increased frequency of MN in HIV-positive patients could be due to VPR and ART.

Future need

Further studies are required to validate the rationale behind the increased frequency of MNs in HIV infection and to establish a correlation between increased MNs in HIV infection with the depletion in CD4 count. Studies are also required to correlate the increased frequency of MN with the development of various malignancies of the oral cavity so that increased frequency of MN can be used as a monitoring tool to prevent the development of such lesions in HIV-infected patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and Maxillofacial Pathology. 3rd ed. Philadelphia, Pennsylvanai: Saunders, an Imprint of Elsevier Inc.; 2009.
2. Qadir S, Naseem N, Nagi A. Oral mucosal changes in patients of HIV/AIDS Taking antiretroviral therapy in Pakistan. J Cytol Histol 2014;S4:019.
3. Umadevi KM, Ranganathan K, Pavithra S, Hemalatha R, Saraswathi TR, Kumarasamy N, *et al*. Oral lesions among persons with HIV disease with and without highly active antiretroviral therapy in Southern India. J Oral Pathol Med 2007;36:136-41.
4. Shiboski CH, Patton LL, Webster-Cyriaque JY, Greenspan D, Traboulsi RS, Ghannoum M, *et al*. The oral HIV/AIDS research alliance: Updated case definitions of oral disease endpoints. J Oral Pathol Med 2009;38:481-8.
5. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta Cytol 2011;55:42-7.
6. Schmid W. The micronucleus test. Mutat Res 1975;31:9-15.
7. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: A field test in snuff users. Am J Epidemiol 1991;134:840-50.
8. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutat Res 2008;659:93-108.
9. Palve DH, Tupkarii JV. Clinico-pathological correlation of micronuclei in oral squamous cell carcinoma by exfoliative cytology. J Oral Maxillofac Pathol 2008;12:2-7.
10. Shimura M, Tanaka Y, Nakamura S, Minemoto Y, Yamashita K, Hatake K, *et al*. Micronuclei formation and aneuploidy induced by Vpr, an accessory gene of human immunodeficiency virus type 1. FASEB J 1999;13:621-37.
11. Kogan M, Rappaport J. HIV-1 accessory protein Vpr: Relevance in the pathogenesis of HIV and potential for therapeutic intervention. Retrovirology 2011;8:25.
12. Chang F, Re F, Sebastian S, Sazer S, Luban J. HIV-1 vpr induces defects in mitosis, cytokinesis, nuclear structure, and centrosomes. Mol Biol Cell 2004;15:1793-801.
13. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Summary of Key Features and Recommendations. World Health Organization; 2013.
14. Lewis W, Gonzalez B, Chomyn A, Papoian T. Zidovudine induces molecular, biochemical, and ultrastructural changes in rat skeletal muscle mitochondria. J Clin Invest 1992;89:1354-60.
15. Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. Nat Med 1995;1:417-22.
16. Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004;73:39-85.
17. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, *et al*. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International programme on chemical safety. Mutat Res 2000;463:111-72.
18. Witt KL, Cunningham CK, Patterson KB, Kissling GE, Dertinger SD, Livingston E, *et al*. Elevated frequencies of micronucleated erythrocytes in infants exposed to zidovudine in utero and postpartum to prevent mother-to-child transmission of HIV. Environ Mol Mutagen 2007;48:322-9.
19. Lourenço ED, do Amaral VS, Lehmann M, Dihl RR, Schmitt VM, Cunha KS, *et al*. Micronuclei induced by reverse transcriptase inhibitors in mononucleated and binucleated cells as assessed by the cytokinesis-block micronucleus assay. Genet Mol Biol 2010;33:756-60.
20. Kaushik D, Saini R, Rana AC, Kaushik P. Geno-cytotoxicity study of antiretroviral drug used in HIV-therapy. J Innov Biol 2014;1:63-7.
21. Herd O, Francies F, Slabbert J, Baeyens A. The effect of HIV and antiretroviral therapy on chromosomal radiosensitivity. J AIDS Clin Res 2014;12:397.
22. Singaraju M, Singaraju S, Parwani R, Wanjari S. Cytogenetic biomonitoring in petrol station attendants: A micronucleus study. J Cytol 2012;29:1-5.