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Chromosome Instability and Micronucleus Frequency on the Oral Mucosa of HIV-positive Patients

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***Abstract*—Extranuclear structures known as micronuclei (MN) are composed of whole or fragmented chromosomes that were not incorporated into the nucleus following cell division. The genotoxic impact of HIV infection on oral cavity cancers remains uncertain. This study sought to determine the impact of HIV infection on MN in HIV+ patients' oral mucosa and its correlation with early cytogenetic alterations in oral carcinogenesis. A total of forty-four non-HIV patients and thirty-eight HIV+ patients were assessed in this study. Smears were collected from the oral cavity and stained with 5% methylene blue. The smears were then examined at a ×100 magnification using a standard microscope. For each participant, 100 buccal cells were counted. Further observations of the viral load (VL), lymphocytes, and granulocytes were made to determine the pattern of MN presence in HIV+ patients. Significant differences were observed between HIV+ patients and healthy controls regarding alcohol consumption ($p = 0.004 < 0.05$) and smoking ($p = 0.041 < 0.05$). The relationship between micronucleus and VL is substantial. After calculating the linear regression model, it was discovered that the VL ratio of HIV-positive patients could predict the micronucleus cells (R-Sq = 55%, $p < 0.000$). In conclusion, HIV VL shows increased genomic instability. These findings are relevant to understanding the mechanisms of cellular damage and developing potential strategies to mitigate carcinogenesis in HIV+ patients.**

***Index Terms*—Chromosome instability, DNA damage, HIV, Micronucleus, Viral load.**

I. Introduction

Preserving genome stability is crucial for ensuring optimal cell function and preventing disease. Accumulated DNA damage speeds up the aging process by interfering with cellular metabolism, which can trigger senescence, immune system decline, programmed cell death, stem cell loss, and inflammation. These detrimental effects heighten the risk of age-related illnesses (Ellwanger, et al., 2023). Genomic instability refers to the genome's susceptibility to chemical modification or mutations (Drews, et al., 2022) and can arise from factors such as replication stress, errors in chromosome segregation during faulty mitosis, defective homologous recombination, environmental exposures, and lifestyle habits such as diet, exercise, alcohol consumption, and smoking. This instability can trigger gene amplifications or deletions, structural rearrangements, extrachromosomal DNA formation, and micronuclei (MN) development, all of which contribute to various pathological outcomes, including different forms of cancer (Valverde and Rojas, 2009; Fenech and Bonassi, 2011).

Acquired immunodeficiency syndrome is an infectious disease caused by the HIV, at present, there are 42.3 million people globally who have HIV infection (World Health Organization, 2024). Despite efforts to prevent, diagnose, and treat HIV, mortality rates remain higher than those of the general population. In addition, people living with HIV have an increased risk of developing cancer (Gutierrez-Sevilla, et al., 2021; Poetsch, 2020). HIV-positive individuals are now approximately 500 times more likely to acquire Kaposi sarcoma, 12 times more likely to develop non-Hodgkin lymphoma, and, in the case of women, 3 times the risk of developing cervical cancer when compared to the general population (Hernández-Ramírez, et al., 2017). Furthermore, individuals infected with HIV face an increased risk of various other cancer types. This includes cancers of the liver, oral cavity, and pharynx, as well as the lungs. In addition, those living with HIV are 3 times more likely to receive a liver cancer diagnosis, approximately twice as likely to be diagnosed with oral cavity or pharyngeal cancer, and roughly twice as likely to be identified with lung cancer compared to the general population (Wang, Silverberg and Abrams, 2014; Silverberg, et al., 2015). The impact of HIV infection on oral cancer development is not yet understood (Pradeep, et al., 2014). The presence of the accessory gene Vpr from HIV virus can be attributed to the viral HIV infection in neoplastic processes, causing disruptions in the cell cycle and resulting in changes in ploidy due to accumulation in the G2/M cell division phase. As a result, it is recommended to biomonitor HIV+ patients for early signs of oral squamous cell carcinoma (OSCC), with the potential use of the micronucleus test (Caponio, et al., 2024). The MN test is commonly utilized for

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biomonitoring and risk assessment of populations exposed to various genotoxic agents, as it aids in detecting the early stages of carcinogenesis. During the anaphase of mitosis, MNs are created when fragments of delayed chromatin or chromosomes with abnormalities separate from the nucleus. They apparently reflect chromosomal aberrations that occurred during the proliferation of the basal layer (Luzhna, Kathiria, and Kovalchuk, 2013).

The amount of HIV in the blood that can be detected through testing is referred to as the HIV viral load (VL). VL is a major factor affecting HIV transmission. Many studies have demonstrated that higher VL is associated with a significantly greater risk of HIV transmission compared to lower VL levels (Eisinger, Dieffenbach, and Fauci, 2019). HIV infects the immune system cells of the host and mainly targets monocytes/macrophages and CD4+ T lymphocytes, leading to gradual destruction. Ongoing viral replication results in a gradual decrease in CD4+ cells, leading to weakened immune defenses and heightened vulnerability to opportunistic infections (Veenhuis, et al., 2023). Moreover, there are data indicating that HIV infection causes an imbalance between the production of reactive oxygen species and antioxidants, causing OS (Dravid, et al., 2022). OS can cause genomic instability, increased risk of mutations, disruption of cell cycle control and DNA repair, chromosome rearrangements, and aneuploidy, which can contribute to the onset of specific cancer types (Poetsch, 2020).

This is the first MN analysis conducted in Northern Iraq. The purpose of this study is to examine the effect of HIV infection on the formation of MN in the oral mucosa of HIV-positive patients.

II. Methodology

A. Sample Collection

The study involved the enrollment of 38 HIV-infected patients with 44 controls. Patients with documented HIV infection older than 24 years of age were included in this study. The stages of HIV infection were not included because they were not recorded in the patients' profiles. As a result, it was not conceivable to precisely report or analyze the stages in this study. Patients who agreed to participate answered a questionnaire about general data and habits. Lymphocytes (10^9/L), granulocyte (10^9/L), and VL values were registered.

A wooden tongue depressor is used for the collection of the buccal mucosa of the participants. A buccal mucosa sample was collected through swabbing the mucosa from the left and right sides of the cheek. The collected cells are then spread on a clean slide to create a smear, which is then stained with a 5% methylene blue. The smears are then examined under a microscope with a magnification of ×100. Cells were evaluated in a zigzag pattern from one side of the smear to the other, counting solely distinct, nucleated, non-overlapping cells that were not folded. Blinding and inter-observer variability were not applied because of practical limitations. Both male and female participants were included to ensure a comprehensive representation, as HIV affects individuals of both genders. The process of identification of MN involves the counting of 100 cells per slide.

B. Statistical Analysis

The statistical program statistica software was used to analyze the data. The association between the affiliation between factors was carried out utilizing the t-test, which could be a measurable theory test to decide the goodness of fit of whether a variable has a place in a specific dispersion. $p < 0.05$ was considered statistically significant.

C. Ethical Approval

The study was conducted at the protect and Transmission Disease Hospital in Erbil, Kurdistan Region, Iraq, in collaboration with the Department of Medical Microbiology, Faculty of Sciences and Health, Koya University, Iraq. The Ethical Committee of the Faculty of Science and Health, Koya University, Iraq, approved the study. Approval number (021Bio).

III. Results

38 HIV-infected patients and 44 healthy controls admitted at the Infectious Diseases Hospital (Erbil, Iraq) were recruited in this study. To study whether there is homogeneity in the patient group with the healthy group in terms of demographic and clinical characteristics, the differences between the HIV+ patients and the control group are illustrated in Table I. For HIV patients and healthy controls, highly significant differences were observed for smokers ($p = 0.041 < 0.05$), alcohol ($p = 0.004 < 0.05$), and other diseases ($p = 0.02 < 0.05$). However, there is no significant difference in gender ($p = 0.85 > 0.05$). All HIV patients are

TABLE I
Demographic and clinical characteristics between HIV+patients and the control group

	Control (n=44)	Patient (n=38)	p-value	Other diseases treatment	HIV treatment
Male	34	30	0.85 >0.05		Tenofovir+Lamivudine+Dolutegravir
Female	10	8			(300:300:50)
Smoke	19	25	0.04 <0.05		
Alcohol	7	17	0.004 <0.05		
Other diseases	5	12	0.02 <0.05		
Diabetes	2	3		Metformin 500 mg and Glimepiride 2 mg	
Blood pressure	3	4		Valsartan 40 mg	
Anxiety	0	5		Mirtazapine 30 mg	

treated with the same medication (Tenofovir + Lamivudine + Dolutegravir [TLD] [300:300:50]).

Table II illustrates the data provide insights into six variables across a sample size of 38, including their means, standard deviations, and 95% confidence intervals. The mean age of participants is 33.42 years (±5.21), indicating a relatively young group, with an age range estimated between 31.71 and 35.13 years. The mean duration of observation is 42.32 months (±10.90), with a confidence interval of 38.73–45.90 months, suggesting moderate variability in study duration. Micronucleate cell counts average at 11.29 (±4.88), with a range from 9.69 to 12.89, reflecting some degree of cellular variability among the participants. VL shows a significantly high mean of 136,645 IU/mL (±209,376), but its wide confidence interval (33,965–67,825) indicates high dispersion, likely skewed by extreme values. Lymphocyte counts average 2.30 × 10^9/L (±1.14), within a confidence range of 1.92–2.67 × 10^9/L, suggesting relatively low variability. Granulocyte counts are slightly higher, with a mean of 3.66 × 10^9/L (±1.97) and a confidence interval of 3.01–4.31 × 10^9/L. These results reveal differences in variability among the variables, with VL showing the highest dispersion, indicating potential outliers or heterogeneity in the dataset. Further analyses, such as correlation or regression, could explore relationships between these variables.

The results regarding age, Micronucleated cells/100, lymphocyte, and granulocyte (means, standard deviation, standard error, t-value, and p-value) and the comparisons between both groups (patients and control) are shown in Table III.

No significant differences were observed between HIV patients and healthy controls in terms of age and granulocytes with p = 0.777 and p = 0.534, respectively. However, a significant difference was found in the comparison of MN frequency in the oral mucosa and lymphocyte count between HIV patients and controls (Table III). In addition, patient hematological characteristics in the control group and HIV+ patients are shown in Fig. 1.

The study involved counting 100 buccal cells of each of the participants. The presence of MN in these cells was further noted to determine the pattern of existence of MN in the cells of HIV patients and healthy controls (Fig. 1a). It was found that MN were more prominent in the buccal cells of the HIV+ patients. Also, our statistical analysis showed that the granulocyte count was slightly higher in HIV+ patients compared to the control group (Fig. 1b). However, our statistical analysis illustrated that the lymphocyte count is higher in the control group than in HIV patients (Fig. 1c).

To study whether there is a significant relationship between MN and VL, a linear regression model was calculated, and it was found that (R-Sq = 55%, p < 0.000), the VL ratio of HIV-positive patients can predict the MN cells (Fig. 2).

TABLE II
Illustrate Mean, Standard Deviation, and 95% Confidence Interval for Patients Group. IU/ml stands for logarithm (log_{10}) international units per milliliter

Sample size (38)	Mean	Standard deviation	95% confidence interval (C.I.)	
			Lower limit	Upper limit
Age/year	33.421	5.207	31.709	35.133
Duration/month	42.320	10.900	38.73	45.90
Micronucleate cell	11.289	4.876	9.687	12.892
Viral load IU/mL	136,645	209,376	33965.0	67825
Lymphocyte 0.9–5×10^9/L	2.297	1.136	1.924	2.671
Granulocyte 1.2–8×10^9/L	3.661	1.971	3.013	4.308

TABLE III
Age, micronucleated cells, and Laboratory values: Comparison between the Control Group as Healthy Individuals and HIV+Patient Samples

	Sample Size	Mean	SD	SE	t	p-value
Age/year						
Control	44	33	7.76	1.2	−0.28	0.777
Patients	38	33.42	5.21	0.84		
Micronucleated cells/100						
Control	44	0.386	0.655	0.099	−14.69	0
Patients	38	11.29	4.88	0.79		
Lymphocyte 0.9–5×10^9/L						
Control	44	3.14	1.01	0.15	3.53	0
Patients	38	2.3	1.14	0.18		
Granulocyte 1.2–8×10^9/L						
Control	44	3.44	1.24	0.19	−0.62	0.534
Patients	38	3.66	1.97	0.32		

SD: Standard deviation, SE: Standard error

IV. Discussion

This study aimed to evaluate the impact of HIV infection on cytogenetic damage in the oral mucosa, considering that DNA damage could lead to oral cancer. For this purpose, two groups were analyzed: The study group, comprising HIV-positive patients, and the control group, consisting of patients without HIV infection. Fortunately, patients in the Kurdistan region of Iraq, start treatment as soon as they are diagnosed. Therefore, all HIV+ patients in this research were treated with the same antiretroviral TLD (300:300:50). This is a significant step for a developing nation. On the other hand, Ellwanger et al. (2023) and Ivanov et al. (2016) suggest that HIV medications can also contribute to chromosome instability. In Brazil, Lima et al. (2017) examined MN in exfoliated oral cells from HIV+ individuals undergoing antiretroviral therapy and non-infected controls, with 30 participants in each group. The MN were divided into two categories: (I) single MN and (II) multiple MN. There was no significant difference in the total number of micronucleated cells or the number of MN between the groups, nor any correlation with CD4+ T cell counts. However, the study found a significantly higher mean number of single MN in the control group compared to the HIV-positive individuals. The occurrence of multiple MN was also reported in the HIV group compared to the controls, though the difference was not significant.

This is one of the first studies in northern Iraq that focused on identifying individuals posing a high risk of forming MN due to having HIV or a HIV VL. In this study, we found

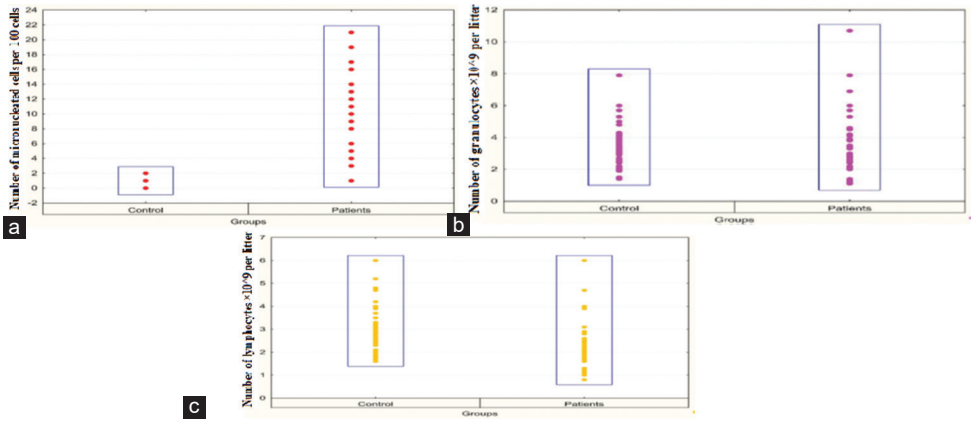

Fig. 1. (a-c) Patient characteristics of micronuclei, granulocytes, and lymphocytes, between the control group and HIV+ patients.

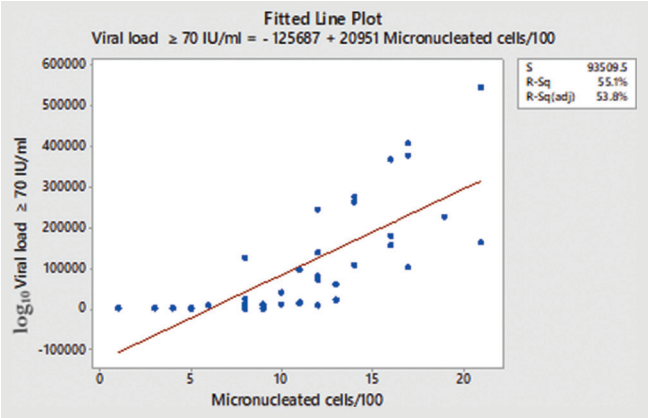

Fig. 2. Graphic representation of the regression analysis between micronucleated cells per 100 cells and the $\log_{10}$ viral load ≥70 IU/mL in HIV-positive patients.

that the VL was associated with the occurrence of MN. A significant correlation was observed between the VL and MN ($p < 0.05$). This indicated that VL might influence the clinical characteristics of patients with HIV. To better understand chromosome instability, we investigated whether VLs are associated with DNA damage. Overall, we concluded there is a modest and consistent linear relationship between VL and MN. However, causality cannot be inferred from cross-sectional data alone. Longitudinal studies are needed to determine whether changes in VL directly contribute to increased MN formation over time. Current clinical observation suggests that VL is a risk factor in the susceptibility to cytogenetic damage. In addition, previous studies have reported that HIV plays a crucial role in the development of MN (Faig Lima, et al., 2017; Zizza, et al., 2019).

Significant differences were observed between HIV patients and healthy controls regarding smoking and alcohol consumption, with $p = 0.041$ ($p < 0.05$) and 0.004 ($p < 0.05$), respectively. Jung and Yoon (2022), and Mohammed et al. (2020), have strongly suggested that the distribution of smoking and alcohol has been correlated with genotoxic effects and acts as a genetic risk factor for different diseases, such as malignancies. Although the exact connection between HIV infection and the development of oral cancer has yet to be determined, the resulting immunosuppression makes individuals more vulnerable to carcinogens and the formation of MN (Lima, et al., 2017). Therefore, HIV-positive patients should be regularly monitored for the early onset of OSCC, for which the MN test is a useful tool. According to Luzhna, Kathiria, and Kovalchuk (2013), the MN test has been widely adopted in biomonitoring and risk assessment of populations exposed to various genotoxic agents, as it serves as an effective method for identifying the initial alterations associated with carcinogenesis.

In our study, 38 HIV-positive patients were evaluated, with 100 buccal cells counted for each participant. Granulocyte and lymphocyte levels were also recorded to help identify the pattern of MN presence in the cells of HIV-positive patients. Our statistical result revealed no significant differences in granulocyte count between healthy individuals and HIV+ patients ($p = 0.534$) (Fig. 1b). On the other hand, there were significant differences in lymphocyte count between the control group and HIV patients ($p = 0.000$) (Fig. 1c). The lack of a significant difference in granulocyte levels ($p = 0.534$) suggests that granulocytes may not play a central role in reflecting HIV-related DNA damage. Unlike lymphocytes, which are more directly affected by HIV infection and are commonly used in genotoxicity assessments due to their higher mitotic activity and susceptibility to DNA damage (Nacher, et al., 2018). Lymphocytes and macrophages, key parts of the immune system, are primarily affected by HIV, which gradually damages these cells. The levels of CD4+ cells decrease gradually as the virus continues to replicate, resulting in a slow deterioration of the host's immune defense system and increased susceptibility to opportunistic infections (Vijayan, et al., 2017).

The MN formation may be associated with various chromosome-breaking events that have a direct impact on the cell cycle (Luzhna, Kathiria, and Kovalchuk, 2013). The existence of HIV accessory genes and infection by oncoviruses derived from human herpesviruses and human papillomavirus (HPV) have been the subject of numerous theories in recent years. The action of the HIV virus accessory gene Vpr, which causes disruptions in the cell cycle, can be used to explain the oncogenic process. Although there is considerable evidence linking the effects of HPV to HIV infection, leading to DNA alterations, this connection

is primarily associated with oropharyngeal cancer (Proulx, et al., 2022; Haworth, et al., 2018) and does not pertain to our study design.

V. Conclusion

Our research lends credence to the idea that HIV infection causes DNA damage, but further research in this area is required. Furthermore, even if the exact period of exposure is unknown, our results indicate that exposure to the virus is a significant factor in the development of chromosome-breaking. Further investigation is required to elucidate the relationship between HIV stages and MN, as the absence of stage data in this study constrained the inclusion of this critical contextual information. Last but not least, many unresolved questions remain regarding the relationship between HIV and MN. In addition, understanding the mechanism of DNA damage is essential for developing therapies aimed at reducing the cancer risk in HIV patients.

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