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Mycobacterium tuberculosis infection and cytogenetic abnormalities among people with HIV

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ABSTRACT

Objective: To compare cytogenetic abnormalities among people living with HIV (PLWH) with and without previous exposure to *Mycobacterium tuberculosis* (*Mtb*) (both latent tuberculosis infection [LTBI] and active tuberculosis [TB]).

Methods: Adult PLWH (≥18 years) were randomly selected at three HIV clinics in Uganda. Previous active TB was confirmed in the clinics' TB records. LTBI was defined as a positive QuantiFERON-TB Gold Plus assay. Participants' buccal mucosal exfoliated cells were examined (per 2000 cells) using the buccal micronucleus assay for chromosomal aberrations (micronuclei and/or nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (normal differentiated cells and basal cell frequency) and/or cell death (condensed chromatin, karyorrhexis, pyknotic and karyolytic cells).

Results: Among 97 PLWH, 42 (43.3%) had exposure to *Mtb*;16 had previous successfully treated active TB and 26 had LTBI. PLWH with exposure to *Mtb* had a higher median number of normal differentiated cells (1806.5 [1757.0 – 1842.0] vs. 1784.0 [1732.0 – 1843.0], p = 0.031) and fewer karyorrhectic cells (12.0 [9.0 – 29.0] vs. 18.0 [11.0 – 30.0], p = 0.048) than those without. PLWH with LTBI had fewer karyorrhectic cells than those without (11.5 [8.0 – 29.0] vs. 18.0 [11 – 30], p = 0.006).

Conclusion: We hypothesized that previous exposure to *Mtb* is associated with cytogenetic damage among PLWH. We found that exposure to *Mtb* is associated with more normal differentiated cells and less frequent karyorrhexis (a feature of apoptosis). It is unclear whether this increases the propensity for tumorigenesis.

1. Introduction

Globally, there are over 38 million people living with HIV (PLWH), of whom 75% are accessing anti-retroviral therapy (ART) [1]. ART has increased the life-expectancy of PLWH by suppressing HIV viral replication and reconstituting immune function [2–4]. As such, PLWH now

Abbreviations: Mtb,, Mycobacterium tuberculosis; PLWH, people living with HIV; TB, tuberculosis; QFT-plus, QuantiFERON-TB Gold Plus assay; LTBI, latent TB infection; ART, anti-retroviral therapy.

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experience non-AIDS-related diseases that are associated with aging. These include several cardiovascular diseases, renal impairment, frailty and non-AIDS defining cancers [5–7]. Moreover, PLWH experience accelerated aging that is partly driven by the HIV, ART, lifestyle factors and viral co-infections [8,9]. These factors are thought to induce DNA damage and chromosomal instability that are characterized by loss or amplification of genes, extra-chromosomal DNA, micronucleus (MN) formation, and defective mitosis [10]. It is unclear whether *Mycobacterium tuberculosis* (*Mtb*) co-infection in HIV contributes to DNA damage.

Tuberculosis (TB) is the leading cause of death among PLWH and is prevalent in 40% of autopsies among PLWH in low-income settings [11]. Several cohorts show that TB, including latent TB infection (LTBI), increases the long term risk of cancer for more than 20 years after TB diagnosis [12,13]. Additionally, TB survivors are thrice as likely as controls to die after TB cure mostly from cancer and cardiovascular disease [14]. The mechanisms to explain the increased TB-related cancer risk are not fully understood. TB is thought to cause inflammation that results in DNA methylation and accelerated cellular senescence [15]. This manifests as a higher number of micronuclei, bi-nucleated cells, condensed chromatin, karyorrhectic and pyknotic cells among people with TB than in controls [16]. Further, macrophages infected with *Mtb* induce oxidative DNA damage and production of a potent epithelial growth factor, epiregulin, which could drive tumorigenesis [17]. Nonetheless, these abnormalities have not been demonstrated among PLWH with *Mtb* exposure. In this study, we hypothesized that, among PLWH, previous exposure to *Mtb* is associated with cytogenetic abnormalities: DNA damage, chromosomal instability, increased cell death and proliferative potential of human buccal mucosal tissue as determined by the buccal micronucleus cytome (BMCyt) assay [18]. We therefore compared cytogenetic abnormalities among PLWH with and without previous exposure to *Mtb* (both LTBI and active TB). We further explored factors associated with these individual cytogenetic abnormalities among PLWH.

2. Materials and methods

2.1. Study design and participants

This was a comparative cross-sectional study conducted among adult PLWH (aged ≥18 years) who were randomly selected at three HIV clinics in Uganda. Eligible participants were adult PLWH receiving ART at Kiruddu National Referral Hospital (KNRH), St. Francis Nsambya Hospital (SFNH) and Mbarara Regional Referral Hospital (MRRH). KNRH is a public tertiary referral facility located in Kampala, the capital city of Uganda. SFNH is private-not-for-profit tertiary facility located in Kampala as well. MRRH is a rural public regional referral hospital in the western region of Uganda. These facilities were purposively selected because they have well established HIV, TB and cancer care programs.

2.2. Study procedures and data collection

Participant were randomly selected, using computer aided techniques, from the HIV care database at each study site, proportional to size. Potential participants were then contacted by telephone to participate in the study on a given HIV clinic day. Study questionnaires were administered through a face-to-face interview upon obtaining written consent. Previous treatment for bacteriologically confirmed TB (either by sputum GeneXpert and microscopy or urine lipoarabinomannan) was ascertained from the HIV care records and the unit TB register at the respective sites. Study questionnaires sought for demographic data, medical history, HIV medical history (baseline CD4 at the point of HIV diagnosis, ART history, and history of opportunistic infections). Thereafter, the participant underwent a brief physical examination. Participants then rinsed the mouth with distilled water and buccal mucosa was harvested from the inside of a patient's cheeks using a small-headed toothbrush for the BMCyt assay [18]. The BMCyt assay is a non-invasive assay for determining DNA damage and chromosomal instability, cell death, and the proliferative potential of buccal mucosa [19]. The assay has been extensively used to show cytogenetic changes arising from exposure to genotoxic agents, nutrients and lifestyle habits [20]. The protocol for this assay has been published elsewhere [18,19]. Cells were examined per 2000 cells for chromosomal aberrations (micronuclei and/or nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (normal differentiated cells and basal cell frequency) and/or cell death (condensed chromatin, karyorrhexis, pyknotic and karyolytic cells).

We obtained blood samples that were tested for the full haemogram, serum creatinine and urea, liver transaminases, serum electrolytes, plasma HIV RNA (viral load) by polymerase chain reaction (COBAS 8800), CD4 T-cell count by flow cytometry (BD Facs Prestio), and the QuantiFERON TB Gold-Plus assay (QFT-plus) according to manufacturer's instructions [21]. Viral suppression was defined as a viral load of < 100 copies/ml.

2.3. Study outcomes

Latent tuberculosis infection (LTBI) was defined as a positive QFT-plus assay in an individual without previous treatment for active TB according to manufacturer's instructions [21]. Exposure to *Mtb* was defined as either LTBI or previous treatment for bacteriologically confirmed TB. Cytogenetic abnormalities among people with previous exposure to *Mtb* were defined as a higher number (compared to those without *Mtb* exposure) of any of micronuclei, nuclear buds, binucleated cells, normal differentiated cells, basal cell frequency, condensed chromatin, karyorrhetic, pyknotic and karyolytic cells.

2.4. Data analysis

Data were entered in KoboToolBox and analysed with Stata 17.0 for analysis. Continuous variables are presented as medians with the corresponding interquartile ranges (IQR). Categorical data are presented as proportions. Group comparisons were performed using the Mood's median test. We used multiple linear regression models to determine correlates of the cytogenetic abnormalities among PLWH. All socio-demographic, clinical and laboratory variables were tested at bivariate analysis as potential correlates. Factors with $p < 0.2$ at bivariate analysis were entered in a multivariable model to determine independent correlates of the cytogenetic abnormalities.

3. Results

3.1. Characteristics of study participants

Among the 97 PLWH enrolled, the median (IQR) age was 46.0 (38.0 – 51.0) years, 72 (74.2%) were female and all were on antiretroviral therapy for a median of 144 (101 − 180) months with a CD4 count of 956 (745 – 1251) cells/mm^3. Additionally, 88 (91.7%) were virally suppressed and 79 (81.4%) had completed TB preventive therapy. Table 1 shows characteristics of the study participants.

3.2. Exposure to Mycobacterium tuberculosis among PHW in Uganda

Forty-two (43.3%) PLWH had exposure to *Mtb*. Of these, 16 (38.1%) had previous successfully treated active TB and 26 (61.9%) had LTBI. Seven participants (7.2%) had previous successfully treated active TB and a positive IGRA. Table 2 shows a comparison between PLWH exposed and those not exposed to *Mtb*.

3.3. Cytogenetic abnormalities among PLWH with and without exposure to Mycobacterium tuberculosis

PLWH with exposure to *Mtb* had a higher median number of normal

Table 1
Characteristics of the study participants.

Characteristic	Number	%
Hospital site		
Mbarara Regional Referral Hospital	36	37.1
St. Francis Nsambya Hospital	32	33
Kiruddu National Referral Hospital	29	29.9
Urban residence	70	72.2
On antiretroviral therapy (ART)	97	100
Drugs in ART regimen		
Tenofovir	89	91.8
Lamivudine	95	97.9
Abacavir	5	5.2
Zidovudine	5	5.2
Efavirenz	7	7.2
Nevirapine	1	1
Dolutegravir	84	86.6
Lopinavir	1	1
Ritonavir	6	6.2
Atazanavir	4	4.1
Past history of HIV opportunistic infections	57	58.8
Clinical stage of HIV		
Stage I	58	59.8
Stage II	14	14.4
Stage III	13	13.4
Stage IV	12	12.4
History of alcohol use	53	54.6
History of cigarette smoking	13	13.4
Use of biofuels for cooking	65	67
Chronic diseases		
None	72	74.2
Diabetes	1	1
Asthma	3	3.1
Other	21	21.6
Current symptoms		
None	55	57.3
Cough	12	12.5
Fever	2	2.1
Weight loss	3	3.1
Night sweats	2	2.1
Dyspnea	4	4.2
Chest pain	7	7.3
Hemoptysis	0	0.0
Bone pain	5	5.2
Anorexia	2	2.1
Body weakness	2	2.1
Eastern Cooperative Oncology Group performance status score		
ECOG 0	95	97.9
ECOG 1	2	2.1
CONTINUOUS VARIABLES, MEDIAN (IQR)		
Age (Years)	46 (38, 51)	
Duration on ART (Months)	144 (101, 180)	
Baseline CD4 Count (cells/mm3)	230 (68, 425)	
Current CD4 Count (cells/mm3)	956.5 (745, 1251)	
Temperature (°Celcius)	36 (35.5, 36.5)	
Systolic blood pressure (mmHg)	124 (115, 136)	
Diastolic blood pressure (mmHg	81 (75, 90)	
Pulse rate (beats per minute)	78 (67, 86)	
Respiratory rate (breaths per minute)	16 (14, 18)	
White blood cell count (x10^9 per litre)	4.8 (3.9, 5.7)	
Absolute Lymphocyte count (x10^9 per litre)	2.3 (1.8, 2.6)	
Absolute Neutrophil count (x10^9 per litre)	2.0 (1.5, 2.5)	
Absolute Eosinophil (x10^9 per litre)	0.12 (0.07, 0.21)	
Absolute Basophil (x10^9 per litre)	0.01 (0.01, 0.02)	
Haemoglobin level (grams per deciliter)	14.4 (13.5, 15.5)	
Mean corpuscular volume (femtolitres)	91.3 (87.3, 95.8)	
Mean corpuscular Hemoglobin (picograms)	30.0 (28.5, 31.3)	
Platelet count (cells/mm3)	263.5 (221.9, 301.8)	
Plateletcrit (%)	0.24 (0.20, 0.27)	
Aspartate amino transferase (IU/L)	27.3 (22.7, 33.5)	
Gamma-glutamyl transferase (IU/L)	46.7 (33.6, 61.2)	
Alanine aminotransferase (IU/L)	20.6 (15.5, 28.1)	
Alkaline amino phosphatase (IU/L)	110 (81, 147)	
Total bilirubin (micromoles per liter)	0.35 (0.23, 0.55)	
Albumin (g/l)	3.94 (3.31, 5.16)	
Total protein (g/l)	7.63 (7.09, 8.04)	
Urea (mmol/l)	3.8 (2.9, 4.64)	
Creatinine (mmol/l)	107.1 (92.6, 123.7)	
Sodium (mmol/l)	142.7 (113, 144)	
Calcium (mmol/l)	2.35 (1.8, 2.83)	
Chloride (mmol/l)	107.3 (83, 109.2)	
Phosphate (mmol/l)	1.42 (1.24, 1.77)	
Uric acid (mmol/l)	312.8 (247, 395.3)	
Lactate dehydrogenase (U/L)	594 (450, 780)	

differentiated cells (1806.5 [1757.0 – 1842.0] vs. 1784.0 [1732.0 – 1843.0], p = 0.031) and fewer karyorrhectic cells (12.0 [9.0 – 29.0] vs. 18.0 [11.0 – 30.0], p = 0.048) than those without. PLWH with LTBI had fewer karyorrhectic cells than those without (11.5 [8.0 – 29.0] vs. 18.0 [11–30], p = 0.006) (Figure 1). There were no significant differences in the number of other cytogenetic abnormalities. Table 3 shows the comparison of cytogenetic abnormalities among PLWH with and without exposure to *Mtb*.

3.4. Correlates of cytogenetic abnormalities among PLWH in Uganda

Correlates of cytogenetic abnormalities are shown in supplementary tables 1 – 7.

HIV viral non-suppression (adjusted regression coefficient (β) = 20.13, 95% confidence interval (CI) 3.03 – 37.23, p = 0.022), self-reported history of diabetes mellitus (β = 67.02, 95% CI 23.33 – 110.72, p = 0.003) and the haemoglobin level (β = 3.97, 95% CI 0.21 – 7.73, p = 0.039) were positively correlated with the number of karyorrhectic cells while the serum calcium level negatively correlated with the same (β = −8.79, 95% CI −16.93 to −0.64, p = 0.035). Efavirenz (β = 7.00, 95% CI 0.25 – 13.75, p = 0.042), and the lactate dehydrogenase level (β = 0.003, 95% CI 0.002 – 0.005, p < 0.001) positively correlated with the number of bi-nucleated cells. HIV clinical stage IV (β = 17.51, 95% CI 1.66 – 33.36, p = 0.031) and bone pain (β = 21.65, 95% CI 1.16 – 42.15, p = 0.039) were associated with nuclear buds. Self-reported fever (β = 69.64, 95% CI 28.18 – 111.09, p = 0.001), and male gender (β = 17.59, 95% CI 1.80 – 33.38, p = 0.029) positively correlated with the number of micronuclei while serum creatinine (β = −0.34, 95% CI −0.57 to −0.11, p = 0.005) negatively correlated with the same. The Eastern Cooperative Oncology Group (ECOG) performance status score of 1 was associated with pyknotic cells (β = 88.07, 95% CI 32.09 – 144.06, p = 0.003) compared to an ECOG score of 0. The mean corpuscular haemoglobin positively correlated with the number of basal cells (β = 1.64, 95% confidence interval [CI] 1.09 – 2.19, p < 0.001).

4. Discussion

In this study we compared the frequency of cytogenetic abnormalities among PLWH with and without *Mtb* exposure. We found that PLWH with exposure to *Mtb* had more normal differentiated cells but fewer karyorrhectic cells than those without. The findings suggest that *Mtb* exposure is associated with increased proliferation of cells and less apoptosis among PLWH; since karyorrhexis is a feature of programmed cell death [22]. The implication of this finding as it relates to cancer risk is not very apparent from our study. However, *Mtb* is thought to induce proliferative signaling and resistance to cell death which if unchecked could result in cancer [23,24]. Several mechanisms can explain why we observed higher proliferative potential (inferred from a higher number of normal differentiated cells) and less cell death. In an in-vivo study, Lochab and colleagues demonstrated that *Mtb* induces persistent DNA double strand breaks that activate the Ataxia telangiectasia mutated-Akt pathway thereby inhibiting apoptosis and accentuating cell growth [25]. Nalbandian et al [17], also showed that *Mtb* up-regulates epiregulin gene expression which epiregulin is a potent epidermal growth

Table 2
Comparison between PLWH exposed and those not exposed to *Mtb*.

Characteristic	Exposure to *Mtb*		P-value[a]
	Not exposedn= 55	Exposedn= 42	
Hospital site			
Mbarara Regional Referral Hospital	19 (34.5)	17 (40.5)	
St. Francis Nsambya Hospital	13 (23.6)	19 (45.2)	
Kiruddu National Referral Hospital	23 (41.8)	6 (14.3)	**0.008**
Urban residence	41 (74.5)	29 (69)	0.549
On antiretroviral therapy (ART)	55 (100)	42 (100)	-
Drugs in ART regimen			
Tenofovir	50 (90.9)	39 (92.9)	0.516
Lamivudine	54 (98.2)	41 (97.6)	0.681
Abacavir	3 (5.5)	2 (4.8)	0.627
Zidovudine	3 (5.5)	2 (4.8)	0.627
Efavirenz	4 (7.3)	4 (9.5)	0.724
Nevirapine	1 (1.8)	0 (0)	0.567
Dolutegravir	45 (81.8)	36 (85.7)	0.784
Lopinavir	0 (0)	1 (2.4)	0.433
Ritonavir	4 (7.3)	2 (4.8)	0.695
Atazanavir	3 (5.5)	1 (2.4)	0.631
Past history of HIV opportunistic infections	21 (38.2)	19 (45.2)	0.484
Current clinical stage of HIV			
Stage I	35 (63.6)	23 (54.8)	0.056
Stage II	10 (18.2)	4 (9.5)	
Stage III	3 (5.5)	10 (23.8)	
Stage IV	7 (12.7)	5 (11.9)	
History of alcohol use	31 (56.4)	22 (52.4)	0.696
History of cigarette smoking	9 (16.4)	4 (9.5)	0.327
Use of biofuels for cooking	34 (61.8)	31 (73.8)	0.213
Chronic diseases			
None	42 (76.4)	30 (71.4)	0.644
Diabetes	0 (0)	1 (2.4)	
Asthma	2 (3.6)	1 (2.4)	
Other	11 (20)	10 (23.8)	
Current symptoms			
Cough	5 (9.1)	7 (16.7)	0.262
Fever	2 (3.6)	0 (0)	0.504
Weight loss	1 (1.8)	2 (4.8)	0.577
Night sweats	1 (1.8)	1 (2.4)	0.681
Dyspnea	2 (3.6)	2 (4.8)	0.584
Chest pain	3 (5.5)	4 (9.5)	0.462
Hemoptysis	0 (0)	0 (0)	-
Bone pain	2 (3.6)	3 (7.1)	0.649
Anorexia	1 (1.8)	1 (2.4)	0.681
Body weakness	2 (3.6)	0 (0)	0.504
Eastern Cooperative Oncology Group performance status score (ECOG)			
ECOG 0	54 (98.2)	41 (97.6)	0.681
ECOG 1	1 (1.8)	1 (2.4)	
CONTINUOUS VARIABLES, MEDIAN (IQR)			
Age (Years)	43 (34, 50)	47 (42, 51)	0.104
Duration on ART (Months)	132 (77, 168)	149.5 (117, 196)	0.163
Baseline CD4 Count (cells/mm3)	230 (60, 489)	231 (90, 420)	0.929
Current CD4 Count (cells/mm3)	881 (686, 1136)	1049 (875, 1372)	**0.040**
Temperature (°Celcius)	36.1 (35.5, 36.5)	35.9 (35.5, 36.4)	0.231
Systolic blood pressure (mmHg)	119 (111, 135)	126.5 (119, 139)	0.135
Diastolic blood pressure (mmHg	81 (74, 89)	83 (77, 91)	0.364
Pulse rate (beats per minute)	78 (66, 90)	78 (69, 85)	0.886
Respiratory rate (breaths per minute)	16 (14, 19)	16 (14, 18)	0.460
White blood cell count (x10^9 per litre)	4.71 (3.81, 5.69)	4.91 (4.04, 5.37)	0.835
Absolute Lymphocyte count (x10^9 per litre)	2.29 (1.75, 2.65)	2.25 (1.76, 2.6)	0.835
Absolute Neutrophil count (x10^9 per litre)	2.01 (1.55, 2.47)	1.87 (1.41, 2.52)	0.533
Absolute Eosinophil (x10^9 per litre)	0.12 (0.05, 0.21)	0.12 (.07, 0.18)	0.619
Absolute Basophil (x10^9 per litre)	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.759
Haemoglobin level (grams per deciliter)	14.17 (13.35, 15.57)	14.7 (13.66, 15.3)	0.145
Mean corpuscular volume (femtolitres)	91.1 (85.9, 96.3)	91.3 (88.3, 94.9)	0.979
Mean corpuscular Hemoglobin (picograms)	29.7 (28.0, 31.2)	30.2 (29.1, 31.3)	0.298
Platelet count (cells/mm3)	244.2 (221.9, 299.8)	282.4 (223.5, 301.8)	0.061
Plateletcrit (%)	0.24 (0.20, 0.28)	0.23 (0.20, 0.26)	0.581
Aspartate amino transferase (IU/L)	28.7 (22.5, 33.9)	26.2 (23.0, 31.9)	**0.044**
Gamma-glutamyl transferase (IU/L)	44.6 (33.6, 66.5)	47.35 (32.1, 60.8)	0.187
Alanine aminotransferase (IU/L)	21.3 (15.5, 29.4)	20.0 (15.4, 26.0)	0.465
Alkaline amino phosphatase (IU/L)	105 (79, 147)	114 (81, 157)	0.094
Total bilirubin (micromoles per liter)	0.38 (0.23, 0.57)	0.30 (0.22, 0.45)	**0.009**
Albumin (g/l)	3.99 (3.55, 5.56)	3.87 (2.75, 4.49)	0.465
Total protein (g/l)	7.68 (7.12, 8.14)	7.58 (7.07, 8.00)	0.465
Urea (mmol/l)	3.73 (2.88, 4.74)	3.94 (2.85, 4.54)	0.364
Creatinine (mmol/l)	109.8 (91.23, 124.4)	106.43 (93.99, 123.71)	0.465
Sodium (mmol/l)	141.3 (111.6, 143.4)	143.3 (141.4, 144.9)	**0.001**
Calcium (mmol/l)	2.4 (1.8, 2.9)	2.24 (1.79, 2.81)	0.254
Chloride (mmol/l)	106 (81.6, 108.5)	108.5 (106.5, 109.8)	**0.002**
Phosphate (mmol/l)	1.51 (1.27, 1.82)	1.38 (1.18, 1.64)	0.254
Uric acid (mmol/l)	312.8 (268.2, 372.3)	302.65 (209.5, 434.5)	0.929
Lactate dehydrogenase (U/L)	573 (427, 768)	605 (490, 828)	0.618

[a] Bolded values indicate a statistically significant difference.

factor that promotes proliferation. Lastly, *Mtb* PtpA, a secreted effector protein, modulates expression of genes that regulate cell proliferation (*GADD45A*) and apoptosis (*TNFRSF21*) [26]. Epidemiological studies could further evaluate whether the high proliferative potential and inhibited apoptosis among PLWH who have been exposed to *Mtb* herald incident metaplasia and cancer. These studies could also evaluate the utility of the BMCyt assay as a screening test for cancer among PLWH.

There are few studies that have evaluated cytogenetic abnormalities in TB using the BMCyt assay, later on among PLWH and *Mtb* exposure. Unlike in our study, da Silva and colleagues [16] found a higher frequency of micronuclei, bi-nucleated cells, condensed chromatin, karyorrhectic and pyknotic cells among people with active TB than in controls. The difference in our findings could be because of the different

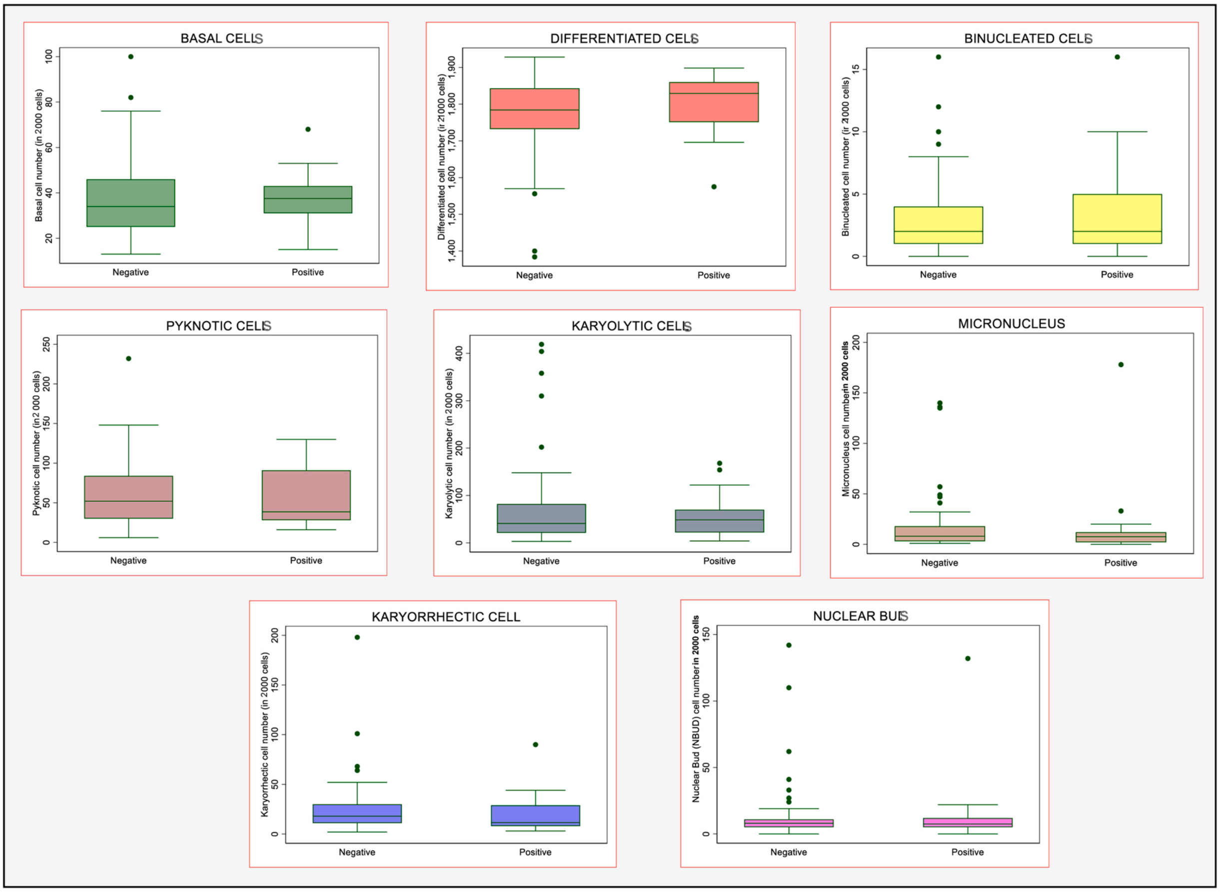

Figure 1. A comparison of cytogenetic abnormalities between people living with HIV with latent TB infection (Positive) and without (Negative).

Table 3
Comparison of cytogenetic abnormalities among PLWH with and without exposure to *Mtb*.

Cell type	Number per 2000 cells (median, IQR)N = 97	Previous TB infection		
		No	Yes	P-value[a]
Basal	35 (26.5, 46)	34 (25,46)	39 (31,46)	0.183
Differentiated	1795.5 (1744, 1842.5)	1784 (1732,1843)	1806.5 (1757,1842)	**0.031**
Bi-nucleated	2 (1, 4.5)	2 (1,4)	2 (1,5)	0.318
Pyknotic	52 (30, 89.5)	52 (30,84)	49 (30,94)	0.889
Karyorrhectic	15 (10, 30)	18 (11,30)	12 (9,29)	**0.048**
Karyolytic	43 (21.5, 72)	41 (21,82)	45 (22,70)	0.271
Micronucleus	8 (3, 15.5)	8 (3,18)	7.5 (2,12)	0.404
Nuclear Buds	8 (4.5, 12)	8 (5,11)	7.5 (4,12)	0.775

[a] Bolded values indicate a statistically significant difference.; IQR – interquartile ranges

study populations (active TB vs. previous exposure to *Mtb*). Increased cell proliferation and reduced apoptosis could be an enduring effect of *Mtb* despite TB and LTBI treatment as is the case in our study [17]. Moreover, our study population was PLWH who could have several potential causes of cytogenetic abnormalities. From our study, HIV viral non-suppression, comorbidities (diabetes), poorer performance status (ECOG score and advanced clinical stage) and certain ART (efavirenz) correlated with cytogenetic abnormalities. Gutiérrez-Sevilla and colleagues also found that efavirenz-based ART regimens were associated with binucleated cells which they attributed to oxidative stress [27]. HIV viral non-suppression can lead to DNA damage and chromosomal instability by direct interaction between HIV proteins with host DNA while comorbidities contribute to the chronic inflammation [10]. The correlates of cytogenetic abnormalities among PLWH emphasize the need for ensuring viral suppression, use of safer ART regimens and optimizing care of comorbidities and organ dysfunction among PLWH.

Our study has limitations. First, the BMCyt assay is prone to bias that is inherent to visual interpretation of images [28]. The histopathologist was blinded to the *Mtb* exposure status of the participants in our study. This reduced on the possible misclassification bias. Secondly, the small sample size could have affected our ability to control for all confounders in evaluating correlates of cytogenetic abnormalities. Notably, the number of PLWH using efavirenz and those with diabetes mellitus was very small. Moreover, the multiple comparisons of variables increase the risk of a type 1 error. Nonetheless, we attempted to control for co-founders in the multivariable linear regression models and this analysis is mostly exploratory. These correlates should be interpreted with caution and need to be validated by a larger study. We also did not evaluate for other current co-infections which could cause cytogenetic changes. Lastly, majority of PLWH had completed TB preventive therapy (TPT) and this could have affected our association of LTBI with cytogenetic abnormalities. Nonetheless, treatment with isoniazid, as is done in TPT in Uganda, does not seem to alter the effect of *Mtb* on cell proliferation and apoptosis [17].

5. Conclusion

PLWH with previous exposure to *Mtb* had a higher number of normal differentiated cells and fewer karyorrhectic cells than those without. These findings suggest that exposure to *Mtb* is associated with increased cell proliferation and less frequent cell death (karyorrhexis is a feature of apoptosis). While animal and in-vivo studies suggest that this could promote tumorigenesis, large prospective studies among PLWH are needed to determine the predictive utility of these findings.

Ethical approval statement

Study participants provided written informed consent before study measurements were undertaken. The study protocol was approved by the Mildmay Uganda Research and Ethics Committee (#REC REF MUREC-107–2022). Further, the Uganda National Council of Science and Technology provided additional approval as required by the guidelines for conducting research in Uganda (HS2328ES).

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CRediT authorship contribution statement

BBJ- conceptualization, study design, data accrual, data analysis, data interpretation, drafting article, revising article, approval of final version. SN, BN, SK, DN, RN, TO, CN, NN, NB, EN, PS - data accrual, data interpretation, revising article, approval of final version. MN- data analysis, data interpretation, drafting article, revising article, approval of final version. IAB, WW, RS, SM, SG, BK – study design, data interpretation, revising article, approval of final version.

Declaration of Competing Interest

The authors declare no relevant conflict of interest.

Data availability

Data will be made available on request.

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None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mrgentox.2023.503640.

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