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Exploring the associations of tobacco smoking and serum cotinine levels with selected inflammatory markers in adults with HIV in South Africa

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This study examined the associations between tobacco smoking and serum cotinine levels, an objective biochemical measure of tobacco smoke exposure, with markers of inflammation, i.e., interferon-gamma (IFN-γ), interleukin 10 (IL-10), interleukin 2 (IL-2) and tumour necrosis factor-alpha (TNF-α) in people living with HIV (PLWH).These specific markers were selected because of their hypothesised associations with smoking, PLWH and their outcomes. In a random sample of ≥18-year-old PLWH receiving care at 17 public healthcare facilities across the Western Cape Province in South Africa, data collection included self-reported smoking history, and serum levels of cotinine and selected inflammatory markers. The inflammatory marker data were log transformed because of the skewedness of their distribution. Linear regression models (1) adjusted for age and gender, and (2) fully adjusted for age, gender, current alcohol use, body mass index and CD4 counts were used to examine the associations between smoking tobacco or serum cotinine and inflammatory markers. Level of significance was $p < 0.05$. Among 749 PLWH who were mainly women (79%), the mean age was 38.5 (8.9) years and similar when stratified by smoking status. Serum cotinine levels exhibited a striking discrepancy, with a median of 154 ng/mL among current smokers, in stark contrast to the consistent median values of 10 ng/mL observed among past and never smokers. In regression models adjusted for age and gender, current smoking and frequent smoking were associated with lower IL-2 but higher TNF-α. Log-cotinine exhibited associations with IFN-γ, IL-10, and TNF-α, while cotinine levels ≥10 ng/mL compared to <10 ng/mL were associated with higher IFN-γ and TNF-α. In fully adjusted models, log-cotinine and cotinine levels ≥10 ng/mL displayed significant associations with higher IFN-γ and lower IL-2. This study underscores the importance of investigating the interplay between smoking tobacco or serum cotinine levels with pro-inflammatory cytokines in PLWH. It signals the need for comprehensive research to unravel the potential synergistic impacts of smoking tobacco and HIV infection on chronic inflammation and immune dysregulation, shedding light on critical avenues for intervention and management strategies.

Keywords Interferon-gamma, Interleukin 10, Interleukin 2, Tumour necrosis factor-alpha, Inflammation, Smoking, Tobacco, Cotinine, HIV

Tobacco smoking is a leading preventable cause of mortality accounting for 15% of mortality worldwide or over eight million deaths annually[1,2]. The latter occurs primarily in low- and middle-income countries where

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the majority of the globally 1.3 million tobacco users reside[1]. The predominant tobacco-related morbidity and mortality in general populations is on account of lung cancer, the primary cancer death globally, and other cancers, cardiovascular diseases (CVDs) including ischaemic heart disease (IHD), stroke and peripheral vascular disease, and respiratory diseases such as chronic obstructive pulmonary disease and tuberculosis[3].

Furthermore, cohort studies have consistently reported higher mortality rates from CVD and non-AIDS malignancies among smokers compared to non-smokers in people living with HIV (PLWH)[4,5]. Smoking has been linked to a poorer quality of life among PLWH, as evidenced by lower scores in general health perception, physical functioning, pain, energy levels, and cognitive functioning[6,7]. PLWH who smoke are at heightened risk of opportunistic infections such as bacterial pneumonias, acute bronchitis, and tuberculosis, compared to non-smokers with HIV[8–10].

The ability to identify tobacco users most susceptible to adverse outcomes may allow targeted preventative measures to be introduced. The possible mechanisms in which tobacco smoking inflicts harm include the detrimental effects on the immune system which are extensive and complex with both pro-inflammatory and suppressive effects being activated[11]. These lead to immune deficiencies and promote inflammation by increasing various pro-inflammatory cytokines[12]. There is increasing evidence of a correlation between several inflammatory markers and smoking status[13]. In a comprehensive investigation conducted in the United Kingdom, researchers delved into the intricate interplay between serum biomarkers and smoking status, revealing profound differentials in the levels of crucial cytokines. The study illuminated a stark contrast in the serum concentrations of tumour necrosis factor-alpha (TNF-α), interleukin 10 (IL-10), and interleukin 2 (IL-2) among smokers compared to non-smokers, showcasing a notable upsurge in inflammatory markers in the former cohort. Among smokers, the levels of TNF-α were observed to be approximately 70 pg/ml, significantly higher than the corresponding 25 pg/ml in non-smokers. Similarly, IL-10 exhibited a substantial elevation in smokers at around 70 pg/ml in contrast to the comparatively lower 30 pg/ml in non-smokers. Furthermore, IL-2 levels were markedly heightened in smokers, measuring approximately 250 pg/ml, while non-smokers registered a notably lower concentration of 30 pg/ml[14]. Moreover, in tobacco smokers, inflammatory markers have been correlated with the subsequent development of lung cancer[15], and have been linked to increases in atherosclerosis and CVD risk[12]. Therefore, identifying tobacco users with raised markers of inflammation may offer an opportunity to detect those at high risk for adverse tobacco-related outcomes[15]. The ability to reduce inflammation may perhaps potentially contribute to preventing or treating tobacco-related conditions[16]. This elucidation underscores the intricate relationship between smoking and systemic inflammation, shedding light on the potential mechanistic pathways through which smoking exerts its deleterious effects on health.

The possible link between smoking and inflammation reported in general populations may not be generalisable to PLWH who are already immunocompromised. Inflammatory markers are generally high in PLWH even in those on antiretroviral therapy (ART)[17]. Several studies have found associations between markers of inflammation, coagulation, and HIV mortality. For example, interferon-gamma (IFN-γ) plays a central role in inflammation and autoimmune disease where it displays characteristics as a main regulator of immune responses and inflammation, and functions as both inducer and regulator[18]. Initially, the role of IFN-γ is to clear the primary infection, but in conjunction with other inflammatory cytokines, chronic immune activation occurs which worsens the clinical disease[19].

The effects of cytokines on HIV can be inhibitory, stimulatory or both underscoring the complexity of cytokine networks[20]. The complex inflammatory processes induced by cigarette smoking in PLWH involve several overlapping key cytokines and chemokines, including TNF-α. However, the precise mechanistic contributions of each pathway in the inflammatory process among PLWH who smoke remain largely unknown. Understanding the relative risk associated with comorbid disease phenotypes in the context of smoking provides targets for intervention given that disease risk evolves over time. Although increased immune activation has been reported in PLWH who smoke compared with their counterparts[17], there is a dearth of literature on inflammatory markers in PLWH, particularly in Africa.

This is highly particularly relevant in South Africa which has the greatest number of PLWH and the largest ART programme worldwide[21]. Almost eight million adults have HIV[22], equivalent to about one in five (19%) South Africans aged ≥ 15 years with HIV[23]. HIV magnifies and accelerates the harmful effects of smoking compared with smokers without HIV while, among PLWH, smokers fare worse than non-smokers[24,25]. Consequently, despite advances in treating HIV, the benefits accrued by combination ART are negated in tobacco smokers[7].

Therefore, the aim of this study was to examine the associations of smoking status and serum cotinine levels, an objective biochemical measure of smoke exposure, with markers of inflammation, i.e., IFN-γ, IL-10, IL-2 and TNF-α in PLWH.

Methods

Study design and sampling

This cross-sectional study was conducted from March 2014 to February 2015 in a random sample of ≥ 18-year-old PLWH treated at public healthcare facilities across the Western Cape Province in South Africa. To ensure optimal recruitment within an acceptable timeframe, facilities that provided ART to ≥ 325 PLWH per month were included. From a sample frame of 62 healthcare facilities (42 in Cape Town and 20 in the surrounding rural municipalities), among 30 healthcare facilities that were randomly selected with an equal distribution of urban and rural sites, 17 facilities, including four rural clinics, consented to participate in this study, and were included. Thereafter, 15–60 patients were randomly sampled from these 17 healthcare facilities for inclusion in the study; originally 30 participants per 30 clinics was planned with a target sample of 900 participants. Further details are provided elsewhere (Nguyen, 2016).

PLWH who were ≥ 18-year-old, attending primary or secondary healthcare facilities with specific HIV-directed clinics and provided informed consent were included in the study. Those who were bedridden, had active

malignancy or were undergoing treatment for malignancies, on chronic corticosteroid therapy, or pregnant were excluded. Viral load data were not collected and did not inform eligibility for inclusion in the study.

Data collection

The data collection process involved a comprehensive approach, combining administered questionnaires and clinical evaluations conducted on the day of recruitment, followed by biochemical assessments carried out the subsequent day after an overnight fast. Trained clinicians, nurses, and fieldworkers meticulously recorded data using electronic case report forms on personal digital assistants (PDAs), equipped with built-in mechanisms for quality control. To ensure data integrity, all information was encrypted at the point of collection and transmitted securely via mobile connection to a dedicated server. Upon arrival, the data underwent thorough checks, after which it was downloaded and securely stored.

Sociodemographic data and medical history were gathered using the World Health Organization's STEPwise approach to Surveillance (STEPS) tool. Additional self-reported data encompassed the duration of HIV diagnosis, while details regarding antiretroviral therapies (ARTs) were sourced from medications brought to the clinic by participants either on the day of interview or at their next visit when blood samples were taken. CD4 counts were obtained from patient charts, ensuring accuracy and reliability.

Anthropometry of heights and weights were measured using standardised procedures. Ensuring that the participant was barefoot, standing and wearing light clothing, height was measured to the nearest millimetre with a Leicester Height Scale (Seca, Liverpool, UK) and weight to the nearest gram using A&D Personal Scale (Model UC-321, Toshima-Ku, Tokyo, Japan).

Following an overnight fast of at least eight hours, blood samples were drawn for lipid profiles, glycated haemoglobin, ultrasensitive C-reactive protein, and cotinine levels. These biochemical specimens were analysed at an ISO 15,189 accredited pathology laboratory (PathCare, Reference Laboratory, Cape Town, South Africa). Serum cotinine testing was measured by immunoassay (Immulite, Siemens Healthcare, Germany). Total cholesterol, high-density lipoprotein cholesterol and triglycerides were measured using the enzymatic colorimetric methods. Low-density lipoprotein cholesterol was calculated using the Friedewald Equation[26]. Ultrasensitive C-reactive protein was read. All colorimetric measurements were conducted using a Beckman Coulter AU 500 spectrophotometer. Glycated haemoglobin levels were determined using high-performance liquid chromatography techniques in accordance with the National Glycohaemoglobin Standardisation Programme (NGSP).

Inflammatory markers of IFN-γ, IL-10, IL-2 and TNF-α were measured on blood samples stored at -80 degrees Celsius. The concentrations of the inflammatory biomarkers were measured using reagent kits purchased from R&D Systems Inc., BioTechne, Minneapolis, Minnesota, USA, on the Bio Plex 200 platform (Bio Rad Laboratories, Hercules, USA). The Bio-Plex Manager Software (ver. 6·1, Bio Rad laboratories) was used for bead acquisition and analysis.

Comprehensive assessment of smoking status, alcohol use, and health parameters among individuals living with HIV

Smoking status was categorised as current, past or never a smoker and encompassed all forms of smoked tobacco, including cigarettes, cigars or pipes. Current smokers comprised daily and occasionally smokers. Past smokers referred to participants who reported they had quit smoking irrespective of the duration of quitting. Smoking status was also categorised as daily smoking i.e., smoking at least once a day versus not-a-daily smoking i.e., occasional, past and never smokers. Current alcohol use (CAU) was defined as consuming any alcohol in the past 30 days. Duration of HIV diagnosis was determined from the time of being diagnosed with HIV. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and categorised as underweight/normal weight (BMI < 25 kg/m^2), overweight (BMI 25–29.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2).

Utilizing serum cotinine levels to assess tobacco exposure in PLWH

Serum cotinine, the primary metabolite of nicotine in the blood, was the biomarker used to determine exposure to tobacco; it is considered the most objective biochemical assessment of smoke exposure i.e., the gold standard for the assessment of smoking status[12]. Smoking categories, based on serum cotinine levels were defined as < 10 ng/mL: no tobacco exposure, 10–100 ng/mL: light smoking or environmental smoke exposure, and > 100 ng/mL: moderate to heavy smoking that were used previously in studies in South Africa[27].

Statistical analysis

Statistical analyses were conducted using the R statistical software version 3.0.3 (the R Foundation for Statistical Computing Platform, Vienna, Austria). Continuous variables were summarized as means (± standard deviation [SD]) for normally distributed data and as medians (interquartile range [IQR]) for skewed data, ensuring comprehensive representation. Categorical variables were meticulously presented as counts and percentages, offering a clear understanding of the sample characteristics. Descriptive statistical analyses were done to compare the differences by smoking status using Analysis of the Variance (ANOVA), Kruskal-Wallis tests, and chi-square tests where appropriate. Linear regression models, meticulously adjusted for age and gender, along with essential covariates including CAU, BMI, and CD4 counts, were proficiently employed to unveil the intricate associations of smoking status and serum cotinine levels with inflammatory marker data. A significance threshold of $p < 0.05$ was utilised to determine statistical significance.

Log transformation of cytokine data

To effectively address the skewed data distribution inherent in the cytokine data, log transformation was applied. In this study, pro-inflammatory marker data exhibited skewed distributions, characterized by a clustering of

observations at lower values with a notable tail of higher values. The log transformation method involved taking the natural logarithm of each data point, compressing the range of values and mitigating the skewness observed.

Ethics approval

The study was conducted in accordance with principles of the International Declaration of Helsinki, 2013. Ethical approval was provided by the SAMRC Ethics Committee (protocol ID EC021-11/2013). The Health Research Office of the Western Cape Department of Health provided permission to conduct the study, as did the relevant healthcare facilities. Informed consent was obtained from all individual participants included in the study.

Results

Only participants who returned for biochemical assessments and had adequate blood samples for analyses were included in this study i.e., 749 out of 831 participants who were interviewed and clinically assessed. Among 749 PLWH, the mean age was 38.5 (8.9) years and similar between participants stratified by smoking status (Table 1). Despite men comprising 21% of the sample, 47% of current smokers and 44% of past smokers were men compared with only 8% of never smokers; the majority of never smokers, at 92%, were women ($p < 0.001$). Serum cotinine levels, at a median of 154 ng/mL was highest among current smokers compared with past smokers and never smokers (median 10 ng/mL for both) ($p < 0.001$). CAU was most common in current smokers (51%) compared with past smokers (34%) and never smokers (16%) ($p < 0.001$). Mean BMI and the prevalence of obesity, however, were the highest in never smokers (29.3 kg/m^2 and 41%) compared with past smokers (25.5 kg/m^2 and 25%) and current smokers (23.6 kg/m^2 and 14%) ($p < 0.001$ for all). Median duration of diagnosed HIV was longer in never smokers at 6 years, IQR: 3–9, compared with past smokers 4 years (IQR: 2–6) years) and current smokers 4 years (IQR: 2–8 years) ($p = 0.002$).

Assessment of clinical measures of inflammation indicated that median CD4 count was lower in past (253 cells/μL, IQR: 164–399) and current smokers (316 cells/μL, IQR: 175–462) compared with never smokers (435 cells/μL, IQR: 268–654) ($p < 0.001$). Median IL-10 was higher in past smokers at 22 (IQR: 16-28.5) pg/ml compared with current smokers (19, IQR: 15–25 pg/ml) and never smoker (17, IQR: 14–22 pg/ml) ($p < 0.001$).

Variables	Overall	Never smoker	Past smoker	Current smoker	*P*-value
Number	749	500	73	176	
Age, years: mean (SD)	38.5 (8.9)	38 (8.4)	41 (10.6)	38 (9.6)	0.054
Male, n (%)	154 (20.6)	39 (7.8)	32 (43.8)	83 (47.2)	< 0.001
Serum cotinine, ng/mL: median (P25-P75)	9.9 (9.9–83.2)	9.9 (9.9–9.9)	9.9 (9.9–30.8)	153.5 (79.3-239.2)	< 0.001
Serum cotinine categories, n (%)					< 0.001
< 10 ng/ml	472 (63)	414 (82.8)	49 (67.1)	9 (5.1)	
10–100 ng/ml	176 (23.5)	41 (8.2)	14 (19.2)	121 (68.7)	
> 100 ng/ml	101 (13.5)	45 (9)	10 (13.7)	46 (26.1)	
Current alcohol use	196 (26.2)	82 (16.4)	25 (34.2)	89 (50.5)	< 0.001
Body mass index (BMI), kg/m^2: mean (SD)	27.6 (6.8)	29.3 (6.6)	25.5 (5.7)	23.6 (5.5)	< 0.001
Adiposity, n (%)					< 0.001
Underweight/normal weight: BMI < 25 kg/m^2	320 (4.3)	154 (30.8)	39 (53.4)	127 (72.1)	
Overweight: BMI:25–29.9 kg/m^2	180 (24)	139 (27.8)	16 (21.9)	25 (14.2)	
Obese: BMI ≥ 30 kg/m^2	249 (33.2)	207 (41.4)	18 (24.6)	24 (13.6)	
HIV duration in years, median (P25-P75)	5 (2–9)	6 (3–9)	4 (2–6)	4 (2–8)	0.002
CD4 count (cells/μL), median (P25-P75)	400 (244–620)	435 (268–654)	253 (164–399)	316 (175–462)	< 0.001
TC, mmol/l: mean (SD)	4.5 (1.0)	4.4 (3.7–5.1)	4.2 (3.9–4.7)	4.5 (3.8-5.0)	0.700
LDL-C, mmol/l: mean (SD)	2.6 (0.9)	2.5 (2.1–3.1)	2.4 (2.0-2.8)	2.5 (2.1–3.1)	0.492
HDL-C, mmol/l: mean (SD)	1.3 (0.4)	1.2 (1.0-1.5)	1.3 (1.1–1.5)	1.2 (1.0-1.5)	0.888
TG, mmol/l: median (P25-P75)	1.0 (0.7–1.4)	1.0 (0.8–1.4)	1.0 (0.7–1.3)	1.0 (0.7–1.4)	0.476
HbA1c, %: mean (SD)	5.6 (0.9)	5.6 (0.8)	5.8 (1.5)	5.5 (0.6)	0.660
hs-CRP, mg/L: mean (SD)	5.5 (2.4–14.4)	5.4 (2.3–13.6)	4.2 (2.4–11.1)	6.4 (2.7–16.9)	0.249
IFN- γ, pg/ml: median (P25-P75)	14 (12–16)	14 (12–16)	15 (11–17)	14 (12.5–17)	0.079
IL-10, pg/ml: median (P25-P75)	18 (14–24)	17 (14–22)	22 (16-28.5)	19 (15–25)	< 0.001
IL-2, pg/ml, median ((P25-P75)	9 (9–10)	9.5 (9–10)	10 (9–10)	9 (9–10)	0.433
TNF-α, pg/ml, median (P25-P75)	19 (15.5–23.5)	18 (15–23)	20 (16–26)	21 (16.5–26.6)	< 0.001

Table 1. Participants' characteristics by self-reported smoking status (3-level-category variable). TC: Total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; HbA1c: glycated haemoglobin; IFN- γ: interferon-gamma; IL-10: interleukin 10; IL-2 interleukin 2; TNF-α: tumour necrosis factor alpha. P-values are from Analysis of the Variance (ANOVA), Kruskal-Wallis tests, or chi-square tests.

Median TNF-α was highest in current smokers at 21 (IQR: 16.5–26.6) pg/ml compared with past smokers (20, IQR: 16–26 pg/ml) and never smokers (18, IQR: 15–23 pg/ml).

Unadjusted linear regression models were conducted to evaluate the crude association between smoking status and pro-inflammatory biomarkers. Current smokers when compared with never smokers had higher log-IFN-γ (0.006), log-IL-10 ($p=0.042$) and log-TNF-α ($p<0.001$) (Table 2). Furthermore, smoking tobacco ≥ 1 time/day was associated with log-TNF-α only ($p<0.001$). When adjusted for other covariates including age, gender, current smoking compared with never smoking, was negatively associated with log-IL-2 (β: -0.011, $p=0.042$) and inversely related to log-TNF-α (β: 0.043, $p=0.004$) as was smoking tobacco ≥ 1 time/day compared with less smoking: log-IL-2 (β: -0.012, $p=0.034$) and log-TNF-α (β: 0.043, $p=0.006$). In regression models adjusted for age, gender, CAU, BMI and CD4 counts, neither current nor daily smoking were related to makers of inflammation.

In unadjusted models, log-cotinine was significantly associated with log-IFN-γ (β: 0.023, $p=0.001$), log-IL-10 (β: 0.036, $p=0.012$) and log-TNF-α (β: 0.037, $p<0.001$) (Table 3). Upon adjusting for age and gender in regression models, these associations persisted, with log-IFN-γ (β: 0.020, $p=0.004$), log-IL-10 (β: 0.032, $p=0.030$) and log-TNF-α (β: 0.024, $p<0.015$). Furthermore, in fully adjusted models accounting for age, gender, CAU, BMI and CD4 count, log-cotinine was significantly associated with log-IFN-γ (β: 0.021, $p=0.034$) and log-IL-2 (β: -0.014, $p=0.013$).

In the unadjusted models, cotinine levels of 10–100 ng/ml compared with cotinine < 10 ng/ml were significantly associated with all markers of inflammation tested, while cotinine > 100 ng/ml compared with cotinine < 10 ng/ml was associated with log-IFN-γ (β: 0.035, $p=0.002$) only (Table 3). These positive associations persisted in models adjusted for age and gender. In the fully adjusted model, only cotinine 10–100 ng/ml was significantly associated with log-IL-2 (β: -0.024, $p=0.002$).

In unadjusted models, cotinine ≥ 10 ng/ml vs. <10 ng/ml was significantly associated with log-IFN-gamma (β: 0.031, $p<0.001$), log-IL-10 (β: 0.039, $p=0.024$) and log-TNF-α (β: 0.040, $p=0.001$) (Table 3). In models adjusted for age and gender, cotinine ≥ 10 ng/ml vs. <10 ng/ml was significantly associated with log-IFN-gamma (β: 0.026, $p=0.001$), and log-TNF-α (β: 0.026, $p=0.029$). In the fully adjusted model containing age, gender, CAU, BMI and CD4 count, cotinine ≥ 10 ng/ml vs. <10 ng/ml was significantly associated with log-IFN-gamma (β: 0.024, $p=0.042$) and log-IL-2 (β: -0.015, $p=0.034$).

Predictors	Outcomes			
	Log-IFN- γ β (*p*-value)	Log-IL-10 β (*p*-value)	Log-IL-2 β (*p*-value)	Log-TNF-α β (*p*-value)
Unadjusted models				
Model 1:				
Never smoker	1.00	1.00	1.00	1.00
Past smoker	0.015 (0.262)	0.076 (0.007)	0.001 (0.868)	0.054 (0.005)
Current smoker	0.026 (0.006)	0.040 (0.042)	-0.006 (0.197)	0.066 (< 0.001)
Model 2:				
Smoke < 1 time/day	1.00	1.00	1.00	1.00
Smoke ≥ 1 time/day	0.020 (0.051)	0.009 (0.691)	-0.008 (0.117)	0.065 (< 0.001)
Models adjusted for age and gender				
Model 1:				
Never smoker	1.00	1.00	1.00	1.00
Past smoker	0.010 (0.456)	0.076 (0.010)	-0.002 (0.742)	0.031 (0.121)
Current smoker	0.018 (0.081)	0.035 (0.108)	-0.011 (0.042)	0.043 (0.004)
Model 2:				
Smoke < 1 time/day	1.00	1.00	1.00	1.00
Smoke ≥ 1 time/day	0.012 (0.259)	-0.004 (0.860)	-0.012 (0.034)	0.043 (0.006)
Models adjusted for age, gender, current alcohol use, body mass index and CD4 count levels				
Model 1:				
Never smoker	1.00	1.00	1.00	1.00
Past smoker	-0.015 (0.413)	0.068 (0.111)	-0.003 (0.787)	0.023 (0.427)
Current smoker	-0.002 (0.869)	-0.012 (0.731)	-0.016 (0.061)	0.009 (0.704)
Model 2:				
Smoke < 1 time/day	1.00	1.00	1.00	1.00
Smoke ≥ 1 time/day	0.013 (0.408)	-0.045 (0.222)	-0.012 (0.220)	0.016 (0.507)

Table 2. Linear regressions for the associations of self-reported smoking status with selected inflammatory markers in South African adult men and women. *Model 1*: self-reported smoking was analysed as 3-level categorical variable: never smoker, past smoker and current smoker; *Model 2*: self-reported smoking was analysed as a binary variable: not-a-daily smoker vs. daily smoker.

Predictors	Outcomes			
	Log-IFN- γ β (*p*-value)	Log-IL-10 β (*p*-value)	Log-IL-2 β (*p*-value)	Log-TNF-α β (*p*-value)
Unadjusted models				
Model 1:				
Log-serum cotinine	0.023 (0.001)	0.036 (0.012)	-0.005 (0.115)	0.037 (< 0.001)
Model 2:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine 10–100 ng/ml (*n* = 176)	0.028 (0.002)	0.053 (0.008)	-0.009 (0.041)	0.050 (< 0.001)
Cotinine > 100 ng/ml (*n* = 101)	0.035 (0.002)	0.014 (0.575)	0.000 (0.970)	0.024 (0.158)
Model 3:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine ≥ 10 ng/ml (*n* = 277)	0.031 (< 0.001)	0.039 (0.024)	-0.006 (0.135)	0.040 (0.001)
Models adjusted for age and gender				
Model 1:				
Log-serum cotinine	0.020 (0.004)	0.032 (0.030)	-0.007 (0.051)	0.024 (0.015)
Model 2:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine 10–100 ng/ml (*n* = 176)	0.024 (0.011)	0.048 (0.019)	-0.012 (0.017)	0.035 (0.013)
Cotinine > 100 ng/ml (*n* = 101)	0.031 (0.009)	0.008 (0.759)	-0.002 (0.788)	0.012 (0.481)
Model 3:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine ≥ 10 ng/ml (*n* = 277)	0.026 (0.001)	0.033 (0.060)	-0.008 (0.059)	0.026 (0.029)
Models adjusted for age, gender, current alcohol use, body mass index and CD4 count levels				
Model 1:				
Log- serum cotinine	0.021 (0.034)	-0.003 (0.883)	-0.014 (0.013)	-0.007 (0.643)
Model 2:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine 10–100 ng/ml (*n* = 176)	0.021 (0.105)	0.048 (0.874)	-0.024 (0.002)	-0.009 (0.670)
Cotinine > 100 ng/ml (*n* = 101)	0.027 (0.109)	-0.034 (0.372)	0.002 (0.804)	-0.039 (0.135)
Model 3:				
Cotinine < 10 ng/ml (*n* = 472)	1.00	1.00	1.00	1.00
Cotinine ≥ 10 ng/ml (*n* = 277)	0.024 (0.042)	0.009 (0.736)	-0.015 (0.034)	-0.019 (0.281)

Table 3. Linear regressions for the associations of serum cotinine levels with selected inflammatory markers in South African adult men and women. *Model 1*: serum cotinine was log-transformed and analysed as a continuous variable; *Model 2*: serum cotinine was analysed as a 3-level categorical variable; *Model 3*: serum cotinine was analysed as a binary variable.

Discussion

This study represents a novel effort in elucidating the associations between inflammatory markers, tobacco smoking, and cotinine levels in PLWH. Particularly in the context of South Africa, where literature on this topic remains limited, our findings significantly contribute to advancing understanding in this field. We specifically shed light on the intricate relationship between tobacco smoking, cotinine levels, and select inflammatory markers, namely TNF-α, IFN-γ, and IL-2.

Our analysis revealed notable associations between TNF-α and various smoking-related factors, including current smoking, smoking tobacco ≥ 1/day, as well as log-serum cotinine and cotinine levels ≥ 10 ng/ml, consistently across unadjusted and age- and gender-adjusted models. Similarly, IFN-γ exhibited significant relationships with log-serum cotinine and cotinine levels ≥ 10 ng/ml in all models, including the fully adjusted model. In contrast, IL-2 displayed a negative association with current smoking and smoking tobacco ≥ 1 time/day in the age- and gender-adjusted models, and with log-serum cotinine and cotinine levels ≥ 10 ng/ml in the fully adjusted models.

However, despite the meticulous examination, none of the four inflammatory markers examined showed associations with current smoking or smoking tobacco ≥ 1 time/day in the fully adjusted models, underscoring the complexity of these relationships and highlighting avenues for further exploration. There are likely several plausible explanations for the lack of associations between inflammatory markers and cotinine or tobacco smoking. Firstly, the specific contribution of nicotine, of which cotinine is a metabolite, compared to the myriad of other harmful components present in smoked tobacco products remains enigmatic[28]. This study could not adjust for the effects of other harmful substances found in tobacco products when examining the specific correlations of inflammatory markers with cotinine. Additionally, discrepancies may arise between self-reported smoking status and objectively measured cotinine levels, potentially due to factors such as concealment

of smoking status or exposure to second-hand smoke[29]. This notion is supported by findings from a nationally representative study in South Africa, where nearly half (47%) of non-smokers reported exposure to second-hand smoke[30]. The latter, which was not examined in this study, may have contributed to the absence of significant findings; 17% of non-smokers had cotinine levels ≥ 10 ng/ml. Further research is required to understand the poor agreement between self-reported smoking and cotinine levels.

Together with high levels of second-hand smoke exposure in South Africa, the similar levels of inflammatory markers demonstrated in current and former smokers in this study may be due to prolonged intervals required after quitting for these markers to return to levels reported in never smokers. Wang and colleagues explain that while continuous smoking increases some inflammatory factors, quitting smoking may disturb this status and lead to increases in inflammatory factors[13]. The specific time course for decreases in inflammation after smoking cessation remains unclear and requires further investigation[13,28]. Further, the rate of reduction in inflammation is unlikely to be uniform across markers e.g., a study demonstrated slower rates of declines for TNF-α and IL-2 levels compared with other markers at two weeks[28]. Nevertheless, these declines in inflammatory marker levels are expected to occur from approximately 14 days to 20 years[28,31].

In this study, we observed higher levels of IL-10 and TNF-α in current smokers compared to never smokers; aligning with findings reported in previous literature[12,14,32,33]. Notably, pro-inflammatory cytokines such as IL-10 and TNF-α, irrespective of smoking status, exhibited elevated levels in PLWH compared with normal values documented in the literature[34,35]. The heightened TNF-α levels observed in PLWH who smoke, in our study are consistent with findings from other independent studies[17].

The negative association of IL-2, a pro-inflammatory cytokine, with smoking and cotinine has been described in the literature[36,37]. The interplay of IL-2, tobacco smoking and HIV is multifaceted and dependent on many variables[11]including several not explored in this study. These include the number of tobacco products smoked, the diversity of tobacco chemical components, the duration of smoking, the use of multiple tobacco products and additional influences on the immune system e.g., the presence of medical conditions such as HIV, etc[11,38].

IFN-γ levels in this sample of PLWH were higher than that suggested for normal populations (< 8.6 pg/mL) (Eurofins Viracor, 2023), and accords with the literature[19,34,39,40]. Across smoking categories, we found that there were no differences in the median levels of IFN-γ in PLWH, in keeping with a study conducted in an Iranian general population[41]. Nevertheless, most cotinine models and the unadjusted model for current smoking in this study demonstrated associations with IFN-γ in PLWH. Therefore, this study represents a novel effort to unravel the inflammatory pathways underlying disease phenotypes in the context of smoking among PLWH, offering invaluable insights for future research and clinical management.

PLWH who smoke are at heightened risk of opportunistic infections such as bacterial pneumonias, acute bronchitis, and tuberculosis, along with exhibiting higher viral loads and lower CD4 cell counts compared to non-smokers with HIV[8–10]. The suppression of local lung defences serves as a common aetiological pathway that may explain the elevated rates of pulmonary illnesses observed in HIV-positive individuals who smoke[7,42]. Understanding the underlying pro-inflammatory mechanisms of these processes holds promise for identifying novel targets for clinical care and intervention.

Study strengths and limitations

The relatively large sample size which was randomly selected is a strength of this study and allows for generalisability of the study findings albeit that participants were predominantly women and from a single South African province. Men comprised almost half the number (47%) of current smokers in this study and PLWH are likely to be similar across provinces. Study limitations include the absence of data on duration of quitting and exposure to second-hand smoke such as living with a smoker, which may have provided greater insights into the study findings. The inclusion of a comparative group without HIV would have further enriched these analyses, and this absence is a study limitation. Further, this study did not ascertain adverse clinical outcomes related to tobacco use in participants. Hence, the associations of raised markers of inflammation with adverse tobacco-related outcomes cannot be established in this study and is speculative.

Conclusions

The correlations of TNF-α, IFN-γ, and IL-2 with tobacco smoking and cotinine in this study illustrate the potential utility of these inflammatory markers. They may serve as indicators of adverse tobacco-related outcomes in PLWH in South Africa, but more studies are needed to support the use these biomarkers in the clinical context. This study highlights the intricate interplay between tobacco smoking, inflammation, and adverse health outcomes among PLWH. Unravelling this complex relationship necessitates a multifaceted approach, as the synergistic effects of tobacco smoking, HIV infection, chronic inflammation, immune dysregulation, and their subsequent impact on health outcomes remain enigmatic. Further exploration through comprehensive research endeavours is imperative to disentangle the intricate web of interactions shaping the health trajectory of PLWH.

Our study represents an initial step towards deciphering this intricate nexus. Yet, the depth of our understanding remains in its infancy, urging the need for continued investigation into this complex interplay. The scarcity of studies delineating the disparities in inflammatory markers across various smoking categories among PLWH, juxtaposed with those without HIV, underscores the pressing need for further studies. Addressing this research gap is pivotal in illuminating the nuances of tobacco-smoking-mediated inflammation within the context of HIV, facilitating the development of targeted interventions aimed at ameliorating the adverse health consequences.

Data availability

Data used for this study are available from Prof Andre-Pascal Kengne (Andre.Kengne@mrc.ac.za) on reasonable request.

Received: 12 March 2024; Accepted: 22 October 2024
Published online: 28 October 2024

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Author contributions
Manuscript conception: EP, APK; Data acquisition: NNC, APK; Writing of the main manuscript text: NP; Data cleaning, analysis, and preparation of tables: KAN; Critical revision of the manuscript: NP, KAN, EP, HX, TEM, NNC, APK; Approval of the final version: all co-authors.

Funding
This study was supported by Grand Challenges Canada, through the Global Alliance on Chronic Diseases Initiative (Hypertension Grant #0169-04); and the South African Medical Research Council (SAMRC) through baseline allocation to the Non-communicable Diseases Research Unit (NCDRU).

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
Ethics approval was obtained from the South African Medical Research Council's (SAMRC) Human Research Ethics Committee (EC021-11/2013). All participants signed informed consent to be included in this study. All research procedures were performed in accordance with the Declaration of Helsinki.

Additional information
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