Ndlovu Research Consortium

THE ASSOCIATION BETWEEN HIV-RELATED STIGMA AND ART ADHERENCE ON CARDIOVASCULAR DISEASE RISK IN PEOPLE LIVING WITH HIV.

by

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LAYMAN'S SUMMARY

Sub-Saharan Africa is home to only 12% of the world's population, and South Africa to 0.76%. But most of the human immunodeficiency virus (HIV) infections are found there. HIV is a retrovirus that changes the immune system, and this makes people living with HIV (PLWH) to be at high risk of other infections. It is a serious public health issue causing high rates of death and disease. If the virus remains untreated, the disease can progress into a more complex stage, acquired immunodeficiency syndrome (AIDS). The availability of HIV medicine (anti-retroviral treatment, ART) has reduced the disease progression and increased the life span in PLWH. However, there has been an increase in age-related, chronic diseases such as cardiovascular disease. The increase has been observed to be two times higher in PLWH, compared to HIV-uninfected people. More cases of cardiovascular diseases are seen in low/middle-income countries (LMIC) such as South Africa when compared to high-income countries (HIC). Furthermore, death rates due to chronic diseases are almost twice as high in LMIC as it is in the HIC. The process behind cardiovascular disease in HIV infection is still unclear. However, it is suspected to be involving multiple factors, and long-term inflammation is likely to be a very important factor. A South Asian study looking at the effect of social and cultural obstacles on the risk of developing atherosclerosis (a condition in which a person develops a sticky substance called plaque, therefore causing their blood vessels to be hard) showed that cardiovascular disease risk may be due to HIV itself and possibly HIV stigma. HIV stigma refers to negative behavior and judgments faced by PLWH. In Sub-Saharan Africa, particularly South Africa, HIV stigma has been studied but to date not within the context of cardiovascular disease risk. Therefore, this study aimed at investigating the relationship between HIV-related stigma, and ART adherence on heart disease risk. In our study, we hypothesised that HIV-related stigma and ART adherence were independently associated with cardiovascular diseases in PLWH.

Lifestyle, biological, and socioeconomic data were collected from 1927 participants. Pulse wave velocity, a measure of the stiffness of the blood vessels, was collected at 12 and 36 months. In this study, we included 325 participants with full PWV data at both time points, of which 67% were women, and 33% were men. Statistical techniques were used to assess the relationship between HIV stigma, ART adherence, and CVD risk. We adjusted for other potential factors such as age, sex, blood pressure, waist circumference, etc. Our results showed that HIV-related stigma is not associated with PWV at 12 months and that PWV did not increase over time.

Abstract

Introduction: In Sub-Saharan Africa, HIV/AIDS remains a leading cause of morbidity and mortality. While HIV/AIDS emerged to the public's awareness in the early 1980s, HIV-related stigma remains a major problem owing to fear of transmission, misperceptions, and misinformation concerning HIV. HIV stigma and discrimination may limit adherence to antiretroviral treatment (ART), thus increasing viremia, inflammation, and cardiovascular disease (CVD) risk. This study aims to investigate the association between stigma and ART adherence on CVD risk among PLWH.

Methods: A longitudinal study was conducted among 325 participants living with HIV, from the Ndlovu Cohort Study in Limpopo province, South Africa (2014 to 2017). A 12-item short-version HIV stigma scale questionnaire was used to measure HIV-related stigma. Pulse wave velocity (PWV, CVD risk predictor) measurements and laboratory assessment of viral load (VL) were performed at 12 and 36 months. VL was considered a surrogate marker of ART adherence. Poor/no adherence = High viremia (viral load, VL) > 1000 copies, suboptimal adherence = low viremia (VL 50-1000 copies), and good adherence = undetectable viral load (<50 copies). Multiple linear regression was used to assess the relationship between stigma, ART adherence, and CVD risk. Beta coefficients with a 95% confidence interval were used to determine the strength and direction of the association, with a p-value of 0.05 declared as statistically significant.

Results: Of the 325 participants living with HIV, 67% were females. Mean age was 42.1 [SD (10.2)] years. Participants with VL in the undetectable, low, and high categories were 78%, 15%, and 7%, respectively. Overall stigma [-0.05 (-0.14_0.04), p=0.27] and high VL [-0.33 (-0.88_0.21), p=0.23] were not associated with PWV. However, low VL [1.06 (0.37_1.84), p=0.003] was significantly associated with PWV. PWV did not increase over the 36-month follow-up period [-0.12 (-0.33_0.08), p=0.24].

Conclusion: In this study, suboptimal ART adherence was associated with higher PWV. Overall stigma was low and not associated with PWV.

Keywords: HIV-related stigma ART adherence, cardiovascular disease, viral load, Pulse wave velocity, and people living with HIV.

1. Background:

Sub-Saharan Africa comprises 70% (> 26 million) of people living with HIV (PLWH), with South Africa standing at approximately 8.45 million cases (1). HIV /AIDS was brought to public awareness in the early 1980s. However, there are still misconceptions and misinformation about HIV which cause HIV-related stigma and discrimination against people living with HIV (PLWH). Fear of causal transmission is seen in people with little/no knowledge regarding HIV which gives rise to HIV stigma (2,3). In many instances, PLWH excluded themselves for fear of being identified as HIV+(4).

Usually, the HIV-related stigma is associated with discrimination, an aspect of stigma in which PLWH are excluded and denied e.g., access to medical care. In healthcare sectors, HIV-related stigma can manifest in numerous ways, including mandatory HIV tests with no prior consent from the patient and no proper counselling (4,5). The stigma and discrimination are well-known for preventing PLWH from having proper healthcare services, medical treatment, and psychosocial support, and consequently, failure to ART adherence (4,6,7). Other studies have shown that stigma and discrimination not only affect PLWH but also people vulnerable to HIV. Both stigma and discrimination are well-recognised barriers to seeking HIV testing and act as major challenges to public efforts whose objective is to prevent further infections(8). Stigma and discrimination may also lead to anxiety and depression in PLWH (7)).

Recent statistics showed that 75% of PLWH in South Africa are on antiretroviral therapy (ART)(1). The availability of ART has reduced disease progression and positively increased life expectancy in PLWH (1,9). Consequently, chronic care in PLWH has increasingly shifted towards age-related non-communicable diseases such as cardiovascular diseases (CVD) (10,11). CVDs are becoming a public challenge, especially among PLWH who are on ART, with PLWH having been reported to be at 2-fold increased risk for CVD compared with age- and sex- matched HIV-negative (10,12,13) In spite of this increased risk of CVD in PLWH the HIV-CVD pathogenesis is not yet clear (1,14). HIV alongside other factors is suspected to be involved in the pathogenesis of CVD in PLWH, with HIV-associated chronic inflammation suspected to be the principal determinant (15). Chronic inflammation in HIV has been associated with poor adherence to ART(16).

A South African study revealed that despite ARTs having been made easily accessible and available in local medical facilities, PLWH avoided these healthcare centres (17). Their reasons included maintaining their HIV status undisclosed and minimising stigma from people in their family and community. ART adherence refers to the commitment by PLWH to the use of ART following the instructions given by the treatment giver. This includes taking the correct dosage, being consistent, and taking medication on time (18) . PLWH should at least have 95% adherence levels to achieve a successful treatment outcome (19). ART compliance is a crucial element in disease management and

reduction of HIV-related morbidity and mortality (7,17). However, there seem to be limitations on disease interventions and adherence to the treatment due to HIV stigma and discrimination.

Studies on ART compliance demonstrated that PLWH with non-compliance to the treatment have up to 4 times increased mortality rates compared to those who adhere, to similar treatment (14,20). Both short and long-term interruption of ARTs can result in decreased CD4+ count, HIV replication, increased susceptibility to opportunistic infections, and a higher risk for ART resistance (21). Short-term ART discontinuation might cause functional monotherapy, especially in ARTs with the longest half-life, for instance, non-nucleoside reverse transcriptase inhibitor (NNRTI), by resulting in NNRTI-resistant mutation (21,22). Drug resistance due to long-term non-compliance might require chemoprophylaxis against opportunistic infections due to a reduction in CD4. ART discontinuation has also been linked to increased immune activation, inflammation, and a greater risk of CVD (4,17).

Cultural or social determinants such as stigma are suspected to be playing a role in the CVD risk, however, not enough is understood about its impact (14). A possible mechanism of HIV stigma-ART adherence on CVD pathogenesis may involve inadequate or lack of adherence to the ART due to stigma, which results in an increased level of viremia. Subsequently, elevated immune activation through lymphocytes and macrophage occurs. This causes excessive secretion of pro-inflammatory cytokines (C-reactive proteins, interleukins) and chemokines that recruit other immune cells, and lead to increased inflammation (23). The resultant inflammation promotes the development of atherosclerosis/arterial stiffness, endothelial dysfunction, platelet activation and coagulation cascade by altering vascular smooth muscle phenotype(23,24). Elevated arterial stiffness results in higher pulse pressure and pulse wave velocity (the frequency with which the pressure waves circulate through the cardiovascular system)(24–26). Consequently, arterial stiffness results in increased left ventricular afterload, causing left ventricular impairment, stroke, myocardial infarction and heart failure (24,26,27). In sub-Saharan Africa, stigma-ART adherence has not yet been examined for CVD risk.

This study aimed to investigate the association between stigma and ART adherence on CVD risk among PLWH in a cohort of middle-aged PLWH living in rural South Africa. We hypothesized that stigma and/or lack of ART adherence were independently associated with higher CVD risk as measured by PWV.

2. Methods:

Study setting:

This study formed part of the Ndlovu Cohort study (NCS), Elandsdooorn, a rural area within the Moutse area, in Limpopo province, South Africa.

Study population:

The NCS is a longitudinal study which began in 2014 with annual follow-ups at 12, 24, 36 and 48 months (26). The study population was enrolled from December 2014 to May 2017 and included 887 participants living with HIV (PLWH). All recruited participants provided informed consent, they were 18 years or older, and committed to long-term follow-up. At baseline, in the PLWH 479 (54%) were women and 408 (46%) were men, a total of 690 PLWH (78%) were on ART, with 89% of those on first line and 11% on second-line therapy (28) while 197 (22%) were ART naïve. HIV viral load was measured at baseline, 12, 24 and 36 months by Realtime PCR (qPCR) at the TOGA laboratory on-site. Any participants lost to follow-up and those who did not meet the inclusion criteria were excluded from this analysis. In this study, PLWH with full PWV data at both 12 and 36 months were included.

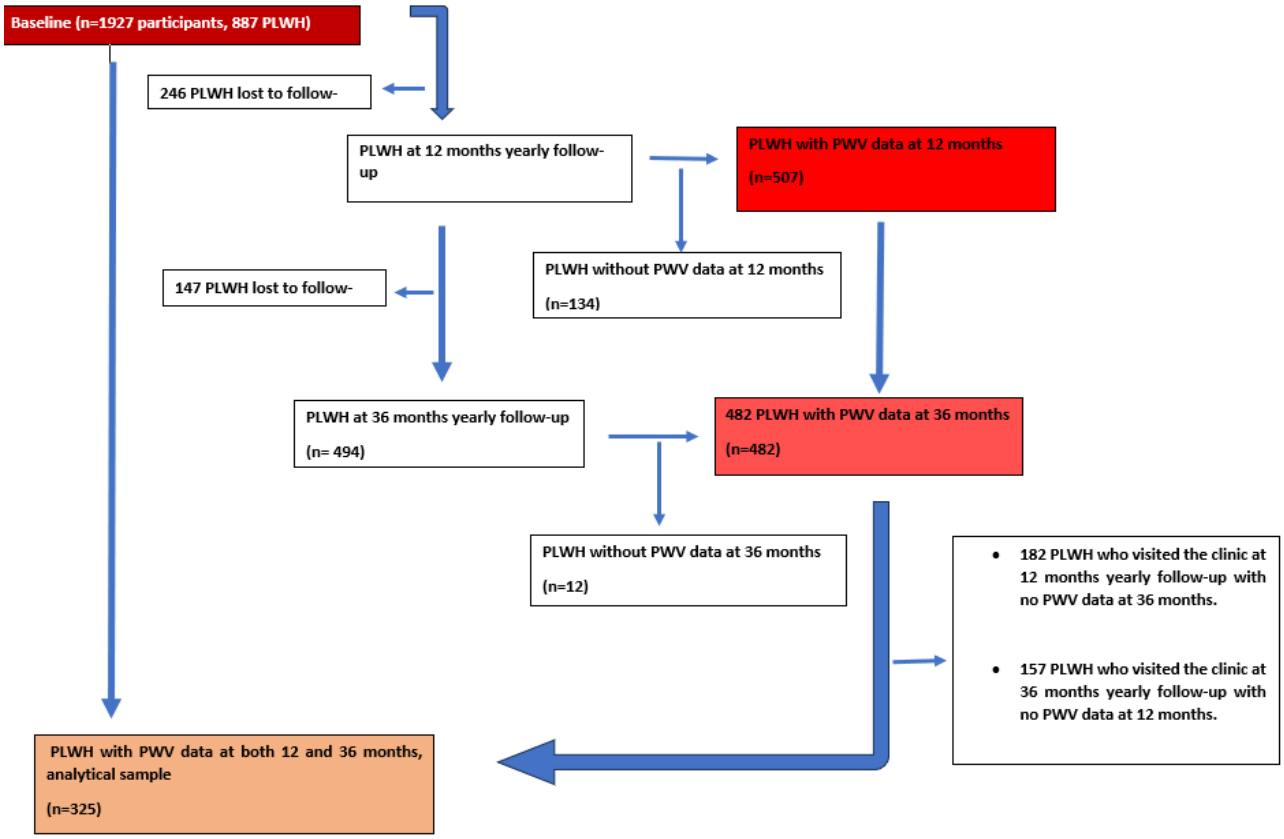

Figure 1: flow chart showing enrolment and screening of eligible participants for this study.

Data collection:

Socio-demographic and clinical characteristics

Questionnaires were used to capture information about stigma, social support, sexual relationships, ART adherence, socioeconomic factors (education, income, employment status) , health status and medical and family history of CVD, lifestyle (smoking, alcohol usage, diet) by trained Ndlovu research centre (NRC) staff at baseline and during yearly follow-ups ((26,28)).

Physical measurements

For physical measurements, trained NRC staff carried out all the physical measurements (blood pressure, pulse rate and anthropometric information) at baseline and follow-up visits. Height, weight, hip, and waist circumference measurements were taken in a standing position as per the standardised procedure. Blood pressure was measured in a sitting position after five minutes of rest with a sphygmomanometric device on both arms and repeated on the side with the highest values. The mean of all measurements was used in the analysis. For 12-36 months follow-up, the participants were invited to the NCR when the data was collected. At 48 months, half the cohort was followed-up through a phone, half in person at the study site.

Lipid and glucose measurements

Blood samples to measure lipid profiles and glucose plasma concentration were taken at each clinic visit. They were only assayed at the participant's enrolment.

Pulse wave velocity

Pulse wave velocity (PWV) measurements were performed at 12- and 36-months follow-ups. The participants were in a supine position for the PWV test. Three electrodes were attached to record the heart rhythm. Subsequently, the carotid-femoral distance was measured with a measuring tape. The value was then multiplied by 0.8(29–31). Quality was based on operator index(%SD), ECG tracing and standard deviation <10%. The tonometer was used to obtain a stable pulse waveform for 12 heartbeats on the right CCA. The same procedure was performed on the right femoral artery. The results of pulse waveforms that satisfy the quality requirements were stored after every arterial pulse measurement on the sphygmocor software (32–34). In this study, PWV, a surrogate marker of arterial stiffness/function was assessed from 12- and 36-months data to investigate CVD risk.

HIV-related stigma

A short 12-item HIV/AIDS stigma questionnaire/scale was used at the participants enrolment to assess thoughts and feelings of being stigmatised(35). The 12 questions were used to grade HIV-related stigma on 4-point Likert scale response (never=1, sometimes=2, often=3 and always=4). The scale response was transformed into 48 scores of overall stigma and considered as a continuous variable. A higher score indicated a stronger HIV stigma.

Viral load

Viral load (VL) was measured at baseline and during yearly clinic follow-up. VL > 1000 copies and low-level viremia (LLV) (<1000 copies)) was used as surrogate markers of ART adherence. Additionally, VL of greater than 1000 copies/ml was considered as very high-level viremia indicating no adherence. On the other hand, LLV was further sub-classified into 2 groups: **1**. Low viremia (50-1000 copies/ml) = suboptimal adherence and **2.** Undetectable viremia (<50copies/ml) = good adherence.

Ethical consideration:

Ethical approval was obtained from the Ethics Committee of the University of Pretoria (NO: 227/2014) and from the University of Witwatersrand (M230850). Informed consent was obtained from each of the participants prior to study inclusion. The questionnaires were anonymous, and participation was voluntary.

3. Data analysis

Statistical data analysis was performed using the R studio programme (R.4.2.2) (36,37). Basic descriptive statistics for the categorical variables (viral load, sex, ART and duration of diagnosed HIV infection, alcohol, and tobacco use) included frequency, percentage and for continuous variables (Age, PWV, stigma, diastolic and systolic blood pressure) it included mean and standard deviation for normal distribution. For skewed distribution, median and interquartile range were included. To assess the relationship between stigma and ART adherence=viral load, one-way ANOVA was performed with the dependent variable being overall stigma score and viral load status as the 3 groups (VL<50, 51-1000, and >1000 copies/ml). Bivariable models were run in R, on possible covariates and PWV at 12 and 36 months. Multicollinearity was assessed using a correlation matrix and variance inflation factor (VIF) between the independent variables. Subsequently, covariates significantly associated with PWV and not highly correlated with each other were included in successive models on outcome-explanatory variable relationships. Multiple linear regression was used to assess the association between the independent variables (stigma and ART adherence=viral load) and the dependent variable, PWV. Age, sex, systolic blood pressure (SBP), time on ART, smoking, and waist circumference were included as confounders/covariates in the multiple linear models. Model 1 had an overall stigma, and viral load as a categorical variable, with a viral load of <50 copies/ml as a reference (ref). In model 2_4, covariates were added where age (ref=18_29 yrs.), sex (ref=female), and smoking (ref=never smoked) were treated as categorical variables. Lastly, model 5 consisted of the independent variables of interest, covariates, and interaction terms between viral load and age, where viral load<50 copies/ml, and age 18_29 yrs., were considered as a ref. To respect the rule of parsimony in multilinear regression, we did not include in those models' variables which were p>0.05 in the 12-month bivariate analyses, or collinear with other variables.

To assess trends in PWV over time, mixed models using the restricted maximum likelihood method (REML) were fitted. Yearly-clinic visits (time), overall stigma, and viral load (surrogate marker of ART adherence) were specified as fixed effects in model 1. Subsequently, potential covariates such as age, sex, systolic blood pressure, time on ART, smoking, waist circumference were included (Mixed Models 2, 3, and 4). Additionally, interaction terms between age and viral load were added (Mixed Model 5). Yearly clinic visits were treated as a categorical variable with two time points, 12 and 36 months.

About 36% (182 of the 507) of PLWH at 12 months did not have PWV data at 36 months, and a total of 33% (157 of the 482) had PWV data at 36 months but not at 12 months follow-up. Complete case analysis was done for the PWV data, with 325 PLWH having PWV data at both 12- and 36-months follow-up. Of the 325 included participants in the study, the missingness on the variables (viral load and alcohol intake in the past 30 days) varied from 0.6 to 8.9%; namely BMI, glucose, LDL, duration of diagnosed HIV infection, and viral load. There was 8.9% missingness for viral load at 12 months,

and only 1.5% of the participants did not have viral load at 36 months. The missing data was regarded as missing at random (MAR), and multiple imputation was performed using multivariate imputation by chained equations (MICE) to generate 10 imputations and 10 iterations, Subsequently, imputation checks (convergence) were done to inspect the imputed dataset. The imputed dataset was then analysed and combined using Rubin's rules(38). PWV, viral load, BMI, glucose, LDL, waist circumference, systolic and diastolic blood pressure, age, sex, alcohol and tobacco use, 12-item stigma questionnaire, HIV and time on ART were included in the imputation model.

4. Results

The study consisted of 325 PLWH, of which 33% (108) were males. The median age was 41.0 (35.0-49.0) years at 12 months. The median systolic and diastolic blood pressure increased by 4.9mmHg and 6.8mmHg at 36 months compared to 12 months, respectively. Median waist circumference was 88.0 [80.0-90.0] cm at 12 months, and 86.0 [78.0-95.0]cm at 36 months. Most participants had never used tobacco and less than half had used alcohol at both time points (table 1).

Table 2 below shows the HIV characteristics of the participants at 12 and 36 months. The percentage difference of undetectable viremia (good adherence) between the two time points was 1%. About 5% people transitioned from high viremia to low viral load (ART suboptimal adherence) at 36 months. The percentage of people with high viral load (poor adherence) decreased from 15% at 12 months to 10% at 36 months. Overall stigma was low with a median of [20.0 (18.0-21.0). The pulse wave velocity median at 12 and 36 months was 7.3 (6.3-8.5) and 7.6 (6.7-8.4), respectively (table 2). There was no significant effect of overall stigma on ART adherence (viral load) at the $p>0.05$ for the 3 groups [$F(2, 322) = 0.59$, $p=0.55$].

Table 3 shows the results of the bivariate analysis at 12 and 36 months. Stigma was not associated with PWV (index of CVD risk) in the crude models at 12 months -0.07 ($p=0.17$) and 36 months -0.05 ($p=0.27$). At both 12- and 36-months, age [0.10 (0.08-0.12), $p<0.001$; 0.09 (0.07-0.10), $p<0.001$] and male sex [1.30 (0.87- 1.73), $p<0.001$; 0.83 (0.47-1.18), $p<0.001$**]** were positively related with PWV. Time on ART and duration of HIV infection were significantly positively associated with PWV at 12 months and 36 months. Furthermore, systolic, and diastolic blood pressure had significant positive association with PWV at both 12 months and 36 months. Smoking and/or tobacco use was significantly associated with PWV at both 12 and 36 months. Alcohol use was not associated with PWV at both 12 and 36 months follow- up. Glucose was significantly associated with PWV at both time points. LDL was not associated with PWV at 12 months 0.15 ($p=0.28$), however at 36 months, there was a positive association 0.23 ($p=0.03$). HDL was associated with PWV at 12 months ($p=0.01$), but not at 36 months. Lastly, triglycerides were significantly associated with PWV at both 12 months ($p=0.01$), and 36 months ($p=0.02$). Details are provided in table 3.

At 12 months, multivariable regression analyses on continuous variables of cardiovascular diseases risk (PWV) showed that HIV-related stigma was not associated with PWV, after adjustment of viral load, age, sex, systolic blood pressure, time on ART, smoking, and waist circumference [$\beta= -0.04$, $p=0.38$]. To examine the non-linear associations between PWV and viral load, regression models were estimated using dummy-coded variables for viral load, where undetectable viral load was used as reference. Low viremia (VL=50-1000) showed a significant positive association with PWV as compared to the undetectable viremia (VL<50) in models 2-4. High viremia was not significantly associated with PWV as compared to the undetectable viremia. Interaction terms between age (as factor with 3 levels) and

viral load to get a better understanding of the finding on significant association of low viremia and PWV, showed a positive significant association [β= 4.21, p=0.001] when compared to age group 1(18-29 years) * (undetectable viremia, VL<50).Low viremia was not significantly associated with PWV, whereas high viremia became significantly associated with PWV when an interaction term was added in model 5 (table 4).

To assess changes in arterial functions/PWV overtime (from 12 to 36 months), a mixed model was used. There was a significant increase in PWV 0.21 (p=0.04) between 12 and 36 months in a model with only overall stigma, and viral load. Overall stigma, and viral load (VL 50-1000 copies/ml, and >1000) were not associated with PWV. After adjusting for age and sex, clinic visit (time) was not associated with PWV. Age group (30-49 years, and 49 years) and male sex had a significant positive association with PWV in model 2-4 (p=0.05). Systolic blood pressure, and time on ART was positively associated with PWV in model 3 and model 4. Furthermore, waist circumference had a significant inverse association with PWV in model 3 and 4. Lastly, the interaction term between age, and viral load in model 4 did not show an association with PWV (table 5).

5. Discussion

The aim of this study was to investigate the association of stigma and ART adherence at baseline with arterial function among HIV-infected participants at 12 months and changes in arterial function (as measured by PWV) from 12 to 36 months with viral load (VL) > 1000 copies/ml or low viremia (VL 50-1000 copies/ml) considered surrogate markers for poor/suboptimal ART adherence. To the best of our knowledge this is the first study in Sub-Saharan Africa (and South Africa) to look at HIV-related stigma and ART adherence, on CVD risk. Previous studies focused on only HIV-stigma and its impact on ART adherence. Our findings showed that HIV-related stigma was low and not associated with CVD risk (PWV). Suboptimal adherence (low viremia) was associated with PWV, while poor adherence (high viremia) was not. In the multiple regression model with interaction, the results showed that suboptimal adherence (low viremia) in the age group >49 years and older was associated with higher PWV at 12 months. In models examining PWV changes over time (12-36 months), we observed a non-significant increase. This increase may be due to age, blood pressure or any other factors. HIV-related stigma and ART adherence were not associated with PWV. However, age, male sex, lifestyle factors (smoking), clinical characteristics such as systolic blood pressure, waist circumference, and time on ART were significantly associated with PWV.

Our findings are not consistent with previous studies(14,39). A south Asian study investigating HIV-stigma, perceived social support and risk of premature atherosclerosis on 119 participants cross-sectionally, found that PLWH felt lower perceived social support (14). Furthermore, they observed presence of high values of carotid intima media thickness (CIMT, a marker of subclinical cardiovascular disease) in HIV positive patients to be associated with increased HIV stigma. The low stigma in our study might be to a large part attributed to the extensive HIV education provided by the Ndlovu Care Group at Elandsdooorn, Limpopo, South Africa (40–43). HIV-related stigma has been linked with the misinformation about spread of the disease (44,45) and increased HIV education has been shown to decrease stigma (46,47). A cross-sectional study conducted in Zambia, and South Africa (39), assessing the relation between HIV stigma, and ART adherence is in alignment with our study findings, indicated that the South African population had low stigma and good ART adherence when compared to the Zambian population. The study further explains that HIV stigmatisation and its consequent negative impact on ART adherence might have been significantly minimised by strong history of community-based advocacy and awareness regarding HIV and HIV treatment in the South African context.

Like our study, other factors than HIV-stigma, such as male sex and systolic blood pressure were associated with CVD risk in the study by Bergman et al. (13). Other longitudinal studies conducted in South Africa, which were investigating CVD risk using carotid-femoral pulse wave velocity (cfPWV) and CIMT, in PLWH and controls, showed that there was no difference in either cfPWV or CIMT between both groups. The studies further demonstrated that age, and sex were positively associated with large artery stiffness/cfPWV (48,49). Similar to our findings, a cohort study in Namibia found that

duration on ART/on long-term ART was associated with greater arterial stiffness through increased carotid-femoral augmentation index (cfAiX), a marker of arterial stiffness(50). To date there are not many longitudinal studies on the topic, especially in South Africa.

Limitations and strength:

This study had some limitations. The stigma information was gathered through self-reports by use of questionnaires, so there may have been bias in reporting. Furthermore, stigma data was collected cross-sectionally, therefore may not give precise causal inference. Our sample size is likely under-powered to detect differences and may therefore result in underestimation of the causal inference and may not be generalisable to other Sub-Saharan African settings especially where HIV education is limited. Selection bias refers to a form of systematic error which arise due to the methods applied on selection of participants or by selective loss-to-follow up (36). In this study, non-response may have occurred in participants experiencing/in fear of stigma. When loss-to-follow up is related to both the exposure and the outcome it results in selection bias (37). However, in the NCS, a prospective study, the participants recruited did not have the outcome during the enrolment. Selection bias might also arise because PLWH experiencing high level of stigma, may not have enrolled in the NCS, therefore, the exposure-outcome relationship could have been underestimated. In our study, there was loss to follow, which may also have had an impact on the true association.

6. Conclusion and future directions.

Our analysis showed low HIV-related stigma in our population. The HIV-related stigma was not associated with the risk of CVD. Suboptimal adherence in those aged >49 years was significantly associated with PWV, suggesting age may have an impact on CVD risk meanwhile low viremia may still be triggering inflammation/increased immune activation in these elderly people. Presence of CVD risk may be related to complex interactions between sex, age, and other CVD risk factors. Longitudinal HIV stigma information would be important to learn about the true HIV-stigma PLWH face and to investigate the association of HIV-stigma, ART adherence and CVD risk.

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7. Conflict of interest

The author declares no conflict of interest.

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9. Appendices

Table 1: Demographics of the 325 participants living with HIV from the Ndlovu Cohort Study with full case pulse wave velocity data at 12 and 36 months.

Characteristics	12 months (N=325)	36 months (N=325)
Age, median (IQR), years	41.0 (35.0-49.0)	43.0 (37.0-51.0)
Men (n, %)	108 (33)	108 (33)
Women (n, %)	217 (67)	217 (67)
CARDIOVASCULAR MEASUREMENTS		
SBP (mm Hg)	113 (101.0-126.0)	118 (107.0-129.0)
DBP (mm Hg)	72.0 (66.0-81.0)	79.0 (72.0-87.0)
Pulse rate per min (bpm)	77.0 (67.0-80.0)	76.0 (69.0-84.0)
ANTHROPOMETRIC MEASUREMENTS		
BMI (kg/m^2) *	23.0 (20.0-26.0)	24.3 (20.5_28.4)
Waist circumference (cm)	88.0 (80.0-90.0)	86.0 (78.0-95.0)
LIFESTYLE FACTORS		
Alcohol use (n, %)		
Never	182 (56)	185 (57)
Ever	143 (44)	140 (43)
In the past 30 days	82 (57)	83 (59.2)
Tobacco use (n, %)		
Never	255 (78)	249 (77)
Current	70 (22)	76 (23)
OTHER CVD RISK FACTORS		
Glucose (mmol/L) *	4.7 (4.3-5.2)	
LDL (mmol/L)) *	2.1 (1.7-2.7)	
HDL* (mmol/L)	1.4 (1.2_1.7)	
Triglycerides* (mmol/L)	1.0 (0.7_1.40)	

***Abbreviations**: IQR, Inter Quartile Range; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LDL, low-density lipoproteins, CVD, cardiovascular disease**;** HDL**,** High density lipoproteins.*

Data is expressed as median (IQR) and count(percentage) unless stated otherwise.

** Indicates imputed value.*

Table 2: Clinical characteristics of the Ndlovu Cohort Study participants living with HIV with PWV at both 12 and 36 months.

	12 MONTHS (N=325)	36 MONTHS (N=325)
	HIV RELATED FACTORS	
VIRAL LOAD N (%) *		
<50	254 (78)	256 (79)
50-1000	23 (7)	37 (11)
>1000	48 (15)	32 (10)
DURATION OF DIAGNOSED HIV INFECTION*		
In Months Median (IQR)	72.0 (26.0-113.0)	96.0 (50.0-137.0)
TIME ON ART		
In Months (Median, IQR)	55.0 (15.0-106.0)	79.0 (39.0-130.0)
PULSE WAVE VELOCITY		
Median (IQR)	7.3 (6.3-8.5)	7.6 (6.7-8.4)
HIV-RELATED STIGMA		
Overall stigma Median (IQR)	20.0 (18.0-21.0)	

Abbreviations: *HIV, human immunodeficiency virus; IQR, Inter Quartile Range; ART, Antiretroviral therapy.*

Data is expressed as median (IQR) or count (%) unless otherwise specified.

**Indicates imputed data.*

Table 3: Bivariate analysis for study participants living with HIV at 12 and 36 months (outcome=PWV).

	12 months (N=325)		36 months (N=325)	
	β (95%CI)	**P-value**	**β (95%CI)**	**P-value**
Overall stigma	-0.07 (-0.17_0.03)	0.17	-0.05 (-0.13_0.04)	0.27
Viral load (copies/ml) *				
<50	REF	REF	REF	REF
50-1000	1.17 (0.30_2.05)	0.01	0.01 (-0.54_0.57)	0.96
>1000	-0.49 (-1.12_0.16)	0.14	-0.27 (-0.86_0.32)	0.37
Age (years)	0.10 (0.08_0.12)	<0.001	0.09 (0.07_0.10)	<0.001
Sex (ref= female sex)	1.30 (0.87- 1.73)	<0.001	0.83 (0.47_1.18)	<0.001
Time on ART (Months)	0.01 (0.005_0.013)	<0.001	0.01 (0.005_0.012)	<0.001
Duration of diagnosed HIV* infection (Months)	0.006 (0.002_0.010)	0.004	0.006 (0.002_0.009)	0.001
BMI (kg/m²) *	-0.04 (-0.08_-0.01)	0.02	-0.05 (-0.08_-0.02)	0.001
Waist circumference (cm)	-0.02 (-0.04_-0.004)	0.02	-0.006 (-0.02_0.008)	0.4
Systolic BP (mmHg)	0.03 (0.02_0.04)	<0.001	0.04 (0.02_0.05)	<0.001
Diastolic BP (mmHg)	0.05 (0.03_0.06)	<0.001	0.06 (0.04_0.07)	<0.001
Smoking				
Never	REF	REF	REF	REF
Ever	0.62 (0.11_1.14)	0.02	0.56 (0.16_0.97)	0.001
Alcohol				
Never	REF	REF	REF	REF
Ever	-0.003 (-0.43_0.43)	0.99	0.06 (-0.30_0.41)	0.75
Glucose (mmol/L) *	0.18 (0.00002_0.37)	0.05	0.17 (0.02_0.32)	0.02
LDL (mmol/L) *	0.15 (-0.12_0.41)	0.28	0.23 (0.02_0.44)	0.03
HDL	0.64 (0.11_1.16)	0.01	-0.10_0.76)	0.13
Triglycerides	0.34 (0.07_0.62)	0.01	0.26 (0.04_0.48)	0.02

Abbreviations: *CI, confidence interval; ART, Antiretroviral therapy; HIV, Human Immunodeficiency virus; BMI, body mass index; BP, blood pressure; LDL, low-density lipoproteins; HDL, high-density lipoproteins.*

** Indicates imputed data.*

Table 4: Association between HIV-related stigma, ART adherence on CVD risk (outcome=PWV) at 12-months.

	Model 1	Model 2	Model 3	Model 4	Model 5
	β (95%CI)	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
Overall stigma	-0.07 (-0.17_0.03)	-0.08 (-0.18_0.02)	-0.04 (-0.12_0.05)	-0.05 (-0.14_0.04)	-0.04 (-0.13_0.05)
VL<50 copies/ml		REF	REF	REF	REF
VL 50-1000 copies/ml		1.15 (0.28_2.02)	1.25 (0.52_2.00)	1.06 (0.37_1.84)	0.31 (-1.64_2.79)
VL>1000 copies/ml		-0.51 (-1.15_0.12)	-0.36 (-0.91_0.17)	-0.33 (-0.88_ 0.21)	-1.54 (-2.95_-0.13)
18-29 (in yrs.)			REF	REF	REF
30-49 (in yrs.)			0.96 (0.28_1.63)	0.81 (-0.13_1.50)	0.46 (-0.34_1.27)
>49 (in yrs.)			2.92 (2.16_3.69)	2.43 (1.60_3.27)	1.88 (0.95_2.81)
Female			REF	REF	REF
Sex (male)			0.89 (0.48_1.31)	0.65 (0.18_1.28)	0.59 (0.13_1.05)
Systolic BP (mmHg)				0.004 (-0.003_0.008)	0.01 (0.003_0.02)
Time on ART				0.02 (0.005_ 0.03)	0.004 (-0.0004_0.008)
Never smoked				REF	REF
Ever smoked				0.15 (-0.38-_0.67)	0.004 (-0.51_0.52)
Waist Circumference				-0.017 (-0.03_-0.002)	-0.02 (-0.03_-0.004)
Triglycerides				0.09 (-0.15_0.34)	0.14 (-0.11_0.37)
VL<50:18-29yrs.					REF
VL 50-1000: age 30-49yrs.					0.04 (-2.11_2.18)
VL>1000: age 30-49yrs.					1.39 (-0.17_2.17)
VL 50-1000: age>49yrs.					4.21 (1.64_6.79)
VL>1000: age>49					1.54 (-0.27_3.37)

Multivariable models at 12 months, outcome=PWV (CVD risk predictor)
Model 3: Independent variables of interest (Overall stigma, and Viral load) and covariates (sex, and age)
Model 4: Adjusted for sex, age, systolic BP, time on ART, smoking, waist circumference, and triglycerides.
Model 5: Covariates in model 3, and interaction term between age and viral load.
***Abbreviations:** CI, confidence interval; VL, viral load; BP, blood pressure; ART, Antiretroviral therapy.*

Table 5: Estimates of the fixed effects for the association of HIV-Related stigma, ART adherence and CVD risk at 12 and 36 months.

Pulse wave velocity			
		β (95%CI)	**P-values**
Model 1: Unadjusted			
Clinic visit	12 months	REF	REF
	36 months	0.21 (0.01-0.40)	0.04
Overall stigma		-0.06 (-0.14-0.02)	0.17
Viral load	VL<50 copies/ml	REF	REF
	VL 50-1000 copies/ml	0.24 (-0.20-0.68)	0.28
	VL>1000 copies/ml	-0.35 (-0.76-0.06)	0.09
Model 2: Adjusted (age and sex)			
Clinic visit	12 months	REF	REF
	36 months	0.10 (-0.09- 0.29)	0.31
Overall stigma		-0.03 (-0.11- 0.03)	0.29
Viral load	VL<50 copies/ml	REF	REF
	VL 50-1000 copies/ml	0.31 (-0.10-0.72)	0.14
	VL>1000 copies/ml	-0.27 (-0.64-0.10)	0.16
Age	18-29 yrs.	REF	REF
	30-49 yrs.	1.06 (0.56-1.56)	<0.001
	>49	2.54 (1.98-3.09)	<0.001
Sex	Female	REF	REF
	Male	0.76 (0.45-1.07)	<0.001
Model 3: Adjusted (age, sex, SBP, Time on ART, smoking, and waist circumference)			
Clinic visit	12 months	REF	REF
	36 months	-0.12 (-0.33_0.08)	0.24
Overall stigma		-0.03 (-0.10_0.03)	0.31
Viral load	VL<50	REF	REF
	VL 50-1000	0.28 (-0.11_0.68)	0.17
	VL>1000	-0.29(-0.65_0.07)	0.12

Age	18-29 yrs.	REF	REF
	30-49 yrs.	0.87 (0.38_1.35)	0.001
	>49	2.03 (1.47_2.59)	<0.001
Sex	Female	REF	REF
	Male	0.53 (0.21_0.84)	0.001
Systolic BP		0.02 (0.01_0.03)	<0.001
Time on ART		0.005 (0.002_0.008)	0.001
Smoking	Never	REF	REF
	Ever	0.20 (-0.13_0.52)	0.25
Waist circumference		-0.01 (-0.02_-0.004)	0.01
Triglycerides		0.12 (-0.06_0.29)	0.20
Model 4: Adjusted for model 3, and interaction term (viral load: age).			
Clinic visit	12 months	REF	REF
	36 months	-0.13 (-0.34_0.08)	0.23
Viral load	VL<50	REF	REF
	VL 50-1000	-0.03 (-0.10_0.03)	0.30
	VL>1000	-0.93 (-1.64_1.03	0.66
Age	18-29 yrs.	REF	REF
	30-49 yrs.	0.68 (-1.91_0.04)	0.07
	>49	1.75 (0.13_1.24)	<0.001
Sex	Female	REF	REF
	Male	0.51 (1.13_2.38)	0.002
Systolic BP		0.02 (0.20_0.82)	<0.001
Time on ART		0.01 (0.002-_0.008)	0.001
Smoking	Never	REF	REF
	Ever	0.17 (-0.16_0.50)	0.31
Waist Circumference		-0.01 (-0.02_-0.005)	0.004
Triglycerides		0.11 (-0.6_0.28)	0.22
Interaction terms	VL<50:18-29yrs.	REF	REF
	VL 50-1000: age 30-49yrs.	0.50 (-0.91_1.92)	0.49
	VL>1000: age 30-49yrs.	0.61 (-0.45_1.68)	0.27
	VL 50-1000: age>49yrs.	1.00 (-0.54_2.56)	0.21
	VL>1000: age>49	1.12 (-0.12_2.37)	0.08

Mixed models with random intercepts.
Model 2: Overall stigma, viral load, clinic visit, age, and sex.
Model 3: Overall stigma, and viral load, adjusted for clinic visit and covariates (age, sex, systolic blood pressure, time on ART, smoking, waist circumference, and triglycerides.
Model 4: model 3, and interaction terms (age: viral load)
Abbreviations: *CI, confidence interval; VL, viral load, BP, blood pressure; ART, antiretroviral therapy.*