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The Effect of HIV Infection on Glycaemic Control and Renal Function in Type 2 Diabetic Patients

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A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, in partial fulfillment of the requirements for the degree of Master of Medicine

Johannesburg, 2018

Declaration

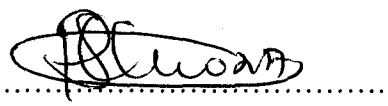
I Siyabonga Khoza declare that:

a) This Research Report is my own, unaided work.

b) It is being submitted for the Degree of Master of Medicine in the branch of Chemical Pathology at University of the Witwatersrand, Johannesburg.

c) It has not been submitted before for any degree or examination at any other university.

d) This research report does not contain other people's data, pictures, graphs or information, unless specifically acknowledged, and sources are listed in the referenced section.



(Signature of candidate)

06 day of September 2018

Abstract

Background: Infection with, and treatment of HIV is associated with effects on glycaemia and renal function. The purpose of this study was therefore to compare glycaemic control and renal function in HIV-positive and HIV-negative type 2 diabetic patients.

Methods: Diabetic patients with and without HIV infection were recruited from a diabetic clinic at Chris Hani Baragwanath Academic Hospital in Soweto, South Africa. Data were collected on weight, height, HbA1c, fasting glucose, urine albumin:creatinine ratio, HIV status, CD4 counts, viral load and concomitant therapies. Multivariable regression analysis was used to isolate the determinants of fasting glucose and HbA1c levels and risk factors for albuminuria.

Results: Data were collected from 106 HIV-positive and 214 HIV-negative diabetics. All HIV infected subjects were receiving anti-retroviral therapy. The determinants of fasting glucose levels (log) were HIV infection (β=0.04, p=0.01) and use of anti-hypertensive agents (β=0.07, p=0.0006), whilst for HbA1c levels (log) they were HIV infection (β=-0.03, p=0.03), BMI (β=0.004, p=0.0005), statin use (β=0.04, p=0.002) and glucose levels (β=0.01, p<0.0005). In HIV-positive subjects, CD4 counts was negatively associated with glucose levels (β=-0.0002, p=0.03). The risk factors for albuminuria were (odds ratio [95% CIs]) dyslipidaemia (1.94 [1.09, 3.44], p=0.02) and HbA1c levels (1.24 [1.12, 1.38], p<0.0001).

Conclusions: These data suggest that glycaemic control is poorer in type 2 diabetic subjects with HIV infection and that HbA1c underestimates glycaemia in these patients. Albuminuria was not associated with HIV-positivity. Improved immunological function would seem to enhance glycaemic control in diabetic subjects with HIV. Statins and anti-hypertensive therapy were associated with worse glycaemic control suggesting that care should be taken when choosing from among such agents for use in subjects with type 2 diabetes.

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Note on the Contents and the Format of this Research Report

This research report comprises of an article that has been submitted for publication in the journal *PLoS One*.

Before this research project was undertaken a research protocol was written describing the procedures to be used in this study and also including a review of the appropriate literature. This protocol was submitted to an assessor panel from the School of Pathology at the University of the Witwatersrand Faculty of Health Sciences, where it was reviewed and approved. All postgraduate students at masters and PhD level at the University of the Witwatersrand must go through this process before they can start their research projects. A copy of this protocol is included in the appendices of this research report.

The appendices also contain a copy of the ethics approval form for this project. The project was approved by the Ethics Committee (Human Research) of the University of the Witwatersrand.

The format of this research report is as stipulated by the Graduate Studies Committee of the University of the Witwatersrand Faculty of Health Sciences.

1. Introduction

Accessibility to highly active antiretroviral therapy (HAART) has dramatically improved the life expectancy of HIV-infected individuals, however studies have shown that this is associated with an increased prevalence of non-communicable diseases, including type 2 diabetes [1]. Thus, Brown et al. [2] observed that the prevalence of diabetes was up to four fold higher in HIV-infected men exposed to HAART compared to HIV-negative men. A recent meta-analysis confirms the strong association between the use of anti-retroviral therapy (ART) and increased risk for diabetes [3]. Furthermore, HIV-infected individuals have been found to have higher rates of metabolic syndrome, dyslipidaemia and lipodystrophy when compared to subjects without HIV infection [4].

The pathogenesis of type 2 diabetes in HIV-positive subjects involves several factors including the inflammatory response to the virus, co-infection and the use of HAART [5]. Not only does HAART contribute to the aetiology of diabetes but studies have also shown that HIV-infected diabetic subjects receiving HAART appear to have poor glycaemic control [6]. However, only a limited number of such studies investigating the effect of HAART on glycaemia have been undertaken.

Kidney dysfunction, including both acute and chronic renal failure, is a known complication of HIV infection. The aetiology of HIV-associated acute kidney disease is thought to involve the virus itself, co-morbid diseases or infections and anti-retroviral therapy [7]. Thus, acute renal failure has been linked to the use of indinavir and tenofovir, and can progress to chronic kidney disease if not treated [8, 9]. Indinavir is associated with nephrolithiasis, papillary necrosis, and dysuria [8, 10] while tenofovir has been implicated in tubular toxicity and Fanconi syndrome

[11]. With regard to chronic kidney disease (CKD), the prevalence of this disorder has been reported to be between 6.0 - 45% in HIV infected, ART-naïve subjects in sub-Saharan Africa [12]. The development of CKD in those infected with HIV is thought to be mediated by viral proteins, host genetic variants, and environmental factors [7].

The association of poorly controlled type 2 diabetes with kidney disease [13] suggests that HIV infection in diabetic subjects may further increase the risk of renal dysfunction. Very few studies have actually measured the prevalence of kidney disease in diabetic subjects with HIV however, 2 studies have shown that albuminuria was more prevalent in such subjects when compared to diabetic subjects who were HIV-negative [14,15].

It is therefore clear that HIV infection and its therapy may lead to both poor glucose control and an increased risk of kidney dysfunction in subjects with type 2 diabetes. This overlap of diseases and the resulting pathology is of significant concern in South Africa where both type 2 diabetes [16] and HIV infection [1] are common. Indeed, South Africa has the highest number of subjects living with HIV infection, with a national prevalence level of 12.2% [1]. In addition, a national survey has shown that the prevalence of type 2 diabetes in South Africa is 9.5% [16]. The aim of the present study was therefore to compare glycaemia and renal function in African type 2 diabetic patients with and without HIV infection, and to isolate the determinants of fasting glucose and HbA1c levels and albuminuria in this population.

2. Materials and methods

2.1 Study population

The study was conducted at Chris Hani Baragwanath Academic Hospital diabetic clinic. The hospital is the referral centre for clinics in Soweto and surrounding communities. Consecutive type 2 diabetic patients of both genders were recruited over a period of 4 months. Patients above 30 years were included in the study while pregnant women and non-diabetic subjects were excluded from the study.

2.2 Data collection

All participants provided written informed consent before any study procedures were conducted. A data sheet was used to collect information from the patient and also from the patient's file. During clinic visits fasting glucose, HbA1c and urine albumin:creatinine ratio were measured using point-of-care (POC) devices.

2.3 Diabetes mellitus diagnosis

Diabetes was defined from self-report of ever being diagnosed with diabetes, and documentation that the patient was receiving anti-diabetic medication based on information in the patient files.

2.4 HbA1c, urine albumin:creatinine ratio and glucose measurements

The HbA1c and urine albumin:creatinine measurements were performed using a Siemens DCA Vantage analyzer (Tarrytown, NY, USA), which is a cartridge-based analyzer. The DCA Vantage HbA1c assay method is based on a latex immunoagglutination inhibition methodology and it is certified by the National Glycohemoglobin Standardization Program (NGSP). The DCA

Vantage has been assessed according to NGSP-certified HbA1c POC device guidelines and was found to have a 2.3% coefficient of variation (CV) at HbA1c levels of 5.26-5.34%, a CV of 2.5% over a HbA1c range of 6.10-6.18%, and a CV of 2.7% in the 8.01-8.09% range [17] and a CV of 3.1% for values of 9.5% [18]. The assay has a reportable range from 2.5-14.0%.

The DCA Vantage albumin:creatinine assay is a quantitative method measuring albumin, and creatinine and reports an albumin:creatinine ratio. Urine albumin is linear between 5 to 300 mg/l and for creatinine linearity is between 1.3 to 44.2 mmol/l. The albumin:creatinine measurement covers the following range: 0.11 to 226 mg/mmol. Evaluation of the performance of this point-of-care (POC) device in terms of measurement of urine albumin, creatinine and albumin:creatinine ratio against a laboratory-based method demonstrated excellent correlations (R^2=0.989, 0.987 and 0.991, respectively) with total imprecision of < 8.7%, and an inter-and intra-day imprecision of < 2.9% [19]. Albuminuria was used as a surrogate marker for nephropathy, and was defined as a urine albumin:creatinine ratio of > 2.5 mg/mmol for men and >3.5 mg/mmol for women [20].

Fasting glucose levels were measured using a point-of-care device, the Accu-Chek Active glucometer (Roche Diagnostics, Mannheim, Germany). It has been noted that this POC instrument has an imprecision of less than 5.5%, and based on a Clarke error grid analysis it demonstrated adequate clinical accuracy [21].

Quality control and calibration of the analysers were performed prior to measuring the patients' samples according to the manufacturer's recommendations.

2.5 Other variables

Further data were obtained from the patient's files and included information on the use of ARTs, therapies for diabetes, dyslipidaemia and hypertension, CD4 counts and viral loads, duration of diabetes, duration since diagnosis of HIV, duration of ART and smoking. Hypertension was defined based on a physician's diagnosis documented in the patient's files and/or self-reported use of anti-hypertensive agents. Dyslipidaemia was defined as a self-reported history of use of lipid lowering agents and/or a diagnosis made by a physician and noted in the patient's file. Weight and height measurements were recorded at the study visit.

2.6 Statistical analyses

Data with a normal distribution were expressed as mean ± standard deviation in tables and text whilst data that is non-parametric is expressed as median (interquartile range). The χ^2 test was used to compare categorical variables between groups whilst the Student non-paired t test was used for continuous variables following log transformation if required.

Multivariable linear regression models were developed to find the principal determinants of glucose and HbA1c levels and albumin:creatinine ratio (dependent variables). Univariate regression analyses were performed to determine the relationship of each study variable (independent variable) with each of the 3 dependent variables. Independent variables that correlated with the dependent variable at $p \leq 0.20$ were included in the multivariable model. Backward, stepwise removal of non-significant variables was then performed until a final model was obtained in which all remaining independent variables correlated with the dependent variable at $p<0.10$. Collinearity was assessed in all the initial multivariable models using the

variance inflation factor (VIFs). Any variable with a VIF≥5 was removed from the model until all variables had VIF<5.

The sample size for this study was calculated based on the use of multivariable linear regression models for identifying the principal determinants of glucose and HbA1c levels in diabetic subjects with and without HIV infection. Assuming a maximum of 12 independent variables in the initial regression models, with a statistical power of 90%, a p-level of 0.05 and an effect size of 0.10, the estimated sample size was 230. We chose to use a sample size greater than this (N=320) to ensure the study was fully powered to achieve its aims.

3. Results

3.1 Comparison of HIV-positive and HIV-negative subjects

The HIV-negative subjects were older ($p<0.005$), had a significantly higher BMI ($p<0.005$), and a longer duration of diabetes ($p<0.05$) than the HIV-positive subjects (Table 1). The higher HbA1c levels in the HIV-negative group ($p<0.05$) were found to be due to higher BMI and age as adjusting for either of these variables in an ANCOVA attenuated the p-value ($p=0.21$ and $p=0.13$, respectively). All subjects were receiving metformin in combination with insulin and/or sulphonylureas. The majority of the HIV-positive patients were receiving an ARV triple therapy of tenofovir, lamivudine, and efavirenz, with a very small number of subjects receiving stavudine or zidovudine. Fewer of the HIV-positive subjects were being treated for hypertension ($p<0.005$) when compared to the HIV-negative subjects, but this relationship became non-significant after adjusting for age ($p=0.24$). No difference in the prevalence of albuminuria was noted between the groups.

Table 1. Comparison of HIV-positive and HIV negative subjects

Variable	HIV-negative	HIV-positive
N	214	104
Females (%)	65.4	63.5
Age (years)	59.7 ± 9.83	53.9 ± 8.5**
BMI	33.1± 6.18	30.4 ± 5.04**
Smoker (%)	22.9	20.6
Diabetes duration (years)	13.3 ± 4.53	11.9 ± 5.04*
Diabetes therapy: Metformin (%) Insulin (%) Sulphonylurea (%)	 100 91.1 31.8	 100 87.8 32.7
Lipid therapy: Statins (%) Fibrate (%)	 64.0 3.27	 57.9 1.90
Use of anti-hypertensives (%)	82.2	67.3**
Anti-retroviral therapy: Tenofovir (%) Lamivudine (%) Efavirenz (%) Stavudine (%) Zidovudine (%)	 - - - - -	 86.0 87.8 87.8 0.93 0.93
Duration of ART (years)	-	5.35 ± 4.19
CD4 count (cell/mm^3)	-	633 ± 215
Viral load (copies/ml)	-	46.0 (0.50,111)
Viral load < 40 copies/ml (%)	-	48.8
HbA1c (%)	8.70 (7.20, 11.0)	8.20 (7.10, 10.0)*
Glucose (mmol/L)	8.20 (6.80, 10.0)	8.80 (6.90, 11.2)
Albuminuria (%)	31.3	35.2

Data expressed as mean ± SD, median (interquartile range) or %; *p<0.05, **p<0.005 vs. HIV-negative

3.2 Multiple linear regression models for glucose and HbA1c

Stepwise, backward multivariable linear regression analysis demonstrated that HIV infection and the use of anti-hypertensive therapy were both independently associated with higher glucose levels (Table 2). Thus, HIV-positive subjects had glucose levels that were 4.0% higher than in HIV-negative subjects and subjects receiving anti-hypertensive therapy had glucose levels that were 7.0% higher than those not receiving such therapy (Table 2). With regards to HbA1c levels, BMI and use of statins were both independently associated with higher HbA1c levels. This, a 1 unit increase in BMI was associated with a 0.40% increase in HbA1c, whilst subjects on statins had a 4.0% higher HbA1c level than those not taking statin therapy (Table 2).

Table 2. Multiple linear regression models for glucose and HbA1c

Dependent variable	Independent variables with β-coefficient[a] (p-value)	Adjusted R^2 (p-value)
Log glucose (mmol/L)	HIV-positive: 0.04 (0.01) Use anti-HT: 0.07 (0.0006)	0.04 (0.0005)
Log HbA1c (%)	BMI: 0.004 (0.0005) Use statins: 0.04 (0.002)	0.06 (<0.0005)

Anti-HT=anti-hypentensive therapy; [a]unstandardised β-coefficient

These data demonstrate that glucose levels are determined by HIV status, and therefore we repeated the multivariable linear regression analyses for both glucose and HbA1c in HIV-negative and HIV-positive subjects. The results of these analyses are shown in Table 3. In terms

of glucose levels, within the HIV-negative group only anti-hypertensive therapy was associated with glucose, with those using such therapy having a 6.30% higher glucose level than non-hypertensive subjects. In the HIV-positive group, BMI and CD4 counts were both associated with fasting glucose levels. Thus, a 1 unit increase in BMI was associated with a 0.60% increase in the glucose level, however this relationship just missed statistical significance (p=0.084). A 1 unit increase in CD4 count was associated with a 0.02% fall in glucose concentration. With regard to HbA1c, in the HIV-negative group BMI, glucose and statin use were all correlated to this marker of glycaemia. Thus, a 1 unit increase in BMI was associated with a 0.30% rise in HbA1c, a 1 mmol/L increase in glucose was associated with a 1.40% rise in HbA1c and statin use was associated with a 4.00% higher HbA1c level. Within the HIV-positive group, gender, BMI, glucose and duration of HIV infection were all associated with the HbA1c level. Thus, females had a HbA1c level that was 4.00% lower than in males, a 1 unit increase in BMI was associated with a 0.40% rise in HbA1c, a 1 mmol/L increase in glucose was associated with a 0.90% rise in HbA1c and a 1 year higher duration of HIV infection was associated with a 0.30% increase in HbA1c. This last association just missed statistical significance (p=0.074).

Table 3. Multiple linear regression models for glucose and HbA1c in HIV-negative and HIV-positive subjects

Dependent variable	HIV status	Independent variables with β-coefficient[a] (p-value)	Adjusted R^2 (p-value)
Log glucose (mmol/L)	HIV-negative	Use anti-HT: 0.063 (0.012)	0.025 (0.012)
	HIV-positive	BMI: 0.006 (0.084) CD4 counts: -0.0002 (0.031)	0.050 (0.028)
Log HbA1c (%)	HIV-negative	BMI: 0.003 (0.022) Glucose: 0.014 (<0.0005) Use statins: 0.040 (0.009)	0.179 (<0.0005)
	HIV-positive	Gender: -0.040 (0.050) BMI: 0.004 (0.047) Glucose: 0.009 (0.0005) HIV duration: 0.003 (0.074)	0.185 (<0.0005)

Anti-HT=anti-hypentensive therapy; [a]unstandardised β-coefficient; gender was coded as females=1 and males=0

3.3 Use of statins and anti-hypertensive agents

Due to the effects of statins on HbA1c and anti-hypertensive (HT) agents on glucose levels, these drugs were analysed in more detail. Patient files showed that simvastatin was the only statin type used in this population whereas 8 different types of anti-HT agents were used. The main agents used for treating hypertension (and their % use in subjects with hypertension) were: the diuretic, hydrochlorothiazide (38.6%); the ACE inhibitor, enalapril (45.3%); the calcium channel antagonist, nifedipine (25.6%). All these agents were used in combination with each other and with other anti-HT drugs. Due to the reported elevation of glucose levels by thiazide diuretics

[22], these agents were compared against all other anti-HT drugs for their effects on glucose. Thus, in non-hypertensives, the median (interquartile range) fasting capillary glucose level was 7.10 (6.25, 9.30) mmol/L, in those using hydrochlorothiazide it was 8.65 (6.90, 11.0) mmol/L (p=0.028 versus non-hypertensives) and in those using all other forms of anti-HT therapy it was 8.80 (7.10, 10.5) mmol/L (p=0.033). Due to the large number of drug combination therapies (12) used in the non-thiazide group, we were not able to determine which individual therapies were the main contributors to the higher glucose level. No significant effects were observed for the anti-HT drugs on HbA1c levels.

3.4 Multiple logistic regression models for albuminuria

The data in Table 4 show the results of backward, stepwise multivariable logistic regression analysis of albuminuria risk. The results demonstrate that gender, dyslipidaemia and HbA1c levels are all independent risk factors for albuminuria. Thus, the risk for albuminuria is 48% lower in females than males, and the risk of albuminuria is 94% higher is subjects with dyslipidaemia than in those with normal lipid levels. In addition, a 1 unit increase in HbA1c levels is associated with a 24% increase in the risk of albuminuria (Table 4).

Table 4. Multiple logistic regression model for albuminuria

Dependent variable	Independent variables with odds ratio (95% CIs) and p-value
Albuminuria	Female gender: 0.52 (0.31, 0.89); 0.02 Dyslipidaemia: 1.94 (1.09, 3.44); 0.02 HbA1c: 1.24 (1.12, 1.38); <0.0005

4. Discussion

The data from this study show that HIV-positive diabetic subjects had higher glucose levels than HIV-negative subjects whilst HbA1c levels were lower in the former group after adjusting for possible confounding factors. Furthermore, within the HIV-positive group higher CD4 counts were associated with lower glucose levels. The use of anti-hypertensive agents was found to be associated with poorer glycaemic control whilst the use of statins was linked to higher HbA1c levels. Kidney function, as assessed via albuminuria, was not different between the HIV-positive and HIV-negative diabetic groups. Albuminuria was found to be more prevalent in subjects with dyslipidaemia and in those with higher HbA1c levels.

This study has demonstrated that in diabetic subjects lower HbA1c levels are observed in HIV-positive compared to HIV-negative subjects, but higher glucose levels in the former compared to the latter group. This has also been observed in other studies [23-25], only 1 of which was undertaken in diabetic subjects [24]. These data demonstrate that HbA1c measurement in HIV-positive diabetic subjects underestimates the level of glycaemia. Whilst HbA1c is an excellent tool for monitoring glycaemic control, there are several factors that can affect its level by influencing the lifespan of red blood cells. These factors include iron/vitamin B12 deficiency, chronic renal failure, splenectomy, splenomegaly, increased haemolysis, cirrhosis and drugs such as ribavirin and dapsone [26]. It has been suggested that in HIV positive subjects, the lower HbA1c levels may be as a result of a faster turnover of red blood cells due to increased haemolysis [23]. The haemolysis may be due to the use of ART, and a number of studies have shown that the discrepancy between glucose and HbA1c levels is related to such therapy [23-25]. Furthermore, a meta-analysis of studies conducted in sub-Saharan Africa has shown that ART-

use is associated with lower HbA1c measurements [27]. It is therefore advised that fasting glucose measurements be used for the diagnosis of diabetes in HIV positive subjects [28], and this is in line with the American Diabetes Association guidelines which discourage the use of HbA1c as a diagnostic or a glycaemia monitoring tool in HIV infected subjects [29].

The current study demonstrated that glucose levels were higher in diabetic subjects with HIV infection when compared to those who were not infected. A recent South African study also demonstrated poor glycaemic control in diabetic patients living with HIV [6], using data collected from patient files. A study from the USA showed that serum glucose levels were higher in diabetic, HIV infected subjects compared to those without HIV but the difference missed statistical significance ($p=0.09$) [24]. Furthermore, a longitudinal investigation demonstrated that newly diagnosed diabetic subjects with HIV, when initiated onto anti-diabetic therapy showed a poorer improvement in glycaemic control than a group of HIV negative subjects [30]. However, a further study showed no difference in fasting glucose levels between female diabetic subjects with or without HIV infection [31], whilst a study from Malawi demonstrated lower fasting glucose levels in HIV-positive compared to HIV-negative diabetic subjects [14]. It can therefore be seen that there are only a few studies that have analysed the effect of HIV infection on glycaemic control in type 2 diabetic subjects. The majority of these studies suggest that HIV infection is associated with poorer glycaemic control. It has been suggested that this is due to the effects of ART [30], and it is interesting to note that in the study showing lower glucose levels in HIV-positive compared to HIV-negative diabetic subjects, only 16.9% (11 of 65) were receiving ART [14].

We were able to show that within the diabetic subjects with HIV infection, the CD4 count correlated negatively with glucose levels. A similar association has been observed in a previous study, which was conducted in non-diabetic subjects [32]. These data suggest that improved immune system functionality may enhance glycaemic control, which may be mediated by a reduction of the viral load and/or attenuation of the inflammatory response to viral infection. It is known that inflammation may play an important role in the aetiology of type 2 diabetes [33]. Conversely, the use of ART can give rise to immune reconstitution inflammatory syndrome (IRIS) in a sub-population of HIV infected subjects [34]. The effect of IRIS on the development of type 2 diabetes in subjects with HIV infection has not been investigated, but should be the focus of future studies. The present study also demonstrated that in HIV-positive subjects a longer duration of HIV infection was associated with higher HbA1c levels. However, this association was very weak.

The use of anti-HT agents was associated with higher glucose levels whilst statin use was associated with higher HbA1c levels. Eriksson et al found that higher glucose levels were associated with hydrochlorothiazide therapy compared to placebo in hypertensive subjects [35], whilst a meta-analysis has shown a significant glycaemic effect of both thiazide diuretics and non-selective beta blockers in subjects with diabetes [22]. The present study also demonstrated an association of both thiazide and non-thiazide therapy with higher glucose levels. With regard to statin therapy, Liew et al reported an increase in HbA1c levels in patients receiving simvastatin regardless of the presence of diabetes [36]. This parallels the current study where simvastatin use was associated with higher HbA1c levels. In addition, a meta-analysis has demonstrated a modest increase of HbA1c in diabetic patients receiving statin therapy [37].

The current study demonstrated that albuminuria was not related to HIV infection. However, it must be noted that only 2 previous studies have investigated this relationship [14,15], both of which showed a higher prevalence of albuminuria in diabetic subjects that were HIV-positive. One of these studies concluded that albuminuria was related to the use of abacavir [15], whilst the other study involved HIV-positive subjects of whom only 16.9% were receiving ART [14]. Within the current study, all HIV-positive subjects were receiving ART but none were using abacavir. These data suggest a complex interaction between HIV infection, its therapy and kidney function, with lack of ART and use of particular anti-retroviral agents both being related to albuminuria. The present study showed that albuminuria was associated with the presence of dyslipidaemia and with raised HbA1c levels. Similarly, a study from Korea demonstrated that dyslipidaemia was associated with an increased risk of albuminuria in pre-diabetic female subjects [38]. Furthermore, it is known that kidney disease can lead to dyslipidemia which in turn can contribute to the development of diabetic nephropathy [39].

Limitations of this study include the use of a single urine albumin-creatinine ratio as a surrogate marker of renal function. In addition the use of POC devices for measuring glucose, HbA1c and the albumin-creatinine ratio may be considered a drawback, however such devices are increasingly being used in both clinical and epidemiological studies and show good agreement with laboratory-based methodologies [17,19,21]. A number of variables in this study were based on data taken from patient files and this may limit the conclusions that can be drawn when using such information. All HIV-positive subjects were receiving ART and therefore we were not able to discern whether any differences between subjects with and without infection were due to the virus or its therapy. Lastly, this study was conducted in a single out-patient clinic in an urban setting and our findings may therefore not be applicable to all HIV-positive diabetics within

South Africa. The strengths of this study include the assessment of a broad range of appropriate variables and that it was sufficiently powered to detect differences in glucose and HbA1c levels between the 2 study groups. Furthermore, this is the first cross-sectional study to specifically assess glycaemic control and kidney function in sub-Saharan African diabetic subjects with HIV infection and receiving HAART.

In conclusion, these data suggest that within this population of type 2 diabetic subjects those receiving statin and/or anti-hypertensive therapy should be regularly monitored with regards glycaemic control and if necessary their therapies switched to those that have less detrimental effects on glycaemia. Furthermore, glycaemic control could be improved in HIV-positive diabetic subjects by improvements in CD4 counts. Further studies are required to determine the effect of HIV infection and its therapy on kidney function in type 2 diabetic patients. Due to the high prevalence of both HIV infection and type 2 diabetes in sub-Saharan Africa it is important that future studies further investigate the clinical consequences of the overlap of these 2 epidemics and put in place programs to help ameliorate these effects.

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Research Protocol

The effect of HIV infection on glycaemic control and renal function in type 2 diabetic patients

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INTRODUCTION

South Africa is in the midst of two epidemics of HIV and diabetes. The national prevalence for HIV infection is 12.2%[1], whilst that for type 2 diabetes mellitus (T2DM) is 9.50%[2]. The high

prevalence of T2DM is being driven by the high rates of obesity. In addition, infection with HIV and its therapy have been implicated with renal disease[3] and glucose intolerance[4]. It is therefore feasible that due to the high prevalence of both HIV and T2DM in South Africa that the disease overlap is high and that HIV infection in T2DM may worsen both glycaemic control and kidney function. However, no prospective studies have been undertaken to test this hypothesis.

LITERATURE REVIEW

HIV and glycaemic control

The prognosis for subjects infected with HIV is continuously improving. This is due to improved testing procedures, earlier diagnosis and accessibility to better methods of therapy[4]. This has helped to improve the survival of these patients, and this has translated into an increase in the chronic complications related to the HIV itself or antiretroviral therapy (ART) as well as increasing prevalence of chronic diseases of aging[5].

Current recommendation for first line HIV regimens in South Africa includes tenofovir (TDF) plus emitricitabine or lamivudine and efavirenz in adults[6]. Brown et al has shown that diabetes can be up to four fold more common in HIV infected men exposed to highly active antiretroviral therapy (HAART) compared to HIV negative men[7]. The introduction of HAART has significantly improved the outcomes of HIV infected patients, but is also associated with metabolic dysfunction. Lipodystrophy is the major metabolic abnormality observed. It is characterized by the presence of insulin resistance, abnormal lipid profiles, central fat (visceral) accumulation and peripheral fat wasting. The aetiology of type 2 diabetes in subjects with HIV infection is not fully understood, and there is no evidence linking HIV infection directly to the development of diabetes[8]. However, it has been suggested that the increased visceral fat

accumulations seen with HAART creates higher systemic levels of inflammatory cytokines such as tumour necrosis factor α (TNFα) and consequently leads to diabetes or impaired glucose tolerance by increasing the levels of insulin resistance[7,8].

Other mechanisms for the aetiology of ART-related dysglycaemia have been suggested. Thus, some classes of anti-retrovirals (ARVs) have been linked with an increased likelihood of developing diabetes in HIV infected individuals[9]. These include protease inhibitors (PIs). This group of ARVs interferes with GLUT-4 mediated uptake which is a rate limiting step in glucose uptake in adipose tissue and skeletal muscle, thus resulting in insulin resistance. In addition, PIs have been shown to have varied metabolic effects. Thus, indinavir induces insulin resistance with no effect on lipid profiles whilst other agents such as ritonavir increases fasting triglyceride and free fatty acid levels but seem not to worsen insulin sensitivity. Stavudine, which is a nucleoside reverse transcriptase inhibitor (NRTI) is associated with the highest risk of diabetes[9].

Based on the link between HIV and diabetes, the American Diabetes Association (ADA) 2016 guidelines state that fasting glucose and lipid levels (cholesterol and triglycerides) should be tested in HIV positive individuals prior to starting ARV therapy and that screening tests for diabetes should be performed 3 months after starting HAART[10]. In HIV-negative subjects it is recommended that HbA1c be used for long term monitoring of diabetes control, however it is unreliable in HIV-infected individuals due to the faster turnover of red blood cells. In view of this limitation, ADA recommends fasting blood glucose as a preferred tool for the diagnosis and monitoring of glycaemic control in diabetic patients with HIV infection[10].

Not only does ART contribute to the aetiology of type 2 diabetes but the use of PIs and NRTIs by HIV-infected type 2 diabetic patients may theoretically pose a challenge in achieving adequate glycaemic control. However, very few studies of glucose levels within HIV-positive

compared to HIV-negative diabetic patients have been performed. Thus, Satlin et al found that the prevalence of inadequate glycaemic control among HIV-infected patients with diabetes mellitus was 33%, which was comparable to that in HIV-negative subjects stated in the literature[11]. Poor glycaemic control was associated with all forms of anti-diabetic therapy. However, the study by Satlin et al did not include a group of HIV-negative diabetic patients for comparison. In a South African study, HIV-infected diabetic patients were found to have worse control of glucose concentrations compared to HIV-negative diabetic patients[12], but all data was taken from patient files.

HIV and renal function

Chronic kidney disease is defined as the presence of abnormalities of kidney structure or function for a duration of greater than 3 months. It is generally accepted that glomerular filtration rate (GFR) is the best marker of renal function. A GFR <60 ml/min/1.73m^2 indicates reduced kidney function and GFR <15ml/min/1.73m^2 is regarded as kidney failure. Albuminuria is defined as the presence of albumin in the urine, which can be seen in normal individuals but is found in high concentrations in patients with kidney disease. Albuminuria is used as a surrogate marker of kidney function. An albumin: creatinine ratio (AER) of ≥ 3mg/mmol for > 3 months is associated with renal dysfunction[13].

Kidney disease is a recognized complication of both HIV/AIDS and diabetes. The aetiology of diabetes associated nephropathy has been well studied and shows that the metabolic and haemodynamic abnormalities seen in diabetes may interact with each other, possibly through shared cell signaling pathways, leading to the enhanced production of reactive oxygen species (ROS)[14]. The consequences of this process are the structural and functional changes seen in

diabetic nephropathy. With HIV infection, the aetiology of kidney disease may be due to the virus itself, co-morbid disease/infections or as the result of therapy[3]. The prevalence of chronic kidney disease (CKD) in HIV-infected ARV-naïve subjects in sub-Saharan Africa is reported to be between 6.0 – 45.0%[15]. In South Africa, 3 screening studies have indicated that HIV-associated nephropathy (HIVAN) is present in 5.0 - 83.0% of patients using biopsy findings[13]. Most cases of HIVAN require renal biopsy to make a diagnosis and exclude other causes such as non-HIV related renal diseases and the use of nephrotoxic medications[16].

Kidney biopsies of HIV-infected individuals with CKD have predominantly reported HIV-immune complexes. The explanation of such findings include abnormal immune responses secondary to viral infection or responses to co-infections[17]. Development of CKD is mediated by factors related to viral proteins, genetic predisposition, and environmental factors. In mice models, expression of the viral proteins *vif, nef, tat, vpr*, and *rev* in renal podocytes leads to many of the clinical features of HIVAN[3]. Genetic predisposition to HIVAN in individuals of African descent has been linked to variants in the APOL1 gene. This gene accounts for an increased risk of HIVAN in subjects of African descent[3].

The impact of HAART on kidney function includes both the benefit on HIV disease progression and HIVAN, however there are potential adverse renal effects associated with exposure to HAART. Tenofovir (TDF) is a nucleoside reverse transcriptase inhibitor which is considered as one of the first line agents for HAART[18]. There are reports that tenofovir may cause acute nephrotoxicity which may progress to CKD if not treated. The mechanism of tenofovir-associated acute nephrotoxicity is not clear but it is suggested that it may interfere with mitochondrial function in the renal tubules[19].

Very few studies have compared kidney function in diabetic patients with or without HIV infection. However, one study which used albuminuria as a marker of kidney damage found an increased prevalence of albuminuria in HIV infected adults with diabetes compared to adults with either diabetes or HIV. This study therefore demonstrates that HIV-infected diabetic subjects may have added risk for renal dysfunction[20].

AIMS and OBJECTIVES

The aim of the study is to determine the effect of HIV on glycaemic control and renal function in type 2 diabetic patients.

The study objectives are:

- To recruit HIV-positive and HIV-negative diabetic subjects from the diabetic outpatients clinic at Chris Hani Baragwanath Academic Hospital
- To compare fasting blood glucose and HbA1c levels between these 2 groups.
- To compare urine albumin-creatinine ratio levels between these 2 groups
- To find the principal determinants of glucose, HbA1c and albumin-creatinine ratios in the full cohort using multivariable regression analyses

METHODS

Recruitment of study subjects

Consecutive, consenting type 2 diabetic patients of both genders will be recruited into this study from the Diabetic Clinic at Chris Hani Baragwanath Academic Hospital. Recruitment will be done on clinic days when type 2 diabetic patients attend for follow-up visits.

Blood measurements and viral status

During clinic visits fasting glucose, HbA1c and urine albumin:creatinine are routinely measured. Patients will be asked about their HIV status. Fasting glucose levels, HbA1c and urine albumin: creatinine ratio will be measured using point-of-care (POC) instruments as part of the clinic visit. Fasting glucose will be measured using the Accu-Chek Active glucometer. It has been noted that this POC instrument has imprecision of less than 5.5% but discordant results were seen when compared to a laboratory reference method[21]. However, based on a Clarke error grid analysis it demonstrated adequate clinical accuracy[21]. The HbA1c and urine albumin:creatinine ratio will be measured using a Siemens DCA Vantage Analyzer. It is a cartridge-based analyzer which is capable of analyzing both HbA1c and the urine albumin:creatinine ratio. The DCA Vantage HbA1c assay method is based on latex immunoagglutination inhibition methodology and it is certified by the National Glycohemoglobin Standardization Program (NGSP). The DCA Vantage has been assessed according to NGSP-certified A1c POC device guidelines and was found to have a 2.3% coefficient of variation (CV) at HbA1c levels of 5.26-5.34%, a CV of 2.5% over a HbA1c range of 6.10-6.18%, and a CV of 2.7% in the 8.01-8.09% range[22].

The DCA Vantage albumin/creatinine assay is a quantitative method measuring albumin, and creatinine and reports a albumin:creatinine ratio. Evaluation of the performance of this POC device in terms of measurement of urine albumin, creatinine and albumin:creatinine ratio against a laboratory-based method demonstrated an excellent correlation (R^2=0.989, 0.987 and 0.991, respectively) with total imprecision of < 8.7%[22]. Method comparison studies also showed inter- and intra-day imprecision of < 2.9[23]. Albuminuria will be used as surrogate marker for nephropathy. Albuminuria will be defined as a urine albumin:creatinine ratio of > 2.5 mg/mmol

for men and >3.5 mg/mmol for women[24]. A patient data collection sheet is included at the end of this document.

The HbA1c and urine albumin/creatinine measurements are performed by the medical technologists in the Department of Chemical Pathology at Chris Hani Baragwanath Academic Hospital. Quality control and calibration of the analysers are performed prior to measuring the patients' samples according to the manufacturer's recommendations.

Data collection from patient files and anthropometric measurements

Further data will be obtained from the patient's files and will include information on body mass index (BMI), use of ARVs, therapies for diabetes, dyslipidaemia and hypertension, CD4 counts and viral loads, duration of diabetes, duration since diagnosis of HIV, duration of ART and smoking. Both weight and height measurements are recorded by nurses with every visit.

Data was collected from 107 HIV-positive and 214 HIV-negative diabetic patients

Inclusion criteria

1. Confirmed type 2 diabetes.
2. Patients above 30 years old.

Exclusion criteria

1. Patients less than 30 years of age
2. Non-diabetics
3. Pregnancy
4. Presence of other diseases that would compromise kidney function, glucose or HbA1c levels

Data Analysis

Appropriate descriptive statistics will be performed depending on the distribution of data. If the data is normally distributed, mean and standard deviation will be used but if the data is non-parametric, median (interquartile range) will be used.

Chi-squared test will be used to compare categorical variables between groups whilst Student non-paired t test will be used for continuous variables following log transformation if required.

Multivariable linear regression models will be set up to find the principal determinants of glucose and HbA1c levels and albumin:creatinine ratio (dependent variables). Univariate regression analyses will be performed to determine the relationship of each study variable (independent variable) with each of the 3 dependent variables. Independent variables that correlate with the dependent variable at $p \leq 0.20$ will be included in the multivariable model. Backward, stepwise removal of non-significant variables will then be performed until a final model is obtained in which all remaining independent variables correlate with the dependent variable at $p \leq 0.05$. Collinearity will be assessed in all the initial multivariable models using the variance inflation factor (VIFs). Any variable with a $VIF \geq 10$ will be removed from the model until all variables have $VIF < 10$.

Ethics

An ethics application has already been submitted to the University of Witwatersrand Ethics Committee, and has been approved (clearance no: M140746).

Timing

Ethics application: May 2014-July 2014

Data collection and analysis: August 2014-January 2018

Preparation of paper/research report: December 2017-February 2018

Funding

No funds required.

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R14/49 Dr PS Khoza

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140746

NAME: (Principal Investigator) Dr PS Khoza

DEPARTMENT: Chemical Pathology
Chris Hani Baragwanath Academic Hospital

PROJECT TITLE: Metformin and Human Immunodeficiency Virus Suppression

DATE CONSIDERED: 25/07/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof N Crowther and Dr S Bhana

APPROVED BY: _____
Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 01/10/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES