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Inappropriately low glycated hemoglobin values and hemolysis in HIV-infected patients

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Abstract

In order to test the accuracy of glycated hemoglobin (HbA1c) to predict mean glycemia in HIV-infected patients, we recorded consecutive HbA1c measurements from 1238 non-HIV-infected and 112 HIV-infected patients, all devoid of any hemoglobinopathy, in a retrospective, transversal study. Mean fasting glycemia from the six previous weeks (Measured-Gly) and HbA1c-estimated glycemia [HbA1c-Gly, (1.85 x %HbA1c – 4.78) mM] were compared. Mean hemoglobin, red cell volume, serum creatinine, CD4 count, and HIV-viral load from the same period were collected in HIV-infected patients. Although Measured-Gly was not significantly different between non-HIV-infected (6.95 ± 3.23 mM) and HIV-infected patients (6.62 ± 2.42 mM), HbA1c underestimated the mean fasting glycemia by 12.3% in HIV-infected as compared to non-HIV-infected patients (p<0.0001). The difference "Measured-Gly - HbA1c-Gly" was correlated with the red cell volume (p=0.0001) in HIV-infected patients. We then searched for the presence of sub-clinical hemolysis, a cause of both macrocytosis and reduced HbA1c levels, in HIV-infected patients. To this end, we prospectively measured serum haptoglobin in 249 consecutive samples from HIV-infected subjects without any known cause of hemolysis. A very low haptoglobin level, a marker of hemolysis, was frequent and negatively correlated with the red cell volume in these patients. Treatment with nucleoside analogueswas significantly associated with macrocytosis and low haptoglobin. In conclusion, HbA1c could be inappropriately low in HIV-infected patients. Its underestimation of mean fasting glycemia could be due to an antiretroviral-induced sub-clinical hemolysis, but further studies are needed to explore this hypothesis. Self-monitoring of blood glucose should be promoted in diabetic HIV-infected patients.

MESH Keywords Adult ; Anti-Retroviral Agents ; adverse effects ; Blood Glucose ; analysis ; metabolism ; Female ; HIV Infections ; blood ; drug therapy ; metabolism ; Haptoglobins ; analysis ; Hemoglobin A, Glycosylated ; analysis ; metabolism ; Hemolysis ; drug effects ; physiology ; Humans ; Male ; Middle Aged ; Predictive Value of Tests ; Prospective Studies ; Retrospective Studies

Introduction

Glycated hemoglobin (HbA1c) is considered as the best marker of metabolic control in diabetes mellitus and patient-management strategies are based upon its measurement.[1] HbA1c is an objective retrospective index of blood glucose concentrations during the preceding six to eight weeks. However, some pathological states like hemoglobinopathies, anemias, chronic hemolysis, liver cirrhosis and end stage renal failure result in misinterpretation of HbA1c values because of the presence of abnormal hemoglobin or the shortening of erythrocyte lifespan.

HIV-infected patients under highly active antiretroviral therapy (HAART) are at risk of developping diabetes, mainly because of the recent emergence of the lipodystrophy syndrome associated with insulin resistance.[2] Indeed, the prevalence of diabetes, adjusted for age and body mass index, has recently been shown to be 4.6 times greater in HIV-infected men under HAART than in HIV-seronegative men.[3] Therefore, accuracy of HbA1c measurements in this population has important medical care consequences.

In our clinical experience, we observed several times that glycemia values were recurrently higher than predicted by HbA1c in HIV-infected subjects with diabetes. Four HIV-infected patients with such inaccurate HbA1c values, possibly due to hemolysis induced by some medications (dapsone, ribavirin, trimethoprim-sulfamethoxazole), have been previously reported.[4]

Therefore, to test the accuracy of HbA1c to predict mean glycemia in HIV-infected patients, we compared the glycemia estimated from HbA1c (HbA1c-Gly) and the mean fasting glycemia during the six previous weeks (Measured-Gly) in 1350 consecutive samples from HIV-infected and non-HIV-infected patients. We show here that HbA1c underestimated mean glycemia in HIV-infected patients but not in non HIV-infected subjects. Results from a complementary prospective analysis of 296 serum samples from consecutive HIV-infected patients showed that this could be due to sub-clinical hemolysis, as assessed by low serum haptoglobin values.

Patients and Methods

Patients

We performed a retrospective transversal study using data from 1699 consecutive HbA1c measurements performed in our Biochemistry Department between December 2002 and October 2003. We excluded samples with abnormal hemoglobin revealed by High Precision Liquid Chromatography (HPLC) (n=53), and repeated samples originating from the same patient (in this case only the last determination was considered for each patient). Data from 1350 patients (1238 non-HIV-infected and 112 HIV-infected) were studied. For each patient, the results of the fasting glycemias measured during the six weeks preceding the HbA1c assay were collected and their mean value calculated (Measured-Gly). The HbA1c-estimated glycemia (HbA1c-Gly) was calculated using the equation published by Nathan et al. in 1984:[5] HbA1c-Gly (mM)= 1.85 x (%HbA1c) – 4.78). In the HIV-infected group, the mean values of hemoglobin (Hb), red cell volume, serum creatinine, CD4 count and HIV RNA viral load during the six weeks preceding the HbA1c assay (all performed in the Biochemistry, Hematology, or Virology Departments from Tenon Hospital) were collected. In addition, information regarding any known hemolytic disease or treatment, or the presence of liver cirrhosis was collected from each medical follow-up record.

In a second study, we prospectively measured serum haptoglobin in 296 consecutive samples from all the HIV-infected patients referred to our Biochemistry Department for determination of lipid profile between March and April 2005. We also collected the clinical and biological data described above, as well as the treatments taken by the patients at the time of the blood testing.

Study methods

Fasting glycemia was measured by enzymatic (glucose oxidase) method on a Hitachi 747® (Roche- Boehringer, Meylan, France). HbA1c was measured by ion-exchange High Precision Liquid Chromatography (HPLC) technique on a Variant II Hemoglobin A1c Program, automated analyser (Bio-Rad Diamat System, Marnes La-Coquette, France) and expressed as percentage of total hemoglobin. This method is certified by the National Glycohemoglobin Standardisation Program (NGSP) and is traceable to the Diabetes Control and Complications Trail (DCCT). The CD4 lymphocyte count was calculated using standard flow cytometry. HIV RNA viral load was assessed using the branched DNA technique (Quantiplex HIV, Chiron, Paris-La Défense, France) with a lower limit of detection of 0.5 10^3 copies/ml. Serum creatinine were measured with conventional methods (Hitachi 747®, Roche-Boehringer, Meylan, France). Haptoglobin was determined by immunonephelemetric methods (IMMAGE®, Beckman –Coulter, Villepinte, France).

Statistical analysis

Results are means ± SD except for CD4 count and HIV RNA viral load which are expressed as median and range. Comparisons between groups were made by using the Student t test for continuous variables and the χ test for categorical data. Significance of the correlations was examined using the linear regression analysis for continuous variables. Logistic regression analysis was used in the multivariate analysis to determine the factors associated with macrocytosis (defined by red cell volume above 100 fl) and hemolysis (defined by haptoglobin <0.5 g/l). P values of <0.05 were considered significant.

Results

HbA1c underevaluated mean fasting glycemia in HIV-infected patients

Measured-Gly and HbA1c-Gly were first studied in the 1350 patients included in our retrospective study. As expected, HbA1c-Gly was significantly correlated with Measured-Gly both in non-HIV-infected (r=0.665, p<0.0001, n=1238) and in HIV-infected patients (r= 0.708, p<0.0001, n=112). Despite an insignificant difference between the means of the Measured-Gly in the two groups, HbA1c and HbA1c-Gly were significantly lower (p<0.005) in the HIV-infected patients when compared to the non-HIV group (Table 1). Measured-Gly was slightly but significantly lower than HbA1c-Gly in the non-HIV-infected group (6.95 ± 3.23 versus 7.29 ± 3.41 mmol/L, p<0.0001, mean variation : -0.2%). By contrast, Measured-Gly was significantly higher, with a mean variation of +12.1%, than HbA1c-Gly in the HIV-infected group (6.62 ± 2.42 versus 6.29 ± 2.87 mmol/L, p<0.0001). Therefore the HbA1c values underestimated the mean fasting glycemia in the HIV-infected as compared to the non-HIV-infected patients (p<0.0001).

The difference between Measured-Gly and HbA1c-Gly was positively correlated with the red cell volume

We then focused our study on the HIV-infected patients by searching for any relation between the difference « Measured-Gly - HbA1c-Gly » and clinical or biological parameters available for these patients. In order to exclude any known cause leading to HbA1c misinterpretation, we excluded subjects with Hb <10 g/l (n=2), creatininemia >160 mmol/l (n=4), treated with by dapsone (n=0), ribavirin (n=2) or pyrimethamine (n=2) (drugs that have been associated with hemolysis) or suffering from liver cirrhosis (n=1) or a hemolytic disease (n=0). When considering the remaining group of 101 HIV-infected subjects, the analyses gave similar results to those obtained in the initial group of 112 HIV-infected patients (Tables 1 and 2).

In the group of 101 HIV-infected patients, the difference “Measured-Gly - HbA1c-Gly” (mean 0.40 mmol/l ± 2.01) was neither correlated with age, Hb, creatininemia, CD4 count, nor with HIV RNA viral load (Table 2). In contrast, the difference “Measured-Gly - HbA1c-Gly” was negatively correlated with HbA1c and positively correlated with the red cell volume (Table 2).

The red cell volume is negatively correlated with serum haptoglobin

We then wondered whether the positive correlation between the difference “Measured-Gly - HbA1c-Gly” and the red cell volume could be linked to a hemolytic process, through both a shortened lifespan and an increased regeneration of erythrocytes. To test this hypothesis, we took advantage of 296 consecutive samples from all the HIV-infected patients referred to our laboratory for determination of lipid profile between March and April 2005. We systematically measured serum haptoglobin, and collected the clinical and biological data. After exclusion of 47 patients for any known cause of hemolysis or renal failure (see above), we considered a group of 249 patients for our analyses. The clinical and biological data from these subjects are shown in Table 3.

The prevalence of low haptoglobin levels (<0.5 g/l), which is a marker of hemolysis, was higher in these HIV-infected patients (54/249, 21.7%) than in all other patients referred for haptoglobin measurements in our laboratory in the same period (26/201, 12.9%, $p=0.01$). The red cell volume (mean 98 fl ± 11) was not correlated with Hb in the HIV-infected patients ($r=0.05$, $p=0.43$, $n=249$). On the other hand, the red cell volume was negatively correlated with serum haptoglobin levels ($r=-0.175$, $p<0.01$, $n=249$) in this population.

Macrocytosis is associated with treatment with zidovudine (ZDV), stavudine (d4T), and lamivudine (3TC)

The relation between macrocytosis (red cell volume above 100 fl) and the different treatments in this population of HIV-infected patients is complex to analyze since each patient is given several antiretroviral medications. However, all patients had a stable antiretroviral treatment for at least 3 months, and the duration of the total antiretroviral exposure (mean 67.8 months ± 52.1) was not related to macrocytosis ($p=0.25$). When considering the different pharmaceutical classes, our studies showed that the treatment with any nucleoside reverse transcriptase inhibitor (NRTI) was significantly associated with macrocytosis, and that several individual NRTI molecules were associated with macrocytosis (Table 4). When we performed a logistic regression analysis including all the NRTIs used by more than 10 patients, only zidovudine, stavudine and lamivudine remained significantly associated with macrocytosis (Table 4).

Treatment with lamivudine (3TC) is associated with hemolysis in the group of 249 HIV-infected patients

Considering the haptoglobin values, Table 5 showed that hemolysis, as defined by serum haptoglobin <0.5 g/l, is significantly associated with NRTI treatment ($p<0.0001$) and in particular, as shown by the logistic regression analysis, with the use of lamivudine. The duration of the total antiretroviral exposure (mean 67.8 months ± 52.1) was not related to hemolysis ($p=0.13$).

As macrocytosis has previously been shown to be associated with the use of stavudine and zidovudine, and as lamivudine is frequently associated with these two drugs, we also performed analyses grouping together stavudine and zidovudine. The results, both on macrocytosis and on hemolysis, were not significantly altered (data not shown).

Discussion

The medical follow-up of HIV-infected patients now routinely includes the prevention, screening and monitoring of metabolic complications associated with HAART, characterized by insulin resistance, dyslipidemia and hyperglycemia.[2] Assessment of blood glucose control in patients with diabetes is based upon HbA1c measurements.[1] However, in HIV-infected patients, inaccuracy of HbA1c values has been suggested.[4] Polgreen et al. have reported a persistent discordance between glycemias and HbA1c in four patients under HAART.[4] A hemolytic process due to a hemoglobinopathy, and/or treatment with dapsone, ribavirin or, less likely, with trimethoprim-sulfamethoxazole, has been suggested.[4] As we also regularly observed such a lack of correlation between HbA1c and laboratory or self-monitoring expected glycemias in HIV-infected patients with diabetes followed in our Infectious Diseases Department, we compared the glycemia evaluated from HbA1c (HbA1c-Gly) and the mean fasting glycemia from the six previous weeks (Measured-Gly) in HIV and non-HIV-infected patients. To calculate HbA1c-Gly, we used a formula published by Nathan et al.[5] that allows an estimation of average glycemia very similar to that based from the Diabetes Control and Complications Trial results.[6] Indeed, HbA1c-Gly and Measured-Gly were significantly correlated in all patients and we observed only a slight overestimation of mean fasting glycemia by HbA1c (+0.2%) in non-HIV-infected patients. However, although the mean Measured-Gly was not significantly different between the two groups of patients, we found that HbA1c significantly underestimated mean fasting glycemia in HIV-infected patients. Since HbA1c is a time-averaged index of chronic glucose level, such a discrepancy could be due to lower non-fasting glycemia in HIV-infected patients. This hypothesis seems unlikely since several studies have reported that non-fasting post-glucose load glycemia is increased preferentially to fasting glycemia in HIV-infected patients treated with HAART,[7–9] which is in accordance with a predominantly peripheral insulin resistance in these patients.[10,11]

The difference “Measured-Gly - HbA1c-Gly” was positively correlated with the mean red cell volume. We wondered whether this correlation could be linked to a sub-clinical hemolytic process that could lead to a reduced level of hemoglobin glycation of young erythrocytes. Indeed, a decreased level of HbA1c can be considered as a marker of hemolysis in patients without diabetes.[12,13] In addition, an inverse relationship between the red cell volume and HbA1c was found in two papers.[14,15] As we had previously excluded from our study patients with known causes of hemolysis, we hypothesized that the antiretroviral treatment could be involved. Indeed, we

observed that hemolysis, as diagnosed by a very low haptoglobin level (<0.5 g/l), was more prevalent in the HIV-infected patients as compared to subjects referred for haptoglobin measurements in our laboratory in the same period. In addition, when haptoglobin levels were expressed as a percentage of the normal values according to sex and age, we found that 36.1% (90/249) of HIV-infected *versus* 20.4 % (41/201) of non-HIV-infected patients had a haptoglobin level lower than 60% of normal values (p<0.0005). The eventual relationship with drugs used in the treatment of HIV infection is difficult to study since antiretrovirals are used in association. However, our results show that both macrocytosis and latent hemolysis were related to the use of nucleoside analogues, but not to the total duration of exposure to antiretroviral treatment. This suggests that nucleoside analogues could have a short-term toxicity on erythrocytes, responsible for subclinical hemolysis. The increased number of young erythrocytes could secondarily reduce the percentage of HbA1c. Macrocytosis has previously been shown to be associated to treatment with zidovudine and stavudine, but the pathophysiological mechanisms have not been clearly elucidated.[16] In our study, logistic regression analyses are in favor of a predominant role of lamivudine, but it should be interpreted with caution in the context of multiple drugs association. Specifically designed studies are required to clarify the relationships linking hemolysis, macrocytosis and treatment with nucleoside analogues. Nevertheless, our study shows that HbA1c could underestimate glycemia in HIV-infected patients. This should lead clinicians to promote the self-monitoring of blood glucose in diabetic HIV-infected patients.

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Table 1

Characteristics of patients included in the initial retrospective study

	Non-HIV infected patients	HIV-infected patients	p
Number of patients	1238	112	
Age of patients (years)	57 ± 15	49 ± 10	< 0.05
Sex (M/F) (n)	736/502	88/24	< 0.0001
Sex (M/F) (%)	59.5/40.5	78.6/21.4	
HbA1c (%)	6.52 ± 1.85	5.98 ± 1.55	< 0.005
Measured-Gly (mM)	6.95 ± 3.23	6.62 ± 2.42	0.28
HbA1c-Gly (mM)	7.29 ± 3.41*	6.29 ± 2.87†	< 0.005

Values are expressed as mean ± SD.

Measured-Gly was significantly different from HbA1c-Gly in the non-HIV (*, p< 0.0001) and in the HIV-infected (†, p<0.0001) patients.

Table 2

Correlations between the difference “Measured-Gly - HbA1c-Gly” and several biological parameters in the group of 101 HIV-infected patients

	r	p
Age (years) 49 ± 10	0.094	0.35
HbA1c (%) (5.95 ± 1.55) or HbA1c-Gly (mM) (6.23 ± 2.87)	− 0.55	<0.0001
Measured-Gly (mM) 6.63 ± 2.44	0.177	0.08
Hb (g/dl) 14.1 ± 1.4	− 0.00006	0.99
Red cell volume (fl) 101 ± 10	0.38	0.0001
Creatininemia (μM) 91 ± 23	− 0.37	0.72
CD4 count (cells/μl) 580 (41–2072)	− 0.12	0.24
HIV RNA viral load (10^3 copies/ml) 0.05 (0.05–338)	− 0.13	0.21

Values are expressed as mean ± SD except for CD4 count and HIV RNA viral load expressed as median (range).

Table 3

Characteristics of HIV-infected patients included in the prospective study

Number of patients	249
Age of patients (years)	42 ± 9
Sex (M/F) (n)	170/79
Sex (M/F) (%)	68.3/31.7
Hb (g/dl)	13.8 ± 1.6
Red cell volume (fl)	98 ± 11
Creatininemia (μM)	89 ± 18
CD4 count (cells/μl)	468 (4–4532)
HIV RNA viral load (10^3 copies/ml)	0.05 (0.05–500)
Serum haptoglobin (g/l)	0.971 ± 0.59

Values are expressed as mean ± SD except for CD4 count and HIV RNA viral load expressed as median (range).

Table 4

Associations between macrocytosis and treatments in the group of 249 HIV-infected patients

Drugs used by more than 10 patients	Number of patients treated	p (χ)	Logistic regression analysis		
NRTI : any	**197**	**<0.0001**	**Odds ratio**	**Range**	**p**
zidovudine (ZDV)	83	<0.0001	16.3	6.9–38.6	<0.0001
stavudine (d4T)	21	0.0139	8.7	2.9–26.0	0.0001
lamivudine (3TC)	150	<0.0001	2.5	1.1–5.6	0.03
didanosine (ddI)	33	0.032	0.7	0.2–2.0	0.52
abacavir	84	0.0009	1.4	0.7–3.0	0.36
tenofovir	60	0.0001	0.7	0.3–1.7	0.47
Protease inhibitors : any	**115**	**0.24**			
ritonavir	99	0.12			
ritonavir/lopinavir	36	0.06			
atazanavir	47	0.07			
NNRTI : any	**47**	**0.42**			
efavirenz	36	0.15			
viramune	11	0.37			
Trimethoprim-sulfamethoxazole	**45**	**0.23**			

Macrocytosis was defined by red cell volume >100fl.

NRTI : Nucleoside reverse transcriptase inhibitors ; NNRTI : Non nucleoside reverse transcriptase inhibitors.

Table 5

Associations between hemolysis and treatments in the group of 249 HIV-infected patients

Drugs used by more than 10 patients	p (χ)	Logistic regression analysis		
NRTI : any	**0.006**	**Odds ratio**	**Range**	**p**
zidovudine (ZDV)	0.10	0.90	0.4–2.1	0.81
stavudine (d4T)	0.76	0.66	0.2–2.4	0.52
lamivudine (3TC)	0.0003	3.97	1.7–9.2	0.001
didanosine (ddI)	0.70	1.2	0.5–3.1	0.72
abacavir	0.37	1.2	0.6–2.3	0.64
tenofovir	0.99	1.2	0.5–2.6	0.73
Protease inhibitors : any	**0.12**			
ritonavir	0.08			
ritonavir/lopinavir	0.34			
atazanavir	0.94			
NNRTI : any	**0.94**			
efavirenz	0.93			
viramune	0.77			
Trimethoprim-sulfamethoxazole	**0.20**			

Hemolysis was defined by serum haptoglobin values<0.5 g/l.

NRTI : Nucleoside reverse transcriptase inhibitors ; NNRTI : Non nucleoside reverse transcriptase inhibitors.