Is HIV a Contributing Factor to Occurrence of Hyperglycemia: A Case of People Living with HIV Attending Nyeri Referral Hospital, Kenya?

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ABSTRACT

Background: The hallmark of advanced HIV infection is the progressive loss of a specific type of immune cells called the CD4 cells weakening the immune system and leaving individual vulnerable to various opportunistic infections (OIs) and other illnesses, ranging from pneumonia to cancers. Complications such as hyperglycemia/ diabetes mellitus (DM) have lately been associated with onset of HIV infection. Minimal information is available on whether the occurrence of Diabetes Mellitus is associated with HIV. This study aims to look at the correlation of hyperglycemia (a risk factor of DM) and HIV infection among the HIV infected individuals in Nyeri County Hospitals-Kenya.

Methods: This was a case control study involving 193 individuals who were grouped into two groups of 97 subjects of HIV individuals (also referred to as study control group) and 96 participants who were HIV positive and based on CD4 count 308.8 ± 249.8 cells per cubic millimeter, Viral load-Not detectable and fasting blood glucose level 4.4 ± 2.2mmol/L.

Results: A total of 193 individuals were enrolled, grouped into 97 subjects of HIV negative individuals (control group) and 96 participants who were HIV positive and based on CD4 count 308.8 ± 249.8 cells per cubic millimeter, Viral load-Not detectable and fasting blood glucose level 4.4 ± 2.2mmol/L. In the study 13.54% of HIV positive were hyperglycemic compared to 6.18% HIV -ve individuals (mean glucose level 7.6 ± 5.1 and 4.4 ± 1.1mmol/L, respectively (P>0.05) r=0.023). CD4 mean 888.8 ± 244.1 HIV and 308.8 ± 249.8 cells per cubic millimeter respectively (P>0.05) r=0.057). The correlation between hyperglycemia and viral loads was significant (P>0.05) (r=0.35) while the correlation between hyperglycemia and CD4 was not significant (P>0.05) (r=0.023).

Conclusion: The study identified Hyperglycemic as a likely complication in HIV infected individuals but resulting from a complex interaction of a variety of diabetes mellitus risk factors.

Key words: HIV, Diabetes Mellitus, blood glucose, viral load, adults

INTRODUCTION

Human immunodeficiency virus infection occurs alongside other infections including diabetes. Diabetes and HIV have the same prognosis and the two diseases have similar symptoms which include weakening of immunity, reduction of body weight (body fat waste) and hyperinsulinemia with insulin resistance, glucose metabolism abnormalities, abnormal liver function with elevated activities of alanine amino transferases, neurovasculopathies with non-healing wounds, dementia, candidiasis, chest infections among others. AIDS and diabetes are both independent diseases in that the occurrence mechanisms are different. AIDS is caused by the human immunodeficiency virus (retrovirus) that causes destruction of immune cells in the body leading to serious immune suppression and hence an onset of
many other illnesses referred to as opportunistic infections (OIs). Diabetes on the other hand is a medical complication as a result of glucose metabolism abnormality with a diverse causative agents and it can also be one of the complications of advanced HIV infection.\(^2\)

Uncontrolled HIV replication have been suggested to cause diabetes mellitus in some patients;\(^3\) Similarly, the incidence of diabetes mellitus among HIV infected individuals was observed to be high compared to the incidence in individuals not HIV infected.\(^4\) In this case clients with advanced HIV infection had high blood sugar which resolved after viral replication was suppressed with antiretroviral therapy (ART).\(^4\)

DM has been classified into three categories, this depend on the circumstances present at the time of diagnosis i.e. the pathogenesis. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency), these is further subdivided into two subtypes as immune mediated diabetes and idiopathic diabetes.

Immune-mediated diabetes results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65) and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis.\(^5\)

In idiopathic diabetes, type 1 diabetes have no known etiologies. Some of these patients have permanent insulinogenic and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity.\(^5\)

Type 2 diabetes, range from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance. This form of diabetes which account for 90–95% of those diabetic encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur.\(^6\)

Other specific types of diabetes include; Genetic defects of the β-cell diabetes that is associated with monogenetic defects in β-cell function, genetic defects in insulin action diabetes that result from genetically determined abnormalities of insulin action, diseases of the exocrine pancreas diabetes which involves any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy and pancreatic carcinoma, Endocrinopathies diabetes-Several hormones e.g., growth hormone, cortisol, glucagon and epinephrine, antagonize insulin action, drug or chemical-induced diabetes. Many drugs and toxins can impair
in insulin secretion they include Vacor (a rat poison) and intravenous pentamidine which can permanently destroy pancreatic β-cells. Infections diabetes is diabetes resulting from infection by certain viruses have been associated with β-cell destruction e.g. Congenital rubella, coxsackievirus B, cytomegalovirus, adenovirus and mumps have been implicated in inducing certain cases of the diabetes.\[^{7,8}\]

The long-term consequences of uncontrolled diabetes include neuropathy, retinopathy and heart disease. It is also associated with vascular disease and is one of the major reasons for amputations.\[^{10}\] Insulin is a hormone that enables the body cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. The principal biochemical function of glucose is to provide energy for life processes. Glucose oxidation by the glycolytic and tricarboxylic acid pathways is the primary source of energy for the biosynthesis of ATP.\[^{9}\]

Independent risk factors in diabetes are old age, larger BMI category, liver damage and diseases and viral infections. In type 1 diabetes the pancreatic cells that make the insulin are destroyed causing severe lack of insulin (hypoinsulinemia). It is not clear what causes type 1 diabetes which account for about 5-10 % of diagnosed diabetes and develops most often in children and young adults, but the disorder can appear at any age, symptoms usually develop over a short period although beta cells destruction begin months, even years earlier. Symptoms appear when at least 80% of the cells are affected.\[^{10}\]

The destruction of beta cells is thought to be the result of the body attacking and destroying its own cells in the pancreas, also known as autoimmune reaction. It is not clear why this happens, but a number of explanations and possibilities could trigger this reaction. These may include infection with a specific virus or bacteria (e.g. Cytomegalovirus whose induction of DM in mice has proven to be an excellent experimental model for the pathogenesis of viral diseases). Other include exposure to food-borne chemical toxins, disorder in the immune systems caused by virus infection whereby immune system cannot kill infectious agents or autoimmune systems response, as seen in type 1 diabetes mellitus, where by lymphocyte infiltration of the islet cells of the pancreas occur with concomitant beta cells destruction and the appearance of antibodies to islet cells components before the manifestation of diabetes.\[^{11}\]

Other rare causes of diabetes (type 1 and 2) can include any other illness or disease that damages the pancreas affecting its ability to produce insulin, e.g. pancreatitis, alteration of the hormone or cytokine levels and interactions of the HIV's protein VPr with proteins responsible for glucose transport within cells.\[^{10}\] Liver cirrhosis can cause insulin resistance and glucose intolerance\[^{13}\] a sign of developing diabetes and liver cirrhosis is common HIV infected individuals.\[^{12}\]

Depression a common problem in HIV infected persons may trigger the body turning on itself (autoimmune response), causing pancreatitis as in case of type 1 diabetes. More often HIV is accompanied by depression and patients with HIV infection, clinical depression is the most frequently observed psychiatric disorder affecting as many as 1 in 3 HIV infected people.\[^{12,14}\] Stress in HIV infected patients elicits a complex hormonal and immunological response that may alter various biochemical pathways, including glucose metabolism.

Clinical signs for diabetes include polyuria, polydipsia and blurred vision while thirst develops because of osmotic effects. Sufficiently high glucose in the blood is excreted by the kidneys, but this requires water to carry it and causes increased fluid loss, which must be replaced. A rarer but equally severe
presentation is hyperosmolar non-ketonic state, which is more common in type 2 diabetes. Diabetes mellitus is also characterized by recurrent or persistent hyperglycemia and is diagnosed by demonstrating; Fasting plasma glucose level at or above 7.0 mmol/l. Plasma glucose at or above 11.1 mmol/l two hours after glucose load in a glucose tolerance test. This is as well as random plasma glucose at or above 11.1 mmol/l. In this study the hyperglycemia was diagnosis was based on random plasma glucose level estimation only.

A relationship between HIV infection and diabetes onset has been suggested by several other authors, for example, a study indicated a prevalence of diabetes of 2-7% among HIV infected individuals receiving protease inhibitors. The incidence of diabetes in HIV patients has been estimated to range from 1% to 10%. Another study showed an increased incidence of insulin resistance among HIV positive patients and hence a concern that this population in general and individual with evidence of fat redistribution common in HIV infected, in particular may be at increased risk of development of diabetes.

It has been observed that sometimes type 1 diabetes occurs when islet of Langerhans cells of the pancreas are destroyed probably as consequence of genetic susceptibility caused by the onset of autoimmune destruction triggered by environmental factors such as viral infection e.g. HI-virus, this’s further compounded by metabolic complications and disorders common with diabetics which include hyperlipidemia, insulin resistance and fat redistribution, which are also common in HIV infected patients. Minimal information exist on whether the occurrence of Diabetes Mellitus is associated with HIV. It is therefore important to assess the difference in the diabetes prognosis between HIV infected and non-HIV infected individuals. The objective of this study was therefore to evaluate the existence of any relationship between HIV infection and hyperglycemia.

**MATERIALS AND METHODS**

A cross-section case control study was conducted at Nyeri Referral Hospital, Nyeri County Kenya with a bed capacity of 320 of which 7% of these beds are occupied by HIV positive patients. Ethical approval was sought and granted by the Nyeri Provincial General Hospital. The participants were reassured of confidentiality in the handling of information and procedures involved in this study.

**Study participants**

The HIV infected individuals who have lived in Nyeri district for 6 months prior to the study, not on ARVs and with no history of DM previous to infection and not obese were included. For the healthy group, (Control group) in addition to the above criteria, the subjects were excluded from the study if they had regularly consumed drugs with potential nephrotoxicity (such as analgesics/anti-inflammatory agents and aminoglycosides) and also those that could affect the blood sugar level. At the end only 193 subjects who were systematic selected (the 10th subject) were involved in this study. They were categorized into two groups i.e. healthy (control group) and HIV positive group consisting of 97, 96 subjects, respectively.

The sample size was calculated using the formulae given by Fisher *et al.* as quoted in Mugennda and Mugenda, method this derived a sample of 91 per group (case and control). This was adjusted the number to 100 per group in order to complement for any error due to chance variations.

**Data Collection and Analysis**

Four ml of venous blood sample was collected using aseptic technique where, 2 ml was put in EDTA vacutainer for CD4 count and the remaining sample, about 2ml, was put in another EDTA vacutainer and
was used for blood sugar analysis and then spun to obtain about 1ml of plasma. The plasma was for viral load analysis and it was stored at -20°C. The containers were labeled with the study number of the participant and the date of birth was also marked to tally with all the required demographic information. This was matched with the demographic information on the questionnaire form to avoid any risk of mix-ups or incorrect identification of samples. [23,24]

Blood sugar analysis was based on enzymatic method and results read spectrometric ally. [23] The FACSCOUNT™ machine was used to determine the CD4 cells count where fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens and a fluorescent nucleus dye binds to the nucleated blood cells. [25] About 1ml sample of serum a sample was sucked into ExaVirload-Quantitative Determination version 2 machine™. The principle of the test is that the lysates contains reverse Transcriptase activity (RT) enzyme will synthesis a DNA-strand whose product is detected with alkaline phosphatase (AP) conjugate alpha BrdU anti body. The product is then quantified by addition of a colorimetric AP substrate on the polyA plate and read calorimetrically at A405 as the number of the cells/uL. [21]

Statistical analysis was carried out using SPSS program version 11.0. Pearson’s correlation coefficients (r) were calculated to determine relationship between means of the studied markers. Results were considered statistically significant at p < 0.05. The reference ranges for the various markers were calculated using the normal healthy individuals (reference group).

RESULTS
Out of the targeted 100 per group, those who responded were 96 in the case and 97 in the control group.

Demographics characteristics
The study noted that majority of the respondents, 41.7% for case group and 46.4% for control group were aged between 31-40 years (Table 1). However, there was a significant difference in the ages of those who were either HIV positive or HIV negative where those below 40 years were more.

| Table 1: Demographics characteristics among people living with HIV |
|--------------------------|--------------------------|--------------------------|---------------------|
|                          | Case group              | Control group            | P value |
| Age (years)              | n (96) | %     | n (97) | %     |       |
| <30                      | 36   | 37.5  | 26    | 26.8  | <0.05 |
| 31-40                    | 40   | 41.7  | 45    | 46.4  |       |
| 41-50                    | 16   | 16.7  | 16    | 16.5  |       |
| > 50                     | 4    | 4.2   | 10    | 10.3  |       |
| Gender                   | 47   | 48.9  | 50    | 51.5  |       |
| Male                     | 18   | 18.8  | 42    | 43.3  | <0.05 |
| Female                   | 78   | 81.1  | 55    | 56.7  |       |

Blood glucose level and CD4 count among people living with HIV
The mean blood glucose was higher for the control group than in the case. The mean CD4 count was higher for the case group than in the control group (Table 2). However, no difference noted between male and female for both groups and for both glucose levels and CD4 count (P>0.005).

| Table 2: Blood glucose level and CD4 count among people living with HIV |
|--------------------------|--------------------------|--------------------------|---------------------|
|                          | Mean                     | Reference point          | Case group         | Control group     | P value |
| Blood glucose levels     |                         |                          |                    |                    |         |
| Male                     | 2.13-6.5                 | 7.8±1.4                  | 4.3±1.3           | <0.005            |
| Female                   | 2.54-6.5                 | 7.6±5.3                  | 4.5±1.2           |                    |
| CD4 count                |                          |                          |                    |                    |         |
| Male                     | 423.49-1,308.1           | 368.8±244.1              | 865.8±225.7       | P<0.005           |
| Female                   | 401-1410.2               | 381.1±204                | 305.6±257.5       |                   |

Correlation between Blood glucose, viral load and CD4 count among people living with HIV
The study noted a significant correlation between Blood glucose versus viral load (r=0.35; p<0.05). However, no significant correlation was found between Blood glucose versus CD4 count and CD4 count versus viral load (p>0.05) (Table 3).
Table 3: Correlation between Blood glucose concentration versus viral load and CD4 count parameters

<table>
<thead>
<tr>
<th>parameters</th>
<th>r</th>
<th>p</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose vs CD4</td>
<td>-0.023</td>
<td>P=0.826</td>
<td>Not significant correlation (r=-0.023; p&gt;0.05)</td>
</tr>
<tr>
<td>Blood glucose vs Viral load</td>
<td>0.000</td>
<td>P=0.733</td>
<td>Significant correlation (r=0.35; p&lt;0.05)</td>
</tr>
<tr>
<td>CD4 vs Viral load</td>
<td>-0.678</td>
<td>P=0.000</td>
<td>Significant negative correlation (r=-0.678; p&lt;0.05)</td>
</tr>
</tbody>
</table>

Those who were positive were 2.3 (OR=2.014) times more likely to have high blood glucose than those who were HIV negative (Table 4). This is an indication of a high likelihood of getting diabetes mellitus.

Table 4: The prevalence of hyperglycemia-diabetes in control and in HIV positive participants

<table>
<thead>
<tr>
<th>Glucose status</th>
<th>Case</th>
<th>Control</th>
<th>Total</th>
<th>OR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glucose</td>
<td>13</td>
<td>90</td>
<td>103</td>
<td>2.014</td>
<td>0.000</td>
</tr>
<tr>
<td>High glucose</td>
<td>83</td>
<td>7</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>97</td>
<td>193</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Strength and Limitations
There are few studies and literature on the status of diabetes in HIV infected individuals or even on the relationship between HIV infection and diabetes. This relationship has been proposed, suggesting that elevated blood sugar levels could be a complication of untreated HIV infection. [4]

This study will go a long way in adding up to the literature on this subject that deals with two major diseases that also affect a significant number of people in Kenya.

DISCUSSION
The mean blood glucose concentration was higher for the case group than in the control in this study and the correlation between CD4 count and viral load was significantly, which indication of active HIV infection in the case group (r=0.35; p<0.05). This can explain the findings of a number of studies including; A study that showed a strong association between diabetes and active HIV infection, [19] [3] another study found that uncontrolled HIV replication have been suggested to cause diabetes mellitus in some patients. [1]

Another study noted the incidences of diabetes mellitus among HIV infected individuals to be higher compared to the incidence in individuals not HIV infected, [4] still another study that confirmed the correlation between CD4 count and viral load in HIV positive groups significantly, is an indicative of active HIV infection noted that there’s a high likelihood of getting diabetes mellitus in case of uncontrolled HIV infection. [20, 22]

CONCLUSION
The study observed that the mean blood glucose concentration in HIV positive was more than double that of the control group 7.7±2.3 and 4.4±1.1, respectively. There was a significant correlation between blood sugar levels and viral load counts (r=0.35; P>0.05). There was however, was no correlation between blood sugar levels and CD4 counts (P>0.05) (r=-0.023). This may suggest that abnormality in glucose metabolism in HIV infected individuals is as a result of a complex interaction of many diabetes mellitus risk factors.

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