Identification of recent HIV infections and of factors associated with virus acquisition among pregnant women in 2004 and 2006 in Swaziland

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ABSTRACT

Background: HIV continues to spread at high rates in sub-Saharan Africa. In particular, Swaziland is one of the countries most affected by the HIV/AIDS pandemic. Monitoring of HIV infection in Swaziland is being made by periodical investigations on HIV prevalence in pregnant women. However, knowledge of proportion of recent HIV infections is important for epidemiologic purposes to assess HIV transmission patterns.

Objectives: To evaluate the proportion of recent HIV infections among pregnant women and its change overtime and to analyze factors associated with recent HIV infection in Swaziland.

Study design: HIV-positive sera from pregnant women were collected during the 2004 and 2006 National HIV Serosurveys conducted in Swaziland and tested for the HIV antibody avidity, in order to identify recent HIV infections. Socio-demographic and clinical information was also collected. A multivariate analysis was conducted to assess the association between recent HIV infection and socio-demographic and clinical factors.

Results: A total of 1636 serum samples were tested for HIV antibody avidity. The overall proportion of recent infections was 13.8%, with no significant difference between 2004 and 2006 (14.6% vs. 13.1%, P > 0.05, respectively). At the multivariate analysis, the younger age [14–19 vs. ≥ 20 years; adjusted odds ratio (aOR) 2.17, 95% CI: 1.45–3.24], as well as being at first pregnancy (1 vs. ≥ 2; aOR 1.61, 95% CI: 1.10–2.35) was independently associated with recent HIV infection.

Conclusions: This study shows no significant difference in the proportion of recent infections between 2004 and 2006 and suggests that young women and women at their first pregnancy are currently high-risk groups for HIV acquisition, highlighting the importance of developing targeted youth programmes to reduce the spread of HIV infection in the country.

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ARTICLE INFO

Article history:
Received 15 January 2010
Received in revised form 12 April 2010
Accepted 30 April 2010

Keywords:
HIV/AIDS
Recent infections
Risk factors
Swaziland
Africa

1. Background

Despite national and international efforts to curb the AIDS pandemic, HIV continues to spread at high rates in sub-Saharan Africa. WHO and UNAIDS estimate that in 2008 two thirds of the global total of 33 million people with HIV were living in sub-Saharan Africa and that an estimated 2.7 million new cases of HIV infection occurred there in that year.1

Abbreviations: AI, avidity index; aOR, adjusted odds ratio; ANC, ante-natal care; ELISA, enzyme linked immunosorbent assay; MGH, Mbabane Government Hospital; NHS, national HIV serosurvey; NRL, national referral laboratory; RPR, rapid plasma reagin; STI, sexually transmitted infections.

1 Both these authors contributed equally to the manuscript.
Thus, the broad HIV spreading is continuing to slow down progress and hard won gains in most of the sub-Saharan countries. Among these, Swaziland is one of the countries most affected by the HIV/AIDS pandemic. The HIV prevalence among pregnant women attending Ante–Natal Care (ANC) services, as measured by National HIV Serosurveys (NHS), carried out every 2 years since 1992, has steadily risen from 3.9% in 1992 to 42.6% in 2004 and 39.2% in 2006.\(^2,7\) In addition, a Demographic and Health Survey (DHS), performed in 2006 on the general population, has shown a total prevalence of 18.8%. However, the prevalence was 31.1% among women aged 15–49 years.\(^2\) These data depicts Swaziland as the country with the highest HIV prevalence in the world.

Thus, it is important to plan strategies of prevention and control of HIV spreading, in Swaziland. This is being obtained through periodical investigations of HIV prevalence in pregnant women attending ANC, in the attempt of continuously monitoring HIV epidemic. However, HIV prevalence data alone cannot provide a complete picture of the dynamics of the epidemic\(^4–6\) that, instead, can be obtained by measuring the proportion of recent HIV infections occurring in a given population.

### 2. Objectives

The aim of this study was to evaluate, for the first time in Swaziland, the number of recent HIV infections occurring in pregnant women enrolled in the framework of the 2004 and 2006 NHS.

In addition, socio-demographic and clinical factors that are associated with recent HIV infection in the same population were evaluated.

### 3. Study design

#### 3.1. Participants enrolment and samples collection

The present cross-sectional study was conducted on plasma samples from HIV positive pregnant women enrolled in the framework of 9th and 10th NHS conducted in Swaziland between August and October in 2004 and 2006, respectively.\(^2,7\) Women were aged 15–49 years and attended Swaziland national ANC services for the first prenatal visit.

For the NHS, 17 clinics were selected in order to be representative of the four regions of the country and of both urban and rural areas. The number of participants per site was proportional to the average number of ANC clients. The required sample size was calculated using the formula for random sampling population surveys. A total of 5131 women at first prenatal visit were selected. In particular, 2665 women were enrolled in 2004 and 2466 in 2006.\(^2,7\)

Blood samples were collected from all women enrolled in NHS to measure the presence of anti-HIV antibodies.

In this study sera from women who were HIV positive at the HIV antibody testing were further tested to evaluate the proportion of recent infections.

At the time of enrollment of HIV positive women, an individual questionnaire was filled out by each participant in order to collect basic socio-demographic information: nationality, residence, age, level of education, marital status, and number of pregnancies. In addition, clinical data regarding syphilis serology and self-reported information on genital complaints in the previous 6 months were included in the questionnaire as indicators of sexually transmitted infections (STI).

The study was approved by Swaziland Ethics Committee and an informed consent was obtained from each woman for the questionnaire and blood collection.

#### 3.2. Laboratory testing

All blood specimens collected from the selected clinics were sent to the National Referral Laboratory (NRL) for HIV/AIDS at the Mbabane Government Hospital (MGH) in Mbabane, for serum separation and laboratory testing. All sera were then tested for HIV antibodies and syphilis serology using standard assays.

HIV1/2 antibody testing for the NHS was done using Access ELISA (enzyme linked immunosorbent assay) from Beckman Coulter (Fullerton, CA, USA). Sera were deemed positive for HIV if they were tested reactive by the Access method. No confirmatory testing of the HIV positive specimen was performed, following the UNAIDS/WHO recommendations for HIV surveillance in populations with a HIV prevalence higher than 10%.\(^8\)

Syphilis antibodies were detected by a quantitative Rapid Plasma Reagin (RPR) screening test. HIV and syphilis test results were anonymously linked to participant questionnaire by means of client identification numbers.

To identify recent HIV infections, the Avidity Index (AI) assay has been used, which was recently standardised and validated in serum samples from individuals infected with either B or non-B clade HIV–1 viruses.\(^9,10\) This assay was intended only for epidemiological studies and has a sensitivity of 87.5% and a specificity of 98.3%.\(^10\) The AI assay was performed on the residual of the HIV-positive sera collected during the 2004 and 2006 NHS. According to previously reported criteria for identification of recent infections, AI values \(\leq 0.80\) identified samples derived from individuals with recent HIV infection (\(\leq 6\) months from seroconversion).\(^9,10\)

#### 3.3. Data analysis

The proportion of recent infections was calculated as the ratio between the number of specimens from individuals with recent infection and total number of specimens tested for AI. The age and the number of pregnancies variables were categorized. In the univariate analysis, chi square test was used to assess statistically significant associations between recent HIV infection and socio-demographic/clinical characteristics, for the years 2004 and 2006, separately. In the multivariate analysis only variables with a \(P < 0.10\) were included. A logistic regression model was constructed using backward elimination methods. The fit of the model was assessed using the Hosmer–Lemeshow test. Rural/urban residence and year of survey were included in the multiple logistic regression model as control variables because these characteristics were significantly associated with HIV prevalence in the NHS source database. Data were analyzed using the statistical package SPSS 17.0.

### 4. Results

From the 2102 total HIV-positive women identified in the 2004 and 2006 NHS, a sufficient amount of serum for AI testing was available for 1636 women. In particular, 729 sera, representing 64% of the total HIV positive samples in the 2004 NHS, and 907 sera, representing 93% of the total HIV positive samples in the 2006 NHS, were tested. The socio-demographic and clinical characteristics of the 1636 women included in the AI study were not significantly different from those of the 466 women not included in the study (\(P > 0.05\)). The median age of the 1636 women was 24 years (interquartile range 21–29); of these, 75% resided in a rural area and 98.8% had Swazi nationality; 51.1% reported being married or cohabiting, 22.3% was pregnant for the first time, 29.2% reported having had no education and 30.6% reported to have completed primary school education. Test for syphilis was positive in 7.5% of women. Finally, the percentage
Table 1

Socio-demographic and clinical characteristics of HIV positive pregnant women in Swaziland, years 2004 and 2006.

<table>
<thead>
<tr>
<th>Year 2004</th>
<th>Year 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent infections (%) in row</td>
<td>Total infections$^a$</td>
</tr>
<tr>
<td>107 (14.6%)</td>
<td>729</td>
</tr>
</tbody>
</table>

- **Age groups**
  - 14–19: 35 (29.2%) | 120 | <0.001 | 35 (25.5%) | 137 | <0.001 |
  - 20–24: 37 (13.7%) | 271 | 40 (12.7%) | 315 |
  - 25–29: 20 (9.8%) | 202 | 28 (10.4%) | 251 |
  - 30–34: 12 (13.3%) | 90 | 7 (5.7%) | 123 |
  - 35–39: 1 (3.6%) | 28 | 11 (16.7%) | 66 |
  - ≥40: 1 (7.7%) | 13 | 0 (0.0%) | 14 |

- **Residence**
  - Urban: 24 (12.8%) | 188 | Ns | 23 (11.1%) | 208 | Ns |
  - Rural: 82 (15.3%) | 536 | 96 (13.7%) | 699 |

- **Nationality**
  - Swazi: 102 (14.6%) | 697 | Ns | 117 (13.3%) | 878 | Ns |
  - Other: 1 (16.7%) | 6 | 1 (7.1%) | 14 |

- **Number of pregnancies**
  - 1: 42 (25.3%) | 166 | <0.001 | 40 (20.7%) | 193 | <0.01 |
  - 2: 31 (13.4%) | 231 | 38 (13.6%) | 279 |
  - 3: 17 (10.9%) | 156 | 16 (7.9%) | 203 |
  - ≥4: 13 (7.8%) | 166 | 24 (11.1%) | 217 |

- **Marital status**
  - Married/co-habitant: 47 (12.4%) | 380 | <0.10 | 44 (9.7%) | 453 | <0.01 |
  - Single: 59 (17.2%) | 344 | 75 (16.6%) | 452 |

- **Educational level (completed level)**
  - No formal education: 33 (14.2%) | 232 | Ns | 36 (14.7%) | 245 | Ns |
  - Primary school: 27 (12.8%) | 211 | 35 (12.2%) | 288 |
  - Secondary school: 42 (16.4%) | 256 | 40 (12.7%) | 316 |
  - Tertiary school: 2 (7.4%) | 27 | 7 (12.5%) | 56 |

- **Genital ulcers (≤6 months)**
  - Negative: 91 (14.1%) | 644 | Ns | 105 (13.3%) | 789 | Ns |
  - Positive: 12 (18.8%) | 64 | 6 (8.7%) | 69 |

- **Offensive discharge (≤6 months)**
  - Negative: 85 (14.9%) | 572 | Ns | 94 (13.2%) | 710 | Ns |
  - Positive: 18 (13.2%) | 136 | 17 (11.6%) | 147 |

- **Syphilis serology**
  - Negative: 95 (14.4%) | 660 | Ns | 111 (13.2%) | 839 | Ns |
  - Positive: 11 (15.9%) | 69 | 7 (13.2%) | 53 |

Ns, not significant.

$^a$ Number of infections in subgroups is different from the number of total infections because for some variables there are some missing data.

$^b$ P value of $\chi^2$ test for association with recent infections. Missing data was not included in the statistical calculation.

of women reporting having had genital ulcers or offensive genital discharges in the previous 6 months was 8.5% and 18.1%, respectively.

Of the total 1636 women tested for AI, 226 (13.8%) were classified as recently HIV infected. According to the year of collection, 107 out of 729 women (14.6%; 95% CI 12.2–17.5%) in 2004 and 119 out of 907 (13.1%; 95% CI 11.0–15.5%) in 2006 were classified as recently infected. No significant difference in the percentage of recent infections was observed between 2004 and 2006 ($P > 0.05$).

Table 2 reports the characteristics of the HIV-infected women in 2004 and 2006. There is a decreasing proportion of recent infections with the increasing of age, both in 2004 (chi square for linear trend 15.5, $P < 0.001$) and in 2006 (chi square for linear trend 8.7, $P < 0.01$). A significantly higher proportion of recent HIV infections

<table>
<thead>
<tr>
<th>Recent HIV infections</th>
<th>Total HIV infections</th>
<th>%</th>
<th>cOR</th>
<th>95% CI</th>
<th>aOR$^a$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–19 years</td>
<td>70</td>
<td>257</td>
<td>27.2</td>
<td>2.95</td>
<td>2.14–4.07</td>
<td>2.17</td>
</tr>
<tr>
<td>≥20 years</td>
<td>155</td>
<td>1378</td>
<td>11.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>82</td>
<td>360</td>
<td>22.8</td>
<td>2.37</td>
<td>1.75–3.21</td>
<td>1.61</td>
</tr>
<tr>
<td>≥2</td>
<td>139</td>
<td>1256</td>
<td>11.1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/co-habitant</td>
<td>91</td>
<td>835</td>
<td>10.9</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Single</td>
<td>134</td>
<td>799</td>
<td>16.8</td>
<td>1.65</td>
<td>1.24–2.19</td>
<td>1.21</td>
</tr>
</tbody>
</table>

cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

$^a$ Adjusted for rural and urban residence, year of survey, and syphilis serology.
is observed among women who are pregnant for the first time compared to those who have had more than one pregnancy \((P < 0.001)\) and among women who report being single compared to those who report being married or cohabiting \((P < 0.10)\). Given that no differences were observed in factors associated with recent HIV infection between 2004 and 2006, a multivariate analysis was conducted pooling data obtained from both years.

The multivariate analysis is reported in Table 2. In particular, the probability to acquire HIV infection is two folds higher among women aged 14–19 years compared to other age groups and about 60% higher for women at their first pregnancy.

Finally, in women in the age group 14–19 years, who were positive for syphilis, the proportion of recent HIV-1 infections was two-fold higher than in women in the same age group who were negative for syphilis (47.1% vs. 25.9%, respectively \(P < 0.001\)). No difference was observed in the older age groups.

5. Discussion

The present study provides for the first time a description of recent HIV infections and a detailed analysis of the characteristics of the very aggressive HIV epidemic among pregnant women in Swaziland.

In this study, we used the AI assay to distinguish recent from chronic HIV infections in pregnant women attending ANC clinics in 2004 and 2006 in Swaziland. Data from the Swaziland NHS have shown that the prevalence of HIV infection among pregnant ANC women rose steadily from 3.9% in 1992 to 42.6% in 2004.\(^7\)

In 2006, for the first time, the HIV prevalence slightly declined to 39.2%.\(^2\) Our results show no significant difference in the proportion of recent infections between 2004 and 2006, thus suggesting that a decline in HIV prevalence in 2006 may not imply a decrease in newly acquired HIV infections. This finding is in agreement with previously published data showing that a decline in HIV prevalence may be observed also in the presence of a high and stable incidence rate.\(^11\)

Our results show that young women (14–19 years) and women at their first pregnancy currently represent groups at high-risk for HIV infection in Swaziland. Data are in agreement with those reported recently in a study conducted among the general population in Uganda,\(^12\) where the probability of acquiring the infection was higher among young individuals. As in our results, the same study showed that other demographic and clinical variables did not correlate with incident HIV infection. However, we observed a higher number of recent HIV infections among young women with syphilis.

In generalized epidemics,\(^13\) as the HIV epidemic in Swaziland, surveillance of HIV infection among pregnant women is considered a good proxy to estimate prevalence and incidence of HIV infection in the sexually active population.\(^6,14\) Nevertheless, caution has to be taken in extrapolating the ANC results to the general population. In fact, antenatal clinic surveillance does not provide information about HIV epidemic in men, children and older people since it is representative of only women of reproductive age. In addition, since the rate of contraceptive use among women is not easily assessable, this study is not representative of women who use contraceptive methods. Therefore, population-based surveys are needed to provide further data to depict the epidemic in the general population. Nevertheless, data obtained in ANC women can be of help in understanding the dimension of HIV spreading in the country.

Swaziland can turn the epidemic around. Certainly, it will be essential to continue monitoring the spread of HIV and the epidemic trends by means of a nation-wide surveillance. Survey outcomes are of extreme importance in better planning and strengthening initiatives for prevention treatment, support and care for HIV infections. In particular, this study has pointed out that young women, pregnant for the first time, represent one of the most vulnerable social groups for HIV acquisition. Therefore, the already existing efforts by the Swaziland Government to develop youth programmes to limit HIV spreading among young people need to be strengthened further on.

Funding

The funding organizations and sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Conflict of interest

Authors declare that no conflict of interest exists and that ethical approval has been obtained by the Swaziland Ethics Committee.

Acknowledgements

This study has been partially funded by the project “Estimates of HIV prevalence and incidence and molecular virological studies in Swaziland and South Africa, two sub-Saharan countries highly hit by HIV infection” of the 2006 Italian HIV/AIDS Research Programme and by grant from EC Commission under the VI Framework Programme of Research and Technological Development (2002–2006), Project No. LSHP-CT-2004-503487, AIDS Vaccine Integrated Project (“AVIP”), Authors thank P. Sergiampieti and L. Ronci for the excellent editorial assistance.

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