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Lack of education and early age of coital debut are strongly associated with increased HIV seroprevalence in a cohort of women in Durban, South Africa

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Abstract:

Background: Young women are at high risk for HIV infection in many parts of the world, but particularly in South Africa. We aimed to investigate the association between early age at first sex and lack of education on HIV seroprevalence.

Method: This cross sectional study used demographic, sexual behavior, biologic risk factors and HIV seroconversion results from 3,492 women who were screened for the Methods for Improving Reproductive Health in Africa (MIRA) trial from Durban were included in the current study. Univariate and multivariate logistic regression models were used to determine independent predictors of HIV seropositivity at the screening visit. Trends in key risk factors for HIV infection were investigated by age at sexual debut.

Results: Overall HIV prevalence in this study population was 39%. Early age at sexual debut was significantly associated with increased HIV seropositivity. Not completing high school, lack of cohabitation, a higher number of lifetime partners and diagnosis with HSV2 and sexually transmitted infections (STIs) were also determined to be the independent predictors for HIV infection at the screening visit. The highest rates of all these risk factors were observed in the lowest quintiles of age at sexual debut compared to the higher quintiles ($P_{trend} < 0.001$ all).

Conclusion: The association of HIV status with younger age at sexual debut was likely due to an increased number of lifetime partners. This increase could result from longer duration of sexual life. Prevention of HIV infection should include efforts to delay age at first sex in young women.

INTRODUCTION

Forty percent of all adult women living with HIV reside in southern Africa [1]. In many Southern African countries more than 30% of young women are infected with HIV [2, 3]. Adolescent and young women may be especially prone to HIV infection in comparison with older women due to the occurrence of larger areas of cervical ectopy in young women and an increased likelihood of trauma to the immature genital tract during sex [4, 5]. Early age at sexual debut has been shown to correlate with subsequent risky sexual behaviors [6, 7].

Early sexual debut (commonly defined as having had first sexual intercourse at or before age 14) and experience of sexual coercion or violence have been reported to contribute unintended adolescent pregnancy [8, 9]. In South Africa, data from a nationally representative household survey of young people showed that 7.8% of women aged 15-24 had had sexual intercourse by age 14, and risk of HIV infection was significantly raised if a woman had been sexually active for more than 12 months at the time of the survey [2].

Age at first sex is an important indicator of sexual risk, as it can be used as a proxy for the onset of exposure to HIV infection [10]. Early sexual debut has been associated with increased sexual risk-taking behavior, such as having multiple partners and decreased contraceptive and condom use, and with incidence of sexually transmitted infections [7, 11]. Another study has shown an association between having sex at an early age and incidence of HIV infection in Malawi [12]. Delaying sexual debut may have been one of the key changes in behavior which lead to a decline in HIV infection in Uganda [13, 14].

There is also particular interest in the role of schooling in sexual risk behaviors [10, 15]. The association between level of schooling completed and sexual debut is complex. Although a woman's level of education may be an indicator of socio-economic status, poor socio-economic conditions limiting school access or contributing to poor performance may also correlate with an increase in sexual activity at an early age. In Tanzania, an analysis of the relationship between education level and HIV prevalence has indicated a steep decline over time in HIV infection among people who had a secondary education; of particular interest, the lowest HIV prevalence was found in young women (15-24 years) who had attended secondary school [16]. Overall, a decline in HIV prevalence over time in the most educated group was seen [16]. Earlier meta-analysis of education and HIV prevalence data from across sub-Saharan Africa showed that the relationship between education and risk of HIV infection has changed over time, with more educated individuals initially at higher risk, and with a later shift of risk towards the less educated, the inflection point being around 1996 [17]. Higher education levels are hypothesized to affect the risk of HIV infection through effects on self-efficacy to use prevention technologies, empowerment, and greater exposure to HIV prevention messages [17].

In an attempt to better understand the risk factors that may increase South African women's risk of HIV infection, we first determined the independent risk factors and their population level impact on HIV infection and then investigated the association between age at sexual debut, schooling and other key risk factors for HIV infection in a cohort of sexually active women in rural and semi-rural neighborhoods of Durban.

METHODS:

Study Population:

A total of 3,492 sexually active women who consented to screening for Methods for Improving Reproductive Health in Africa (MIRA) trial of the diaphragm for HIV prevention [18] (September 2002-September 2005; undertaken in Umkomaas and Botha's Hill, southern Durban) were included in this study. The MIRA study was a randomised controlled open-label study comparing the effectiveness of the latex diaphragm plus lubricant gel with provision of condoms alone for prevention of heterosexual HIV acquisition among women. The methodology for the MIRA study has previously been published [18]. Eligibility criteria included: being sexually active; between 18 and 49 years old; HIV negative; *Chlamydia trachomatis* and *Neisseria gonorrhoeae* negative at the screening visit (or willing to be treated if positive); not pregnant and willing to follow the study protocol requirements. All women provided written informed consent at screening.

Study procedures

At screening, an interviewer-administered questionnaire covering topics on demographics and sexual behaviour was undertaken. Participants were also offered HIV pre-test counselling before HIV and STI testing at the screening visit. HIV diagnostic testing was accomplished using two rapid tests on whole blood from either finger-prick or venipuncture: Determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan) and Oraquick (Orasure Technologies, Bethlehem, PA, USA). Those found to be HIV infected were referred to appropriate referral clinics for HIV care. A urine specimen was collected for testing for *Neisseria gonorrhoea*, *Chlamydia trachomatis* and

Trichomonas vaginalis infections, and a blood specimen for syphilis and HSV2 testing. STI testing was completed using the following methodologies: *Chlamydia* and gonorrhea were assessed using PCR (Roche Pharmaceuticals, Branchburg, NJ, USA); syphilis by rapid plasma reagin (RPR) in combination with *Treponema pallidum* haemagglutination (TPHA) (Randox Laboratories, Crumlin, UK); HSV2 by ELISA (FOCUS Diagnostics, Cypress, CA, USA); and *Trichomonas vaginalis* by PCR (Roche Pharmaceuticals, Branchburg, NJ, USA).

The MIRA study protocol was reviewed and approved by the University of California at San Francisco Institutional Review Board Committee on Human Research. The study received ethical approval from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, as well as the ethics review committees at all other local institutions and collaborating organisations. This study is registered with ClinicalTrals.gov, number NCT00121459.

Measurements

The primary outcome was proportion of women who tested positive for HIV infection at the screening visit. Among covariates under consideration: age (<25, 25-34, 35+), education level <12 years (less than high school), employed/earning income (yes/no), cohabitation status (single/not cohabitating vs. married/cohabiting), condom used (during last seven days at baseline; yes/no), using any contraception (yes/no), frequency of sex acts (last seven days), diagnosis with HSV2 and STIs (*Chlamydia*, gonorrhea, syphilis or *Trichomonas vaginalis*) (baseline). Current study only used the available data.

Statistical Analyses

Univariate and multivariate analyses of risk factors associated with HIV infection were assessed using logistic regression, taking the HIV serostatus as the primary outcome. The total study population was ranked by age at sexual debut and divided into quintiles. The relationships between demographic, sexual risk behaviors and quintiles of age at sexual debut were investigated. Prevalence of HIV seropositivity, HSV2 and STIs were also assessed across the quintiles of age at first sexual debut. Tests for linear trend were conducted by using the chi-square test.

To assess effect modification we performed stratified analyses and evaluated the statistical significance of multiplicative interaction terms by comparing the −2 log-likelihood statistics of the main effect model and the model including the interaction term. All P values are two-sided and significance set at the 0.05 level.

Population Attributable Risk

The population attributable risk (PAR) and 95% confidence interval (CI) were calculated to estimate the population level impact of the risk factors associated with HIV infection [19]

Results

A total of 3,492 women consented to be screened for the MIRA trial from Durban clinical research sites. The median age was 26 (inter-quartile range (IQR): 22-33), and the majority of women (96%) reported speaking Zulu at home. Only 26% of the women had completed 12 years of schooling. Approximately 86% were either single or not living with their sexual partners. The median age at sexual debut was 17 (IQR: 16-18) (data not shown). Overall prevalence of HIV

infection, STIs (Chlamydia, gonorrhea or syphilis) and HSV2 were 39%, 16% and 72% respectively (data not shown).

Risk factors for HIV seropositivity

Table 1 presents univariate and multivariate logistic regression results. Age at the screening visit had a inverted U-shape association with risk of HIV seropositivity: compared to the youngest group (i.e. <25 years old), those aged 25-29 years showed increased risk (OR: 1.52, 95% CI: 1.23, 1.87) and those aged 35 or older showed decreased risk for HIV infection. The first four quintiles of age at sexual debut were consistently associated with increased HIV seropositivity. Less than high school education, a higher number of lifetime sexual partners and lack of cohabitation, being diagnosed with HSV2 and STIs were all determined to be independent predictors for HIV seroconversion.

Characteristics of women by quintiles of age at sexual debut

Table 2 presents the distribution of various demographic characteristics, sexual behaviors and biological risk factors according to the quintiles of age at sexual debut. Significant associations were found between age at sexual debut and key sexual risk behaviors. There was a linear decreasing trend observed between the proportion of women who had less than high school education and age at first sex ($P_{trend} < 0.001$). For example, compared with the oldest age at first sexual debut (>20 years), those women in the earliest age at first sexual debut quintile (<15 years) were less likely to have completed high school (62% vs. 86%, p-value for trend <0.001). Women were similar in terms of religion across the quintiles of the age at first sex ($P_{trend} = 0.827$). A slightly higher proportion of women reported speaking Zulu at home in the earlier

quintiles of the age at first sex compared with the higher quintiles ($P_{trend} = 0.037$). Women who initiated sexual activity at an early age were more likely to report not living with their husband or sexual partners compared to those who were older. With regard to the number of sexual partners during their lifetime, early initiators were significantly more likely to have had multiple partners over their lifetimes (having at least four lifetime sex partners; $P_{trend} < 0.001$). However, other sexual risk behaviors such as proportion of women using any contraception or using condoms as their main form of contraception, and frequency of sexual intercourse were similar across the quintiles ($P_{trend} = 0.784$ and $P_{trend} = 0.347$ respectively). The proportion of women who tested positive for HIV infection, STIs and HSV2 was the highest among those who had early onset sexual activity ($P_{trend} < 0.001$, all).

Table 3 presents the proportion of HIV infections attributable to the independent predictors for HIV infection. Delaying age at sexual debut accounted for 26% (95% CI: 21%, 31%) of the HIV infections. If all the participants had completed at least high school education, 13% (95% CI: 10%, 17%) of the HIV infections could theoretically be prevented. Not cohabiting with a sexual partner and reporting at least four or more lifetime sexual partners accounted for 39% (95% CI: 35%, 43%) and 41% (95% CI: 39%, 43%) of the cases respectively. Diagnosis with HSV2 accounted for 82% (95% CI: 80%, 83%) of the HIV cases while STIs accounted for only 5% (95% CI: 4%, 6%) of the cases. The high prevalence and high odds ratio (OR) of HSV2 and relatively low prevalence and low OR of STIs were responsible for these impacts among women.

Discussion:

Not surprisingly, key independent predictors for HIV seropositivity included early initiation of sex, less than high school education, lack of cohabitation, diagnosis with HSV2 and STIs. An inverse association was found between having less than high school education and increasing quintiles of age at sexual debut. Our results showed that women who initiated sexual activity early were more likely to engage in risky sexual behaviours and a clear trend emerged indicating that early onset sexual activity was associated with increased HIV infection, STIs and HSV2, higher number of lifetime sexual partners and lower education level. The strong association between higher number of lifetime sexual partners and decreased age at sexual debut (also noted for young men in rural South Africa by Harrison et al [20]) is important because a higher number of partners, particularly concurrent partners, is believed to play a critical role in the spread of HIV [21, 22].

Several studies have found a correlation between early onset of sexual activity and higher HIV prevalence among young people, who may not be biologically or psychologically ready for sex [23, 24]. In fact, increasing age at first sex appears to be one contributing factor to declines in HIV prevalence among youth in sub-Saharan countries with generalized epidemics [14, 25]; each year of delay in sexual debut has been found to increase the chances that a condom would be used at debut by 1.44 times, which in turn increased the chances of subsequent consistent use substantially (among young men in Kenya) [26].

The proportion of women who had less than high school education was 86% in the lowest quintile of age at sexual debut, compared to 76-62% across other quintiles ($P_{trend} < 0.001$). Early age at sexual debut and not completing high school were also determined to be independent

predictors for HIV infection in multivariate analysis. Although impact of schooling on age at sexual debut is undoubtedly a complex phenomenon [6], the current study presents clear evidence of an inverse association between these two factors (ie. the older a woman is at sexual debut, the less likely it is that she will have less than high school education) and their strong association with HIV infection. The relationship between lack of education, early sexual debut and HIV risk may be indicative of a social milieu in which young women are made vulnerable to HIV infection through interacting factors, with poverty or depressed socio-economic status [27] as one of a number of mediating forces. A number of authors have analysed the relationship between educational attainment and risk of HIV infection, and an emerging dynamic pattern indicates that the correlation differs according to the stage of the epidemic in the country in question [16, 17, 28, 29] – the highest risk shifts from populations with higher to lower educational levels as the epidemic progresses. In the South African context in particular, recent evidence shows that higher levels of education are protective [30], in agreement with our findings.

This study is exploratory, raising questions about the dynamics of women's vulnerability and sexual risk. Lack of education may indeed be the key factor and pathway to risk among women with early sexual debut, although we could not determine the direction of the association. However, recent data from northern Malawi appears to indicate that early sexual debut may lead to school drop-out since girls were viewed as "ready for marriage" [31]. Whatever the reasons, these findings highlight a troubling lack of preparation for sexual activity among women in this setting. Further research should be directed toward better understanding young women's early relationships and sexual experiences, and to the design of interventions for pre-adolescent young

women and men, before sexual debut. Interventions for young women should start in late primary (10-12 years old) [32] or early high school (13-15 years old) to emphasize condom promotion, as well as delay in sexual activity. Reaching young women prior to sexual debut is important since it has been shown that better outcomes may be expected from programmes which establish protective behaviours, rather than attempting to change existing behaviour patterns [33]. A "life-skills orientation" programme addressing HIV prevention education (among other needs) was intended to be fully implemented in South African high schools by 2005, and thus the women in this study may not have directly benefited from this intervention. The need for this programme to be fully and urgently implemented is also supported by decreases in the age of sexual debut in later birth cohorts and increases in risky behavior found in a study conducted among rural South Africans aged 14-35 years during the period 2001-2004 [34]. Continued assessment of the impact of the life skills orientation programme on HIV protective behaviours and especially biological outcomes among young people will be necessary for an effective tailored response to the epidemic – early research indicated that the programme had positive effects on reported condom use among adolescents in two centres in KwaZulu-Natal, but that other effects were at best modest [35]. Also of concern in light of this is evidence from a large scale prevention/education programme in Tanzania which found no effect on HIV or STI prevalence, although changes in behavior were reported [36].

Delay of sexual debut is an important tactic in HIV prevention among youth, resulting in fewer years at high risk. Sexual health education programmes may have positive impacts on delay of sexual debut and other protective and risk taking behaviours among young people, with several found to have an enduring effect [37], but the delivery of such programmes in schools mired in

highly patriarchal and didactic contexts severely undermines their usefulness [38]. Effective school-based HIV prevention education is likely to require wholesale structural changes to the educational system in the countries most affected by the HIV epidemic. However, it can be conclusively stated that "abstinence-only" programs, which promote complete sexual abstinence as the only effective method for preventing unintended pregnancy and sexually transmitted infections including HIV, have not been demonstrated to reduce risk of HIV infection as assessed by self reported outcomes [39]. Recommended instead are comprehensive sexual education programs that include sex education and information on abstinence, delay of sexual debut, partner number limitation, condom use, contraception and empowerment of young women.

Potential limitations of study

Several limitations need to be borne in mind when considering the interpretation of our results: the nature of the research conducted required that the population selected have an established moderate-to-high risk of HIV infection. Current study was cross sectional and only used the data from those who were screened for the study. The study population may have limited representativeness. We cannot rule out the effects of unmeasured characteristics such as multiple or concurrent sex partners and commercial sex on our findings. No data concerning migration, socio-economic status at the time of sexual debut, or sexual behavior data from male partners were collected or included in these analyses. We could not determine whether young women were in school at the time of sexual debut.

Conclusion

Our results indicate that a theory-based, contextually appropriate HIV/STD risk-reduction programme may be important in shaping the sexual behavior of young adolescents before or at the beginning of their sexual lives. Particularly, efforts to delay sexual debut should be incorporated into comprehensive sexual education programs and should begin early, offering age-appropriate messages over time. Comprehensive sexual education programs are typically targeted towards youth and are predominately school-based. Opportunities to reach out-of-school youth, who may be at heightened vulnerability, should be identified as well. These programs may include messages such as reinforcing safer sex practices for young people who are already sexually active.

ARTICLE SUMMARY:

Article focus:

- Early sexual debut and experience of sexual coercion may increase women's vulnerability to HIV infection.
- Early sexual debut has been associated with increased sexual risk-taking behavior, such as having multiple partners and decreased contraceptive and condom use, and with incidence of sexually transmitted infections.
- Delaying sexual debut may have been one of the key changes in behavior which lead to a decline in HIV infection in the past.

Key Messages:

- Our results showed that women who initiated sexual activity early were more likely to engage in risky sexual behaviours.

- A clear trend observed indicating that early onset sexual activity was associated with increased HIV infection, STIs and HSV2.
- Comprehensive sexual education programs should reach out-of-school youth, who may be at heightened vulnerability, should be identified as well.

Limitations and Strengths:

- Current study was cross sectional and only used the data from those who were screened for the study.
- We cannot rule out the effects of unmeasured characteristics such as multiple or concurrent sex partners and commercial sex on our findings. No data concerning migration, socio-economic status at the time of sexual debut, or sexual behavior data from male partners
- Nevertheless, current study used the data from the region where the HIV epidemic is severely high among particularly young women.

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Competing interests: The authors declare that they have no competing interests.

Authors' contributions: GR was the principal investigator of the MIRA Durban clinical research sites. HW performed the statistical analysis. GR, HW and CW interpreted and drafted the manuscript. All authors read and approved the final manuscript.

Role of funding source: The sponsor of the study had oversight of the trial through regular progress reports and meetings with investigators. The sponsor had no role in the data collection, data analysis, data interpretation, or writing of the report.

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Table 1: Multivariate logistic regression: Odds Ratios (OR) and 95% confidence intervals (CI) for HIV infection

	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (years)				
<25 year	1		1	
25-29 years	1.98 (1.66,2.38)	<0.001	1.52 (1.23,1.87)	<0.001
30-34 years	1.60 (1.32,1.95)	<0.001	1.19 (0.94,1.50)	0.142
35 plus	0.70 (0.58,0.85)	<0.001	0.50 (0.40,0.63)	<0.001
Age at first sex (years)				
<15 year (1st quintile)	1.87 (1.47,2.36)	<0.001	1.96 (1.53,2.51))	<0.001
15-16 years (2nd quintile)	1.50 (1.17,1.90)	0.001	1.61 (1.25,2.08)	<0.001
17-18 years (3rd quintile)	1.41 (1.13,1.76)	0.002	1.49 (1.19,1.87)	0.001
19-20 years (4th quintile)	1.16 (0.87,1.54)	0.316	1.18 (0.89,1.59)	0.251
20+ (5th quintile)	1		1	
At least high school education				
Yes	1		1	
No	1.21 (1.04,1.41)	0.010	1.37 (1.15,1.62)	<0.001
English spoken at home				
Yes	1		-	
No	1.91 (1.53,2.39)	<0.001	-	
Religion				
Other	1			
Christian	1.34 (1.06,1.71)	0.015	-	
Number of lifetime sex partners				
One	1		1	
Two	2.49 (2.01,3.10)	<0.001	1.73 (1.37,2.20)	<0.001
Three	4.35 (3.47,5.44)	<0.001	2.82 (2.20,3.62)	<0.001
Four or more	6.65 (5.35,8.26)	<0.001	4.09 (3.20,5.22)	<0.001
Cohabitation status				
Married/living w/ a sexual partner	1		1	
Not married/not living w/ a sexual partner	7.02 (5.20,9.47)	<0.001	4.32 (3.11,6.00)	<0.001
Have you ever had vaginal sex with condom?				
No	1			
Yes	1.40 (1.21,1.63)	<0.001	-	
Using any contraception [1]				
Yes	1			
No	1.36 (1.16,1.60)	<0.001	-	
HSV diagnosis				
No	1		1	
Yes	7.20 (5.88,8.82)	<0.001	6.19 (4.98,7.70)	<0.001
STI diagnosis[2]				
No	1		1	
Yes	1.34 (1.12,1.61)	0.001	1.24 (1.02, 1.51)	0.038

[1]any of the following: male condom, female condom, pills, injectables, spermicides, withdrawal, long term (partner vasectomy, tubal ligation) other.

[2] *Chlamydia*, gonorrhea, syphilis or *Trichomonas vaginalis*

Table 2: Association of various socio-demographic and risk characteristics with age at first sex.

	Age at first sex (quintiles)					
	1st quintile <15 years	**2nd quintile** 15-16 years	**3rd quintile** 17-18 years	**4th quintile** 19-20 years	**5th quintile** >20 years	**p-value**
Demographic factors						
Less than high school education	86%	76%	71%	67%	62%	<0.001
Zulu speaking at home	96%	95%	95%	92%	92%	0.037
Religion- Christian	91%	90%	90%	90%	91%	0.827
Single/Not living with a regular sex partner	88%	89%	87%	85%	81%	<0.001
Sexual risk behaviors						
Using condom (as current contraception)	45%	42%	47%	45%	46%	0.347
Using any contraception	77%	77%	77%	80%	78%	0.784
At least 4 life time sex partners	39%	29%	25%	18%	14%	<0.001
Biological risk factors						
HIV prevalence	48%	43%	41%	37%	33%	<0.001
STI prevalence	20%	15%	14%	13%	12%	<0.001
HSV prevalence	79%	74%	71%	70%	68%	<0.001

Table 3: Theoretical Prevention strategies

Modifiable risk factors	PAR% (95% CI)
Full PAR	**0.85 (0.84,0.87)**
Age at first sex (<15)	0.26 (0.21-0.31)
Less than high school	0.13 (0.10,0.17)
Age at first sex	
+ less than high school	**0.32 (0.27,0.38)**
Not cohabiting	0.39 (0.35,0.43)
Number of life time male sex partners	
Two	0.12 (0.11,0.13)
Three	0.20 (0.18,0.21)
Four or more	0.41 (0.39,0.43)
Biological risk factors	
Tested positive for STI [1]	0.05 (0.04,0.06)
HSV 2	0.82 (0.80,0.83)

[1] *Chlamydia*, gonorrhea, syphilis or *Trichomonas vaginalis* at screening

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (page 1)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 1-2)
Objectives	3	State specific objectives, including any prespecified hypotheses (page 3)
Methods		
Study design	4	Present key elements of study design early in the paper(pages 4-6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 4)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (N/A)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed (N/A)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (4-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (4-6)
Bias	9	Describe any efforts to address potential sources of bias (page 12)
Study size	10	Explain how the study size was arrived at (N/A)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (4-5)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Pages 4-6)
		(*b*) Describe any methods used to examine subgroups and interactions (N/A)
		(*c*) Explain how missing data were addressed (5)
		(*d*) If applicable, explain how loss to follow-up was addressed (N/A)
		(*e*) Describe any sensitivity analyses (N/A)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (N/A)
		(b) Give reasons for non-participation at each stage (N/A)
		(c) Consider use of a flow diagram (N/A)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (6)
		(b) Indicate number of participants with missing data for each variable of interest (5)
		(c) Summarise follow-up time (eg, average and total amount) (N/A)
Outcome data	15*	Report numbers of outcome events or summary measures over time (6)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (6-7)

		(*b*) Report category boundaries when continuous variables were categorized (pages 5-6 and page 7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (N/A)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (6)
Discussion		
Key results	18	Summarise key results with reference to study objectives (pages 12-13)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (pages 13-14)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (page 12-13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (12)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (14)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Early age of coital debut is associated with increased HIV seroprevalence and incidence in a cohort of women in Durban, South Africa

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Early age of coital debut is associated with increased HIV seroprevalence and incidence in a cohort of women in Durban, South Africa

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Abstract:

Objective: We aimed to investigate the impact of early sexual debut on HIV seroprevalence and incidence rates among women a cohort of women.

Method: A total of 3,492 women who were screened for the Methods for Improving Reproductive Health in Africa (MIRA) trial from Durban were included in the current study. Trends in key risk factors for HIV infection were investigated by the quintiles of the age at sexual debut. Univariate and multivariate logistic regression models were used to determine independent predictors of HIV seropositivity at the screening visit. Impact of early sexual debut was investigated among women who enrolled in the trial using Kaplan-Meir curves, unadjusted and adjusted Cox regression models.

Results: Lowest quintiles of age at sexual debut, less than high school education, a higher number of lifetime sexual partners and lack of cohabitation, being diagnosed with HSV2 and other sexually transmitted infections were all significantly associated with prevalent HIV infection in multivariate analysis. During follow-up, 148 (6.8 per 100 person year) women seroconverted. Highest seroconversion rate was observed among women who had reported to have had sex 15 years or younger (12.0 per 100 person year). Overall, impact of risk factors considered in this study was associated with considerable potential reductions in HIV prevalence and incidence rates (85% and 77% respectively).

Conclusion: The association of HIV status with younger age at sexual debut may likely due to an increased number of lifetime partners. This increase could result from longer duration of sexual life. Prevention of HIV infection should include efforts to delay age at first sex in young women.

INTRODUCTION

Forty percent of all adult women living with HIV reside in southern Africa [1]. In many Southern African countries more than 30% of young women are infected with HIV [2, 3]. Adolescent and young women may be especially prone to HIV infection in comparison with older women due to the occurrence of larger areas of cervical ectopy in young women and an increased likelihood of trauma to the immature genital tract during sex [4, 5]. Early age at sexual debut has been shown to correlate with subsequent risky sexual behaviors [6, 7].

Early sexual debut (commonly defined as having had first sexual intercourse at or before age 14) and experience of sexual coercion or violence have been reported to contribute unintended adolescent pregnancy [8, 9]. In South Africa, data from a nationally representative household survey of young people showed that 7.8% of women aged 15-24 had had sexual intercourse by age 14, and risk of HIV infection was significantly raised if a woman had been sexually active for more than 12 months at the time of the survey [2].

Age at first sex is an important indicator of sexual risk, as it can be used as a proxy for the onset of exposure to HIV infection [10]. Early sexual debut has been associated with increased sexual risk-taking behavior, such as having multiple partners and decreased contraceptive and condom use, and with incidence of sexually transmitted infections [7, 11]. Another study has shown an association between having sex at an early age and incidence of HIV infection in Malawi [12]. Delaying sexual debut may have been one of the key changes in behavior which lead to a decline in HIV infection in Uganda [13, 14].

There is also particular interest in the role of schooling in sexual risk behaviors [10, 15]. The association between level of schooling completed and sexual debut is complex. Although a woman's level of education may be an indicator of socio-economic status, poor socio-economic conditions limiting school access or contributing to poor performance may also correlate with an increase in sexual activity at an early age. In Tanzania, an analysis of the relationship between education level and HIV prevalence has indicated a steep decline over time in HIV infection among people who had a secondary education; of particular interest, the lowest HIV prevalence was found in young women (15-24 years) who had attended secondary school [16]. Earlier meta-analysis of education and HIV prevalence data from across sub-Saharan Africa showed that the relationship between education and risk of HIV infection has changed over time, with more educated individuals initially at higher risk, and with a later shift of risk towards the less educated, the inflection point being around 1996 [17].

In an attempt to better understand the risk factors that may increase South African women's risk of HIV infection, we investigated the independent risk factors and their population level impact on HIV infection among sexually active women in rural and semi-rural neighborhoods of Durban. A sub-analysis was performed to investigate the association between the age at sexual debut and HIV seroconversion during the study by including only women who were HIV seronegative at the screening and enrolled in the study.

METHODS:

Study Population:

A total of 3,492 sexually active women who consented to screening for Methods for Improving Reproductive Health in Africa (MIRA) trial of the diaphragm for HIV prevention [18]

(September 2002-September 2005; undertaken in Umkomaas and Botha's Hill, southern Durban) were included in this study. The MIRA study was a randomised controlled open-label study comparing the effectiveness of the latex diaphragm plus lubricant gel with provision of condoms alone for prevention of heterosexual HIV acquisition among women. The methodology for the MIRA study has previously been published [18]. Eligibility criteria included: being sexually active (defined as average of 4 sexual acts per month); between 18 and 49 years old; HIV negative; *Chlamydia trachomatis* and *Neisseria gonorrhoeae* negative at the screening visit (or willing to be treated if positive); not pregnant and willing to follow the study protocol requirements. All women provided written informed consent at screening.

Study procedures

At screening, an interviewer-administered questionnaire covering topics on demographics and sexual behaviour was undertaken. Participants were also offered HIV pre-test counselling before HIV and STI testing at the screening visit. HIV diagnostic testing was accomplished using two rapid tests on whole blood from either finger-prick or venipuncture: Determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan) and Oraquick (Orasure Technologies, Bethlehem, PA, USA). Those found to be HIV infected were referred to appropriate referral clinics for HIV care. A urine specimen was collected for testing for gonorrhoea, chlamydia and trichomonas vaginalis infections, and a blood specimen for syphilis and HSV2 testing. STI testing was completed using the following methodologies: Chlamydia and gonorrhea were assessed using PCR (Roche Pharmaceuticals, Branchburg, NJ, USA); syphilis by rapid plasma reagin (RPR) in combination with *Treponema pallidum* haemagglutination (TPHA) (Randox Laboratories, Crumlin, UK); HSV2 by ELISA (FOCUS Diagnostics, Cypress, CA, USA); and *Trichomonas vaginalis* by PCR (Roche Pharmaceuticals, Branchburg, NJ, USA).

At the enrolment visit, participants provided written informed consent, underwent a pelvic examination, provided a blood sample for testing for syphilis (rapid plasma reagin [RPR] and *Treponema pallidum* haemagglutinin [TPHA], Randox Laboratories, Crumlin, UK) and herpes simplex virus 2 (HSV2; ELISA, FOCUS Diagnostics, Cypress, CA, USA), and provided a urine sample for pregnancy testing. After enrolment, at each quarterly follow-up visit, HIV and STI testing were conducted.

The MIRA study protocol was reviewed and approved by the University of California at San Francisco Institutional Review Board Committee on Human Research. The study received ethical approval from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, as well as the ethics review committees at all other local institutions and collaborating organisations. This study is registered with ClinicalTrials.gov, number NCT00121459.

Measurements

The primary outcomes were proportion of women who tested positive for HIV infection at the screening visit and time to seroconversion among those who tested negative for HIV infection and enrolled in the study. Among covariates under consideration: age at the screening (<25, 25-34, 35+), age at sexual debut (in quintiles: <15 years, 15-16 years, 17-18 years, 19-20 years and 21 years or older), education level <12 years (less than high school), language spoken at home (English versus others), religion (Christian versus other), cohabitation status (single/not cohabitating vs. married/cohabiting), ever had vaginal sex using condom (yes/no), using any contraception (yes/no), diagnosis with herpes simplex virus 2 (HSV2) and other sexually transmitted infections (STIs) (chlamydia, gonorrhea, syphilis or trichomonas vaginalis). Current study only used the available data.

Statistical Analyses

We expressed descriptive data as percentages, medians and interquartile ranges (IQR). Chi-square test for trend (categorical variables) and Kruskal-Wallis test (continuous variables) were used to formally compare the risk factors across the quintiles of age at sexual debut.

We examined the individual associations of the risk factors described above using univariate logistic regression, taking the HIV serostatus as the primary outcome. Next, marginally significant variables with $p < 0.10$ in univariate analysis were included in multivariate analysis. Variables with $p < 0.05$ were retained in the final multivariate logistic model in a forward stepwise manner. We also tested for interactions at $p < 0.10$ for entry into the model. Finally, to test the fitness of the model, we performed the goodness of fit test examined using the Hosmer-Lemeshow criteria. A low chi-square value and a high non-significant *P* value for the Hosmer-Lemeshow test indicated that the fit of the model and observed values was acceptable.

The incidence rate for HIV infection, expressed as time to seroconversion, was estimated for women who were HIV-negative at screening and satisfied the eligibility criteria. The date of seroconversion was estimated using the midpoint between the last negative and the first positive antibody test results within the follow-up period. Kaplan–Meier survival analyses were carried out to estimate the crude HIV incidence rate over time; results were stratified and compared by the five age groups of sexual debut. Cox proportional hazard regression analysis was performed to calculate unadjusted and adjusted HIV incidence rates.

All analyses were performed using Stata 10.0 (College Station, Texas) and SAS 9.2 (Rayleigh, North Carolina).

Population Attributable Risk

Although, odds ratios (ORs) and relative risks (RRs) may be well suited to assessment of causality, they do not provide information about the potential impact on disease occurrence by eliminating these risk factors. The population attributable risk (PAR) provides additional information to determine the public health implications of risk factor reduction/elimination at population level. Briefly, we estimated PARs associated with modifiable (at least theoretically) and their 95% confidence interval (CI) to estimate the population level impact of the risk factors associated with HIV seroprevalence and incidence [19]. We estimated the proportion of HIV seroprevalence and incidence cases could be attributed to the combined effect of these risk factors.

Results

A total of 3,492 women consented to be screened for the MIRA trial from Durban clinical research sites. The median age was 26 (inter-quartile range (IQR): 22-33), and the majority of women (96%) reported speaking Zulu at home. Only 26% of the women had completed 12 years of schooling. Approximately 86% were either single or not living with their sexual partners. The median age at sexual debut was 17 (IQR: 16-18) (data not shown). Overall prevalence of HIV infection, STIs (chlamydia, gonorrhea, syphilis or trichomonas vaginalis) and HSV2 were 41%, 16% and 73% respectively.

HIV seroprevalence and other characteristics of women by quintiles of age at sexual debut

Table 1 presents the distribution of various demographic characteristics, sexual behaviors and biological risk factors according to the quintiles of age at sexual debut. Compared to the highest two quintiles, women in the lowest three quintiles were younger (P<0.001). Significant associations were found between age at sexual debut and key sexual risk behaviors. There was a linear decreasing trend observed between the proportion of women who had less than high school education and age at first sex ($P_{trend} < 0.001$). For example, compared with the oldest age at first sexual debut (>20 years), those women in the earliest age at first sexual debut quintile (<15 years) were less likely to have completed high school (62% vs. 86%, P_{trend} <0.001). Women were similar in terms of religion across the quintiles of the age at first sex ($P_{trend} = 0.827$). A slightly higher proportion of women reported speaking Zulu at home in the earlier quintiles of the age at first sex compared with the higher quintiles ($P_{trend} = 0.037$). Women who initiated sexual activity at an early age were more likely to report not living with their husband or sexual partners compared to those who were older. With regard to the number of sexual partners during their lifetime, early initiators were significantly more likely to have had multiple partners over their lifetimes (having at least four lifetime sex partners; $P_{trend} < 0.001$). However, other sexual risk behaviors such as proportion of women using any contraception and condoms as their main form of contraception were similar across the quintiles ($P_{trend} = 0.784$ and $P_{trend} = 0.347$ respectively). The proportion of women who tested positive for HIV infection, STIs and HSV2 were the highest among those who had early onset sexual activity ($P_{trend} < 0.001$, all).

Risk factors for HIV seropositivity

Table 2 presents univariate and multivariate logistic regression results. Age at the screening visit had an inverted U-shape association with risk of HIV seropositivity: compared to the youngest group (i.e. 18-25 years old), those aged 25-29 years showed increased risk (OR: 1.52, 95% CI: 1.23-1.87) and those aged 35 or older showed decreased risk for HIV infection. Lowest quintiles

of age at sexual debut, less than high school education, a higher number of lifetime sexual partners and lack of cohabitation, being diagnosed with HSV2 and STIs were all significantly associated with prevalent HIV infection in multivariate analysis. The p-value for the interaction between the quintiles of age and education in univariate analysis was calculated to be 0.683, therefore, interaction effect between these factors in the model was not considered further. The p-value for the Hosmer-Lemeshow goodness-of-fit was 0.416 indicating that the fit of the model and observed values was acceptable.

HIV incidence by the quintiles of age at sexual debut

Table 2 summarizes the crude incidence rate ratios and results from the Cox proportional hazard analyses unadjusted and adjusted risk. A total of 1,485 women enrolled in the MIRA trial, from the Durban sites, and 148 (10.0%) HIV infections occurred during the study. Figure 1 presents the Kaplan-Meier curves stratified by the quintiles of the age at sexual debut for HIV seroconversion. The most prominent effect of age at sexual debut was observed among women who had reported to have had sex 15 years or younger compared to those who had their sexual debut after 20 years of age (12.0 per 100 person year versus 6.4 per 100 person year).

We also used unadjusted and adjusted Cox proportional-hazards modelling to examine the associations between the risk of HIV seroconversion and the groups at age first sex. This analysis was not an attempt to quantify causal links for HIV seroconversion, but rather to control statistically for confounding for demographic and sexual risk behaviour differences (Table 3). Women who had their first sexual experience at age 15 year or younger had significantly higher risk of HIV acquisition compared to those 20 years or older (Hazard ratio (HR): 1.97, 95% Confidence Interval (CI):1.07-3.63, P = 0.029). This association was slightly attenuated when the

analysis was adjusted for age, level of education, number of sexual partners, cohabitation status and ever used condom with vaginal sex (HR: 1.78, 95%CI: 0.96-3.29, P = 0.066).

Result from Population attributable risk

Table 4 presents the proportion of HIV infections (prevalence and incidence) attributable to the independent predictors for HIV infection. Delaying age at sexual debut accounted for 26% (95% CI: 21%, 31%) of the HIV infections at the screening. Completing at least high school education, 13% (95% CI: 10%, 17%) of the HIV seropositivity at the screening could theoretically be prevented. Not cohabiting with a sexual partner and reporting at least four or more lifetime sexual partners accounted for 39% (95% CI: 35%, 43%) and 41% (95% CI: 39%, 43%) of the cases respectively. Diagnosis with HSV2 accounted for 82% (95% CI: 80%, 83%) of the HIV cases while STIs accounted for only 5% (95% CI: 4%, 6%) of the cases. The high prevalence and high odds ratio (OR) of HSV2 and relatively low prevalence and low OR of STIs were responsible for these impacts among women.

We also present estimates of the proportion of HIV incidence cases attributable to the five modifiable risk factors – namely, early age at sexual debut (<15 years), cohabitation status, number of sexual partners, and diagnosis with HSV2 and STIs. Level of education was not associated with HIV seroconversion therefore it was not included in this analysis. In the overall study population, these factors accounted for 77% (95% CI: 72%-82%) of cases. Lack of cohabitation appeared to have the largest impact on the risk of HIV seroconversion with 54% (95% CI: 46%-62%) followed by diagnosis with HSV2 (PAR=21%, 95% CI: 14%-31%), early age at first sex (PAR=17%, 95% CI: 10%-21%) and four or more lifetime sexual partners (PAR=16%, 95% CI: 13%-20%).

Discussion:

In this study of sexually active young women who initiated sexual activity early, were significantly associated with prevalent HIV infection in multivariate analysis along with the other factors namely – reporting less than high school education, more than one lifetime sexual partners, single/not cohabiting with a sexual partner, diagnosis with HSV2 and other sexually transmitted infections at the screening. In addition, women 25- to 29- years-old were more likely to be infected compared with those ages <25 years. Several studies have reported associations between early onset of sexual activity and higher HIV prevalence among young people, who may not be biologically or psychologically ready for sex [20-24]. In fact, increasing age at first sex appears to be one contributing factor to declines in HIV prevalence among youth in sub-Saharan countries with generalized epidemics [14, 25]; each year of delay in sexual debut has been found to increase the chances that a condom would be used at debut by 1.44 times, which in turn increased the chances of subsequent consistent use substantially (among young men in Kenya) [26]. The relationship between lack of education, early sexual debut and HIV risk may be indicative of a social milieu in which young women are made vulnerable to HIV infection through interacting factors, with poverty or depressed socio-economic status [27] as one of a number of mediating forces.

A number of authors have analysed the relationship between educational attainment and risk of HIV infection, and an emerging dynamic pattern indicates that the correlation differs according to the stage of the epidemic in the country in question [16, 17, 28, 29] – the highest risk shifts from populations with higher to lower educational levels as the epidemic progresses. In the South African context in particular, recent evidence shows that higher levels of education are protective [30], in agreement with our findings.

This study is exploratory, raising questions about the dynamics of women's vulnerability and sexual risk. Lack of education may indeed be the key factor and pathway to risk among women with early sexual debut, although we could not determine the direction of the association. However, recent data from northern Malawi appears to indicate that early sexual debut may lead to school drop-out since girls were viewed as "ready for marriage" [31].

Our results also showed that women who initiated sexual activity early were more likely to engage in risky sexual behaviours and a clear trend emerged indicating that early onset sexual activity was associated with increased risk of HIV seroconversion during the study follow up.

Overall, impact of risk factors considered in this study was associated with considerable potential reductions in HIV prevalence and incidence rates (85% and 77% respectively). If these results are applied to the target population, majority of the HIV cases among women could potentially have been avoided by effective public health interventions, particularly measures aimed at reducing number of lifetime sexual partners by keeping couples together through changes in labor migrating patterns.

Interventions for young women should start in late primary (10-12 years old) [32] or early high school (13-15 years old) to emphasize condom promotion, as well as delay in sexual activity. Reaching young women prior to sexual debut is important since it has been shown that better outcomes may be expected from programmes which establish protective behaviours, rather than attempting to change existing behaviour patterns [33]. A "life-skills orientation" programme addressing HIV prevention education (among other needs) was intended to be fully implemented

in South African high schools by 2005, and thus the women in this study may not have directly benefited from this intervention [34].

Continued assessment of the impact of the life skills orientation programme on HIV protective behaviours and especially biological outcomes among young people will be necessary for an effective tailored response to the epidemic – early research indicated that the programme had positive effects on reported condom use among adolescents in two centres in KwaZulu-Natal, but that other effects were at best modest [35]. Also of concern in light of this is evidence from a large scale prevention/education programme in Tanzania which found no effect on HIV or STI prevalence, although changes in behavior were reported [36].

Potential limitations of study

Several limitations need to be borne in mind when considering the interpretation of our results: These data were taken from the responses of women participating in a large randomized controlled trial. Therefore they may have limited representativeness. We also cannot rule out the effects of unmeasured characteristics such as poverty, cultural differences, multiple or concurrent sex partners and commercial sex on our findings. No data concerning migration, socio-economic status at the time of sexual debut, or sexual behavior data from male partners were collected or included in these analyses. We could not determine whether young women were in school at the time of sexual debut.

Current study calculated the PAR where both the odds and hazard ratios and population prevalences were estimated from the same study population. In order to interpret a PAR as the proportion of cases caused by a risk factor and thus that could be prevented by its elimination

from the target population, causality needs to be proven. When PARs are estimated for more speculative risk factors, they can be regarded as measuring potential impact on disease incidence and the potential reduction in disease incidence could be attained from their elimination were later proven to be causal.

Conclusion

Our results indicate that a theory-based, contextually appropriate HIV/STD risk-reduction programme may be important in shaping the sexual behavior of young adolescents before or at the beginning of their sexual lives. Particularly, efforts to delay sexual debut should be incorporated into comprehensive sexual education programs and should begin early, offering age-appropriate messages over time. Comprehensive sexual education programs are typically targeted towards youth and are predominately school-based. Opportunities to reach out-of-school youth, who may be at heightened vulnerability, should be identified as well. These programs may include messages such as reinforcing safer sex practices for young people who are already sexually active.

ARTICLE SUMMARY:

Article focus:

- Early sexual debut may increase women's vulnerability to HIV infection.
- Early sexual debut has been associated with increased sexual risk-taking behavior, such as having multiple partners
- Delaying sexual debut may have been one of the key changes in behavior which lead to a decline in HIV infection in the past.

Key Messages:

- Our results showed that women who initiated sexual activity early were more likely to engage in risky sexual behaviours.
- A clear trend observed indicating that early onset sexual activity was associated with increased HIV seroprevalence and incidence.
- Comprehensive sexual education programs should reach out-of-school youth, who may be at heightened vulnerability, should be identified as well.

Limitations and Strengths:

- Current study was cross sectional and only used the data from those who were screened for the study.

- We cannot rule out the effects of unmeasured characteristics such as multiple or concurrent sex partners and commercial sex on our findings. No data concerning migration, socio-economic status at the time of sexual debut, or sexual behavior data from male partners
- Nevertheless, current study used the data from the region where the HIV epidemic is severely high among particularly young women.

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Competing interests: The authors declare that they have no competing interests.

Authors' contributions: GR was the principal investigator of the MIRA Durban clinical research sites. HW performed the statistical analysis. GR and HW interpreted and drafted the manuscript. Both authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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Table 1: Association of various socio-demographic and risk characteristics with age at first sex.

	Age at first sex (quintiles)					
	1st quintile <15 years	**2nd quintile** 15-16 years	**3rd quintile** 17-18 years	**4th quintile** 19-20 years	**5th quintile** >20 years	**p-value**
Demographic factors						
Age (years), median (IQR)	27 (21-34)	25 (20-32)	25 (21-32)	28 (23-34)	30 (25-35)	<0.001†
Less than high school education	86%	76%	71%	67%	62%	<0.001
Zulu speaking at home	96%	95%	95%	92%	92%	0.037
Religion- Christian	91%	90%	90%	90%	91%	0.827
Single/Not living with a regular sex partner	88%	89%	87%	85%	81%	<0.001
Sexual risk behaviors						
Using condom (as current contraception)	45%	42%	47%	45%	46%	0.347
Using any contraception	77%	77%	77%	80%	78%	0.784
At least 4 life time sex partners	39%	29%	25%	18%	14%	<0.001
Biological risk factors						
HIV prevalence	48%	43%	41%	37%	33%	<0.001
STI prevalence	20%	15%	14%	13%	12%	<0.001
HSV prevalence	79%	74%	71%	70%	68%	<0.001

†Kruskal-Wallis test was used

Table 2: Multivariate logistic regression: Odds Ratios (OR) and 95% confidence intervals (CI) for HIV infection

		UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	Prevalence (%)	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (years)					
<25 year	41	1		1	
25-29 years	21	1.98 (1.66,2.38)	<0.001	1.52 (1.23,1.87)	<0.001
30-34 years	16	1.60 (1.32,1.95)	<0.001	1.19 (0.94,1.50)	0.142
35 plus		0.70 (0.58,0.85)	<0.001	0.50 (0.40,0.63)	<0.001
Age at first sex (years)					
<15 year (1^{st} quintile)	20	1.87 (1.47,2.36)	<0.001	1.96 (1.53,2.51))	<0.001
15-16 years (2^{nd} quintile)	20	1.50 (1.17,1.90)	0.001	1.61 (1.25,2.08)	<0.001
17-18 years (3^{rd} quintile)	20	1.41 (1.13,1.76)	0.002	1.49 (1.19,1.87)	0.001
19-20 years (4^{th} quintile)	20	1.16 (0.87,1.54)	0.316	1.18 (0.89,1.59)	0.251
21+ (5^{th} quintile)	20	1		1	
At least high school education					
Yes	26	1		1	
No	74	1.21 (1.04,1.41)	0.010	1.37 (1.15,1.62)	<0.001
English spoken at home					
Yes	5	1		-	
No	95	1.91 (1.53,2.39)	<0.001	-	
Religion					
Other	9	1			
Christian	91	1.34 (1.06,1.71)	0.015	-	
Number of lifetime sex partners					
One	25	1		1	
Two	28	2.49 (2.01,3.10)	<0.001	1.73 (1.37,2.20)	<0.001
Three	21	4.35 (3.47,5.44)	<0.001	2.82 (2.20,3.62)	<0.001
Four or more	26	6.65 (5.35,8.26)	<0.001	4.09 (3.20,5.22)	<0.001
Cohabitation status					
Married/living w/ a sexual partner	15	1		1	
Not married/not living w/ a sexual partner	85	7.02 (5.20,9.47)	<0.001	4.32 (3.11,6.00)	<0.001
Have you ever had vaginal sex with condom?					
No	30	1			

Yes	70	1.40 (1.21,1.63)	<0.001	-	
Using any contraception [1]					
Yes	78	1			
No	22	1.36 (1.16,1.60)	<0.001	-	
HSV diagnosis					
No	27	1		1	
Yes	73	7.20 (5.88,8.82)	<0.001	6.19 (4.98,7.70)	<0.001
STI diagnosis[2]					
No	84	1		1	
Yes	16	1.34 (1.12,1.61)	0.001	1.24 (1.02, 1.51)	0.038

[1]any of the following: male condom, female condom, pills, injectables, spermicides, withdrawal, long term (partner vasectomy, tubal ligation) other.
[2] *Chlamydia*, gonorrhea, syphilis or *trichomonas vaginalis*

Table 3: Estimated crude incidence rates and hazard ratios for time to HIV by the quintiles of age at first sex

	Crude incidence rate per 100 PY (95% CI)	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at first sex (years)					
<15	12.0 (8.0,18.0)	1.97 (1.07,3.63)	0.029	1.78 (0.96,3.29)	0.066
15-16	7.2 (5.7,9.0)	1.11 (0.69,1.80)	0.664	0.90 (0.53,1.46)	0.633
17-18	6.1 (4.1,9.0)	0.95 (0.53,1.69)	0.851	0.82 (0.45,1.47)	0.496
19-20	5.1 (2.9,9.0)	0.78 (0.39,1.60)	0.503	0.72 (0.35,1.67)	0.365
21 +	6.4 (4.2,9.8)	1		1	

[1]adjusted for age, number of lifetime sex partners, cohabitation status, level of education, ever used condom with vaginal sex

Table 4: Estimated PAR (95% CI) for risk factors for the prevalence and incidence of HIV infection in MIRA Trial

Modifiable risk factors	PAR (95% CI)[†] (prevalence of HIV infection)	PAR (95% CI) [†] (incidence of HIV infection)
Combined effect [††]	**0.85 (0.84,0.87)**	**0.77 (0.72,0.82)**
Age at first sex (<15)	0.26 (0.21-0.31)	0.17 (0.10,0.21)
Less than high school [‡]	0.13 (0.10,0.17)	N/A[‡‡]
Age at first sex + less than high school	**0.32 (0.27,0.38)**	-
Not cohabiting	0.39 (0.35,0.43)	0.54 (0.46,0.62)
Number of life time male sex partners		
Two	0.12 (0.11,0.13)	0.11 (0.09,0.15)
Three	0.20 (0.18,0.21)	0.13 (0.11,0.17)
Four or more	0.41 (0.39,0.43)	0.16 (0.13,0.20)
Biological risk factors		
Tested positive for STI [□]	0.05 (0.04,0.06)	0.03 (0.02,0.05)
HSV 2	0.82 (0.80,0.83)	0.21 (0.14,0.31)

[†] Age adjusted
[††] Assumes all the risk factors removed from the target population
[‡] Less than 12 years of education;
[‡‡] Level of education was not determined to be significant predictor of HIV seroconversion
[□] *Chlamydia*, gonorrhea, syphilis or *Trichomonas vaginalis* at screening

Figure 1: Time to HIV seroconversion by the quintiles of age at sexual debut

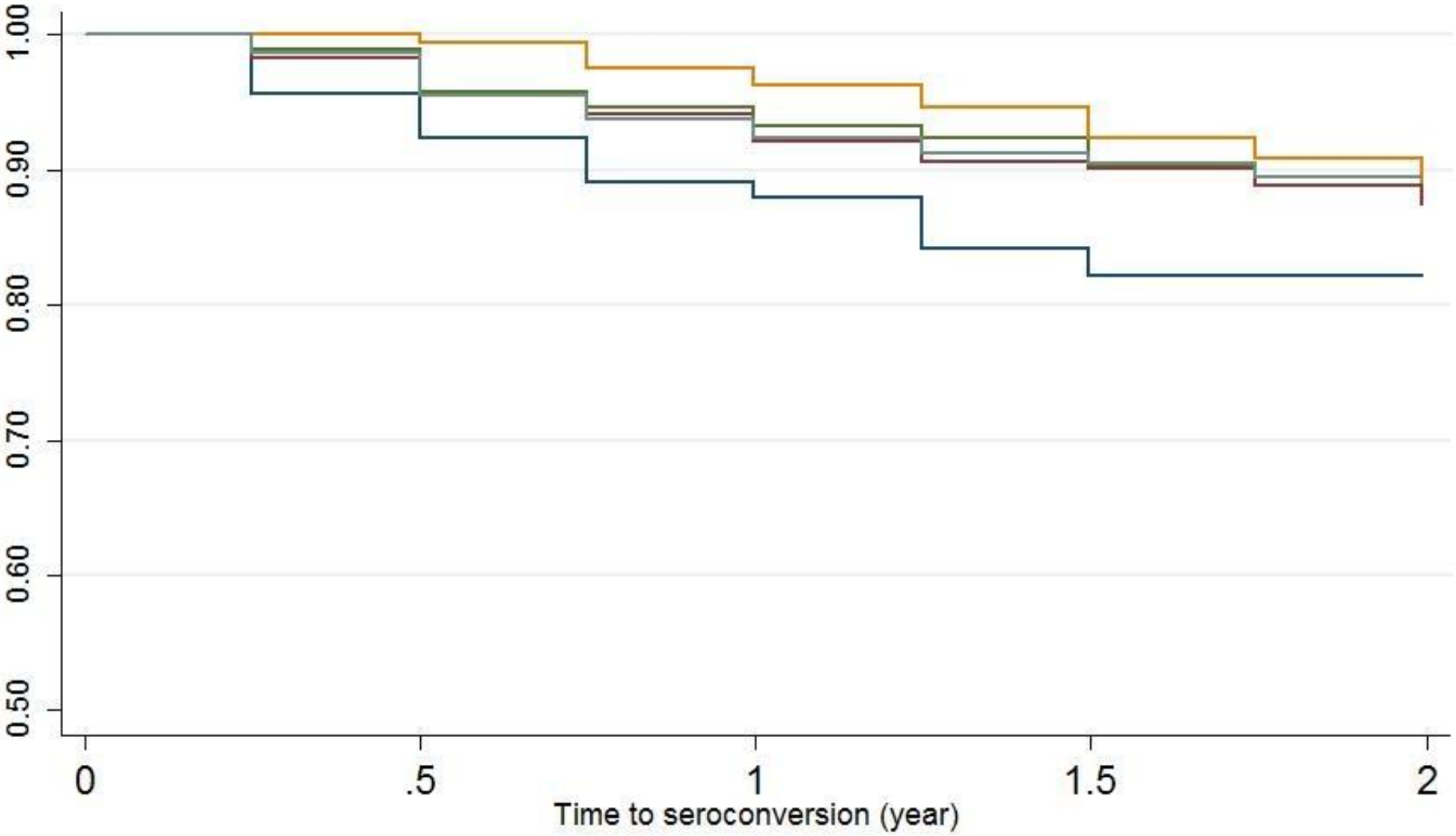
STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (page 1)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 1-2)
Objectives	3	State specific objectives, including any prespecified hypotheses (page 3)
Methods		
Study design	4	Present key elements of study design early in the paper(pages 4-6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 4)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (N/A)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed (N/A)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (4-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (4-6)
Bias	9	Describe any efforts to address potential sources of bias (page 12)
Study size	10	Explain how the study size was arrived at (N/A)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (4-5)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Pages 4-6)
		(*b*) Describe any methods used to examine subgroups and interactions (N/A)
		(*c*) Explain how missing data were addressed (5)
		(*d*) If applicable, explain how loss to follow-up was addressed (N/A)
		(*e*) Describe any sensitivity analyses (N/A)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (N/A)
		(b) Give reasons for non-participation at each stage (N/A)
		(c) Consider use of a flow diagram (N/A)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (6)
		(b) Indicate number of participants with missing data for each variable of interest (5)
		(c) Summarise follow-up time (eg, average and total amount) (N/A)
Outcome data	15*	Report numbers of outcome events or summary measures over time (6)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (6-7)

		(*b*) Report category boundaries when continuous variables were categorized (pages 5-6 and page 7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (N/A)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (6)
Discussion		
Key results	18	Summarise key results with reference to study objectives (pages 12-13)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (pages 13-14)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (page 12-13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (12)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (14)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.


1.00
0.90
0.80
0.70
0.60
0.50
0
.5
1
1.5
2
Time to seroconversion (year)