Delayed puberty in perinatally HIV-infected adolescents in Thailand

MASTER 1 THESIS BASED ON TEEWA SURVEY

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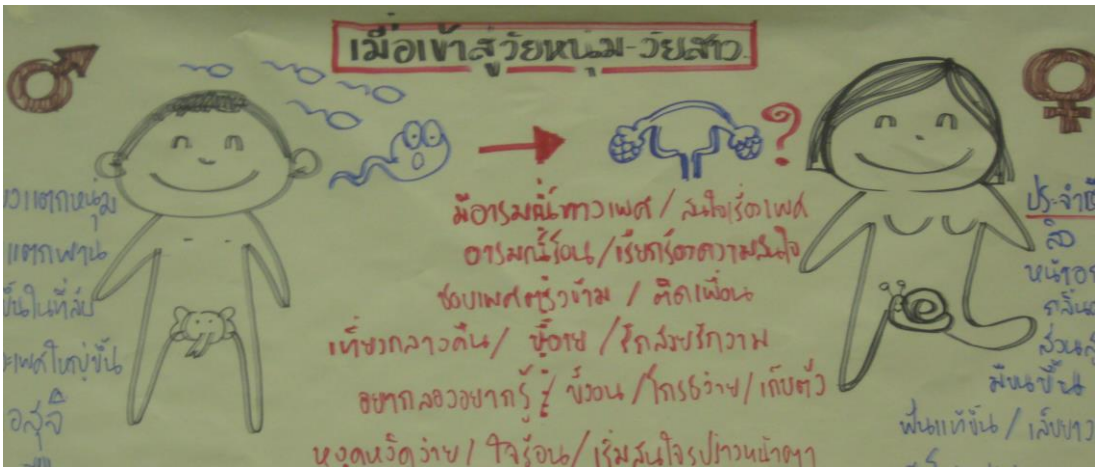

Puberty represented through a drawing produced by a group of HIV-infected adolescents during sexual education session

A thesis presented for the first year of master degree in demography at the University of Strasbourg, conducted during an internship with INED at PHPT, in collaboration with the Faculty of Medical Sciences of Chiang Mai, Thailand, and supervised by Directors of Research Sophie Le Coeur and Didier Breton.

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ABSTRACT

Background: Several studies have pointed out a pubertal delay in HIV-infected children compared to the general population (WILLIAMS, et al., 2013) (Mbwile, 2012). However, few have quantified this delay. The objectives of our analysis were 1) to quantify the puberty delay in adolescents born with HIV compared with a control group of adolescents from the general population of the same age, sex and place of residence; and 2) to analyze the factors associated with pubertal delay in adolescent girls born with HIV.

Methods: For the analysis, we used the data from the perinatally HIV-infected aged from 12 to 19, living in family settings who were interviewed in the TEEWA survey (n=573) as well as the sample from the general population, matched on sex, age and place of residence (n=576). Pubertal onset was defined as menarche in girls and as voice change in boys. The survival analysis of pubertal onset was performed using Kaplan-Meier survival method. The Cox regression was used to assess the factors associated with pubertal delay. Analysis was performed separately for boys and girls.

Results: Perinatally HIV-infected youth were less likely to experience puberty than the controls at each age ($p<0.001$ for girls and $p<0.05$ for boys). However, the difference was clearer in girls than in boys. The estimated delay in mean age at the outcome was 12 months for the HIV-infected girls compared to control girls and 6 months for the HIV-infected boys compared to control boys. Among perinatally HIV-infected girls, initiating antiretroviral treatment (ART), HIV status disclosure and being a lonely child were associated with increased probability of menarche at any age (respectively $p<0.001$, $p<0.05$ and $p<0.05$).

Conclusion: Perinatally HIV-infected children experience a delayed puberty. This delay is reduced among children who started ART in their early childhood. Early ART initiation should be recommended.

KEYWORDS

Perinatally, HIV, aids, puberty, menarche, voice change, ART, adolescence, sibling, disclosure

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1 INTRODUCTION

1.1 The HIV epidemic

1.1.1 The epidemic in the world

Nowadays, more than 35.3 million individuals are living with HIV/AIDS worldwide (UNAIDS, 2013). Although the number of new infections has been decreasing in the recent years, the number of people living with HIV/AIDS (PLWHA) is still rising: indeed, as the use of antiretroviral treatments has been generalized, less people are dying from HIV/AIDS.

1.1.2 The epidemic in Thailand

Thailand has been particularly affected by the epidemic, and currently it is estimated that 450,000 people are living with HIV (UNAIDS, 2013). The epidemic has spread largely through heterosexual transmission which accounts for 88% of the known transmissions (UNAIDS/WHO, 2004). The epidemic is now receding in the country with decreasing numbers of new infections, deaths and PLWHA (Data-Hub, 2013). These progresses are related to the public health efforts to prevent new infections and increased access to antiretroviral (ART) drugs (Ainsworth, et al.). As of 2009, HIV counselling and testing and ART were offered in most medical facilities (Prevention, et al., 2009). By inhibiting viral replication, ART halt disease progression, and also prevent viral transmission (sexual and perinatal). In 2012, 83% of the adult PLWHA eligible for antiretroviral treatment in Thailand were receiving it (UNAIDS, 2013).

1.1.3 Mother-to-child transmission

HIV can be transmitted from mother to child during pregnancy, at delivery and breast feeding. Without any prevented treatment, the rate of transmission is about 30%. But with ART the rate of transmission is decreased to less than 2%.

Thailand has been particularly successful in preventing mother-to-child transmission (PMTCT) of HIV. Under the government's national PMTCT programme, pregnant women are routinely tested for HIV for free, HIV-infected mothers are receiving ART as well as their HIV-exposed newborns (UNAIDS, 2013). As a result, in 2013, more than 95% of HIV-infected pregnant women have received ART for the prevention of mother to child transmission

(UNAIDS, 2014). In 2012, these public health measures have been successful in reducing the rate of mother-to-child transmission under 2.8% (UNAIDS, 2013).

1.2 Adolescence and puberty

1.2.1 Puberty

During adolescence, children experience interconnected physical, emotional and social changes. As a result, several scientific disciplines are studying adolescence. Sociology and anthropology focus on the unusual importance of peers in the child's social environment, as well as the double bind in adult-adolescent relationships (Diasio, et al., 2014). In psychology, the desire to become independent, as well as the capacity to apprehend complex situations, have been considered as factors of troubles, or "storm and stress", since the early twentieth century (Hall, 1904). In biology, the focus is set on puberty: the physical transformation of the child body into an adult one. It includes sexual maturation, growth of the skeleton, and hormonal changes (Kipke, 1999). The onset of these physical transformations is known to depend on the health of the children, but also on social and psychological factors (Kipke, 1999). Conversely, these transformations also have social and psychological consequences on the child (Duclos, 2014) (Diasio, et al., 2014). It has been shown that the timing of puberty depends on the nutritional status of children (Mosley, 1979), but genetic factors (Brown, et al.), stress (Palermo, 2014) and environmental toxins (Howdeshell, et al., 1999) may also play a role.

1.2.2 Puberty indicators

The staging system elaborated by Tanner and Marshall is the most common indicator used in medical sciences to determine the puberty stage in girls as well as boys. Tanner Staging classifies pubertal development according to primary and secondary sexual characteristics. It is based on the development of pubic hair (P stages), breast (B stages), genitalia (G stages), and the occurrence of menarche among girls. Each type of stage (P, B or G) is graded on a scale from 1 to 5, from the initial stage of puberty up to adult sexual characteristics. This evaluation requires an examination by trained health personal and youth are often reluctant to have it.

In girls, age at menarche is a common indicator used in many studies (Rochebrochard, 1999), for it provides an effective puberty assessment while it does not require any examination. Age at menarche is generally well remembered.

Because an easily accessible puberty marker, such as menarche in girls, does not exist in boys, studies on puberty in boys are less numerous than in girls. Serum testosterone levels can be used in endocrinology (Gertner, et al.) but requires a blood draw. The age at first ejaculation and masturbation have been used since the 1940s in some studies on puberty (Kinsey, 1953) but youth may be embarrassed to answer them and may have difficulty dating it. The occurrence of a voice change, a question easier to answer by boys, has also been used in some American and French studies (Kinsey, 1953) (Rochebrochard, 1999). However, dating it may be challenging, as it is a progressive event.

It is worth noticing that growth is often studied along with puberty. Thus, anthropometric measures such as height, weight and BMI, are often included in studies on puberty.

1.3 Adolescence in Thailand

Since the 1970s, Thailand has experienced, in a context of rapid economic and social development and following the Western model, a generalization of the "teenage culture", shaped by American movies, rock stars, brands, etc. Therefore, Thai adolescents enjoy similar hobbies and face similar problems than teenagers in western countries. Thus their culture is mainly based on technologies: in the TEEWA survey, 90% of the adolescents in the sample from the general population declared playing computer games sometimes or often, 90% of them have a mobile phone, 85% reported chatting on the internet and 52% reported watching TV series often. In the meantime, the child-parent relationship changes, for the child seeks independence and experiment new behaviors (Kipke, 1999): in the same adolescents' sample, 55% of the controls declared arguing with their parents sometimes or often. Finally, the development of cosmetic and fashion brands indicates globalization of beauty standards among youth.

However, the traditional Buddhist culture remains strongly anchored in the society and still rules adolescents' lives. In the TEEWA study among the sample from the general population, half of the adolescents reported visiting once a pagoda, church or mosque at least once in the last month. Although ordination of boys has been less common since the 1970s, TEEWA survey reveals that 55% of the male adolescents from the general population had already become novice. Ordination is traditionally a four-month period –often much shorter— during which boys join the monastery and live as a monk. This used to mark the transition from childhood to adulthood. Thai society is also strongly hierarchical, and respect from the younger

to the older is expressed in the language: the formulation is different when addressing an older or a younger person. Obedience from youth to elderly people is very important. Finally, while sexuality of boys is permitted, it is not the case for girls who are supposed to stay virgin until marriage. As everywhere in the world, parents rarely speak about sexuality to their children (Ounjit, 2015).

1.4 Adolescence in HIV-infected children

As a consequence of the successful mother-to-child transmission prevention, perinatal HIV transmission has affected only one generation of children in Thailand, mostly from the beginning of the 1990s until the beginning of the 2000s, when the National prevention program was launched: it therefore comprises children infected before the prevention of mother to child transmission program was implemented, and those infected despite the program. Nowadays, these children are reaching adolescence. The impact of HIV on their puberty needs to be evaluated as it can be considered as a marker of health and wellbeing during this crucial transition period.

1.4.1 Health and social concerns

Medical advances have allowed to extend the life expectancy of children suffering from chronic diseases such as diabetes, sickle cell anemia, asthma, or HIV. Therefore, more and more children with a chronic illness are reaching adolescence (Viner, et al., 2005). Specialists agree that adolescence is a particularly difficult period of life for these children (Bernays, et al.). Adolescents who have a chronic disease need to manage more responsibilities than healthy children of their age, and they often face additional health problems. Indeed, children who have been medicated since a young age often develop treatment fatigue (Haberer, et al., 2009). Furthermore, the differences in terms of health status or lifestyle with their peers can lead to social distress and depression (Christin, et al., 2016).

HIV-infected children born with HIV are experiencing even more burdens: They have experienced the disease or death of their parents and have to face stigma assigned to their disease (Popoola, et al.). Adolescence is also often the time when their HIV status is revealed to them, a diagnosis particularly distressing when disclosed at this age (Bernays, et al.). Finally, at a time when they usually experience sexuality, they are learning that they are carriers of a sexually transmitted disease from which they have to protect their partners. They have to learn

how to manage their social and sexual life as an HIV-infected individual (Chokephaibulkit, et al.). While common body transformations already cause concerns in teenagers, HIV-infected adolescents have to handle additional physical differences such as lipodystrophy – lack of fat tissue in their cheeks affecting their physical appearance (Haberer, et al., 2009). For these reasons, a decrease in ART treatment adherence, can be observed. It leads to drug resistance requiring a change in ART combination. But, since the number of ART drug in Thailand is limited, lack of adherence may endangered adolescent's survival (Haberer, et al., 2009).

1.4.2 Literature and pubertal development in HIV-infected children

To our knowledge, seven studies have analysed the impact of HIV on pubertal development, all using the Tanner scale.

These studies were performed in the United States, Italy, Zimbabwe, Uganda, and Tanzania. The first studies were carried out in the 1990s in the United States among haemophiliac populations (Mahoney, et al., 1999) (Gertner, et al.), when transfusion of blood or blood products was a major route of transmission in children in America (Thiaudière, 2002). The most recent studies were targeting perinatally-infected populations.

All these studies reveal that HIV-infected children experience a pubertal delay of several months compared to the non-infected adolescent population and highlights three characteristics of this pubertal delay.

First, the delay occurs at pubertal onset. In one longitudinal study in the US in 2013, in which 12% of the 2086 HIV-infected children were not on ART, the perinatally HIV-infected girls had reached stage P2 of the Tanner scale, 8 months after the uninfected girls (WILLIAMS, et al., 2013) while the boys experienced a delay of 11 months. Age at menarche has also been used as a puberty indicator, yielding the same conclusions: in a 2012 retrospective study in Tanzania among 330 HIV-infected children aged 8 to 18 years old, girls experienced their first menstruations at 15 years old (median), whereas it happened at 13 year in the general population (Mbwile, 2012). All HIV-infected girls were on ART, for a median duration of 48 months.

Second, it appears that, even after the pubertal onset, the pubertal delay experienced by HIV-infected children extends during the whole pubertal process, at each Tanner stage. In one of the early longitudinal study in the USA, only 57% of the 333 HIV-infected haemophiliac children aged 6-19 had reached stage P5 on the Tanner scale at age 18, compared to 75% in the

general population (Gertner, et al.). However, it should be noted that these children were also haemophiliac and that it may also have an impact on their puberty.

Finally, it has been shown that, in children older than 14 years old, the probability to reach the next Tanner stage decreases with age after 14 years in HIV-infected children compared with uninfected children (Gertner, et al.).

1.4.3 Factors associated with pubertal delay

Not all adolescents living with HIV experience a pubertal delay, and when they do so, the magnitude of the delay may vary (Buchacz, et al., 2003). This is why possible factors associated with pubertal delay in HIV-infected population were investigated.

In four studies, severe immunosuppression was found associated with pubertal delay, and the delay was correlated with the immunosuppression. The lower the CD4 cell count (marker of the immunosuppression) level was, the longer the pubertal delay was. For instance, in a study conducted in the US in 2013 among 2086 perinatally HIV-infected children, it was shown that girls with a CD4 count below 200 cells/mm^3 experienced breast development and pubic hair development 6 months later than the exposed but uninfected girls. For boys, the delay was 7 months for genitalia development and 8 months for pubic hair, after adjustment on ethnicity and birth cohort (WILLIAMS, et al., 2013). Nevertheless, two studies have found no statistical association between pubertal age and immunosuppression (de Martino, et al., 2001) (Szubert, et al., 2015). It should be noted that the design of these studies was different from the 2013 study. In the study by de Martino et al., 1664 perinatally infected Caucasian children were included. The paper does not mention the age of the HIV-infected youth, but only indicates that controls were aged 8 to 14 years old. This age distribution, quite young to study the whole puberty transition, may explain why the authors could not find the statistical association. In the study by Szubert et al. the 582 vertically children included, had only started ART in their late childhood.

Late antiretroviral treatment is another factor found associated with pubertal delay according to two studies. In the study in Uganda and Zimbabwe among in children aged 10 to 17 years old , Szubert came to the conclusion that age at ART initiation was associated with age at reaching all puberty stages (on the Tanner scale) and menarche. For example, boys initiating ART at 9 years old would experience G2 on the Tanner scale at 11.8 years old, while

boys initiating ART at 11 years, would reach the same stage at 12.3 years (Szubert, et al., 2015). Yet, the association between the duration of ART and age at puberty onset was not observed in the de Martino et al. study, probably because lack of power as only 212 children had been included (de Martino, et al., 2001).

1.5 Hypotheses and Objectives

Our hypothesis is that perinatally infected youth experience a delay of pubertal onset as compared to children from the general population.

The objectives of this specific analysis were 1) to quantify the puberty delay in adolescents born with HIV compared with a control group of adolescents of the same age, sex and place of residence; and 2) to analyse the factors associated with pubertal delay in girls born with HIV.

2 METHODS

Because the question on pubertal onset was different for boys and girls (occurrence and age at menstruation for girls versus occurrence and age at voice change for boys), the analysis of the pubertal onset was performed separately for girls and boys.

In a first time, a descriptive analysis was performed: socio-demographic characteristics were compared between the perinatally HIV-infected youth and the controls. Also, health-related characteristics were presented in HIV-infected youth only, since these data were not available in the controls' questionnaire. In a second time, the occurrence of puberty was estimated in a univariate analysis in boys and girls, comparing HIV-infected and control youth. In a third time, a multivariate analysis was performed only in perinatally HIV-infected girls, since the outcome's indicator was estimated less reliable in boys.

2.1 The TEEWA study in Thailand

Between 2010 and 2013, a large nationwide cross-sectional survey was carried out among adolescents (12-19 years old), born with HIV and receiving ART in Thailand, the Teens Living with Antiretrovirals (TEEWA) survey. This study was carried out by researchers from the *Institut National d'Etudes Démographiques* (Ined), PHPT, an international research unit from the *Institut de Recherche pour le Développement* (IRD), and the Faculty of Associated Medical Sciences of Chiang Mai University. The study was designed as a life-event study to assess the family situation of these children and determine their needs while they were entering adolescence and adulthood. The study targeted all perinatally infected adolescents aged 12 to 19 receiving ART from 20 participating hospitals, covering rural, periurban, and urban areas throughout Thailand.

This quantitative survey has three components. First, a self-administered questionnaire filled by the adolescent, which documented aspects of everyday life including household environment, school, work, health/medications, sexual and reproductive life, interpersonal relationships, and daily activities. There was no reference to HIV, AIDS or ART in the adolescent's questionnaire in order to prevent unintended disclosure. Second, a face-to-face interview of the primary caregiver of the adolescent, conducted by two trained interviewers—one psychiatric nurse and one social scientist. This interview collected information on socio-demographic characteristics of the caregiver, and major events in the adolescent's life history

including illness/death of biological parents, HIV-related medical history, ART treatment and adherence, disclosure of HIV status to the adolescent, caregiver-adolescent relationship, and experiences of discrimination. Third a medical component, based on the clinical and biological data collected from the medical records focused on clinical characteristics at HIV diagnosis, initiation of ART and latest CD4, viral load (VL) and ART status. Interviews of the HIV infected adolescents took place in the hospitals where they were receiving their HIV care.

Sample from the general population, "controls". In each district, the village where the greater number of perinatally HIV-infected youth were residing was selected. In the health centre of the village, a control group of adolescents 12-19 years old were randomly selected from the electronic listing of the village population. For each HIV-infected child, a child of the same sex and month of birth was randomly selected and called with his/her caregiver, for an interview. Interviews of the controls took place in the health centres where they were randomly selected based on the population database of the village.

Questions about puberty were collected from the self-administered interview of the adolescents (same questionnaire in HIV-infected adolescents and controls). Children were asked whether they had experience pubertal onset (first menstruations in girls, and change of voice in boys) and the age of its onset. These data will be used for our analysis of the puberty process in children born with HIV.

The study was approved by Ethics Committee, Faculty of Associate Medical Sciences, Chiang Mai University, and the Institutional Review Board of the Harvard University Faculty of Medicine. All participants provided written informed consent/assent to join the study. They were assured that the information provided was kept confidential. All data collected were anonymised using unique study identifiers.

2.2 Data

Three data-set tables were provided:

1) One corresponding to the answers to the adolescent self-administered questionnaire (perinatally HIV-infected youth and controls) composed of 1149 observations: 573 perinatally HIV-infected youth and 576 controls.

2) One corresponding to the answers to the caregiver face-to-face interview (perinatally HIV-infected youth and controls) composed of 1149 observations: 573 perinatally HIV-infected youth's caregivers and 576 controls' caregivers.
3) One corresponding to the information collected from the medical records (only perinatally HIV-infected youth) composed of 941 observations: 573 youth living in family settings and 368 youth living in orphanage. The latter were not included in this analysis.

These three tables were merged. The last table was used for the descriptive analysis of clinical and HIV characteristics of the HIV-infected adolescents and for the analysis of the factors associated with pubertal delay among the perinatally HIV-infected girls. All merges were carried out using the personal identifiers of the children (pid).

2.3 Variables

For the multivariate analysis, variables included in the model were transformed into binary variables. Transformed covariates are presented in this section. Their distribution can be found in section 4.1. Clinical characteristics were available for perinatally HIV-infected youth only.

2.3.1 Outcome

For girls, pubertal onset's indicator was the occurrence of menarche. Girls were considered as pubescent once they had declared having experienced first menstruation. This information was provided in the adolescent questionnaire: "Have you already begun menstruation?". If the answer was positive, the girl was asked at what age she had begun menstruation.

For boys, pubertal onset's indicator was change in the voice. Boys were considered as pubescent once they had declared having experienced change of voice. Such information was provided by the adolescent questionnaire: "Has your voice changed?". If the answer was positive, the boy was asked at what age he had experienced voice change.

2.3.2 Age of the child

The age used in the data was calculated previously by data managers, using the date of birth and the date of the interview. At the time of the survey, only 3 children (0.3%) were under 12 years of age and were grouped with the 12 years old. Similarly, 4 children (0.3%) who were older than 19 years were grouped with the 19 years old.

For the univariate and multivariate analysis, exact ages were used.

2.3.3 Siblings

In Thailand, the fertility rate is very low, about 1.5 child per woman (United Nations Population Division. 2015 Revision of World Population Prospects). Therefore 4 categories were created: having no siblings, one sibling, two siblings and three siblings or more

For the multivariate analysis, two categories were used: having no siblings or having siblings. Indeed, the median number of siblings was 0 in perinatally HIV-infected youth and 1 in controls.

2.3.4 Parental environment

Based on the information about the vital status of the parents, we created a variable to characterize the parental environment of the child, i.e. if the children had 2 parents alive, had lost their mother or their father, or both. However, in some instances, the status of one or both parents was unknown, indicating very loose links with the family. Children whose parents were dead or of unknown status were therefore considered in the same category. Finally we created four categories: both parents alive, father alive and mother dead or of unknown status, mother alive and father dead or of unknown status, father and mother dead or both of unknown status.

For the multivariate analysis, two categories were used: having both parents alive or not.

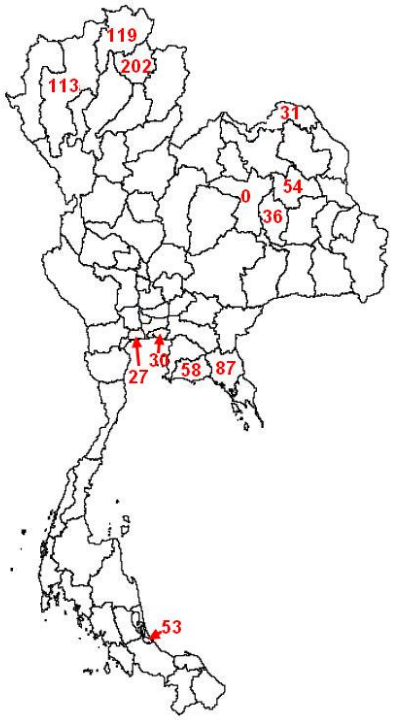
2.3.5 Region

The country was divided in four regions: the North, the North-East, the Center, the South and Bangkok. Northern Thailand was the region with the first and most severely HIV affected region in Thailand (Nelson, 1998).

Table 1. Hospitals by region

Hospital name	*Region*
Bhumibol Adulyadej Hospital	Bangkok
Prapokklao Hospital	Center
Rayong Hospital	Center
Samutsakhorn Hospital	Center
Chiang Dao Hospital	North
Chiang Kham Hospital	North
Chomthong Hospital	North
Doi Saket Hospital	North
Mae Chan Hospital	North
Mae Sai Hospital	North
Nakornping Hospital	North
Phan Hospital	North
Phayao Provincial Hospital	North
Sankhampang Hospital	North
Sanpatong Hospital	North
Sarapee Hospital	North
Kalasin Hospital	North-east
Mahasarakam Hospital	North-east
Nong Khai Hospital	North-east
Hat Yai Hospital	South

Figure 1. Map of the distribution of the perinatally HIV-infected youth sample in the districts in Thailand for the TEEWA study in 2010



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

2.3.6 Caregivers

The adult person who accompanied the child for the interview was considered as the caregiver. Caregivers were grouped into four categories: parents, grandparents, aunt or uncle, other relatives. When parents had died or had left the household, grandparents became most of the time the caregiver of the child.

For the multivariate model, caregivers were grouped into two categories: parents of the child or others.

2.3.7 Education

We used the American school system (grade 1 to 12) to code the education level. Highest completed level of school and current grade were grouped into four categories: first grade to 6th grade (corresponding the primary school), 7th to 9th grade (corresponding to secondary school, 10th to 12th grade (corresponding to High school) and college/university (bachelor degree). For

children who had dropped out of school, we created two categories for the last school level reached: first grade to 6th grade (primary school) and 7th grade to 12th grade.

For the multivariate analysis, we considered the primary school versus the other grades.

2.3.8 Income

The household's income was not provided in the data sets provided. Therefore the socioeconomic status was assessed through income of the caregiver and additional financial supports collected by the household's members. Taking these financial supports into account limited the possible underestimation of the household economic situation. The Thai government provides 500 THB per month to people living with HIV and 500 THB per month to elderly people. However, these supports are not systematic. Overall, the median income was 5,000THB. The lowest tercile of incomes was below 3,000THB, while the highest tercile was over 6,250THB. Three categories were created based on this summary.

- Less than 3,000THB: low category
- Equal or between 3,000 and 6,250THB: median category
- More than 6,250THB: « high » category

  For the multivariate analysis two categories were used: low vs. median and high category.

2.3.9 Viral load

The viral load (VL) is a marker of the number of viruses in the blood. ARV treatment reduces dramatically the viral load in the blood. When children respond to the treatment, the VL becomes undetectable, i.e. usually under 400 copies/mL. If the viral load increases above the 400 copies/mL threshold, it suggests treatment failure. It should prompt a change in the treatment (switch to second-line treatment). Viral load was grouped into two categories: less than 400 copies/mL, and 400 copies/mL or more.

2.3.10 CD4%

CD4 lymphocytes are immunologic cells. The HIV viruses replicate in these cells and destroy them. Immunologic status can be measured by the number of CD4 cells in the blood (Absolute CD4 count), or by the percentage of CD4 T cells among all the lymphocytes (CD4

percentage). In children, CD4 percentage is preferred to CD4 cell count, as the CD4 cell count physiologically decreases with age. In our analysis, we used the CD4 percentage and grouped it into two categories: less than 15% and 15% or more. This percentage is the usual threshold in medical literature to define serious immunologic damage.

2.3.11 HAART

Highly Active Antiretroviral treatment (or ART) is a combination of different types of antiretrovirals. Regimens were grouped in two categories: non-nucleoside reverse transcriptase inhibitors “NNRTI” and protease inhibitor “PI”. In Thailand NNRTI-based regimens are the most common treatment against HIV used, and generally the first line of treatment provided to the patients. Moreover, there is a cheap one pill NNRTI-based combination produced in the country. As a result, treatment based on protease inhibitor (PI) or other drugs usually indicate previous treatment failure (related to drug resistance for example).

2.4 Tests in descriptive analysis

Descriptive tables were created to summarize the distribution of the socio-demographic and clinical key variables for the analysis. The statistical significance of the differences in proportions between the group of perinatally HIV-infected youth and the control group was tested using the chi-square test. Median and interquartile range were used to provide the distribution of continued quantitative variables, and the differences in distributions between groups was tested using the Wilcoxon rank sum test. Differences were considered statistically significant when p-value was ≤ 0.05.

2.5 Univariate and multivariate survival analysis

To study the occurrence of puberty, a survival analysis method was used in univariate and multivariate analysis. It allows the description of the distribution of life time spent between an initial event and a final event (the outcome). In this analysis, the initial event was birth (age zero) and the outcome was the occurrence of pubertal onset.

2.5.1 Survival analysis

The outcome of interest was the occurrence of pubertal onset, i.e. voice change for boys and menarche for girls. Individuals were considered censored when they had not experienced

pubertal onset at the time of the survey. Thus, in the survival analysis, observation time started at birth and ended at the age of pubertal onset or at the time of the interview for censored observations. The age at the occurrence of the outcome (puberty) was reported in years.

MEASURE OF TIME IN SURVIVAL DATA

Table 2, Figure 1 and Figure 2 present some examples of children and how data were considered in the survival analysis. A child aged 14 years old at the time of the survey, but who experienced pubertal onset at 13, would be considered as a complete observation from birth to age 13 in the survival analysis. A child aged 12 at time of the survey and who had not yet experienced survival onset would be considered as a censored observation at 12 years.

Table 2. Fictive sample of children with the variables used for the survival analysis

Observation	Age at survey	Pubertal onset	Age at pubertal onset	Time start	Time stop
1	14	1	13	0	13
2	12	0	NA	0	12

Figure 2. Observed time of each observation until censor or event

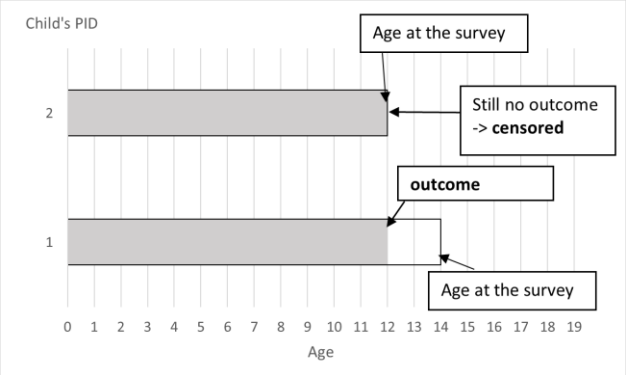
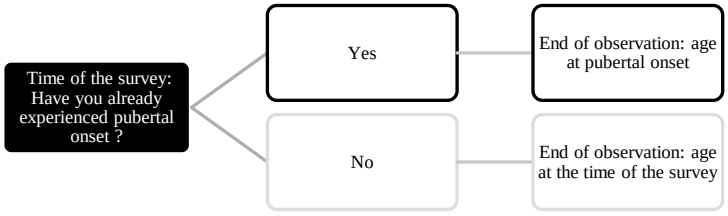


Figure 3. Decision tree for the time attribution



2.5.2 Kaplan-Meier estimator

The nonparametric Kaplan-Meier estimator (KME) is $\hat{S}(t_j) = \prod_{i=1}^{j} \widehat{\Pr}(T > t_i | T \geq t_i)$, with T corresponding to the time at failure (censor or event). S(t) is the probability of "surviving" ("death"=puberty) after t, i.e. the probability of not experiencing the outcome. The complement to the KME was used in the univariate analysis. It is the cumulative density function which is defined by $F(t) = 1 - S(t)$. It measures the probability that the outcome occurs at or before time t. The cumulative density function is the integral of the probability that the event occurs at time t, denoted by $f(t)$.

The hazard rate $h(t)$ is the conditional probability that at time t an individual experiences the outcome in the next instant, given that the outcome has not occurred before. It is the ratio of the probability that an event occurs at time t on the probability of surviving beyond time t: $h(t) = \frac{f(t)}{S(t)}$.

Instead of using the Nelson Aalen estimator, we used the cumulative hazard function H(t) which can be approximated by the equation $S(t) = e^{(H(t))}$, It is the integral of the hazard rate $h(t)$. It measures the probability that the outcome occurs at or before time t, given that it has not occurred before.

2.5.3 Cox Model

In Cox regression, the hazard ratio is: $h(t) = h_0(t).e^{(\beta_1.x_1 + \cdots + \beta_k.x_k)}$, with h the hazard of pubertal onset, h_0 the baseline hazard which is time dependent, t the time, k the number of covariates, β the effect of each covariates. Parameters were estimated using the maximum likelihood method. Individuals who experience the outcome at the same age are considered ex-aequo. They were handled using the Efron approximation method which estimates an average hazard for all events occurring at the same time (Cleves, 2008).

Hazard ratios were used to analyze the impact of each variable on pubertal onset:

$HR(t) = \frac{h_{11}(t)}{h_{10}(t)} = \frac{h_{10}(t).e^{\beta}}{h_{10}(t)} = e^{\beta}$ With h_{11} the hazard of the modality 1 for variable 1, and h_{10} the hazard of the modality 0 for the variable 1.

The model was considered statistically significant when the null hypothesis: $\beta_i = 0$ was rejected and the hypothesis H_1, which stipulates that at least one of the parameters is different from zero, was accepted. Several tests are usually used: the Wald test, the likelihood ratio test and the log-rank test.

2.5.4 Univariate analysis

The analyses were run separately for boys and girls.

In the univariate analysis, we tested whether the hazard rates of the outcome in the group of perinatally HIV-infected youth compared to the controls were equal at all ages or not. The Cox model was carried out, but it included only one explicative binary variable. The null hypothesis "H_0: $HR(t) = 1$ for all t" was tested. The log-rank test compares the observed and expected distribution of the outcome. Results were considered statistically significant when p-value was ≤0.05. Only individuals with complete data on their pubertal time or censor time were included. Were excluded:

- Individuals who did not answer the question on pubertal onset (n=2)
- Individuals who answered having experienced pubertal onset but who did not give the age at the occurrence of pubertal onset (n=15)

Time distributions were summarized in life tables and figures to illustrate the difference between the perinatally HIV-infected child and control groups.

2.5.5 Multivariate analysis on female perinatally HIV-infected youth

Since the range in the age at pubertal onset in boys was very wide and since the voice change was finally considered as an unreliable indicator of puberty in boys, the multivariate analysis was performed on perinatally HIV-infected girls only. We tested whether the hazard rates of the outcome in different sub-groups were equal at all ages. Sub-groups were defined according to a selection of characteristics possibly associated with the age at menarche. Results were considered statistically significant when p-value was ≤0.05.

2.5.5.1 Variables in the Cox model

A Cox model was developed, including the following variables at the time of the survey: type of caregiver, vital status of the parents, number of siblings, caregiver's income and

financial supports, type of antiretroviral treatment, CD4%, viral load, and perceived height. It also included time-dependent variables: age at experience of first symptoms, age at child's HIV status disclosure, age at antiretroviral treatment initiation, and age school drop-out. All variables were transformed into binary variables (see section 2.2 for details). Only observations with complete data for all variables included in the model were kept. As a result, 25 girls had to be excluded from the model (7%) with only 309 individuals remaining in the analysis.

TIME-DEPENDENT COVARIATES IN SURVIVAL DATA

Table 3 presents an example: a female aged 18 at the time of the survey declaring having experienced menarche at 13, having dropped school at 15, having experienced first symptoms at 5, having begun ART at 8 and having been told of her HIV status at 16. Since school dropping and disclosure occurred after menarche, it is does not appear in the new dataset.

Table 3. Fictive individual data

Observation	1
Age at survey	18
Pubertal onset	1
Age at pubertal onset	13
Drop School	1
AgeDropSchool	15
Disclosure	1
AgeDisclosure	16
On ART	1
AgeART	8
First Symptoms	1
AgeSymptoms	5

Table 4. Fictive individual data remastered with time-dependent covariates for survival analysis

Observation	Time start	Time stop	Pubertal onset	Drop School	Disclosure	On ART	First Symptoms	Age at survey
1	0	5	0	0	0	0	1	18
1	5	8	0	0	0	1	1	18
1	8	13	1	0	0	1	1	18

Four variables were considered as time-dependent: age at drop-out of school, age at ART initiation, age at experiencing first symptoms, age at HIV disclosure. Child's work was not introduced in the model because no information was available on the age at first job. BMI was also not considered in the model because we had only the BMI at the time of the survey, and not the BMI at previous ages. Therefore, the dataset was transformed to document the

changes in the school attendance, symptoms, ART, and disclosure situations with time. Instead of having a line per observation, the new dataset presented a line per observation and stable characteristics. For one individual, each time one characteristic had changed, a new line was added to the dataset with the updated characteristics. If several variables changed at the same age, then only one line was added. Therefore, for each individual, we could have a maximum of 5 lines. After remastering the dataset with time-dependent variables, there was 1,017 entries for the 309 individuals.

2.5.5.2 Interactions in the model

However, some covariates were not independent from each other. A characteristic can be apparently not associated with the outcome, but once combined with another attribute it can have an effect on the outcome. On the contrary, an attribute might seem to impact the outcome, but once it was combined with another characteristics the effect was no longer significant. The association of a variable with the outcome timing (earlier or later) can also vary depending on another variable. As a result, 9 interactions were explored and tested:

- Age at Disclosure and at first symptoms
- Age at Disclosure and CD4%
- Disclosure and ART initiation
- Disclosure and caregiver
- Caregiver and parental environment
- Caregiver and income
- ART and first symptoms
- Siblings and parental environment
- CD4% and viral load

The detailed explanations of the expected interactions can be found in Appendix (Text 1, Appendix). Only significant interactions ($p<0.05$) were taken into account in the model. Thus, only the interaction between age at first symptoms and disclosure was introduced ($p<0.01$).

Our final model was:

$$h_{puberte}(t) = h_0(t) + \exp\big(\beta_{caregiver} \times caregiver_i + \beta_{dropschool} \times dropschool_i + \beta_{siblings} \times siblings_i + \beta_{income} \times income_i + \beta_{parent's\ status} \times parent's\ status_i + \beta_{first\ symptoms} \times first\ symptoms_i + \beta_{disclosure} \times disclosure_i + \beta_{height} \times height_i + \beta_{type\ of\ ART} \times type\ of\ ART_i + \beta_{CD4\%} \times CD4\%_i + \beta_{on\ ART} \times on\ ART_i + \beta_{virlan\ load} \times viral\ load_i + \beta_{(disclosure*first\ symptoms)} \times (disclosure * first\ symptoms)_i \big)$$

2.5.5.3 Hypothesis on the model's covariates

It was expected that:

- Having experienced symptoms is associated with lower probability of menarche
- Having experienced disclosure of HIV-infection is associated with lower probability of menarche
- Not being on ART is associated with lower probability of menarche
- Having a CD4% <15% is associated with lower probability of menarche
- Having VL > 400 copies /ml is associated with lower probability of menarche
- Thinking being smaller than children of the same age is associated with lower probability of menarche
- Having a caregiver other than a parent is associated with lower probability of menarche
- Having no siblings is associated with lower probability of menarche
- Dropping school is associated with lower probability of menarche
- Low caregiver's income and financial support is associated with lower probability of menarche
- Having at least one parent dead or whose vital status is unknown is associated with lower probability of menarche
- In children who had experienced first symptoms, being aware of your status was associated with lower probability of menarche

2.5.5.4 Model diagnostic

Since Cox is a semi-parametric model, there is no assumption on distribution of time at pubertal onset. Also, because all variables were categorical, it was not necessary to check for non-linearity.

In Cox regression, the main assumption is that the hazard ratio of two observations do not vary over time. Thus the null hypothesis is: $\frac{h^i(t)}{h^j(t)} = \frac{h_0(t).e^{(\beta_i.x_i)}}{h_0(t).e^{(\beta_j.x_j)}} = \frac{e^{(\beta_i.x_i)}}{e^{(\beta_j.x_j)}}$ is constant in time for two individuals i and j. Proportional hazard (PH) assumption was estimated with Schoenfeld residuals graphics for each variable (Appendix, Figure 1). If the curb is flat, then the hazard ratio is constant in time. If it is curved, then the hazard ratio varies with time. The PH assumption was also tested with the Chi-square test. It tests the null hypothesis $H_0 : \beta^i(t) = \beta^i$. In our analysis, the Chi-square test for the entire Cox model was not statistically significant (p=0.28), indicating that the null hypothesis could not be rejected. The hazard ratio was globally the same at each period for all the covariates included.

Influential points were examined through standardized dfbeta residuals (Annex, Figure 2). These residuals provide the change in coefficient after the removal of each observation. The limit was decided to be +/-0.4. Points which crossed this limit were considered as influential. Only 4 observations crossed this limit. Once they were removed, conclusions were relatively similar to the first model, with the same factors remaining significant. Therefore, the model could be considered as robust.

Outliers were spotted with deviance residuals (Appendix, Figure 3). Outliers are observations poorly explained by the model: the age at the outcome predicted by the model differs greatly from the observed age. In our analysis, there is no outlier, indicating that the ages at outcome were well predicted.

3 RESULTS : DESCRIPTIVE ANALYSIS

3.1 Comparison of the perinatally HIV-infected youth and the control group

A total of 1149 adolescents living in family settings were analysed: among them 573 were perinatally HIV-infected youth and 576 were controls (adolescents from the general population).

The perinatally HIV-infected youth and the controls were individually matched on age, sex and district of residence. Because of the matching, the sex distribution was similar in perinatally HIV-infected youth and controls (p=0.988), as well as the age distribution (p=0.906). Also the distribution of the participants in the five regions was similar in the two groups (p=0.999). As a result, standardisation on sex, age or region was not necessary to compare the socio-demographic characteristics of the two groups.

3.1.1 Sociodemographic characteristics

Table 5 presents the sociodemographic characteristics of participants by group. Of the children, 58% were girls. The median age of children was 14 years (IQR 13-16). The majority of them (58%) were living in Northern Thailand.

Table 5. Demographic characteristics used to match the perinatally HIV-infected child and the control groups

	Perinatally HIV-infected youth		**Controls**		
	N=573		**N=576**		**p.value**
Age (years)					
Median (IQR)	14 (13-16)		14 (13-16)		0.9060
	N	%	N	%	
Age (years)					0.9296
12 or less	68	11.9	62	10.8	
13	135	23.6	141	24.5	
14	106	18.5	117	20.3	
15	90	15.7	85	14.8	
16	77	13.4	72	12.5	
17	45	7.9	53	9.2	
18	34	5.9	32	5.6	
19 or more	18	3.1	14	2.4	
Total	573	100.0	576	100.0	
Sex					0.9882
Boys	239	41.7	239	41.5	
Girls	334	58.3	337	58.5	
Total	573	100.0	576	100.0	
Region					0.9999
Bangkok	21	3.7	21	3.6	
Center	119	20.8	122	21.2	
North	336	58.6	336	58.3	
North-east	66	11.5	66	11.5	
South	31	5.4	31	5.4	
Total	573	100.0	576	100.0	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

3.1.2 Family structure

The family structure of the perinatally HIV-infected youth was different from that of the controls (Table 6).

Table 6. Family characteristics in the perinatally HIV-infected child and control groups

	Perinatally HIV-infected youth		Controls		
	N=573		N=576		
	N	%	N	%	**p.value**
Caregiver					<0.001
Parents	180	32.0	418	72.7	
Grandparents	214	38.1	90	15.7	
Aunt or uncle	109	19.4	37	6.4	
Other relatives	59	10.5	30	5.2	
Total	562	100.0	575	100.0	
Caregiver sex					0.1996
Man	128	22.3	110	19.1	
Woman	445	77.7	466	80.9	
Total	573	100.0	576	100.0	
Parents environment					<0.001
Mother dead or status unknown	119	20.8	13	2.3	
Father dead or status unknown	108	18.8	40	6.9	
Both parents dead or status unknown	268	46.8	12	2.1	
Both parents alive	78	13.6	511	88.7	
Total	573	100.0	576	100.0	
Siblings					
Median (IQR)	0 (0-1)		1 (1-2)		<0.001
	N		N		
Siblings					<0.001
0	405	70.9	161	28.0	
1	145	25.4	291	50.5	
2	15	2.6	95	16.5	
3 or more	6	1.1	29	5.0	
Total	571	100.0	576	100.0	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

First, the caregivers of the perinatally HIV-infected youth' were less likely their parents. Indeed, only 31% of the perinatally HIV-infected youth' caregiver were the father or mother while 73% of the controls' caregiver were parent ($p<0.001$). This difference was explained by the high rate of orphanhood among perinatally HIV-infected youth.

Perinatally HIV-infected youth were more likely to have lost one or both parents. Almost half of them (43%) had lost both their mother and father, whereas it was the case for only 1% of the controls ($p<0.001$).

Moreover, children born with HIV grew up with less siblings than the controls (median 0 vs. 1, respectively, $p<0.01$). Large families are rare in Thailand, as the total fertility rate in Thailand has very rapidly declined from more than 6 in the 1960s to less than 2 children per woman at the beginning of the 1990s (United Nations Population Division. 2015 Revision of World Population Prospects). This dramatic decline in fertility was achieved through a strong, noncoercive family planning program, emphasizing contraceptive methods (Knodel, et al., 1987). The total fertility rate was estimated at 1.53 children per woman over the last five years (United Nations Population Division. 2015 Revision of World Population Prospects). But large families are even less frequent in families with a HIV-infected child. Only 4% of perinatally HIV-infected youth in the TEEWA study have 2 siblings or more, whereas they are 22% in controls ($p<0.001$). This can be explained by the fact that siblings of HIV-infected children may also be HIV-infected and have died. Moreover mothers of children born with HIV are themselves HIV-infected and may have been seriously sick or have died, limiting their offspring. Finally, HIV-infected women, after a first or second delivery, are often proposed to undergo sterilization (Lallemant, et al., 2006).

3.1.3 School attendance and work

Perinatally HIV-infected youth were less likely to attend school than controls (Table 7). Eighteen percent of perinatally HIV-infected youth had dropped-out from school, while they were only 6% in controls ($p<0.001$). Also the perinatally HIV-infected youth dropped school at younger ages than the controls. Nearly 54% of perinatally HIV-infected youth who had dropped out school did it between the first and 6^{th} grade (approximately elementary school). On the other hand, almost 85% of controls who had dropped out did it between the 7^{th} and 12^{th} grade mostly during the high school years ($p<0.001$). The median age of perinatally HIV-infected youth who had dropped out school was 16 years, whereas it was 17 years in the control group. Thus only 18% of perinatally HIV-infected youth had completed 10^{th} to 12^{th} grade, whereas they were 28% in controls ($p<0.001$).

Table 7. Education characteristics in the perinatally HIV-infected child and the control groups

		Perinatally HIV-infected youth		Controls		
		N=573		N=576		p.value
Age of children not attending school						
Median (IQR) (years)		16 (14-18)		17 (15-18)		0.139
		N	%	N	%	
School attendance						<0.001
	Not attending school	100	17.5	33	5.7	
	Attending school	473	82.5	543	94.3	
Total		573	100.0	576	100.0	
Grade at drop out						<0.001
	First grade to 6th grade	52	53.6	5	15.2	
	7th to 12th grade	45	46.4	28	84.8	
Total		97	100.0	33	100.0	
Current grade						<0.001
	First grade to 6th grade	98	20.9	33	6.1	
	7th to 9th grade	277	58.9	345	63.5	
	10th to 12th grade	93	19.8	154	28.4	
	Bachelor	2	0.4	11	2.0	
Total		470	100.0	543	100.0	
Highest grade completed						<0.001
	First grade to 6th grade	150	26.5	38	6.7	
	7th to 9th grade	316	55.9	368	65.1	
	10th to 12th grade	99	17.5	159	28.1	
	Bachelor	2	0.3	11	1.9	
Total		573	100.0	576	100.0	
Work reported by the child						0.0011
	Not regularly work for money	359	62.7	414	71.9	
	Regularly work for money	214	37.3	162	28.1	
Total		573	100.0	576	100.0	
Work reported by the caregiver						0.0182
	Not currently working	513	89.5	539	93.6	
	Currently working	60	10.5	37	6.4	
Total		573	100.0	576	100.0	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Perinatally HIV-infected youth were more likely to work regularly for money than controls (Table 7). When asked whether they were working regularly, almost 40% of perinatally HIV-infected youth declared working for money, whereas there were less than 30% in the control group ($p<0.05$). However, there were some discrepancies with the answers obtained from the caregivers. According to the caregivers, only 11% of perinatally HIV-infected youth

were working regularly and they were 6% in the control group (p=0.0182). However, questions were slightly different in the two questionnaires: "Do you regularly work for money?" was asked to the adolescent in the self-administered questionnaire and "Does the child currently work?" was asked to the caregiver during the face-to-face interview.

3.1.4 Household income

Perinatally HIV-infected youth were more likely to live with a poorer caregiver than the controls (Table 8). The median caregiver's income was 3,000THB [1]per month (IQR 1,667-6,000) in perinatally HIV-infected youth and 6,250THB[2] per month (IQR 3,000-10,000) in controls (p<0.001). Only 23% of perinatally HIV-infected youth' caregivers earned more than 6,250THB, whereas they were 46% in the controls (p<0.001). Perinatally HIV-infected children did not only cope with a chronic disease. They also faced less favorable economic conditions than the controls.

Table 8. Monthly income of the child's caregiver plus financial support in the household

	Perinatally HIV-infected child		**Control**		
	N=573		**N=576**		**p.value**
Median (IQR)	3,692 (1,667-6,000)		5,600 (3,000-10,000)		<0.001
	N	%	N	%	
Monthly income plus financial support in THB					<0.001
Low, below 3,000 THB	244	43.6	115	21.8	
Middle, 3,000-6,250 THB	189	33.8	169	32.0	
High, over 6,250 THB	126	22.5	244	46.2	
Total	559	100.0	528	100.0	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

[1] Approximately 70 euros in 2011
[2] Approximately 149 euros in 2011

3.1.5 Puberty

The overall prevalence of puberty was significantly lower in the perinatally HIV-infected youth compared to the controls. Also, the age distribution at pubertal onset was different in the two groups (Figure 5).

3.1.5.1 Overall rate of puberty in perinatally HIV-infected youth and controls

The results presented in Table 9 and 10 suggest that in both sex, perinatally HIV-infected youth were less likely to have experienced pubertal onset than controls. Only 74% of the perinatally HIV-infected girls declared having already begun menstruation, whereas almost all (91%) of the controls had already menstruated (p<0.001). Overall, only 57% among the perinatally HIV-infected boys had experienced voice change, whereas they were 69% of the boys in the control group (p<0.05).

Table 9. Distribution of menarche in girls: Comparison of perinatally HIV-infected youth versus controls

	Perinatally HIV-infected youth		**Control**		
	N=334		**N=337**		**p.value**
	N	%	N	%	
Menarche					<0.001
No	88	26.4	32	9.5	
Yes	245	73.6	305	90.5	
Total	333	100.0	337	100.0	
Age at menarche					
Median (IQR) (years)	13 (12-14)		12 (12-13)		<0.001
	N	%	N	%	
Menstruated girls in each age-at-the-survey group					
12 years old or less	30	26.7	33	60.6	
13 years old	38	48.7	69	84.1	
14 years old	49	76.6	59	92.2	
15 years old	47	87.0	50	98.0	
16 years old	48	96.0	45	100.0	
17 years old	27	96.4	36	100.0	
18 years old or more	28	96.6	26	100.0	
Total	267		305		

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 10. Distribution of outcome (voice change) in perinatally HIV-infected child and control boys

	Perinatally HIV-infected youth		Controls		
	N=239		N=239		p.value
	N	%	N	%	
Voice change					0,0151
No	102	42.7	75	31.5	
Yes	137	57.3	163	68.5	
Total	239	100.0	238	100.0	
Age at voice change					
Median (IQR) (years)	13 (12-14)		14 (12-14)		0.2737
	N	%	N	%	
Pubescent boys in each age-at-the-survey group					
12 years old or less	38	36.8	29	44.8	
13 years old	19	33.3	41	69.5	
14 years old	23	54.8	34	64.2	
15 years old	29	80.6	29	85.3	
16 years old	18	66.7	21	77.8	
17 years old	14	82.4	13	76.5	
18 years old or more	19	86.4	13	65.0	
Total	136		164		

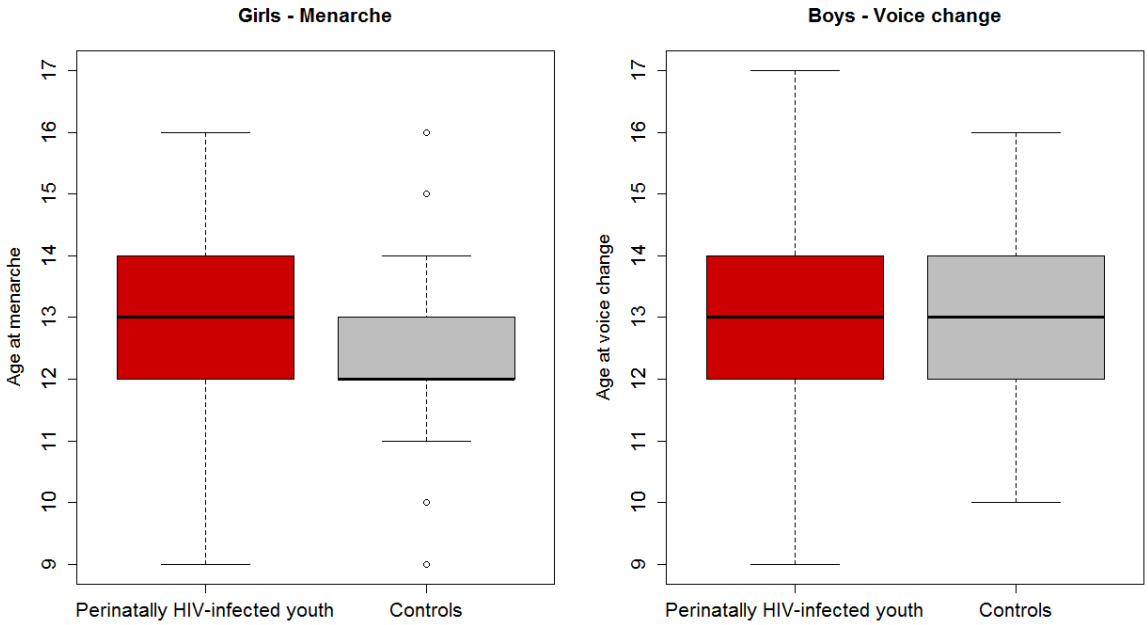
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

3.1.5.2 Age at the outcome in perinatally HIV-infected youth and controls

Figure 5 shows perinatally HIV-infected girls experience the outcome later than the control girls. The median age at the outcome was 13 years old in perinatally HIV-infected girls and 12 in control girls ($p<0.001$). The distribution was symmetrical in perinatally HIV-infected youth with a wide dispersion, as indicated by the Interquartile range from 12 to 14 years old. On the other hand, in the controls the range was narrower, from 12 to 13 years old.

Results in Figure 5 show the age distribution at voice change in boys. In both perinatally HIV-infected youth and controls, the median age at voice change was 13 years old and there is not significant difference in the age distribution ($p=0.2737$). However, as menarche in girls, the age range at voice change was wider (9 to 17 years) in perinatally HIV-infected youth compared to controls (10 to 16 years).

Figure 4. Age at the outcome distribution



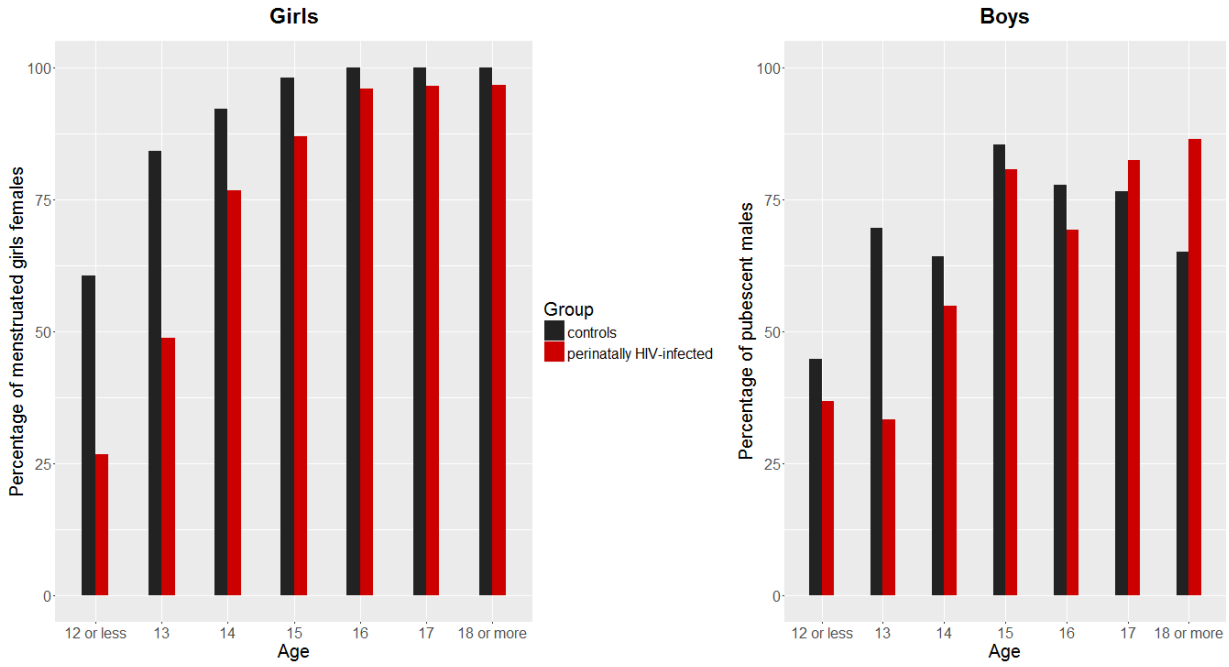
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

3.1.5.3 Rate of pubescent children in each age classes: difference between perinatally HIV-infected youth and controls

Figure 6 provides the percentage of menstruated girls in each age-at-the-survey group. It shows that in girls, in each age group, the rate of pubescent girls was lower in perinatally HIV-infected youth than in controls. In 12-year-old perinatally HIV-infected youth, only 27% had already experienced menarche while it was 61% in the controls. However, in the girls interviewed after 15 years old, the percentage of menstruated perinatally infected girls was closer to the percentage of menstruated controls, with differences below 5 percentage points.

Figure 6 provides the percentage of pubescent boys in each age-at-the-survey group. It shows that in boys, in each age group, the rate of pubescent boys was lower in perinatally HIV-infected youth than in controls. In 12-year-old perinatally HIV-infected boys, only 37% had already experienced voice change while it was 45% in the controls. However, in 17-year-old and 18-year-old boys, frequency of pubescent was higher in perinatally HIV-infected boys.

Figure 5. Percentage of puberty in perinatally infected youth and controls



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

3.1.5.4 Right-censored data

In our study, data were right-censored for 2 reasons: 1) the timing of pubertal onset was not provided for some participants at the time of the survey, 2) some children had not experienced puberty at the time of the survey. This is the reason why, we used survival analysis to study the occurrence of puberty.

3.2 Perinatally HIV-infected youth: health related characteristics

As mentioned earlier, perinatally HIV-infected youth appear to experience the outcome (puberty) later than the control group. Moreover, since the social and demographic characteristics of the infected youth were very different from the controls, there may be an association between these characteristics and the outcome. However, the health status of the perinatally infected youth may also play a role and should be investigated.

3.2.1 Physical Development

Table 11. Clinical and perceived physical development in perinatally HIV-infected youth

	N	%
Body Mass Index		
<15	58	10.2
15-19	407	71.3
20-24	98	17.2
≥25	8	1.4
Total	571	100.0
Perceived height		
Taller	102	17.8
Average	297	51.9
Smaller	173	30.2
Total	572	100.0
Perceived weight		
Chubbier	43	8
Average	339	60
Skinnier	183	32
Total	565	100.0

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 11 presents the clinical and perceived physical development in the perinatally HIV-infected youth. They tended to be skinnier than the general population. Eighty percent of perinatally HIV-infected youth had a BMI below 20, and 10% were severely underweight. One

third of the perinatally infected youth perceived themselves as being smaller and/or, skinnier than other children of their age. They were 15% in the controls.

3.2.2 HIV-related characteristic

Table 12. HIV characteristics in perinatally HIV-infected youth

Age at first symptoms		
Median (IQR) (years)	6 (3-9)	
	N	%
Before 1 year old	36	7.4
1 to 2 years old	68	14.0
3 to 9 years old	280	57.7
10 years old or more	101	20.8
Total	485[a]	100.0
Age at ART initiation		
Median (IQR) (years)		9 (7-11)
	N	%
Before 10 years old	303[b]	55.0
At 10 years old or after	248	45.0
Age at disclosure (years)		
Median (IQR)	11 (9-12)	
	N	%
Before 12 years old	214	37.3
At 12 years old or after	166	29.0
Do not know the age at disclosure	115	20.1
Caregiver not sure of disclosure	19	3.3
No disclosure	59	10.3
Total	573	100.0
Current CD4%		
15% or above	494	86.7
Less than 15%	76	13.3
Total	570	100.0
Current Viral load		
<400 cells/mL	458	80.2
>=400 cells/mL	113	19.8
Total	571	100.0
Current HAART		
NNRTI-based	423	73.8
PI-based or others	150	26.2
Total	573	100.0

(a) 88 no answer; (b) 22 no answer

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

At the time of the survey, almost 80% of the perinatally HIV-infected youth had experienced their first HIV symptoms and the median age at ART initiation was 9 years. At the time of the survey, 74% of them were receiving NNRTI-based HAART, indicating no past treatment failure. Taking other drugs than NNRTI-based treatment is indeed a sign that problems occurred with the treatment. At the time of the survey, most of perinatally HIV-infected youth had no or moderate immunosuppression (87% had a CD4 percent over 15%), while only 13% were presenting with a severe immunosuppression at the time of the survey (CD4 percent below 15%). Also, 80% of the perinatally HIV-infected youth had a viral load below 400 copies per mL. Therefore we were dealing with a population of well controlled, healthy perinatally infected youth. Finally, at least 88% of the youth knew about their HIV status at the time of the survey. The median age at the HIV disclosure was 11 in these youth. Figures 12, 13 and 14 (Appendix) presents the distribution of age at first symptoms, ART initiation and HIV disclosure. They illustrate well the fact that perinatally HIV-infected children underwent diverse HIV histories. While some children experienced their first HIV symptoms at birth, other experienced them in their late adolescence. This is also true for initiating ART and being told about his/her HIV status.

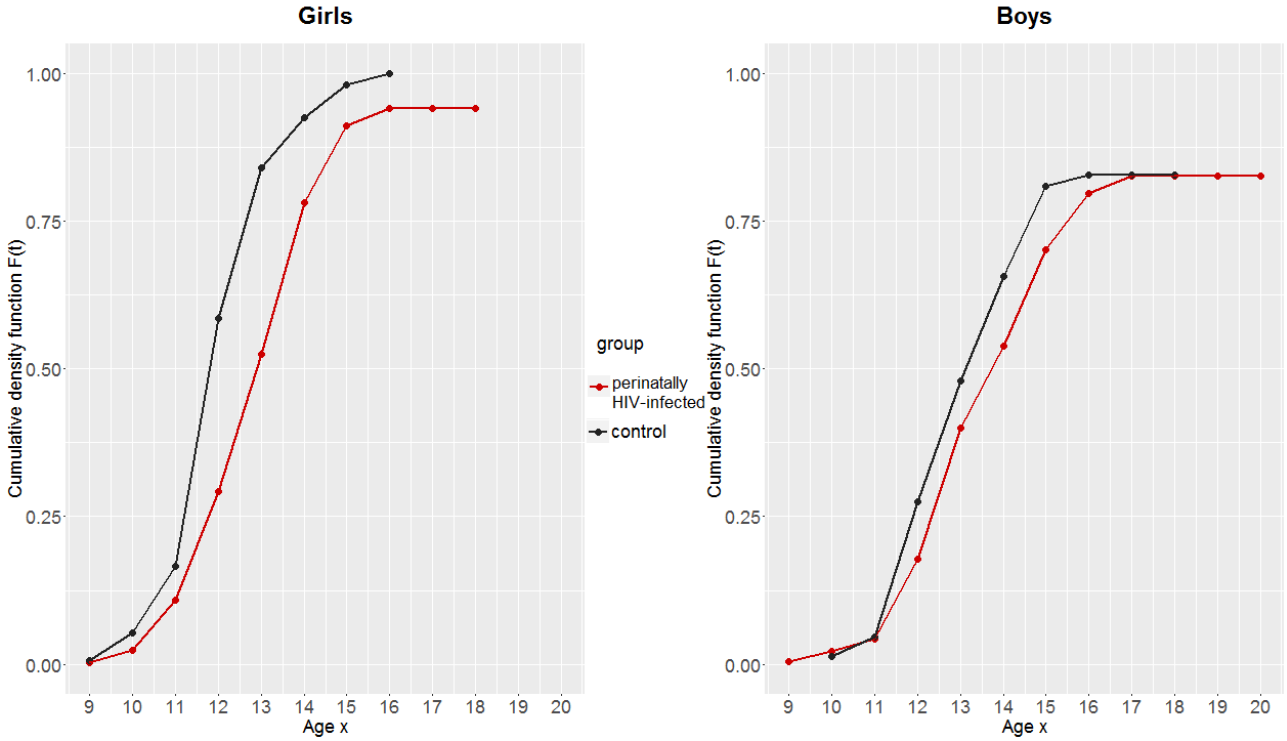
4 RESULTS: SURVIVAL ANALYSIS

4.1 Univariate model

4.1.1 Age at menarche

In both sex and group (perinatally-infected youth and controls), probability of experiencing or having experienced puberty at age *x* increases greatly between 11 and 15 years old and then stabilizes. Figure 7 presents the probability that the event will occur at or before age *x.*

Figure 6 Cumulative density function of the outcome regarding the HIV-infection

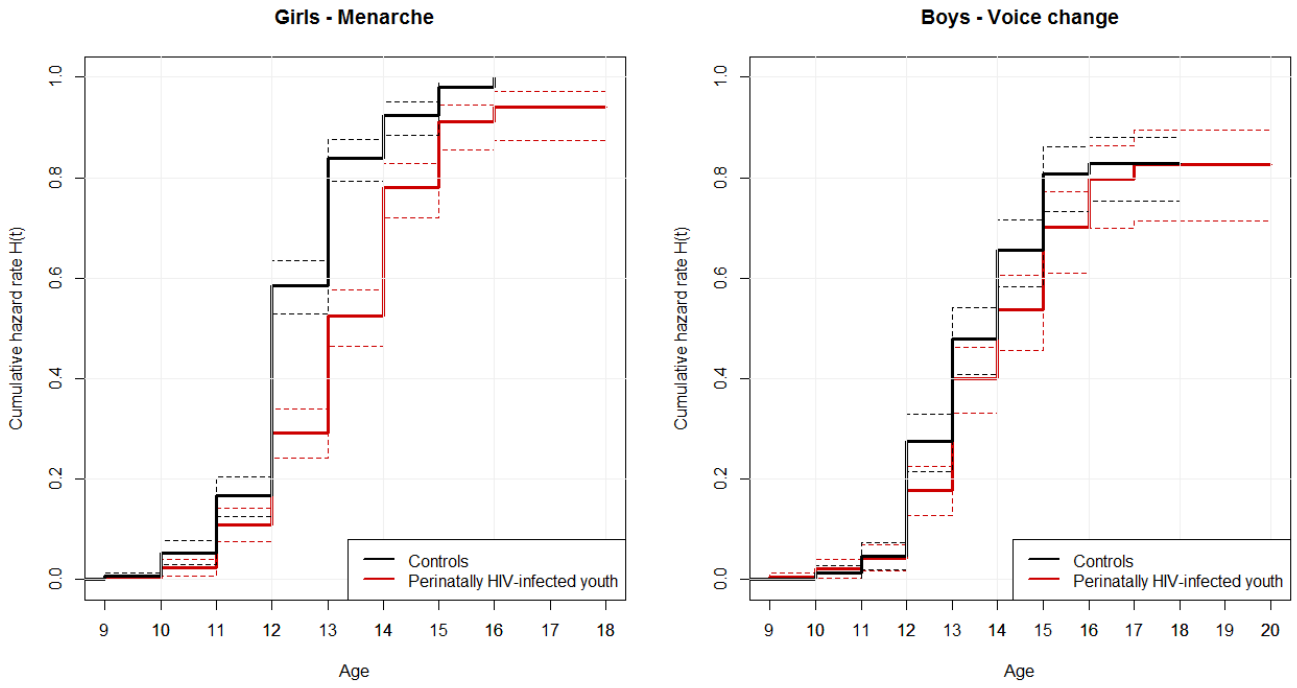


Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

In girls and boys, this probability is lower in perinatally HIV-infected youth than in controls for almost any age group. In girls, the difference in the cumulative density is greater than in boys. At 13 years old, there is a difference of 30 points between perinatally HIV-infected girls and their controls and of 10 percentage points between perinatally HIV-infected boys and

their controls. But the probability that the outcome will occur at or before age *x* does not stabilize at the same level in boys and girls. Probability of being pubescent stabilized after 95% in perinatally HIV-infected girls and 100% in control girls, whereas it stabilized after reaching 80% in boys (same for perinatally infected boys and controls). In perinatally infected girls, the probability that menarche has occurred reached 50% at 12.9 years old, whereas in control girls it reached 50% one year earlier, at 11.8 years old. In boys, the probability that voice change has occurred reached 50% at 14 years old in perinatally infected boys, whereas it reached 50%, also one year earlier, at 13.1 years in control boys.

Figure 7 Cumulative hazard function of the outcome regarding the HIV-infection



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 8 presents the conditional probability that menarche will occur or have occurred at age *x* given that it has not occurred prior age *x*. In girls, for all ages, the probability of having experienced menstruation was lower among the perinatally HIV-infected youth than among the controls (p=0 and Chi-square=72.3). The estimated mean age at menarche was 13.4 years old for the perinatally infected girls and 12.4 years old for the control girls. The difference in probability of having experienced or experiencing the outcome between the perinatally infected girls and controls was greatest between 12 and 15 years old.

In boys, for all ages, the probability of having experienced voice change was lower among the perinatally HIV-infected youth than among the controls. Although the difference was statistically significant (p=0.0282), the difference was less important than for girls. The estimated mean age at voice change was 14.7 years old in perinatally infected boys and 14.2 years old in controls. The difference in probability of having experienced or experiencing the outcome between the perinatally infected boys and controls was greatest between 12 and 15 years old.

4.2 Multivariate models: socio-demographic and clinical characteristics associated with occurrence the outcome in perinatally HIV-infected girls

As mentioned before, due to the wide dispersion in the age at voice change in boys and its progressive occurrence, we thought that this indicator of puberty was not very accurate. Therefore, we decided that we would performed the multivariate analysis only on perinatally HIV-infected girls.

Table 13 summarizes hazard ratios from the Cox regression analysis. All covariates included in the model are presented in the table. Only three covariates were significantly associated with the age at menarche occurrence: the number of siblings ($p<0.05$), being currently on ART ($p<0.001$) and knowing his/her HIV status ($p<0.05$).

Table 13. Hazard ratio of having menarche of socio-demographic and clinical characteristics (N=309)

	Hazard Ratio		95% CI
Socio-demographic characteristics			
Caregiver			
Parents	1.00		
Other	0.84		0.63-1.11
Parental environment			
Both parents alive	1.00		
At least one parent dead or status unknown	0.81		0.54-1.22
Siblings			
1 or more	1.00		
None	0.73	*	1.05-1.76
Education			
At school	1.00		
Not at school	1.00		0.64-1.58
Caregiver's income and financial supports			
High or middle income	1.00		
Low income	0.97		0.76-1.22
Clinical characteristics			
Been told about her HIV status			
No	1.00		
Yes	1.99	*	1.16-3.40
Having experienced HIV symptoms			
No	1.00		
Yes	1.35		0.93-1.96
Being on ART			
No			
Yes	2,21	***	1,55-3,14
Type of Antiretroviral treatment			
Other	1,00		
NNRTI-based	1,07		0,78-1,48
CD4% at time of the survey			
>=15%	1,00		
< 15%	0,85		0,58-1,27
Viral Load at time of the survey			
<400	1,00		
>=400	1,03		0,70-1,52
Perceived herself smaller than average			
No	1,00		
Yes	1,00		0,98-1,01
Interaction between time and disclosure	0,45	**	0,25-0,82

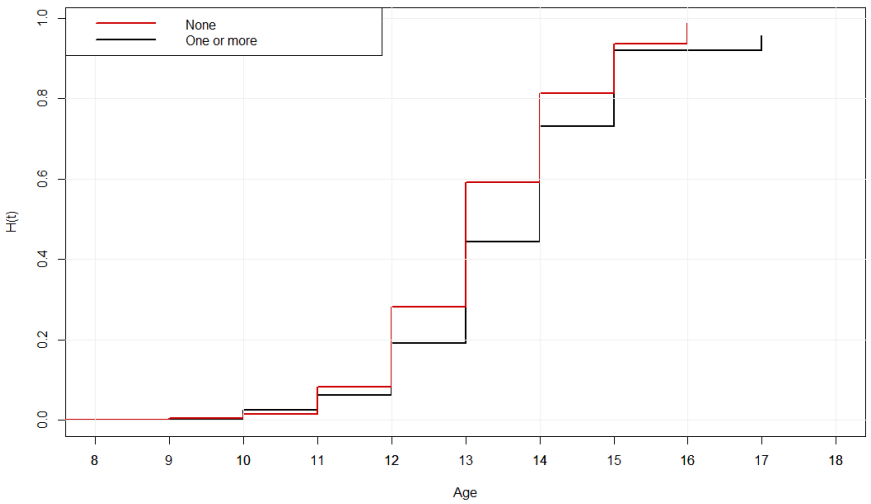
*Notes: Variables in italic are time-varying. *p≤.05; **p≤.01; ***p≤0.001*

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

4.2.1 Siblings

Cox regression analysis showed that being a lonely child was associated with a greater probability of attaining menarche. Figure 10 presents the cumulative hazard function curve according to the siblings' category, after controlling for the aforementioned covariates. Girls with no sibling were 1.36 (95%CI 1.05-1.76) times more likely of experiencing menarche at any time, controlling for the socio-demographic and clinical characteristics than girls with siblings ($p<0.05$). However, it is worth noting that the lower boundary of the confidence interval is close to 1, and thus probability of beginning menstruation may be only 1.05 times greater among lone girls than among girls with siblings. The estimated mean age at menarche (i.e. restricted time survival) was 13.67 among girls with siblings, versus 13.28 among those with no siblings. Other socio-demographic characteristics such as the type of caregiver, the parental environment or the caregiver's income were not statistically significantly associated with menarche in this model.

Figure 8. Hazard Function of menarche in perinatally HIV-infected girls depending on the number of siblings

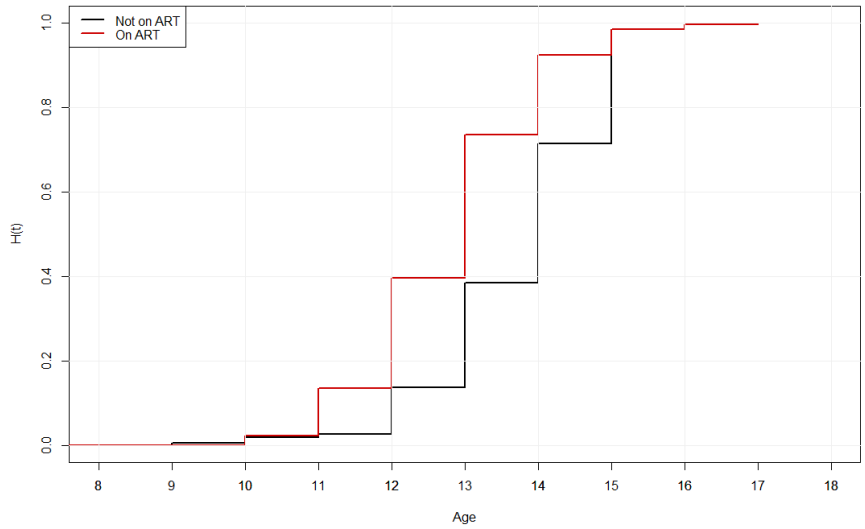


Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

4.2.2 ART and disclosure

There was a positive association between being currently on ART and experiencing pubertal onset. Figure 11 presents the cumulative hazard function curve according to the ART situation. Girls who were on ART were 2.21 (95%CI 1.55-3.14) times more likely to experience pubertal onset at any time, after controlling for the other variables (p<0.001). The estimated mean age at menarche among girls not on ART was one year before among girls on ART, compared to those not on ART (12.8 versus 13.7 years old).

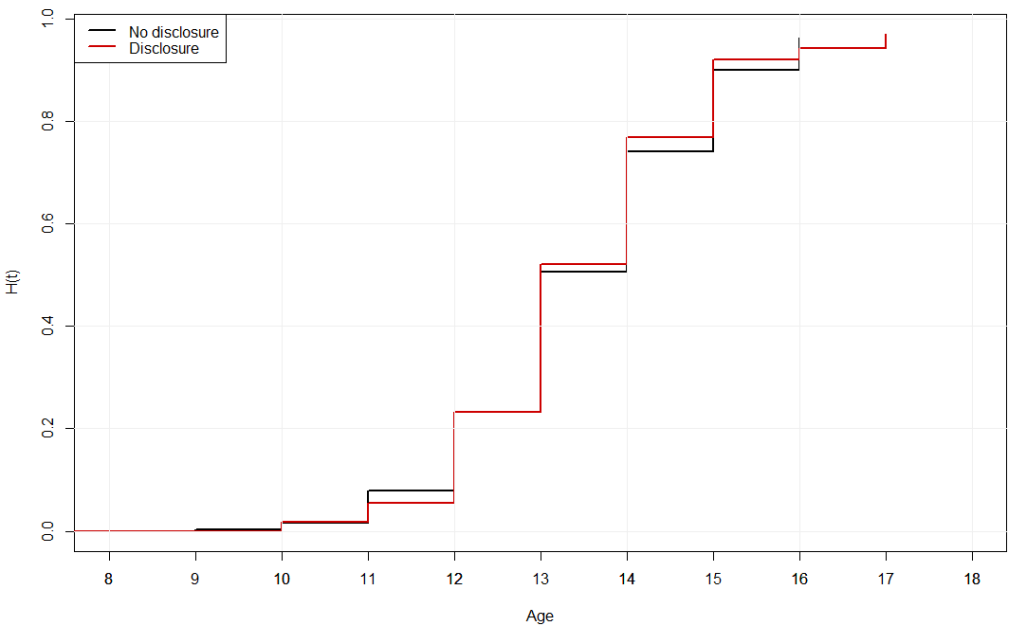
Figure 9. Hazard Function of menarche in perinatally HIV-infected girls depending on ART initiation



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Finally, our results indicate that delay in menarche was associated with ignorance of their HIV status after controlling for other characteristics. Figure 12 presents the cumulative hazard function curve according to the disclosure category. Girls aware about their HIV status were 1.99 (95%CI1: 1.16-3.40) times more likely to experience menarche at any age, after controlling for variables presented before (p<0.05). However, the estimated mean age at menarche was only slightly different between the two groups (13.54 vs. 13.51 years old)(p<0.05).

Figure 10. Hazard Function of menarche in perinatally HIV-infected girls depending on the HIV disclosure



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

4.2.3 Interactions

Nine interactions were tested. Among them only clinically relevant interaction were taken into account in the analysis. Were statistically significant. Interaction between disclosure and first symptoms was statically significant.

Table 14. Hazard ratios menarche with experiencing first symptoms in Cox regression stratified on disclosure variable (N=309)

	Hazard Ratio		95% CI
No first symptoms			
Aware of HIV status			
No	1.00		
Yes	2.27	**	1.35-3.81
First symptoms			
Aware of HIV status			
No	1.00		
Yes	1.08	p=0.553	0.84-1.39

*Notes: Variables in italic are time-varying, *p≤0.05; **p≤0.01; ***p≤0.001*

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

First, first symptoms affect pubertal onset only in perinatally HIV-infected child of ignorance of the HIV status. In girls who were aware about their HIV status, the association between first symptoms and menarche was borderline significant (p=0.0509). However, the relationship between the two covariates was significant in children who were unaware of their HIV infection. In girls who did not know their HIV status, first symptoms increased the likelihood of menarche occurrence. Children who were not aware of their disease but who had experienced their first symptoms were 1.47 (CI95% 1.01-2.12) more likely to experience menarche than those who knew about their HIV status but who had not experienced first symptoms ($p<0.05$). Also, disclosure was associated with menarche only in girls who had not yet experienced symptoms. Relationship between disclosure and pubertal onset was not significant among girls who had experienced their first symptoms. But in girls who did have experienced their first symptoms, disclosure had an effect on attainment of menarche. Girls who were aware of their HIV status were 2.3 (CI95% 1.35-3.81) times more likely to begin menstruation than girls who ignored their disease.

Overall, the fit of the model was satisfactory. The model explained 4.3% of the variation in the occurrence of outcome (menarche). For the likelihood ratio test, Wald test and log-rank test, null hypothesis $\beta=0$ could be rejected with $p<0.001$. Therefore, the H1 hypothesis that at least one of the β_i was significantly different to 0 could be accepted. Concordance ratio showed that the model had a good prediction capacity [3]. However, the model could slightly underestimate time at pubertal onset, as Figure 19 in Annex shows that the poorest predicted observations in the model experienced menarche sooner than predicted.

[3] The concordance ratio is the percentage of actual pairs (outcome vs no outcome, or 1 vs 0) where the observation with the desired outcome (event) has a higher predicted probability than the observation without the outcome (non-event). In our model, the concordance ratio was 66.3%.

5 DISCUSSION

5.1 Delaying effect of HIV-infection among children

Our study indicates that HIV-infected children experience pubertal onset later than non-infected children. However, the pubertal delay observed in our study was more clearly highlighted in girls compared to boys.

5.1.1 Menarche and HIV-infection

The association between HIV-infection and delayed menarche in girls was statistically significant, indicating delayed puberty. This result is consistent with prior research (Buchacz, et al., 2003; Mbwile, 2012; de Martino, et al., 2001).

Menarche appears as a reliable indicator of puberty onset in girls. Indeed, first menstruation is an important event in the life of girls that is usually well memorized. Also, since they were interviewed when aged 9 to 20 years, the event was recent enough to be well remembered.

Moreover, it easily noticeable with no risk of misclassification, unlike secondary sexual characteristics for Tanner staging (i.e. pubic hair development and breast and genital development which need to be assessed by a nurse or a physician). Indeed, the Tanner staging has two major limits. First, it is a visual inspection. Therefore, the staging depends of the subjectivity of the person who does the assessment and is subject to inter-observer variability. Second, the quality of the visual inspection suffers greatly from the embarrassment related to sex in our societies.

Finally, menarche is a onetime event, happening abruptly one day. Although it is a not much subject to recall bias, we must admit that age at menarche may have been occasionally under or overestimated by children. Especially when delayed, girls may be feel embarrassed and may report first menstruations while it did not occurred yet.

5.1.2 Voice change: an unreliable indicator of pubertal onset?

We could demonstrate an association between HIV-infection and age at puberty onset in boys. This result is consistent with the results of other studies (de Martino, et al., 2001; Gertner, et al.). However, the descriptive analysis pointed out unreliable results in age at the

outcome. Notably, as indicated in Figure 6, the percentages of pubescent boys increased or decreased from one age to another. Moreover, the probability of having experienced the outcome remained at a low level and never reached 100%.

This is most probably explained by our indicator of puberty onset in boys, occurrence of voice change, which clearly appears to be an unreliable marker of pubertal onset in our survey. To our knowledge, in the literature, voice change has rarely been used as an indicator of puberty.

In a study conducted in France in the 1990s, boys aged 15 to 18 years old were interviewed on their voice change. It was found that median age at voice change was 14.8 years old (Rochebrochard, 1999). In our findings, the age at voice change was slightly younger: in male controls, the estimated median age at voice change was 14. Moreover, the study found that at 18 years old, 10% of the boys had not experienced voice change yet. It is in agreement with our results, that some boys have still not experience voice changed after 17 years old. As a result we can wonder if some boys will never experience this outcome, did not noticed it or if it could happen at older ages. But in a retrospective survey conducted in the United-States in the 1940s, which used voice change as a puberty indicator, it was estimated that voice change can occur between 8 years old and 25 years old (Kinsey, 1953). In this survey, 100% of the 2,279 boys interviewed had experienced voice change. Thus, our data could be incomplete because our population target was limited up to 19 years old.

To check the validity of the responses, the questionnaires of the boys over 17 years who had declared having not yet experienced voice change (n=7) were qualitatively examined one by one. Their social and clinical characteristics did not differed from the others.

This voice change indicator suffers from several biases. First, voice change does not indicate pubertal maturation, although it is one of the change that occurs during puberty. Second, it generally occurs progressively, therefore it is difficult to date it. Finally, as it usually expends over several months, it is more difficult to remember the time of its occurrence. Kinsey found in his study that voice change lasted 12 to 18 months in his sample (Kinsey, 1953). Thus, our results on pubertal onset in boys, in particular the lack of significant difference between perinatally HIV-infected youth and controls, should be considered with caution, as it may be the consequence of the poor quality of this indicator.

However, voice change was the best puberty indicator for boys that could be provided in the TEEWA survey. Other possible indicators would have been the Tanner staging or questions on the first ejaculation or masturbation. But, as puberty was not the main topic of this social science survey, intrusive questions were considered inappropriate.

5.2 Socio-demographic and clinical characteristics associated with the age at pubertal onset in girls

5.2.1 ART

Our main finding was that ART was the factor with the greatest and most significant effect on menarche. Whatever their age, girls who were not on ART were more likely to experience pubertal delay. These results are broadly similar to a study performed in Ugandan and Zimbabwean (Szubert, et al., 2015) in which initiating ART at older rather than young age was found associated with delayed menarche. Median age at ART initiation was 9.4, compared to 9 in our study.

At the time of the survey, children were initiated on ART when HIV symptoms occurs or when they are immunosuppressed. Although all perinatally infected children who were interviewed were on ART when interviewed, some of them declared having initiating ART after 15 years old. These children who initiated ART at older ages have a different disease history than those who initiated ART at younger ages: they have been exposed to a high viral replication for many years. They are considered as « long-term survivors », a terminology usually used for adults, as they are able to control their disease for several years without any treatment. This long term fight against the virus may constitute a challenge to their body, impacting the physiological process of puberty.

This result could be compared to the various studies pointing out delayed menarche in female athletes (Hata, et al., 1990) (Malina, et al., 1977) (Sharma, et al., 1992). These girls undergo more physical pain and stress during their childhood than other girls of their age.

Finally, not being on ART could indicate a problem in the medical follow-up, exposing the children to HIV consequences, including delayed menarche. Literature indicates that serious disease leads to pubertal delay (Buchacz, et al., 2003) (WILLIAMS, et al., 2013).

5.2.2 Disclosure and its interaction

Disclosure was also found to be associated with menarche. Whatever their age, girls who were aware of their HIV status were more likely to begin menstruation than those who were not aware of their status. To our knowledge, there was no study which included disclosure as a potential factor in pubertal onset. Stress is known to trigger menarche and HIV disclosure is known to be very stressful and there could be psychologic effects leading to increasing risk of menarche. Notably, disclosure could yield to a greater interest from the adolescents for their disease and its care. It could lead for example to a better adherence to ART. It can be considered that once the child is aware of his/her HIV-infection, adherence to the treatment would improve because of the understanding of its importance. Focus on other disclosure covariates confirmed this assumption. Of the 312 caregivers of perinatally HIV-infected youth aware of their HIV-infection, 76% thought that adherence of the child had been improving since the disclosure.

5.2.3 Siblings

The number of siblings was associated with menarche. Whatever t, girls who were lonely child were more likely to begin menstruation than girls who had one sibling or more. This result is in agreement with previous studies in England (Dann, et al., 1984) (James, 1973), in the United-States (Malina, et al., 1997), Portugal (Padez, 2003), Romania (R, et al., 1967) and Poland (Zarow, et al., 2008). Tanner found it was the one factor which was systematically associated with menarche. His assumption was that children born in large families enjoy a poorer nutrition and care (Tanner, 1962). More recent studies argue that the association between menarche and siblings could reflect the effect of the birth rank on menarche (Malina, et al., 1997).

5.3 The study's strengths

The main strength of the study was that it targeted two groups of adolescents matched on age, sex and region, using the same questions to assess pubertal onset. Therefore potential biases are the same in the two groups and should not affect the comparison. Most studies used as controls population from other studies, such as WHO references or the National Health And Nutrition Examination Survey results (Buchacz, et al., 2003) (Szubert, et al., 2015). In the TEEWA survey, controls were not tested for HIV and it could be argued that some could have been infected with HIV. However, it was estimated in 2013 that 6,900 children aged 0-14 were

living with HIV/AIDS in Thailand in 2014 (Unicef, 2013). Thus, the probability of randomly selecting an HIV-infected child was close to zero. In fact, one HIV-infected child was randomly chosen as a control. The child declared being infected and was excluded of the survey.

Also, there were very few missing values in the data set. Only 25 observations were not included in the multivariate analysis. After a qualitative analysis, we checked that these observations were not different from the others included in the analysis. It seemed in some of these cases, the person who accompanied the child could not answer all the questions.

Most of the covariates could be considered as reliable, since, for each child, three persons were interviewed and specific questions were asked to the respondent, able to provide the most appropriate answer. For example, co-morbidities experienced by the child were informed by the nurses. The child or the caregiver has less medical knowledge and could have forgotten a co-morbidity, or misclassified one. The caregiver gave information such as the income of the household, which would have been less accurate if the nurse or the child had answered. Finally, the child answered questions about his own feelings with his family and his friends. These personal information would have suffered of misinterpretation if the nurse or the caregiver had answered instead of the child.

5.4 The study's limitations

The number of perinatally HIV-infected adolescents interviewed in our study, 573, may appear small. However, the sample size was large enough considering that we only used binary variables in the multivariate analysis. It is in the same range as the other studies (Szubert, et al., 2015) (Buchacz, et al., 2003). Due to the small number of children aged over 16, results on pubertal delay at these age could be less reliable than the others. Also, binary variables were used in the Cox regression. Therefore specific small sub-groups could have not been identified (specific small sub-groups could not be identified)

Adolescents included in the survey may be not representative of all perinatally HIV-infected children in the Thai population. Selection bias might have occurred at three levels. First, in each hospitals, only about 80% of the children participated to the study. The non-respondents were slightly older and more likely to live in family settings. Thus the study may miss a sub-group of adolescents, more independent. Second, some children did not answered to the questions about the occurrence of pubertal onset or the age of its occurrence. Since their

number is quite limited (only 15 children) it should not bias substantially the results. Finally, there were some missing data for the covariates introduced in the multivariate analysis of the occurrence of puberty in girls born with HIV. Therefore, among a total of 334 girls who participated in the study, only 309 could be considered in the analysis.

We examined qualitatively questionnaires of the girls who were excluded from the multivariate analysis. There was no difference in the social or clinical characteristics compared with the other girls. So it should not introduce a bias.

Finally, the person called "caregiver" was not necessarily the caregiver of the child in real life. Indeed, the caregiver was the adult who came with the child to the health center the day of the survey. Therefore, the actual caregiver could have other obligations and not come with the child. In this case, the adult who came with the child may not be the most appropriate person to answer all the questions about the life of the child. Also, many children have had several consecutive caregivers, and the current one may not have witnessed all the health events and may have difficulty dating them. This is the case for all the time dependent variables such as the age at HIV diagnosis, HIV symptoms, ART initiation, or disclosure.

We considered the time as a continuous variable while pubertal onset was dated in years. Therefore, data could also have been considered as interval-censored data. Thus non-significant results could be the result of the lack of accuracy in the time information in the data. However, the indicators used could have hardly been more accurate. In two studies at least, it was found that most of girls forget the month of their first menstruation (Rochebrochard, 1999) (Koo, et al., 1997). Thus in a survey conducted in 1997 by Koo and Rohan, only 66% of the girls recalled the month of their first menstruation, whereas they were interviewed in the same year than the occurrence of their menarche (Koo, et al., 1997).

6 CONCLUSION

This retrospective study showed an association between HIV-infection and pubertal onset. At any given age, perinatally HIV-infected youth are less likely to have experienced puberty than their counterparts in the general population. However, this delay was more obvious in girls than in boys. The main interest of the study was to quantify accurately the delay in girls, and maybe less accurately in boys. Among HIV-infected girls, the absence of treatment was

significantly related to delayed menarche. Being aware of one's HIV status and being an only child were also positvely associated with the age at menarche.

In a public health perspective, our results support the early initiation of ART as it is now recommended by WHO/UNAIDS. Longitudinal studies to understand the pubertal delay would allow a better evaluation of the pubertal delay, but they suffer attrition. Puberty assessment using the Tanner staging would be more precise, but would require the intervention of medical personnel. Now that all children are treated with ART as soon as they have been diagnosed with HIV, it would be interesting to assess whether the delay in puberty will fade out in the new generations of perinatally HIV-infected.

REFERENCES

Ainsworth Martha, Beyrer Chris et Soucat Agnes AIDS and public policy: the lessons and challenges of success; in Thailand [Revue] // Health Policy. - [s.l.] : Elsevier. - 1 : Vol. 64. - pp. 13-37. - ISSN: 0168-8510 DOI: 10.1016/S0168-8510(02)00079-9.

Bauermeister Jose A [et al.] Sexual behavior and perceived peer norms: Comparing perinatally infected and affected youth [Revue] // Journal of youth and adolescence. - #aug# 2008. - 8 : Vol. 38. - pp. 1110-1122. - ISSN: 1573-6601.

Bernays Sarah [et al.] Children growing up with HIV infection: the responsibility of success [Revue] // The Lancet. - [s.l.] : Elsevier. - 9925 : Vol. 383. - pp. 1355-1357. - ISSN: 0140-6736 DOI: 10.1016/S0140-6736(13)62328-4.

Brown D.C., Kelnar C.J.H. et Wu F.C.W. Energy metabolism during male human puberty. I. Changes in energy expenditure during the onset of puberty in boys [Revue] // Annals of Human Biology. - [s.l.] : Taylor \& Francis, #jan# 1996. - 4 : Vol. 23. - pp. 273-279. - ISSN: 0301-4460 DOI: 10.1080/03014469600004502.

Brown Kathleen M. [et al.] Changes in self-esteem in black and white girls between the ages of 9 and 14 years [Revue] // Journal of Adolescent Health. - [s.l.] : Elsevier. - 1 : Vol. 23. - pp. 7-19. - ISSN: 1054-139X DOI: 10.1016/S1054-139X(97)00238-3.

Buchacz Kate [et al.] Delayed Onset of Pubertal Development in Children and Adolescents With Perinatally Acquired HIV Infection. [Revue] // JAIDS Journal of Acquired Immune Deficiency Syndromes. - 2003. - 1 : Vol. 33. - pp. 56-65.

Chokephaibulkit Kulkanya [et al.] Characterizing HIV Manifestations and Treatment Outcomes of Perinatally Infected Adolescents in Asia [Revue] // The Pediatric infectious disease journal. - #mar# 2014. - 3 : Vol. 33. - pp. 291-294. - ISSN: 1532-0987.

Chokephaibulkit Kulkanya [et al.] Outcomes of a Comprehensive Youth Program for HIV-infected Adolescents inÂ Thailand [Revue] // Journal of the Association of Nurses in AIDS Care. - [s.l.] : Elsevier. - 6 : Vol. 26. - pp. 758-769. - DOI: 10.1016/j.jana.2015.08.005.

Christin A. [et al.] Parent-adolescent relationship in youths with a chronic condition [Revue] // Child: Care, Health and Development. - 2016. - 1 : Vol. 42. - pp. 36-41. - ISSN: 1365-2214 DOI: 10.1111/cch.12266.

Cleves Mario An introduction to survival analysis using Stata [Ouvrage]. - [s.l.] : Stata Press, 2008.

Dann T. C. et Roberts D. F. Menarcheal age in University of Warwick students [Revue] // Journal of Biosocial Science. - 1984. - 04 : Vol. 16. - pp. 511-519.

Data-Hub AIDS 2013 HIV AIDS Asia Pacific Research Statistical Data Information Resources AIDS Data Hub [Rapport] : unpublished. - 2013.

de Martino Maurizio [et al.] Puberty in perinatal HIV-1 infection: a multicentre longitudinal study of 212 children [Revue] // AIDS. - 2001. - 12 : Vol. 15. - pp. 1527-1534.

Diasio Nicoletta et Vinel Virginie La préadolescence: un nouvel age de la vie [Revue] // Revue des sciences sociales. - 2014. - Vol. 51. - pp. 8-13.

Diasio Nicoletta et Vinel Virginie La salle de bain: reconfiguration des rapports aux autres et Ã soi Ã lâ€™aube de lâ€™adolescence [Revue] // La revue internationale de l'Ã©ducation familiale. - [s.l.] : L'Harmattan, 2015. - 1. - pp. 39-61.

Duclos Karine De l’adolescence à l’exigence du haut niveau : les paradoxes du temps [Revue]. - [s.l.] : LÂ’Esprit du temps, 2014. - 2 : Vol. 66. - pp. 71-80. - ISSN: 2260-2100.

Elkington Katherine S [et al.] Substance Use and Sexual Risk Behaviors in Perinatally Human Immunodeficiency Virus-exposed Youth: the Role of Caregivers, Peers and HIV Status [Revue] // The Journal of adolescent health : official publication of the Society for Adolescent Medicine. - #mar# 2009. - 2 : Vol. 45. - pp. 133-141. - ISSN: 1879-1972.

Frisch Rose E., Wyshak Grace et Vincent Larry Delayed Menarche and Amenorrhea in Ballet Dancers [Revue] // N Engl J Med. - [s.l.] : Massachusetts Medical Society, #jul# 1980. - 1 : Vol. 303. - pp. 17-19. - ISSN: 0028-4793 DOI: 10.1056/NEJM198007033030105.

Gertner Joseph M. [et al.] Delayed somatic growth and pubertal development in human immunodeficiency virus-infected hemophiliac boys: Hemophilia Growth and Development Study [Revue] // The Journal of Pediatrics. - [s.l.] : Elsevier. - 6 : Vol. 124. - pp. 896-902. - ISSN: 0022-3476 DOI: 10.1016/S0022-3476(05)83177-4.

Grmek Mirko Histoire du sida [Ouvrage] / éd. Broché. - [s.l.] : Payot, 1990.

Grmek Mirko Histoire du sida. Début et origine d'une pandémie actuelle [Ouvrage]. - [s.l.] : EHESS, 1989. - Vol. 44 : pp. 1521-1523. - ISSN: 0395-2649.

Haberer Jessica et Mellins Claude Pediatric Adherence to HIV Antiretroviral Therapy [Revue] // Current HIV/AIDS reports. - #nov# 2009. - 4 : Vol. 6. - pp. 194-200. - ISSN: 1548-3576.

Hall GS Adolescence: in Psychology and its relation to physiology, anthropology, sociology, sex, crime, religion, and education [Ouvrage]. - 1904. - Vol. I\&II.

Hata Eiichi et Aoki Kunio Age at menarche and selected menstrual characteristics in young Japanese athletes [Revue] // Research quarterly for exercise and sport. - [s.l.] : Taylor & Francis, 1990. - 2 : Vol. 61. - pp. 178-183.

Howdeshell Kembra L. [et al.] Environmental toxins: Exposure to bisphenol A advances puberty [Revue] // Nature. - #oct# 1999. - 6755 : Vol. 401. - pp. 763-764. - ISSN: 0028-0836.

James William H Age at menarche, family size, and birth order [Revue] // American journal of obstetrics and gynecology. - [s.l.] : Mosby, 1973. - 2 : Vol. 116. - pp. 292-293.

Jitnarin N [et al.] Prevalence of overweight and obesity in Thai population: results of the National Thai Food Consumption Survey. // Prevalence of overweight and obesity in Thai population: results of the National Thai Food Consumption Survey.. - National Development and Research Institutes, Public Health Solutions of NYC, New York, NY 10010, USA. jitnarin@ndri.org FAU - Kosulwat, V : [s.n.]. - pp. --.

Kinsey A. C. Sexual behavior in the human male, Philadelphia (W. B. Saunders Company) 1953. [Ouvrage]. - 1953.

Kipke Michele D Adolescent Development and the Biology of Puberty:: Summary of a Workshop on New Research [Ouvrage]. - [s.l.] : National Academies Press, 1999.

Knodel John E, Chamratrithirong Aphichat et Debavalya Nibhon Thailand's reproductive revolution: Rapid fertility decline in a third-world setting [Ouvrage]. - [s.l.] : JSTOR, 1987.

Koo M.M. et Rohan T.E. Accuracy of short-term recall of age at menarche [Revue] // Annals of Human Biology. - 1997. - 1 : Vol. 24. - pp. 61-64. - DOI: 10.1080/03014469700004782.

Lallemant C [et al.] Choix contraceptifs en post-partum chez les femmes infectÃ©es par le VIH en ThaÃ¯lande: la place de la stÃ©rilisation [Revue]. - 2006.

Mahoney Elizabeth M. [et al.] HIV-Associated Immune Dysfunction and Delayed Pubertal Development in a Cohort of Young Hemophiliacs. [Revue] // JAIDS Journal of Acquired Immune Deficiency Syndromes. - 1999. - 4 : Vol. 21. - pp. 333-337.

Majaliwa E.S., Mohn A. et Chiarelli F. Growth and puberty in children with HIV infection [Revue]. - [s.l.] : Springer International Publishing, 2009. - 1 : Vol. 32. - pp. 85-90--. - ISSN: 0391-4097.

Malina RM [et al.] Family size and age at menarche in athletes [Revue] // Medicine and science in sports and exercise. - Institute for the Study of Youth Sports, Michigan State University, East Lansing 48824-1049, USA. : [s.n.], 1997. - 1 : Vol. 29. - pp. 99-106. - ISSN: 0195-9131.

Malina ROBERT M [et al.] Age at menarche and selected menstrual characteristics in athletes at different competitive levels and in different sports. [Revue] // Medicine and science in sports. - 1977. - 3 : Vol. 10. - pp. 218-222.

Mbwile Gloria Reginald Growth and pubertal Development among HIV Infected children aged 8-18 years in Dar Es Saalam [Rapport] : Master's thesis / Muhimbili University of Health and Allied Sciences. - 2012.

Mosley H. The effects of nutrition on natural fertility [Revue] // Natural fertility. - 1979. - pp. H. Leridon p 83-105.

Murphy Debra A. [et al.] Health literacy and antiretroviral adherence among HIV-infected adolescents [Revue] // Patient Education and Counseling. - [s.l.] : Elsevier. - 1 : Vol. 79. - pp. 25-29. - DOI: 10.1016/j.pec.2009.07.014.

Nahata Leena, DaJusta Daniel et Gupta Basuray Rakhi The Male Genital Examination: Overcoming Barriers to Prevent Missed Diagnoses [Revue] // Clinical Pediatrics. - 2015. - 13 : Vol. 54. - pp. 1237-1239. - DOI: 10.1177/0009922815577962.

Nelson Kenrad E. The demographic impact of the HIV epidemic in Thailand [Revue] // AIDS. - 1998. - 7 : Vol. 12. - pp. 813-814.

Ounjit Wilailak Social and Cultural Dimensions of Adolescent Sexual Relationships in Thailand [Revue] // Procedia-Social and Behavioral Sciences. - [s.l.] : Elsevier, 2015. - Vol. 171. - pp. 606-612.

Padez C Social background and age at menarche in Portuguese university students: a note on the secular changes in Portugal [Revue] // American Journal of Human Biology. - [s.l.] : Wiley Online Library, 2003. - 3 : Vol. 15. - pp. 415-427.

Palermo Davis S Coping with uncertainty: Behavioral and developmental perspectives [Ouvrage]. - [s.l.] : Psychology Press, 2014.

Popoola Tosin et Mchunu Gugu How Later Adolescents With Adult Responsibilities Experience HIV Bereavement in Nigeria: Application of a Bereavement Model [Revue] // Journal of the Association of Nurses in AIDS Care. - [s.l.] : Elsevier. - 5 : Vol. 26. - pp. 570-579. - ISSN: 1055-3290 DOI: 10.1016/j.jana.2015.06.003.

Pozo JesÃºs et Argente JesÃºs Delayed puberty in chronic illness [Revue] // Best Practice \& Research Clinical Endocrinology \& Metabolism. - [s.l.] : Elsevier. - 1 : Vol. 16. - pp. 73-90. - DOI: 10.1053/beem.2002.0182.

Prevention National AIDS et Committee Alleviation RepReport Period January 2008 - December 2009 [Revue] // UNGASS Coutnry Progress Report Thailand. - 2009.

R Stukovsky, JA Valsik et M Bulai-Stirbu Family size and menarcheal age in Constanza, Roumania [Revue] // Human Biology. - [s.l.] : JSTOR, 1967. - pp. 277-283.

Rochebrochard De La Les âges à la puberté des filles et des garçons en France. Mesures à partir d'une enquète sur la sexualité des adolescents [Revue] // Population. - [s.l.] : Institut national d'Ã©tudes dÃ©mographiques, 1999. - 6 : Vol. 54. - pp. 933-962. - ISSN: 0032-4663 DOI: 10.2307/1534716.

Saengwonloey Orapun, Jiraphongsa Chuleeporn et Foy Hjordis Thailand Report: HIV/AIDS Surveillance 1998 [Revue] // JAIDS Journal of Acquired Immune Deficiency Syndromes. - 2003. - Vol. 32. - pp. S63-S67.

Sedlmeyer Ines L. et Palmert Mark R. Delayed Puberty: Analysis of a Large Case Series from an Academic Center [Revue] // The Journal of Clinical Endocrinology \& Metabolism. -

[s.l.] : The Endocrine Society, #apr# 2002. - 4 : Vol. 87. - pp. 1613-1620. - ISSN: 0021-972X DOI: 10.1210/jcem.87.4.8395.

Selevan Sherry G. [et al.] Blood Lead Concentration and Delayed Puberty in Girls [Revue] // New England Journal of Medicine. - 2003. - 16 : Vol. 348. - pp. 1527-1536. - PMID: 12700372. - DOI: 10.1056/NEJMoa020880.

Sharma S S et Shukla N B Menarcheal age among Indian sportswomen. [Revue] // British Journal of Sports Medicine. - #jun# 1992. - 2 : Vol. 26. - pp. 129-131. - ISSN: 1473-0480.

Szubert Alexander J. [et al.] Pubertal development in HIV-infected African children on first-line antiretroviral therapy [Revue] // AIDS. - 2015. - 5 : Vol. 29. - pp. 609-618. - DOI: 10.1097/QAD.0000000000000590.

Thiaudière Claude Sociologie du sida [Ouvrage] / éd. Découverte La. - [s.l.] : Repères, 2002.

UNAIDS HIV in Asia and the Pacific [Revue] // UNAIDS report 2013. - 2013. - p. 60.

UNAIDS The Gap Report [Revue]. - 2014.

UNAIDS UNAIDS report on the global AIDS epidemic 2013 [Revue] // Global Report. - 2013.

UNAIDS World AIDS day report 2011 [Revue] // World AIDS day report. - 2011.

UNAIDS/WHO Thailand 2004 update [Revue] // Epidemiological Fac Sheets. - 2004.

Unicef Towards an AIDS-free generation: children and AIDS: sixth stocktaking report, 2013 [Ouvrage]. - 2013.

Viner Russell et Booy Robert Epidemiology of health and illness [Revue] // BMJ : British Medical Journal. - [s.l.] : BMJ Group, #feb# 2005. - 7488 : Vol. 330. - pp. 411-414. - ISSN: 1756-1833.

WILLIAMS Paige L [et al.] Pubertal Onset in HIV-infected Children in the Era of Combination Antiretroviral Treatment [Revue] // AIDS (London, England). - #jul# 2013. - 12 : Vol. 27. - pp. 1959-1970. - ISSN: 1473-5571.

Zarow Ryszard et Cichocka BA A comparative analysis of estimation of age at menarche by various methods in women participating in the Krakow Longitudinal Growth Study, Poland [Revue] // American Journal of Human Biology. - [s.l.] : Wiley Online Library, 2008. - 2 : Vol. 20. - pp. 146-148.

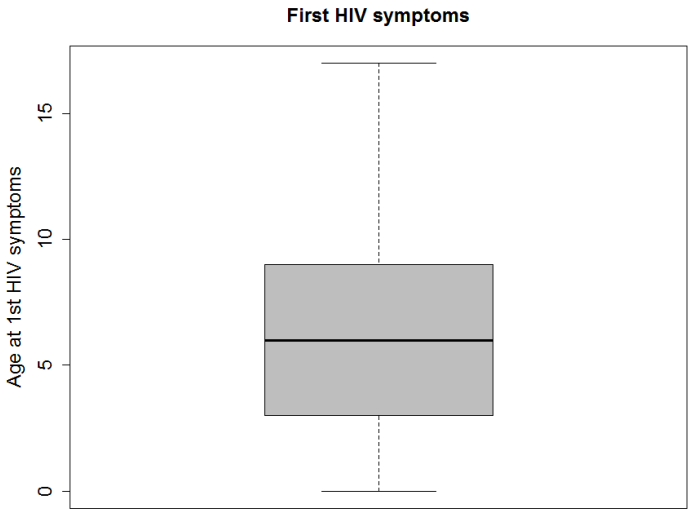
APPENDIX

Table 15. Codes for type of caregiver in the dataset

Codes for type of caregiver	
Code	*Meaning*
M	Mother
F	Father
MS	Mother's sister
MB	Mother's brother
FS	Father's sister
MSL	Mother's sister-in-law
MBL	Mother's brother-in-law
FSL	Father's sister-in-law
FBL	Father's brother-in-law
C	Cousin
ML	Mother-in-law
FL	Father-in-law
SM	Step mother
SF	Step father
MGF	Maternal Grandfather
MGM	Maternal Grandmother
PGM	Paternal Grandmother
PGF	Paternal Grandfather
B	Brother
S	Sister
R	Relative
OT	Other relative

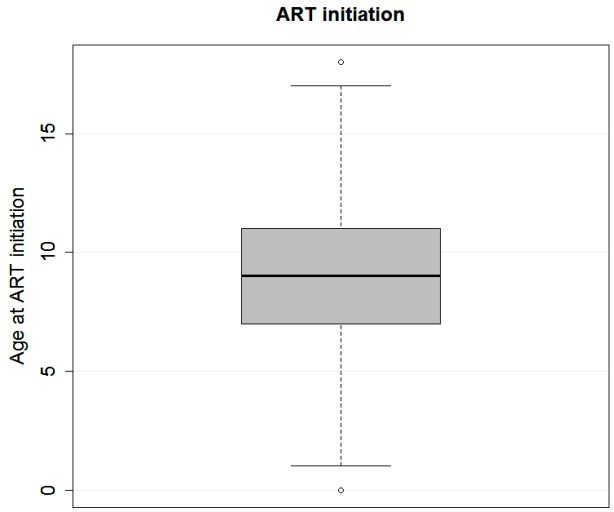
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 11. Age at first HIV symptoms distribution



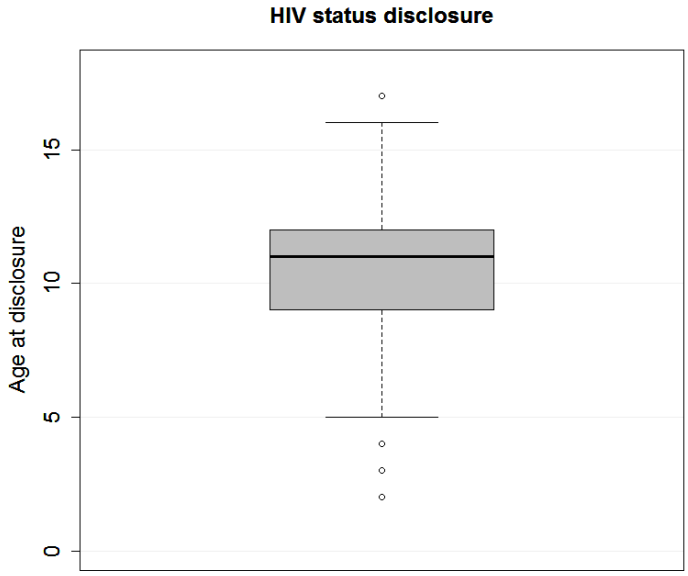
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 12. Age at ART initiation distribution



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 13. Age at HIV status disclosure distribution



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 16. Pubertal onset life table in perinatally HIV-infected girls

				Conditionnal probability	
Age interval x to x+1	Risk population Px	Menarche in year M(x,x+1)	Probability of experience menarche ahx	Surviving pubertal onset asx	Probability of being pubescent ah'x
9	1000	3	3	997	3
10	997	24	21	976	24
11	973	107	87	891	109
12	866	253	205	708	292
13	613	321	327	476	524
14	292	228	539	219	781
15	64	58	594	89	911
16	6	6	333	59	941
17	0	0	0	59	941
18	0	0	59	941	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 17. Pubertal onset life table in female controls

				Conditionnal probability	
Age interval x to x+1	Risk population Px	Menarche in year M(x,x+1)	Probability of experience menarche ahx	Surviving pubertal onset asx	Entering puberty ah'x
9	1000	6	6	994	6
10	994	53	48	947	53
11	941	156	119	834	166
12	785	459	502	415	585
13	326	274	614	160	840
14	52	48	528	76	924
15	4	4	750	19	981
16	0	0	1000	0	1000
17	0	0	0	0	0
18	0	0	0	0	

Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 18. Pubertal onset life table in male perinatally HIV-infected youth

				Conditionnal probability	
Age interval x to x+1	Risk population Px	Voice change in year M(x,x+1)	Probability of experience menarche ahx	Surviving pubertal onset asx	Entering puberty ah'x
9	1000	4	4	996	4
10	996	22	17	978	22
11	974	42	22	957	43
12	932	165	140	823	177
13	767	307	271	600	400
14	460	248	229	462	538
15	212	149	356	298	702
16	63	50	318	203	797
17	13	11	143	174	826
18	2	2	0	174	826
19	0	0	0	174	826
20	0	0	174	826	

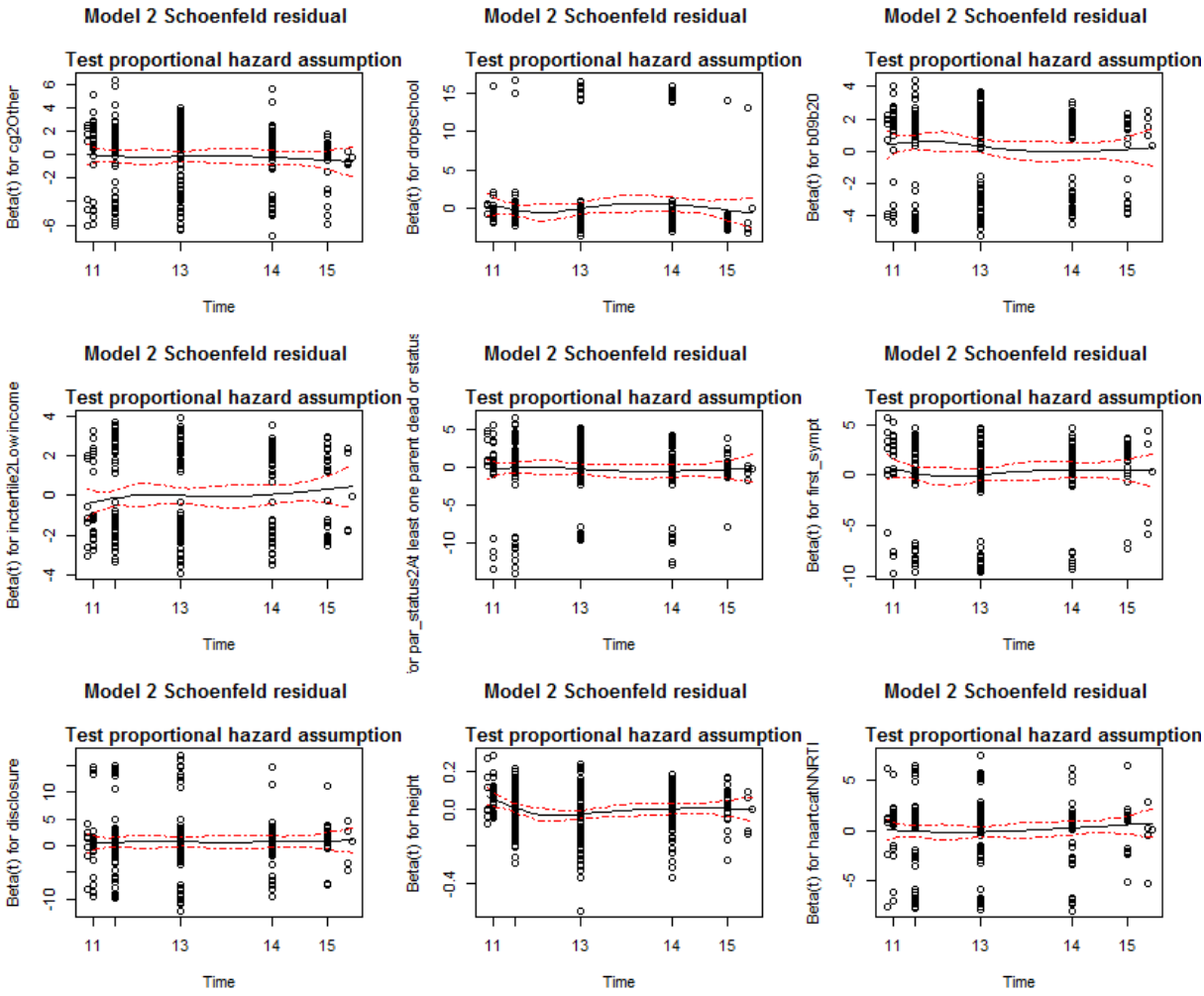
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Table 19. Pubertal onset life table in male controls

				Conditionnal probability	
Age interval x to x+1	Risk population Px	Voice change in year M(x,x+1)	Probability of experience menarche ahx	Surviving pubertal onset asx	Entering puberty ah'x
10	1000	13	13	987	13
11	987	45	34	954	46
12	942	259	239	726	274
13	683	327	282	521	479
14	356	234	340	344	656
15	122	99	442	192	808
16	23	19	105	172	828
17	4	3	0	172	828
18	1	1	0	172	828
19	0	0	0	0	0
20	0	0	0	0	

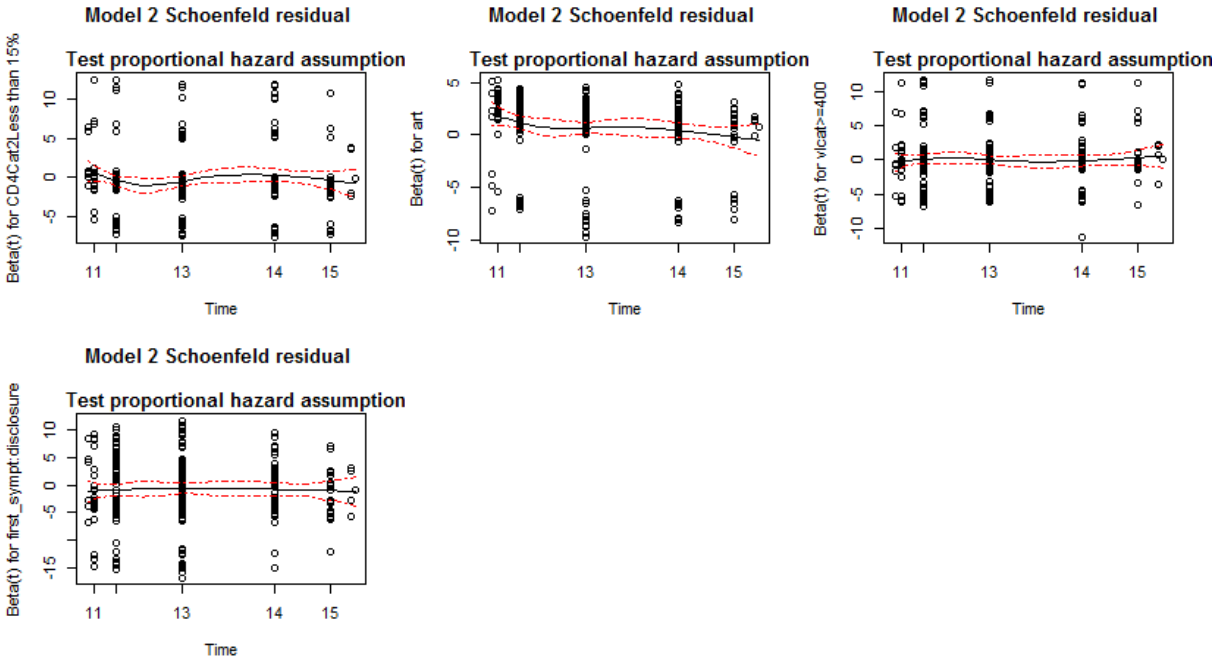
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 14. Schoenfeld residuals – part 1



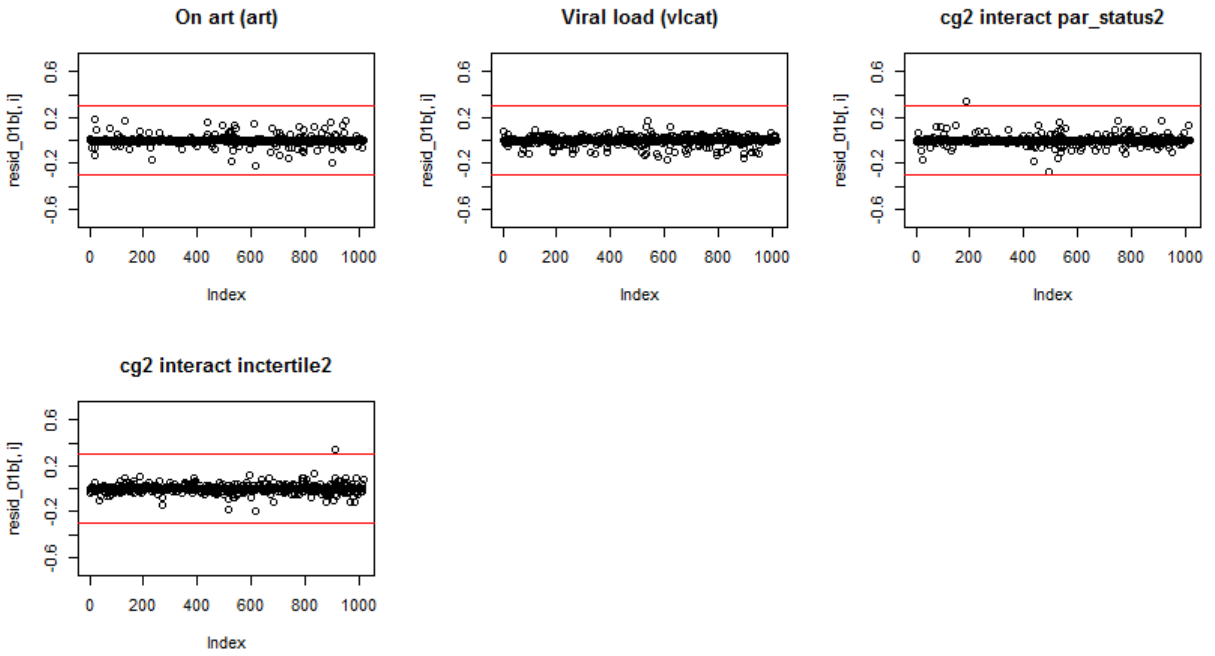
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 15. Schoenfeld residuals – part 2



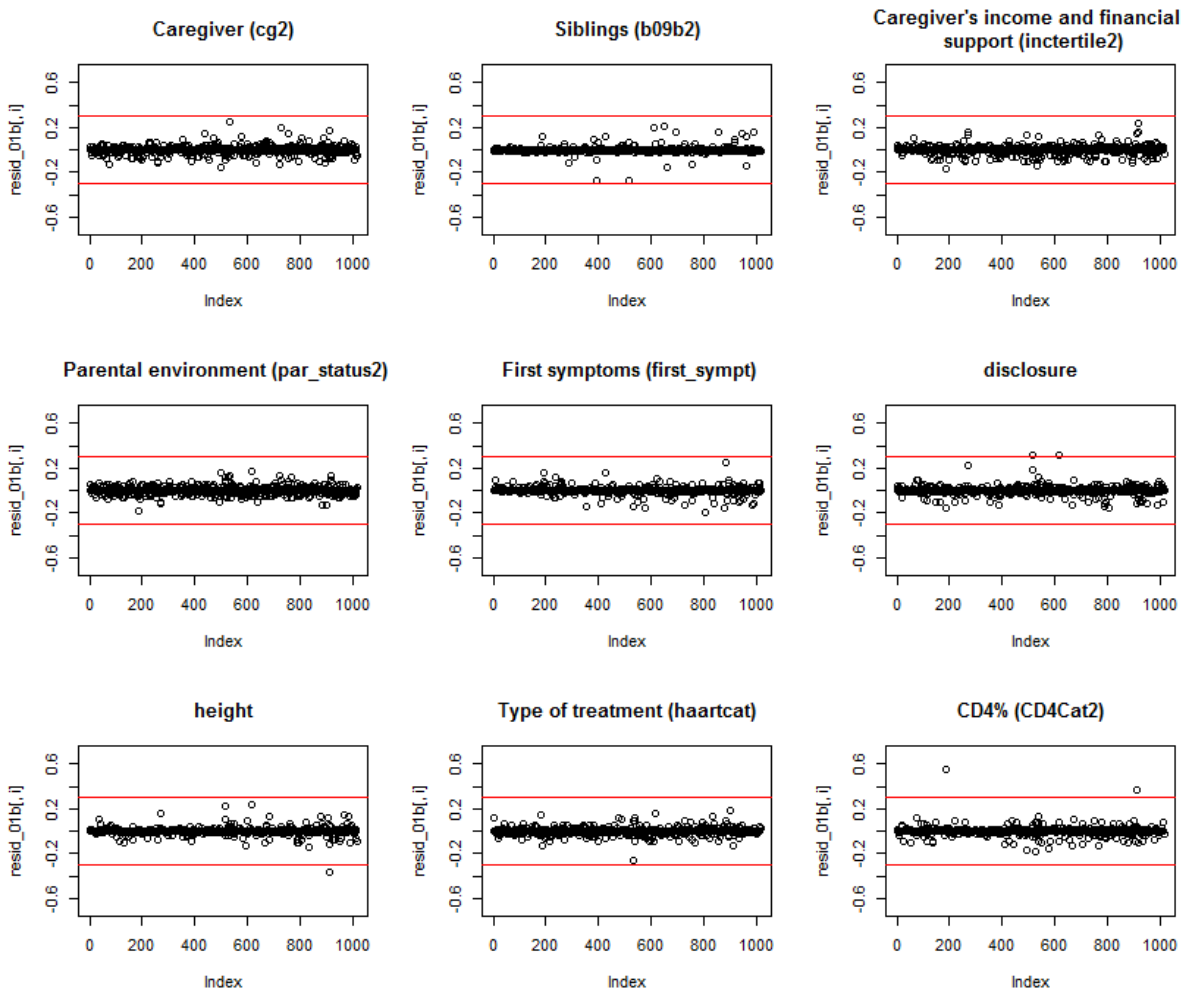
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 16. Standardized dfbetas residuals – part 1



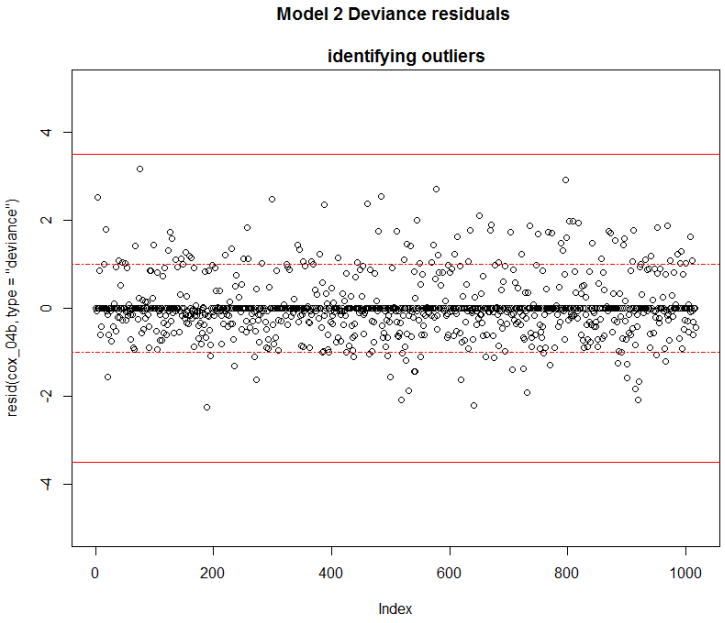
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 17. Standardized dfbetas residuals – part 2



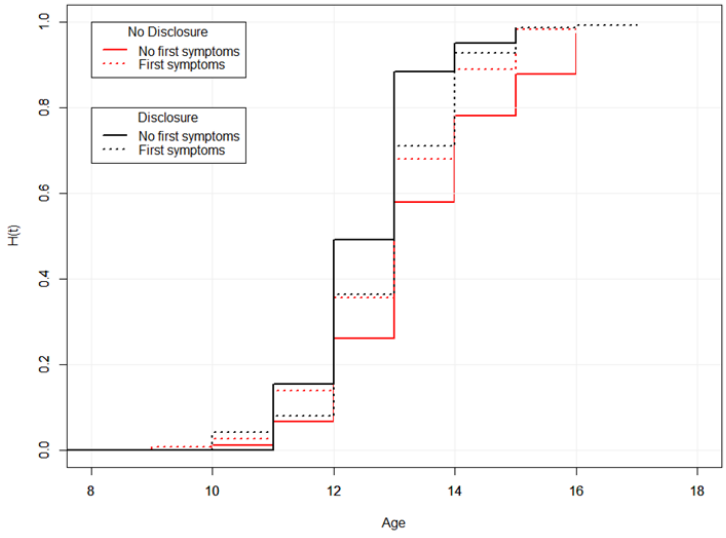
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 18. Deviance residuals



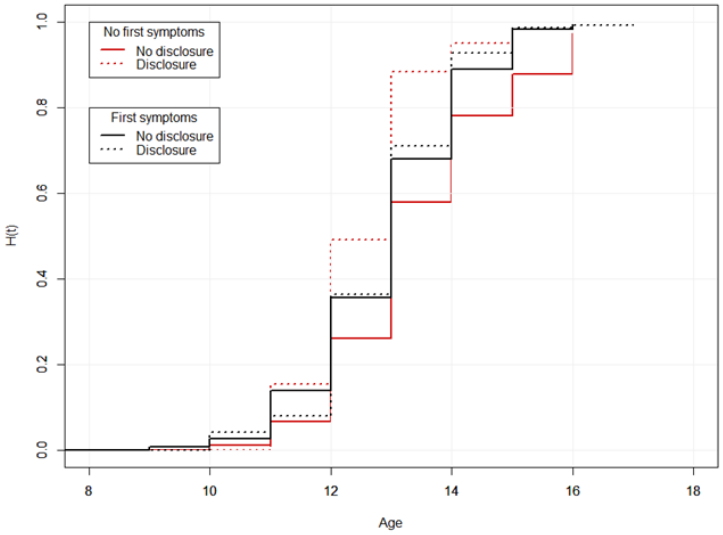
Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 19. Hazard Function of menarche in perinatally HIV-infected girls depending on the first symptoms stratified on HIV disclosure



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Figure 20. Hazard Function of menarche in perinatally HIV-infected girls depending on the HIV disclosure stratified on first symptoms



Source: TEEWA survey of HIV-infected adolescents on ARV, 2010-2013

Text 1. Hypotheses on interactions

First, association between disclosure and menarche was expected to be different regarding the occurrence of first symptoms, the CD4% category and being on ART. Indeed, disclosure might have different consequences according to the severity of the disease. Association between disclosure and menarche might also depend on the caregiver type, for the relationship between the child and his caregiver should be different whether the caregiver is close related or not. Also, while mothers (and certainly fathers) are HIV-infected, other type of caregiver may not be, which imply a different connection to the disease.

Second, association between caregiver and menarche was expected to depend on parent's vital status. Children who lost both parents should be more likely to live with other relatives. Therefore, children who live with their grand-parents while their parents are alive might have a different behavior.

Furthermore, association between income and financial support amounts and menarche may depend on the caregiver. Indeed, grand-parents are more likely to earn low income than parents because they are retired. Therefore, income might be a better indicator of children's social class when they live with their grand-parents than when they do not.

Moreover, association between ART and menarche was expected to depend on first symptoms. Indeed, beginning treatment before any symptoms might improve immunity to the disease. Therefore, it could weaken the effect of HIV on menarche.

Also, association between CD4% and menarche was expected to depend on viral load. Usually having low CD4% indicates a high viral load. Having contradictory results in CD4% and viral load may suggest a problem in the estimation of the immunosuppression.

Association between siblings and menarche was expected to depend on parent's vital status, since the longer you live the greater is your probability of having an additional child. In addition, having children cost a lot to parents for health care and education. However, Chinese migrants are known to have high fertility rates in Thailand. Therefore, having both parent dead but several siblings might reflect upper-class or Chinese migrants group belonging.