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Risk Factors Sexual Behaviour and *C. trachomatis* Infection among Health Sciences University Students in Lisbon

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Abstract

Background: Individuals between 15 and 24 years of age are at higher risk of acquiring *Chlamydia trachomatis* infection. The objectives of this study were to describe risk taking sexual behaviour and the presence of *C. Trachomatis* in health sciences university students in Lisbon.

Methods: We included 308 university students. An anonymous self-administered questionnaire was provided with questions referring to risk taking sexual behaviour. A PCR technique was used in urine samples to identify chlamydial DNA. Blood was taken for anti- *C. trachomatis* antibodies testing.

Results: A significant statistical difference between sexes (p = 0.001) was found for the age of the first sexual contact (minimum age for males - 13 and for females - 15). The median number of sexual partners during the last six months was one, although 23.5% of males and 10.2% of women had more than one sexual partner (difference between genders statistically significant - p = 0.001). Only 33.4%, 5.2% and 34.2% participants referred to use a condom for vaginal, oral or anal intercourse, respectively. Around 40% and 8% of the students admitted to have sexual intercourse under the effect of alcohol or drugs use, respectively. Information on STI was obtained from friends in 51%, followed by books and magazines (47.9%). *C. trachomatis* DNA was not identified. IgG anti-*C. trachomatis* antibodies were present in 14.4%.

Conclusions: The high risk taking sexual behaviour, namely the early age of first sexual contact, the high number of sexual partners, specially between males, the low rate of condom use and the high number of students assuming to have sexual intercourse under alcohol or drugs, suggests basic preventive measures and education on STI are urgently needed.

The finding of IgG anti- *C. trachomatis* antibodies, together with the aspects cited above, also suggests that a high number of females in this group may be at high risk of developing tubal damage.

Keywords

STI risk taking behaviour, Student's sexual behaviour, Knowledge gaps STI

Introduction

Sexually transmitted infections (STI) caused by *Chlamydia trachomatis* are very prevalent worldwide, being the most frequently reported communicable disease in EU/EEA countries during 2011 [1,2]. Young adults with less than 30 years of age are the most affected [2]. This infection is considered a global public health problem, since complications such as pelvic inflammatory disease (PID), ectopic pregnancy, infertility and chronic pelvic pain may occur. It may be transmitted to the neonate during labour [1-5]. These conditions can be potentiated by reinfection [1,3,5,6]. The Fifth Millennium Objective is the reduction of maternal mortality until 2015. The control of *C. trachomatis* genital infections should be included as one of the points of action, especially because ectopic pregnancy, which is mainly caused by *C. trachomatis* is the major cause of first trimester maternal mortality [7].

The number of cases of *C. trachomatis* infection as reported by ECDC increased from 3,48,594 in 2008 to 3,84,105 in 2012 [8]. The majority of cases occur in young adults, from whom 70% are between 15 and 24 years of age (university students belong to this age group). Women between 20 and 24 years of age are the most affected [9]. The comparison of the incidence rate between countries is difficult since there are a lot of differences on surveillance systems, diagnostic methodologies and in the proportion of sub notifications [9].

In Portugal, no official data is available, since this infection was not on the list of notifiable diseases until 2014 [10]. However, there are a few studies in different populations in which the prevalence of *C. trachomatis* varied between 4-11.8% [11-13]. This publication also describes risk factors for this infection, the majority being young age specially females, having a high number of sexual partners during the last six months and being non Caucasian [11-13]. One of these studies [12] was performed in pregnant adolescent women and there was an association with low birth weight.

The importance of STI in the control of the Human Immunodeficiency Virus (HIV) transmission has not reached a very clearly consensus among the scientific community [14]. However, the World Health Organization (WHO) recognizes that the presence of an STI in a very recent HIV infection can potentiate the transmission



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of this virus and that, in general, strategies and interventions which prevent sexual transmission of STI work as well for the prevention of HIV sexual transmission [4].

Control of chlamydial infection is difficult, since many cases are asymptomatic, but capable of transmitting the infection to their sexual partners, which is around 70 % in women and 50% in men [1,15].

Most university students belong to the age group at higher risk of acquiring an infection caused by C. trachomatis. It is also known that they generally underestimate this risk and the use of condoms has been shown to be low in this population group [16-19].

The objectives of the present study were to describe risk taking sexual behaviour, and the presence of C. trachomatis in health sciences university students in Lisbon.

Methods

Study type and population of the study

The present study is an observational study. Approval from participating universities was first obtained. The opportunity to participate was offered to all students with the collaboration of the respective Students Associations. The study was explained to all those that, in a voluntary basis, came to the research team (during the days that they were in the campus), expressing their will to participate.

Four hundred students from three Lisbon universities attending Medicine, Pharmacy, Clinical Laboratory Sciences, Radiology, Physiotherapy and Nursing degrees were enrolled in the study, after informed consent was obtained. An anonymous autofilled questionnaire was given to all students and blood and/or urine were collected from those who agreed to have any of these samples taken. Those who did not answer the questionnaire or answering the questionnaire did not refer their degree were not included in the study.

Analysis

Statistical analysis of questionnaire data was performed using the Predictive Analytics Software Statistics 18.0 (PASW) (IBM, Chicago, USA), for Macintosh.

Descriptive statistic and hypothesis tests as the Mann-Whitney, the Kruskal-Wallis, the chi-square and the Fisher's exact test were used whenever appropriate for a significance level of 5% ($\alpha = 0.05$).

Samples collection

First voided of fresh collected urine samples (15-50 mL) were obtained from 350/383 (91.4%) students after two hours from the last micturition and frozen at the arrival to the laboratory. Blood was drawn from 236/383 (61.6%) students to be tested for the presence of anti-C. trachomatis antibodies.

Detection of C. trachomatis DNA in urine samples by PCR technique

DNA extraction was performed with JetQuick DNA Spin kit (Genomed, Löhne, Germany), following the manufacturer's instructions and after defrosting the urine samples, stepwise until room temperature.

An in house multiplex PCR technique described by Jalal et al. in 2006 was used for the identification of *C. Trachomatis* [20].

Detection of IgG anti- C. trachomatis antibodies

IgG anti-C. Trachomatis antibodies were detected in serum samples with an immunofluorescence assay (anti-C. trachomatis IIFT - EUROIMMUNE; Lubeck, Germany). Manufacturer's instructions were followed.

Results

Sociodemographics

Three hundred and eighty three of the 400 (95.75%) individuals

who volunteered for the study, returned questionnaires suitable for analysis. Of those, 294/383 (76.8%) were females, and 86/383 (22.5%) males. The mean age was 21.7 years (SD - 2.797) and there was no significant statistical difference between sexes (p = 0.728) or degrees attended (*p*= 0.323).

The majority of the participants were not married 377/383 (98.4%) and were of Portuguese nationality 360/383 (94%). Concerning religion, 259/361 (71.7%) participants referred to have a religious faith, with a significant statistical difference between genders (p =0.001) and 211/278 (75.8%) of the females referring to have a religious

Distribution of individuals between the three universities was: Universidade Nova de Lisboa, 84/383 (21.9%); Universidade de Lisboa, 229/383 (59.8%) and Universidade Atlântica, 70/383 (18.3%).

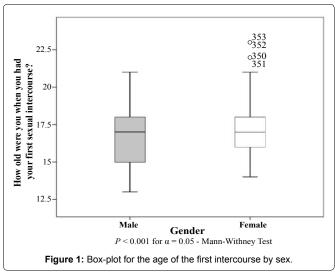
Sexual behaviour

Three hundred and thirty six of 375 participants (89.6%) had already started their sexual life, with no statistically significant differences between genders (p = 0.121). The mean age for the first sexual contact was 17.3 (range 13-23).

A significant statistical difference between sexes (p = 0.001) was found for the age of the first sexual contact. Among males, 34/78 (43.6%), the first sexual contact was before 17 years old, with the minimum age being 13 years. Among females, 22/78 (28.2%) the first sexual contact was before 17 years, with a minimum age of 15 (Figure 1).

The median of sexual partners in the last six months was one (range 0-6), with 12.9% having more than one. A significant statistical difference between sexes was found (p = 0.001). Two hundred and six of the 257 females (69.4%) that answered the question had only one sexual partner and 7.1%, 2.4% and 0.7% referred two, three and four sexual partners, respectively. Among males, 56 of the 80 (65.1%) that answered the question, mentioned only one sexual partner and 14%, 3.5%, 4.7% and 1.3% had two, three, four and five sexual partners, respectively. No sexual partner in the last six months was referred by 7.1% females and 3.5% males.

Concerning condom use for vaginal, oral and anal intercourse, we asked participants to designated one of the following categories "never, rarely, sometimes, almost always, always" for each type of sexual intercourse. Only 108/323 (33.4%), 15/286 (5.2%) and 39/114 (34.2%) participants, respectively for vaginal, oral and anal sex, referred "always" to use it. No statistically significant differences were found for condom use between sex regarding vaginal and anal intercourse (p = 0.448; p = 0.707; p = 0.162, respectively figure 2), as well as between the number of sexual partners and the use of condoms for vaginal, oral or anal intercourse (p = 0.628; p = 0.800; p



= 0.505, respectively),

To evaluate the importance given by the participants to condom use, the following questions were asked: "When I am having vaginal intercourse I must use a condom", "When I am having oral intercourse I must use a condom", "When I am having anal intercourse I must use a condom". "I agree" was answered, respectively, by 359/383 (94.0%), 267/383 (69.9%) e 335/383 (87.7%) of the participants. No statistically significant differences in the answers to those questions were found between sexes for vaginal intercourse (p = 0.400), for oral intercourse (p = 0.094) and for anal intercourse (p = 0.222).

Sexual behaviour and religion

Among the 355/383 (92.6%) participants that answered the questions "Do you have a religion?" and "Did you already had a sexual experience?", no statistically significant difference was found (p=0.088), as well as among the 314/383 (81.9%) participants who answered to the questions "Do you have a religion?" and "What was your age when you had your first sexual intercourse?" (p=0.068). However, a statistically significant difference was found between having a religion and the number of sexual partners during the previous six months (p=0.034).

Sexual behaviour and alcohol and/or drug abuse

The participants were also asked if they ever have had sexual intercourse under the effect of alcohol or drugs, 135/383 (39.9%) and 28/338 (8.3%) participants, respectively, referred "yes".

Male students were found more frequently than females to have these behaviours. This difference was statistically significant ($\mathbf{p} = 0.002$ and $\mathbf{p} = 0.043$, respectively).

Sexual information and help sources when thought to be infected with an STI

The mainly sources for sexual information were friends (51.0%), followed by books and magazines (47.9%), internet (44.5%), the mother (31.4%), the TV (32.2%), the doctor (31.2%), the boyfriend or the girlfriend (28.5%), the teacher (25.4%) and the father (23.2%). Statistically differences were found between sexes ($p=0.008 \ p<0.001$, respectively) in relation to the mother and the doctors as information sources, with females more frequently using both of them (34.8% vs 19.8% and 35.8% vs 15.1%, respectively). These questions were answered by 99.7% of the participants.

When asked "in case you thought to be infected with an STI, to whom will you ask for help?", there was no statistically significant differences between males and females with respect to the various sources referred above, with the exception of the gynaecologist for females and the father for male students. These questions were answered by 99.2% of the participants.

C. trachomatis infection

No active infection with *C. trachomatis* was identified in the 350/383 (91.4%) students who provided urine samples. IgG anti-*C. trachomatis* antibodies were present in 14.4% of 34/236 students tested and no significant statistically differences between sexes (p = 0.557) was found (Figure 3).

A significant statistical difference was not found between participants who referred "always" using condoms and those

who referred an inconsistent use for the presence of IgG anti- C. trachomatis antibodies (p=1.000 for vaginal intercourse, p=0.676 for oral intercourse and p=0.519 for anal intercourse) for the various types of sexual intercourse. However, anti- C. trachomatis antibody titres (IgG) were found to be more prevalent between participants who referred inconsistent condom use (Table 1).

Discussion

The age of sexual initiation in this population follows an identical pattern as the one described in *The Irish Survey of Sexual Health and Relationships* [21], where the beginning of sexual activity occurs before the age of 17 among 22.3% of the females and 31.3% of the males [21], although in the present study percentages of sexual initiation were higher at that age ($\mathbb{Q}=28.2\%$ e $\mathbb{Z}=43.6\%$). In relation to the age of first sexual intercourse, the mean age for this population was 17.3, similarly to the Irish study, in which the first sexual contact was at the same age. In a survey conducted by Durex between 2005 and 2009, the age of first sexual contact in 41 countries varied between 19.8 in India and 15.6 in Iceland. The average age for all countries was 17.3, for Spain 17.5 and for Portugal 16.9 [22].

In the present study, only 12.9% of the students had more than one sexual partner during the last six months, which is a lower percentage compared with the one found by Stocks et al. in 2001 [16] in a cohort study among both Spanish (18.6%) and German students (37.3%). In that study and in a follow-up one, among the Spanish participants and like in the present study, there were no cases of infection with *C. trachomatis*, while in 4.7% of the German participants this infection was detected [16.23].

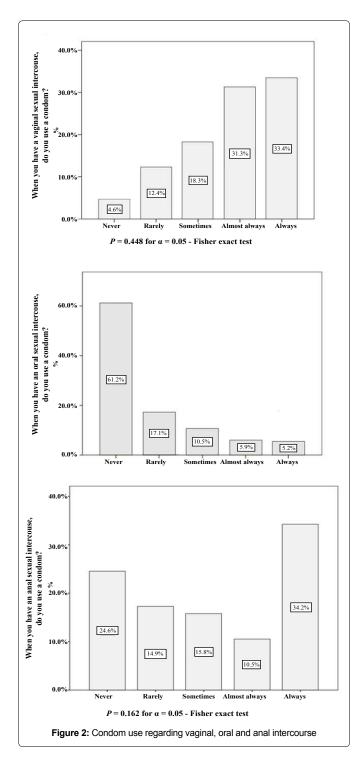
Overall, anti-*C. trachomatis* antibodies were found in 14.4% of the participants in this study. It is known that the presence of these antibodies does not differentiate between active and past infection [6]. However, among females, the level of antibody titters found in the present study (Figure 1) has been observed in previous studies to be related to infertile women with tubal damage. The presence of anti-*C. Trachomatis* antibodies seem to be related to an increased risk of infertility in women [24-26], those antibodies being present in 13.9% of the female participants of our study (Figure 3).

The percentage of participants that referred "always" to use condoms during intercourse was low (33.4% for vaginal intercourse, 5.2% for oral intercourse and 34.2% for anal intercourse), which has also been described by other authors [27,28]. When asked if they should use it, the percentage of students that agreed was much higher (87.7% for vaginal intercourse, 94.0% for oral intercourse and 69.9% for anal intercourse), showing that there is a large gap between knowledge and respective sexual behaviour. Among the participants who referred to have had more than two sexual partners in the last six months, the number who mentioned "always" to use condoms during intercourse was not higher than the one observed among the group that referred to had only one sexual partner in the last six months. There was no significant statistically difference between sexes, which was also observed in other studies [27-29]. In the social adventure study, performed by Matos et al. [30], a decrease on the use of condoms was found when compared with the previous years.

In relation to the influence of religion on sexual behaviour, it seems that having a faith does not interfere with the age of sexual initiation, but it may influence behaviour related to the number of sexual partners,

Table 1: Relative frequencies and disaggregated titers of anti- C. trachomatis antibodies (IgG) in relation to condom use and different types of sexual intercourse

Type of sexual intercourse	Condom	C. trachomatis antibodies titers (IgG)			р
	use	Negative (%)	1:320	1:1000	Р
Vaginal	Inconsistent	119 (57.8)	13 (6.3)	8 (3.9)	1.000
N = 206	Always	56 (27.2)	6 (2.9)	4 (1.9)	
Oral	Inconsistent	146 (78.5)	17 (9.1)	11 (5.9)	0.676
N = 186	Always	10 (5.4)	2 (1.1)	0 (0)	
Anal	Inconsistent	39 (52)	6 (8)	3 (4)	0.519
N = 75	Always	25 (33.3)	1 (1.3)	1 (1.3)	

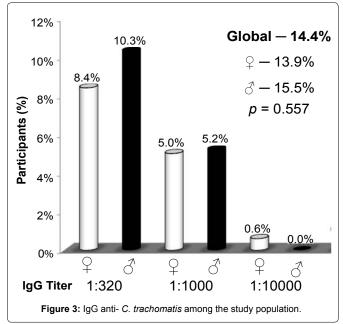


since those participants who professed a faith, also referred to have less sexual partners than those who did not have a religious faith.

A large number of students referred to engage in sexual intercourse under the effect of alcohol (39.9%) and/or drugs (8.4%), which is worrying, since in other studies it was shown that percentages between 12.1% and 20% of absence in condom use were related to the taking of these substances [21,27,31]. Matos et al. [30] also referred that there was an increase of sexual intercourse associated with alcohol consumption during the last years as also an increased association between alcohol consumption and no condom use. This association was also found in other studies [18,19,32-35].

The most frequent sources of information about sexuality for both genders were friends, books, magazines and the internet. In a British study, participants also described friends and magazines as the most important sources for acquisition of this type of information [21]. They also referred less internet use.

In another study undertaken in Eastern Europe, only 8% of the



participants pointed school as an information source and 20% stated to be "educated by friends" [36].

The results found in this and other studies [29,36,37] indicate that the credibility of sexuality sources of information used by the students is questionable. We observed that a low number of participants resort their parents, teachers or doctors for information, which may reflect difficulties of communication between generations for this issue.

Study Limitations

In this study, no participant was found to be infected with *C. trachomatis*. We believe that a bias could have been present due to fact that they were volunteers and that may have selected those more concerned and less risk taking. The majority of participants only had one sexual partner during the last six months previous to this study. It's known that sexual partner change is more frequent in this time of life [29]. As such, the findings in this study might not point the real prevalence of infection in the group, since the latest epidemiological report of the European Centrer for Disease Prevention and Control (ECDC) referred *C. Trachomatis* infection as the most reported STI in Europe and that the notification rate among 20 and 24 years of age is 621 per 100000 [2]. ECDC also recognizes that true incidence of *C. trachomatis* is likely to be higher due to undereporting or asymptomatic disease. Another portuguese study showed prevalences of *C. trachomatis* infections in 10.2% of pregnant adolescents [12].

Response bias may also have happened since the study was based in a questionnaire as some bias other may have resulted from social desiribility to correspond to social expectations.

Conclusions

A low awareness of self-risk seems to be present in this population, since the use of condoms by the students study was low, as well as the engagement in sexual intercourse under the influence of alcohol and drugs, which places these individuals at risk of acquiring any STI.

The high number of students with a high level of anti- *C. trachomatis* antibodies highlights this risk. Females included in this study, may be at a higher risk of infertility due to tubal obstruction in case of having new infections with *C. trachomatis*, since it is known that a reinfection or reactivation of a latent infection induces an exuberant immune response that may damage the Fallopian tubes [24-26].

Concerning information sources about sexuality, the parents, physicians or teachers were less sought than friends, books/magazines and internet, which suggests that this group looks for this kind of information in sources that probably are not the most suitable ones.

On the other hand, parent's knowledge about sexuality and STI may also be scarce. However, individuals cannot know what they ignore or do not have conscience about, and in that case, health authorities must identify needs and promote prevention to minimize individual risk and improve public health [21].

Besides the eventual poor knowledge and prejudice about these issues among parents in Portugal, it is important to know if teachers and health professionals are well prepared to transmit information on this subject, in view of alerting youngsters and young adults to the risk of STI.

Since in this study, mothers and the boy/girl friends were those to whom the students more frequently go to in case they thought they had an STI, and only afterwards the doctors, we believe that information campaigns are urgently needed, not only directed to youngsters, but also to the previous generations, to help parents and those involved in education in transmitting correct and effective messages and to promote dialogue about these issues.

At the same time, and taking into account the great use of internet and television, this campaign should use these means to transmit the information in a pro-active way. Nevertheless, for that to become real and efficient, it is necessary that primary health care services are able to manage patients with STI in all aspects, especially youngsters, who need to be confident in confidentiality. Efficient laboratory diagnosis must also be available and ready to give a quick response, which includes the right treatment. It's also necessary that an overall study or studies are carried out to assess the prevalence of *C. trachomatis* in the population at risk to better understand the real needs.

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