ORIGINAL ARTICLE

Prevalence of toxoplasmosis, HIV, syphilis and rubella in a population of puerperal women using Whatman 903® filter paper

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Submitted on: 07/09/2009
Approved on: 11/06/2009

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This study was approved by the Research Ethics Committee of Hospital Geral and Universidade de Caxias do Sul, and there are no restrictions for publication.

We declare no potential conflicts of interest.

ABSTRACT

Objectives: to determine the seroprevalence rate of toxoplasmosis, HIV, syphilis and rubella in a population of puerperal women. **Methods:** a prospective, cross-sectional study was performed from February 2007 to April 2008 at Hospital Geral, Universidade de Caxias do Sul in a population of 1,510 puerperal women. Women that gave birth to live born or stillborn infants were included in the study; maternal and perinatal variables were analyzed. Descriptive statistics and Pearson's chi-square with occasional Fisher's correction were used for comparisons. Alpha was set in 5%. **Results:** a total of 148 cases of congenital infection (9.8%) were identified: 66 cases of syphilis (4.4%), 40 cases of HIV (2.7%), 27 cases of toxoplasmosis (1.8%) and 15 cases of rubella (1.0%). In ten cases there was co-infection (four cases of HIV and syphilis, two cases of HIV and rubella, one case of HIV and toxoplasmosis, two cases of rubella and syphilis, and one case of toxoplasmosis and rubella). In a comparison between puerperal women with and without infection there was no statistical significance in relation to incidence of abortions, small for gestational age, prematurity, live births and stillbirths, and prenatal care. Need of neonatal intensive care unit (NICU), maternal schooling, maternal age higher than 35 years and drug use (alcohol, cocaine and crack) had statistical significance. **Conclusion:** the prevalence rate of infections was 9.8%. Need of NICU, maternal schooling lower than eight years, maternal age higher than 35 years and drug use were significantly associated with occurrence of congenital infection.

Keywords: perinatal infections, congenital infections, clinical diagnosis, seroprevalence.

[*Braz J Infect Dis 2010;14(1):24-29*]

INTRODUCTION

The relationship between pregnancy results and maternal colonization by a wide range of bacteria, fungi and viral organisms has been studied for many years.[1]

Since the studies by Norman McAlister Gregg,[2] an ophthalmologist from Sydney who in 1941 identified the relationship between occurrence of rubella in early pregnancy and occurrence of congenital cataract in infants born from such pregnancy, interest in intrauterine infections has increased and considerable attention has been given to diagnosis, prevention and management of such infections. Despite the emphasis on treatment of perinatal infections seen in several neonatal protocols, many newborns still suffer the consequences of maternal infections acquired during pregnancy.

Many factors have contributed to the high frequency of these infections. In Brazil the main factors are: (1) little knowledge and low availability of diagnostic methods related to such infections in daily medical practice, which makes treatment and early and accurate follow-up difficult; (2) total lack of knowledge of incidence of the aforementioned infectious diseases in many obstetrical and neonatal populations, except HIV and syphilis in some larger cities; (3) limitations of diagnostic examinations to be offered by the public network.

Therefore, due to a growing severity observed in Obstetrics/Gynecology and Neonatology Departments of Hospital Geral, Universidade de Caxias do Sul, identification of the prevalence rate of these diseases was performed to set guidelines relative to early diagnoses and prevention with the aim of reducing vertical transmission and its consequences for the newborn.

MATERIAL AND METHODS

A prospective, cross-sectional study was performed at the Obstetrics/Gynecology Department and Neonatal Intensive Care Unit of Hospital Geral, Universidade de Caxias do

Sul, which exclusively serve patients from the Brazilian Unified Health System (SUS). It included 1,510 puerperal women from February/2007 to April/2008. The sample was divided into two groups: Group I (n = 148; 9.8%), comprised of puerperal women whose examinations were positive to congenital infection and Group II (n = 1,362; 90.2%), comprised of puerperal women with no infection.

All puerperal women that gave birth to live born or stillborn infants and that signed an informed consent form were included in the study.

The following variables were assessed: (1) maternal: age, schooling, job, family income, prenatal care, incidence of abortion, route of delivery, drug addiction; (2) perinatal: frequency of small for gestational age (SGA), premature, stillborn and live-born newborns; need of neonatal intensive care (NIC).

Women admitted to the study were submitted to collection of blood sample to identify HIV, syphilis, toxoplasmosis and rubella during stay in maternity ward. The samples were collected on filter paper (Whatman 903® dried blood spot) and sent to the Laboratory of HIV/AIDS Research for identification of specific antibodies.

The technique of blood sample collection on filter paper was used in accordance with the norms established in the collection protocol and are in conformity with the Clinical and Laboratory Standards Institute (CLSI) collection manual LA4-A4. A sufficient amount of blood (approximately 250 μL) able to saturate five collection regions on the filter paper was collected by finger prick using a disposable lancet. Samples on filter paper were dried at room temperature for at least three hours and placed in individual zip-lock bags. The bags with patient samples were sent to the laboratory in charge of performing analyses in up to 48 hours. The samples sent to the laboratory were stored under refrigeration (4º C), and their bags were only opened during analysis.

All the samples collected on filter paper were visually assessed as to quality and volume immediately before the analysis. Diagnostic sets were specifically produced to use dried samples collected on filter paper and were properly registered at the National Health Surveillance Agency. The anti-HIV test used (*Q-Preven HIV 1 and 2 – DBS, Prime Diagnostics*) is an enzyme immunoassay to detect antibodies formed by the main antigenic proteins of HIV-1, HIV-2 and HIV-1 subtype O. The test for syphilis (*Q-Preven Sífilis Total – DBS, Prime Diagnostics*) is an enzyme immunoassay to detect IgM and IgG antibodies formed against the *Treponema pallidum* antigenic proteins. The tests used for toxoplasmosis and rubella (*Q-Preven Toxoplasmose IgM – DBS* and *Q-Preven Rubéola IgM – DBS, Prime Diagnostics*) are capture enzyme immunoassays to determine IgM against the corresponding etiologic agent. Analysis procedures, including processing of dried samples, were performed according to instructions provided by the manufacturer of diagnostic sets.

Table 1. Prevalence of congenital infections in 1,510 puerperal women at Hospital Geral, Universidade de Caxias do Sul, February 2007 to April 2008

Type of infection	n	% (95% CI)
Syphilis	66	4.4 (3.4-5.5)
HIV	40	2.7 (1.9-3.6)
Toxoplasmosis	27	1.8 (1.2-2.6)
Rubella	15	1.0 (0.6-1.6)
Total	148	

Table 2. Characteristics of 1,510 puerperal women at Hospital Geral de Caxias do Sul, February 2007 to April 2008

Characteristics	n*	% (95%CI)
Age (years)		
< 20	235	15.6 (13.8-17.5)
20-35	1090	72.3 (69.9-74.5)
> 35	183	12.1 (10.5-13.9)
Schooling		
Illiterate	3	0.2 (0.04-0.6)
Incomplete elementary school	673	44.6 (42.1-47.2)
Complete elementary school	321	21.3 (19.2-23.4)
High school	466	30.9 (28.6-33.3)
Higher education	45	3.0 (2.2-4.0)
Job		
Housewife	772	52.3 (49.7-54.9)
Factory worker	457	31.0 (28.6-33.4)
Housemaid	117	7.9 (6.6-9.4)
Student	38	2.6 (1.8-3.5)
Farmer	36	2.4 (1.7-3.4)
Unemployed	30	2.0 (1.4-2.9)
Self-employed	26	1.8 (1.2-2.6)
Family income (minimum wages)		
< 3	983	79.1 (76.7-81.3)
3-5	224	18.0 (15.9-20.3)
5-10	34	2.7 (1.9-3.8)
> 10	2	0.2 (0.02-0.6)
Prenatal care (at least six visits)		
Yes	876	65.9 (63.9-68.5)
No	453	34.1 (31.5-36.7)
Type of delivery		
Vaginal	810	58.8 (56.1-61.4)
Cesarean	568	41.2 (38.6-43.9)
Drug addiction		
Alcohol/cocaine/crack	34	2.4 (1.7-3.4)
No	1377	97.6 (96.2-97.9)

*Difference in values results from lost or uninformed data.

The study was approved by the Research Ethics Committee of Universidade de Caxias do Sul.

Characteristics of the study population were presented, prevalence of congenital infections was calculated, and results were presented as percentage with respective 95% confidence intervals (95% CI). Later, patients were divided into two groups according to presence or not of perinatal infection and results between both groups were compared using the chi-square test. Statistical procedures were performed using the software SPSS 15.0 (SPSS Inc.); $p < 0.05$ was considered significant for all tests.

Sample size was determined taking into consideration that the lowest estimated prevalence was between 0.5 and 1.5; an approximate number of 1,500 pregnant women were calculated using 95% confidence level and 90% power.

RESULTS

A total of 148/1,510 puerperal women (9.8%) with positive results for infections were identified (Table 1). Ten pregnant women had more than one associated infection: four had HIV and syphilis, one had HIV and toxoplasmosis, two had rubella and syphilis, and one had toxoplasmosis and rubella.

Table 2 shows the distribution of characteristics of the population in relation to some socioeconomic, pregnancy and delivery variables.

From the Table, it can be seen that there was prevalence of patients in the age group between 20 to 35 years, and that 45% of the pregnant women had incomplete elementary school. Most women were unemployed, and 79% had family income lower than three minimum wages. Therefore, most of the patients included in the study had low income and schooling level, without the required qualification to enter the job market. In addition, 78 patients were involved with some type of drug.

Table 3 compared the group of patients with perinatal infection (Group I) and the group without infection (Group II) in relation to some perinatal results.

The difference between both groups was statistically significant as to need of ICU, maternal schooling lower than eight years and maternal drug addiction.

Prevalence of infections was associated with age and was higher in pregnant women aged 35 years or older (Table 4).

Table 3. Comparison of the group of mothers with perinatal infection (Group I) and the group without infection (Group II) in relation to perinatal results, Hospital Geral de Caxias do Sul, February/2007 to April/2008

	Group I (138) n (%)	Group II (1,364) n (%)	p*	OR (95% CI)
Abortions	23 (16.7)	223 (16.3)	0.923	1.02 (0.62-1.67)
SGA**	9 (9.0)	68 (6.7)	0.376	1.39 (0.67-2.87)
Prematurity**	23 (20.7)	164 (15.5)	0.152	1.43 (0.88-2.32)
Need of ICU	38 (27.5)	235 (17.2)	0.003	1.83 (1.23-2.72)
Neonatal death	3 (2.2)	27 (2.0)	0.752	1.10 (0.33-3.68)
Stillborns	1 (0.7)	27 (2.0)	0.507	0.36 (0.05-2.68)
Prenatal care	9 (6.5)	85 (6.2)	0.842	1.08 (0.53-2.20)
Years of schooling < 8 years	82 (59.4)	592 (43.4)	< 0.001	1.91 (1.33-2.72)
Drug addiction**	12 (9.4)	22 (1.7)	< 0.001	5.9 (2.9-12.3)
Syphilis and HIV	12 (34)	84 (6.1)	< 0.001	8.1 (3.9-16.8)

**Difference in values results from lost or uninformed data.

Table 4. Comparison between the groups of mothers with perinatal infection (Group I) and the group without infection (Group II) in relation to age

	Group I (n = 138) n (%)	Group II (n = 1,364) n (%)	p*	OR (95% CI)
< 20 years	9 (3.8)	225 (96.2)	0.006	0.39 (0.18-0.81)
20-35 years	101 (9.3)	983 (90.7)	1	
> 35 years	28 (15.4)	154 (84.6)	0.012	1.77 (1.10-2.84)

DISCUSSION

Knowledge of prevalence of infectious problems during the pregnancy cycle is very important in all population levels, especially in groups of higher risk, in which the maternal and child population undoubtedly has a higher number of complications.

The social impact of this project concerns identification of infectious maternal diseases that can be transmitted during intrauterine life and that, depending on gestational age, cause interruption of pregnancy, congenital malformations and intrauterine death. Research becomes more important as there is no reliable information on prevalence rates of such infections, neither on clinical outcomes resulting from them.

Data are usually obtained from the international literature and are related to specific regions that cannot be extrapolated to studies conducted in Brazil.

This study was carried out in a population of pregnant women with low schooling level and income. Prevalence of infections under study was 9.8%, and syphilis was the most frequently found infection, followed by HIV, toxoplasmosis and rubella.

In the group of pregnant women with infection the number of infected women increased with age. Such increase was statistically significant in women older than 35 years. These pregnant women also had lower schooling level and more involvement with drugs.

Syphilis is still a major challenge to the Brazilian public health and brings serious consequences to the infant when it occurs during pregnancy. Since it is a disease that can be easily diagnosed and treated, untreated syphilis during pregnancy indicates failure in prenatal care and is, therefore, considered as a marker of prenatal quality.

The total number of cases of syphilis in Brazil, from 2005 to 2006, had an 11% increase; early latent syphilis increased 12.4% and late latent and tertiary syphilis increased 9.9%.[3]

In the state of Mato Grosso do Sul, Figueiró Filho *et al*. identified a 0.8% prevalence rate of syphilis in pregnant women.[4]

Data from the Brazilian Department of Health collected from a representative sample of laboring women aged 15-49 years showed a 1.9% prevalence rate in the Northeast, 1.8% in the North, 1.6% in the Southeast, 1.4% in the South, and 1.3% in the Midwest. A national study performed in 2000 showed a 1.7% prevalence rate of syphilis in laboring women.*

This study had a three-fold higher prevalence of syphilis (4.4%). Such prevalence, above the national average, has followed the increase in reports of congenital syphilis in the state of RS, which rose from 0.6 cases/1,000 live births in 1998 to 1.4 cases/1,000 live births in 2006. The highest rates found in RS may not be a reflection of a more unfavorable reality than that of other states, but of intense efforts to detect and report the disease since under diagnosis and underreporting of syphilis are still high.

The World Health Organization had already reported that at least 30-40 million adults should be infected by the human immunodeficiency virus (HIV) by 2000; of these, 90% would be from developing countries and half would be women.[5]

Although the result of the protocol ACTG 076 (*AIDS Clinical Trials Groups 076*), in which zidovudine (AZT) is administered to pregnant women and to their children gave new meaning to our ability of fighting the epidemics of AIDS in children, vertical transmission of HIV is still high worldwide.[6]

In Brazil the proportion of HIV-positive women has increased quickly, especially in the low-income population. The age group with the highest number of HIV/AIDS cases in women reported by the Brazilian Department of Health is between 20 and 39 years, which coincide with reproductive age. As the number of infected women grows, the threat of an increase in number of children exposed to the virus becomes more real. Today 90% of cases of HIV-infected children in Brazil are due to maternal-child transmission. For that reason, the health policy used by the Brazilian Ministry of Health emphasizes the need of early identification of the population vulnerable to HIV transmission during the pregnancy cycle.[7]

The study identified a 2.7% prevalence rate of HIV infection. A study conducted in Porto Alegre, RS, Brazil, showed that HIV seroprevalence ranged between 0 and 8%, depending on city area.[8] Areas with lower income, lower schooling level and higher fertility rate were those with the highest prevalence.[7]

Another study performed in 27 municipalities in Southern Brazil found 0.5% HIV-positive pregnant women in a sample of 8,002 patients. The only variable associated with seropositivity for HIV was schooling level, nearly three times lower in women with eight or more years of formal education than in those that had three or less years of education. Pregnant women that were younger, single, unemployed and had lower schooling level comprised the higher exposure group.[9]

In the current study, low schooling and use of drugs had a statistically significant relationship with a higher number of cases of syphilis and HIV, showing the strong association between such diseases and number of years of study and increase in drug use among pregnant women.

Toxoplasmosis is an infection with considerable public impact. Its prevention is not simple since its occurrence is influenced by the host's genetics, immunity and behavior. The high prevalence of anti-*Toxoplasma gondii* antibodies is attributed to some geographical discrepancies related to climactic conditions, life and eating habits, hygiene and

* Brazil. Ministry of Health. Secretariat of Health Surveillance. STD/Aids National Program. Estudo Sentinela. Parturiente Corte Nacional – October 2000.

cultural differences. The main risk factors for toxoplasmosis during pregnancy are parity, older age, race, immunodeficiency, eating habits, contact with cats and soil.[10]

In addition, developed countries have lower seropositivity rates than developing countries. In the United States approximately 20-25% of women of reproductive age had positive serologic test for previous infection by *Toxoplasma gondii*, which shows that 75% of pregnant women are susceptible to this disease.

In Italy a study performed in 3,426 pregnant women showed seroprevalence of 21.5% and acute infection of 1.2% in samples treated by the ELISA method.[11] Similar seroprevalence rates were described by other authors.[12,13]

In this study the rate of previous infection by *Toxoplasma gondii* was 24.7%, similar to that of developed countries, and acute infection rate during pregnancy was 1.8%. Slightly higher rates were described in European prospective studies, which showed an infection incidence of 2-8%.[12,14-17]

However, when rates of acute infection and seroprevalence were compared with other Brazilian studies, acute infection during pregnancy is higher and seroprevalence is much lower than in other studies. Such differences are probably related to cultural aspects, different life and development habits. A study conducted in the state of Mato Grosso do Sul showed a 0.4% frequency of this disease.[4] Another study performed in the Brazilian Midwest region showed a 0.42% frequency for acute infection by *Toxoplasma gondii* in the population of pregnant women, 92% of whom had been previously exposed to the infection.[18]

A study conducted in Recife, state of Pernambuco, Brazil, showed that 74.7% were seropositive, 22.5% were susceptible and 2.8% had active infection. There was no relationship between infection and age, morbidity and gestational age. However, there was a significant association with schooling and higher susceptibility in pregnant women with eight or more years of education.[19]

In Cali, Colombia, the seroprevalence rate was 45.8%, and acute infection was 2.8%. Seroprevalence significantly increased with age (39% - 14 to 19 years *vs.* 55.3% - 30 to 39 years). Increased seroprevalence in relation to age was more significant in lower socioeconomic classes. There was also lower seroprevalence in higher socioeconomic levels (low: 49% *vs.* high: 29%).[20]

In the area of infectious and contagious diseases, the epidemiological importance of rubella is represented by occurrence of congenital rubella syndrome (CRS), which affects the fetus or newborn whose mothers were infected during pregnancy.[21]

During the worldwide epidemics of rubella in 1962-1965, there were 12.5 million cases in the United States, resulting in 2,000 cases of encephalitis, 11,250 fetal deaths, 2,100 neonatal deaths and 20,000 newborns with CRS, a series of defects that include deafness, blindness and cardiac malformations, generating an economic impact estimated in US$ 1.5 billion in the USA.[22]

In 1997 the Pan-American Health Organization recommended strategies to control the disease. Rubella vaccination was licensed in 1969; since then, the incidence of rubella and CRS has been reduced substantially.[23]

In 2005 the Center for Disease Control and Prevention (CDC) announced the elimination of endemic rubella and CRS in the USA.[24] Such occurrence was probably due to a large vaccination coverage (in more than 95% of school-age children) and high immunity rate in the population (91%).

In Brazil the vaccine against rubella was gradually implemented from 1992 (São Paulo) and 2000, when all states had the **measles-mumps-rubella** (MMR) vaccination implemented in all health units. Data from the Brazilian Department of Health reported that there were 4,408 cases of rubella in the country from 2002 to 2007, which corresponds to a 95% decrease when compared with the incidence in 1997. However, there are still rubella outbreaks.[25] In 2007 the Brazilian Department of Health confirmed rubella outbreaks in 20 states and in 577 towns, representing a total of 8,683 cases. In the state of Rio Grande do Sul, Brazil, the number of cases was 2,852, and there were two confirmed cases of CRS.

In this study the prevalence of rubella in pregnancy was 1% (15 cases), a similar index to that found in Londrina, state of Paraná, Brazil (1.2%).[26]

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