A Study on the Seroprevalence and Risk Factors of Chlamydia trachomatis Infection among Pregnant Women in Japan

日本における妊婦クラミジアトラコマティス感染の疫学と危険因子に関する研究

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Abstract

Background: Chlamydia trachomatis infection is often asymptomatic. This fact promotes its spread among populations. It is considered to be the most common bacterial STD in developed countries. The possible consequences of the infection in women are infertility and pelvic inflammatory disease (PID). There have been several studies on the prevalence and risk factors of C. trachomatis infection globally. However, there have been few studies on the trend in the prevalence of C. trachomatis, and the detailed demographic information associated with this infection in Japan. Further, no study on the loss of C. trachomatis antibodies for the purpose of estimating trends in C. trachomatis seroprevalence has yet taken place.

Methods: To assess the seroprevalence of C. trachomatis antibodies and its relationship to demographic information related to the reproductive and sexual behavior among pregnant women, this study was performed by using the enzyme immunoassay (EIA) and a self-administered questionnaire.

Results: The seroprevalence of C. trachomatis antibodies has decreased in all age groups during the past ten years. The

seroprevalence of C. trachomatis antibodies has decreased conversely with the age of women in each study year, excluding the age group of pregnant women over 40. 24 out of 33 (72.7%) and 23 out of 33 (69.7%) pregnant women seroconverted from positive to negative in ten and five years, respectively. The risk factors of C. trachomatis seropositivity were identified as low level of education ($p<0.0001$), early marriage ($p=0.043$), early pregnancy ($p<0.0001$), the first induced abortion at youth ($p=0.037$) and multiple induced abortions ($p<0.0001$).

Conclusion: The seroprevalence of C. trachomatis antibodies has decreased during the past ten years and is inversely correlated to the age of pregnant women. C. trachomatis antibodies were often found to have seroconverted from positive to negative. Lower levels of education, early marriage, early pregnancy, the first induced abortion at youth and multiple induced abortions were risk factors for C. trachomatis infection.

Key words: Chlamydia trachomatis, Seroconversion, STDs, Pregnant women, Sexual and reproductive behavior

Introduction

In the early 1980s, concern over Chlamydia trachomatis rose in the field of public health in developed countries, as new serological techniques developed (1, 2, 3). Although C. trachomatis is known as a causative agent of non-gonorrhea uritis (NGU) and pelvic inflammatory disease (PID), it has become clear that C. trachomatis infection in women also causes ectopic pregnancy and infertility (4, 5, 6, 7). Infants born from infected mothers become infected, leading to low birth weight, neonatal pneumonia and conjunctivitis (8, 9, 10, 11, 12). However, the majority of infected women are asymptomatic. For this reason, many infected women fail to seek health care, and this promotes the spread of C. trachomatis infection in the population (6, 13, 14, 15).

Sexually transmitted diseases (STDs) including C. trachomatis receive much attention not only in developed countries, but also in developing countries, because of the increased risk of acquiring HIV (16, 17, 18, 19, 20), and it has become clear that control and prevention programs for STDs are also useful in reducing the number of HIV incidences (21).

In Japan, C. trachomatis infection is considered to be the most

common bacterial STD. The number of cases reported from the sentinel surveillance conducted by Ministry of Health since 1987 gradually increased (22). However, it is unknown whether the increased number of cases detected may reflect the extent of testing, due to the increasing availability of diagnostic facilities throughout the 1980s, rather than the real prevalence of infection.

In order to elucidate the seroepidemiological trend in C. trachomatis seropositivity during the past 10 years, this study was performed, retrospectively by using a sensitive diagnostic technique for C. trachomatis antibodies. Changes in the incidence of C. trachomatis may reflect changes in life style, including sexual and reproductive behavior.

Recently, several seroepidemiological studies have been conducted on C. trachomatis in Japan. They showed that seroprevalence for C. trachomatis was about 20-25 percent among pregnant women and about 10-35 percent among college students, and that the antigen positive rate was about 5 percent (23, 24, 25, 26). These studies also suggested that both unmarried pregnant women and teenage pregnant women were at high risk of C. trachomatis infection (25, 26, 27). However, in Japan, there was little detailed demographic information about other risk factors.

Two studies of ours already reported the importance of elucidating the relationship between C. trachomatis infection and sexual behavior (24, 26). Hence, this study was designed to assess the seroprevalence of C. trachomatis antibodies among pregnant women and its relationship to possible risk factors by gathering demographic information and data on reproduction.

Materials and Methods

Subjects

9,652 blood samples were collected from pregnant women in the first trimester and were routinely screened for C. trachomatis infection from June 1996 to May 1997 in Nagasaki prefecture, in the western part of Japan. The average age of the participants was 28.9 ±4.3 (mean±SD).

The survey using questionnaires was conducted from June 1997 to September 1997. The number of respondents to the questionnaires was 1,025 (age: 28.5±4.5: mean±SD). The youngest respondent was 18 years old and the oldest one was 44 years old.

Stocked sera

The age-stratified randomized method was used to collect samples from frozen stock of sera of pregnant women in 1987 and 1992. In 1987, 275 samples were collected and in 1992, 297 samples were collected and tested for C. trachomatis antibodies. The seroprevalence of these samples was then compared with the current seroprevalence of C. trachomatis antibodies.

In order to investigate whether seroconversion of antibodies is seen during the observation period, sample sera were chosen from the frozen stocked blood of 420 pregnant women whose blood samples were collected at least three times between 1983 and 1996. 85 out of 420 pregnant women were randomly chosen to be tested for C. trachomatis antibodies.

C. trachomatis Antibodies testing

The IgG and IgA classes of serum antibodies for C. trachomatis were tested by enzyme immunoassay (EIA), a commercial kit (HITAZYME, Hitachi chemical colt'd, Tokyo, Japan). This method uses purified EB outer-membrane protein of C. trachomatis L2 strain as antigens. The distribution of absorbing capacity of both IgA and IgG antibodies among general population was bimodal. To maximize specificity and sensitivity, the cut-off values of absorbing capacity

of both IgA and IgG antibodies were given by the values of the trough of the bimodal shape and there was mean+2SD of the population who was considered to be negative for C. trachomatis antibodies by the distribution of antibodies. Samples which gave values more than the cut-off index of 1.10 in one or both of the IgG and IgA tests, scored antibody-positive. The detection rates of IgA and IgG class C. trachomatis antibodies for the population whose C. trachomatis antigen was positive were 71.9% in IgA and 77.1% in IgG and their sensitivity was higher than that for microimmunofluorescent assay (28). Further, the coincidence rates of the result between this method and Western Blotting (WB) method are 97.2% in negative cases and 100% in positive cases (28). On the other hand, the estimation on the cross-reaction rate of this testing kit for Chlamydia pneumoniae is less than 3% (28).

Questionnaire survey

Self-administered questionnaires were given to pregnant women in the selected obsterical clinics. The questionnaires asked about age, occupation, history of education, age at first marriage, age at first pregnancy, age at first induced abortion, frequency of pregnancy, number of children, number of induced abortions, and

experience of condom use (Appendix 4, Appendix 5).

Statistical analysis

The chi square test, the t-test and the Mann-Whitney's U test were used for statistical analysis. A p value of less than 0.05 was considered significant. The crude odds ratio was calculated by univariate analysis to compare the grouped demographic, reproductive and behavioral characteristics between women testing seropositive and seronegative for C. trachomatis.

Results

Subjects

The average number of births in Nagasaki prefecture from 1993 to 1995 was 15,500±650 (mean±SD), which is a decline in the average number of births from 1987 to 1995 (Appendix 1). The number of screened participants was 9,652 for one year. However, targeted pregnant women for the screening program of C. trachomatis infection were habitants in mainland of Nagasaki prefecture, which results in coverage population of about 12,000 for one year. Thus, more than 80% of pregnant women took part in this screening program. The questionnaire was given to the pregnant

women in selected obsterical clinics and the number of births in those clinics was 1,251. The number of questionnaire respondents was 1,025 for four months (Table 1). Therefore, respondent rate of questionnaire was 82%.

The average age of the participants who were routinely screened was 28.9±4.3 (mean±SD) and the average age of respondents was 28.5±4.5. A t-test for the average age of the two populations was performed and the p value was 0.928. Further, to compare the composition of the two populations, the chi square test was used. The p value was 0.143. Therefore, the composition of the two populations was not significantly different (Table 1).

Prevalence of C. trachomatis antibodies among pregnant women

Positive rates of C. trachomatis in 1996-97 were 33.1% (44/133) for pregnant women under the age of 19, 30.8% (430/1,397) for 20-24, 23.6% (920/3,898) for 25-29, 22.7% (717/3,156) for 30-34, 22.6% (212/940) for 35-39 and, 19.5% (25/128) for pregnant women over 40 (Table 2). Thus, the trend in seroprevalence of C. trachomatis infection in 1996-7 is that the older the pregnant women were, the lower the positive rate of C. trachomatis

antibodies (Appendix 2).

The positive rates of stocked sera for C. trachomatis antibodies in 1987 and 1992 were 47.8% (11/23) and 41.2% (7/17) for pregnant women under the age of 19, 35.3% (24/68) and 30.5% (18/59) for 20-24 age group, 29.6% (16/54) and 25.8% (17/66) for 25-29 age group, 27.8% (15/54) and 24.2% (15/62) for 30-34 age group, 26.9% (14/52) and 25.0% (13/52) for 35-39 age group, and 33.3% (8/24) and 29.3% (12/41) for the over 40 age group, respectively (Table 2). Regarding the over 40 age group, both of the positive rates in 1987 and 1992 were higher than those of the 25-29 age group, 30-34 age group and 35-39 age group, and at the same level as the 20-24 age group (Appendix 2). Further, excluding the 20-24 age group, positive rates of C. trachomatis antibodies among each corresponding group were always at highest levels in 1987, always at medium levels in 1992 and at lowest levels in 1996-1997. The positive rate of C. trachomatis for the 20-24 age group in 1996-1997 was at the same level as that in 1992 and lower than that in 1987 (Table 2).

The seroconversion of C. trachomatis antibodies

85 pregnant women were tested for C. trachomatis antibodies and 33 out of 85 (38.8%) were positive and 52 (61.2%) were negative

at the time of their first collection of blood. In terms of positive cases at the time of their first collection of blood, positive cases of both IgA and IgG antibodies were 19 out of 33 (57.6%), positive cases of only IgA antibody were 9 out of 33 (27.3%), and positive cases of only IgG antibody were 5 out of 33 (15.2%). At least three blood samples were collected from each pregnant woman. The number of the frozen stocked sera from these pregnant women was 267 in total, and they were tested for C. trachomatis antibodies to investigate whether the negative seroconversion is observed. 24 out of 33 (72.7%) pregnant women seroconverted from positive to negative in ten years and 23 out of 33 (69.7%) seroconverted from positive to negative in five years (Appendix 3). The shortest period of conversion from positive to negative was four months. All pregnant women who did not seroconvert, were positive for both IgA and IgG antibodies (Table 3). One pregnant woman seroconverted twice from positive to negative and from negative to positive in four years. The average values of the cut-off index of C. trachomatis antibodies in the population of pregnant women who seroconverted were 1.53 ± 0.10 (mean$\pm$SE) in IgA and 1.98 ± 0.23 in IgG, and those of IgA and IgG in the population who did not seroconvert were 4.30 ± 1.37 and 5.27

±1.06. However, the average values of the cut-off index of C. trachomatis antibodies in the population of pregnant women who have been seronegative through the observation period were 0.68± 0.26 (mean±SE) in IgA and 0.68±0.25 in IgG. The Mann-Whitney's U test and t test were used for statistical analysis. The differences of the average cut-off index of both IgA and IgG between population who seroconverted and those in the population who did not seroconvert were significant ($p=0.005$, $p=0.026$: Mann-Whitney's U test and $p=0.006$, $p=0.0009$: t test). Further, The differences of the average cut-off index of both IgA and IgG between population who seroconverted and those who have been seronagative were significant ($p<0.0001$, $p<0.0001$: Mann-Whitney's U test and t test) (Fig. 1). In addition, the distribution of the cut-off index of both IgA and IgG were bimodal (Fig. 2-1 and 2-2). On the other hand, 5 out of 52 pregnant women converted from negative to positive in terms of antibodies for C. trachomatis in five years.

The risk factors for Chlamydia trachomatis infection among pregnant women.

The age at first marriage was 18.5±0.25 (mean±SE) among pregnant women under the age of 19, 21.6±0.17 for 20-24 , 25.0±

0.10 for 25-29, 27.0±0.17 for 30-34, 29.6±0.50 for 35-39 and, 30.7 ±2.83 in pregnant women over 40 (Table 4). The age at first pregnancy was 16.5±0.50 (mean±SE) among pregnant women under the age of 19, 20.0±0.21 for 20-24 , 23.9±0.17 for 25-29, 26.8± 0.19 for 30-34, 29.6±0.50 for 35-39 and, 29.7±2.43 for pregnant women over 40 (Table 4). The pregnant frequency and number of children were 0.28±0.14 and 0.11±0.08 (mean±SE) among pregnant women under the age of 19, 0.58±0.06 and 0.29±0.04 for 20-24, 0.95±0.05 and 0.59±0.03 for 25-29, 1.60±0.07 and 1.09±0.05 for 30-34, 2.00±0.14 and 1.46±0.10 for 35-39, and 2.71±0.55 and 1.44 ±0.41 in pregnant women over 40 (Table 4). The average length of their education was 10.9±0.36 years (mean±SE) among pregnant women under the age of 19, 12.3±0.10 for 20-24, 13.0±0.07 for 25-29, 13.2±0.09 for 30-34, 13.3±0.15 for 35-39, and 12.1±0.43 for pregnant women over 40 (Table 4). The average length of education increased with the age of pregnant women, excluding pregnant women over 40. The length of education for the population of pregnant women over 40 was the same as that of the 20-24 age group, and was lower than these of other age groups. The percentage of pregnant women who had induced abortion until the time of the

survey was 15.8% in pregnant women under the age of 19, 12.2% for 20-24, 15.2% for 25-29, 13.4% for 30-34, 13.1% for 35-39, and 30.0% for pregnant women over 40 (Table 4). Concerning condom use, the number of respondents who had never used condoms was 31.6% for pregnant women under the age of 19, 26.5% for 20-24, 20.7% for 25-29, 21.3% for 30-34, 30.0% for 35-39, and 44.4% for pregnant women over 40 (Table 4). The highest rate of the pregnant women who reported to have never used a condom was those pregnant women over 40. The lowest rate was those aged between 25 and 29.

The risk factors associated with positive rates of C. trachomatis antibodies were age ($p=0.043$), education ($p<0.0001$), age at first marriage ($p=0.043$), age at first pregnancy ($p<0.0001$), age at first induced abortion ($p=0.037$), and number of induced abortions ($p<0.0001$). However, occupational status ($p=0.821$), frequency of pregnancy ($p=0.236$), number of children ($p=0.951$), and experience of condom use ($p=0.344$) were not significantly associated with seropositivity of C. trachomatis.

In terms of education, the seroprevalence of C. trachomatis antibodies was 40.0% (14/35) among questionnaire respondents with junior high school education (odds ratio: 1.00), 26.0% (138/530) with

high school education (odds ratio: 95% CI; 0.53: 0.26-1.07), 14.7% (25/170) with vocational school education (0.26: 0.12-0.58), and 11.1% (30/270) with college or university education (0.18: 0.08-0.39) (Table 5). Hence, the seroprevalence of C. trachomatis antibodies was inversely correlated to the level of education. In addition, there was a similar trend in the seroprevalence of C. trachomatis antibodies being that seropositivity decreased conversely with the age at first marriage and pregnancy. As for the age at first marriage, the seroprevalence of C. trachomatis antibodies was 34.3% (12/35) for pregnant women under the age of 19 (odds ratio: 1.00), 22.5% (73/324) for 20-24 (odds ratio: 95% CI; 0.56: 0.27-1.18), 19.3% (88/456) for 25-29 (0.46: 0.22-0.96), 11.8% (10/85) for 30-34 (0.26: 0.10-0.68), 7.7% (1/13) for 35-39 (0.16: 0.02-1.38), and 0% (0/3) for pregnant women over 40 (Table 5). Concerning the age at first pregnancy, the seroprevalence of C. trachomatis antibodies was 44.6% (25/56) in pregnant women under the age of 19 (odds ratio: 1.00), 21.7% (44/203) for 20-24 (odds ratio: 95% CI; 0.34: 0.18-0.63), 17.9% (55/307) for 25-29 (0.27: 0.15-0.49), 8.7% (6/69) for 30-34 (0.12: 0.04-0.32) and, 0% (0/10) for 35-39 (Table 5).

The seroprevalence of C. trachomatis antibodies was inversely correlated to the frequency of induced abortion. Seropositivity was 17.9% (153/857) (odds ratio: 1.00) among questionnaire respondents who had never experienced induced abortion, 32.4% (34/105) (odds ratio: 95% CI; 2.20: 1.41-3.43) among those who had experienced induced abortion once, and 46.3% (19/41) (3.97: 2.10-7.52) among those who had experienced induced abortion more than twice (Table 5). The experience of condom use did not seem to affect seropositivity of C. trachomatis. Positive rates for C. trachomatis were 22.6% (53/235) among those who reported to have never used condoms and 19.7% (154/781) among those who reported to have experienced condom use (Table 5).

Discussion

Trends in seroprevalence of C. trachomatis antibodies among pregnant women.

In terms of C. trachomatis antibodies testing, a study showed that the sensitivity of EIA method (HYTAZYME) was higher than that for microimmunofluorescent assay and the coincidence rates of the result between EIA method and Western Blotting (WB) method were

97.2% in negative cases and 100% in positive cases (28). Therefore, it is possible to think that antibody detection method used in this study had validity to estimate the trend in C. trachomatis and to explain the possibility of the loss of C. trachomatis antibodies.

Although speculations on the loss of C. trachomatis antibodies exist (29, 30), it is unknown how long antibodies are detectable after infection or treatment. C. trachomatis is sensitive to antibiotics such as erythromicine, tetracycline, doxicycline, ofloxacine and azithromicine (31, 32, 33). Failure rates in clinical trials of tetracycline and doxicycline were reported to range from 0% to 8% among women with cervical infection (33). These antibiotics are used in Japan for the treatment of bacterial infections. When these antibiotics are used for the treatment of any other infections, there is a possibility that C. trachomatis infection is cured simultaneously. As a result, C. trachomatis antibodies may decline to undetectable levels. This study was designed and performed to elucidate whether the seroconversion of C. trachomatis antibodies is observable. At least three blood samples were collected from one pregnant woman and tested for C. trachomatis antibodies. As a result, the loss of antibodies for C. trachomatis was observed among 69.7% (23/33) of the pregnant women in five years and 72.7% (24/33) of them in ten

years (Table 3), despite the fact that antibodies for viral STDs such as HIV, Herpes Simplex Virus (HSV) and Human Papilloma Virus (HPV) continued to be detected during the lifetime. On the other hand, 9 out of 33 cases were positive for only IgA antibody at the time of their first collection of blood. The bacterial infection was often localized and did not lead to bacteremia although viral infection often leads to viremia. That evidence might suggest that Immunological reaction for the bacterial infection was different from that for viral infection. Further, IgA antibody functions to prevent local infection. Therefore, it might be possible to think that those cases were mucousal infection localized in vagina or cervix.

Although it is unknown from this study about how the demographic characteristic-age of pregnant women influence the loss of antibodies for C. trachomatis, the negative-seroconversion of C. trachomatis antibodies frequently occurred in this retrospective cohort study. On the other hand, 9.6% (5/52) pregnant women seroconverted from negative to positive in five years (data not shown). These pregnant women were considered to be newly infected with C. trachomatis during the last five years. Hence, these results suggested that the seroprevalence of C. trachomatis

antibodies did not reflect a past history of infection, but reflected the balance between losses of C. trachomatis antibodies and these gained from new infection. Furthermore, it might be possible to think that if the loss of C. trachomatis antibodies occurred at the same rate, irrespective of the age of the pregnant women, the seroprevalence of C. trachomatis antibodies reflects the extent of new infection in the last several years.

Regarding the value of the cut-off index of C. trachomatis antibodies, the average values of both IgA and IgG in the population who did not seroconvert were significantly higher than those in the population that did seroconvert. Further, the average values of both IgA and IgG in the population who seroconverted were significantly higher than those in the population who have been seronagative during the observation periods (Fig. 1). In addition, to exclude the possibility that the population who seroconverted was not different from the population who have been seronegative, the distribution of serum antibodies was investigated. As a result, the distribution of both IgA and IgG revealed to be bimodal (Fig. 2-1 and 2-2). These results showed that in terms of serum C. trachomatis antibodies, population who seroconverted was different from those in the population who have been seronagative during the observation

periods. There may be two sub-populations in the population who are seropositive. One sub-population consists of pregnant women who were actively infected with C. trachomatis, sometimes developed some symptoms. The other sub-population consists of those who are not infected at the time of study but were infected before it, or who are infected mildly, and did not have any symptoms. In this study, three pregnant women had extremely high titer. Although the significance of a serum titer of IgA and IgG in terms of activity of C. trachomatis infection is controversial, some studies showed that a person who had high titer of serum antibodies for C. trachomatis was more likely to have some symptoms (30, 34). Therefore, those three high-titer pregnant women may have been infected actively with C. trachomatis.

Recently, declining trends in the seroprevalence of C. trachomatis antibodies among commercial sex workers, attendants of family planning clinics and college students have been reported in many countries (27, 35, 36, 37, 38, 39, 40). However, there have been few surveys about the trend in C. trachomatis infection in Japan except for the data from sentinel surveillance conducted by the Ministry of Health. The data, however, from sentinel surveillance for C. trachomatis infection is not sensitive to discuss the trend in C.

trachomatis infection because there were some weak points on the methodology such as the techniques used for the screening and confirmation of C. trachomatis infection which were not unified, and the fact that the data were collected from gynecological and urogenital clinics that were not representative of the general population. In this early study, trends in seroprevalence of C. trachomatis antibodies among pregnant women were investigated in 1987, 1992 and 1996-7 by using the same laboratory method. The positive rates of C. trachomatis antibodies were the highest in 1987, medium in 1992 and the lowest in 1996-7 in all the groups excluding pregnant women aged 20-24 (Table 2 and Appendix 2). However, in the 20-24 age group, the positive rates of C. trachomatis antibodies in 1997 were at almost the same levels as those in 1992 and lower than those in 1987 (Table 2 and Appendix 2). Therefore, it might be possible to think that the positive rates of C. trachomatis have decreased year by year. Furthermore, from this study on the seroconversion of C. trachomatis antibodies, it might be possible to think that the new infection of C. trachomatis decreased year by year. The result from this study have also demonstrated that there has been a declining trend in C. trachomatis infection among pregnant women in Japan. This result has greater validity and reliability

because the same laboratory method was used and the stocked sera collected in 1987 and 1992 were randomly chosen.

During the last ten years, concern over HIV/AIDS has risen. AIDS prevention programs have been carried out since the late 1980s in Japan. There is a debate on whether AIDS prevention programs change people's behavior and reduce HIV incidence and other STDs. However, some studies have showed that AIDS prevention programs are effective in reducing HIV incidence and other STDs (41, 42, 43, 44, 45). Therefore, it is possible to speculate that the declining trend in the prevalence of C. trachomatis might be related to changes in their sexual behavior, owing to fear of HIV infection.

There are several reports showing that young pregnant women are more likely to be positive for C. trachomatis antibodies and that the seroprevalence of C. trachomatis antibodies declines conversely with the age of pregnant women (25, 26, 46). In this study, the seroprevalence of C. trachomatis antibodies also decreased conversely with the age of women in all years investigated, except for the age group of pregnant women over 40 in 1987 and 1992 (Table 2 and Appendix 2). Findings from this study showed that the seroprevalences of C. trachomatis in pregnant women over 40 was 32.0% in 1987 and 29.3% in 1992, and they were at the same level as

those for the 20-24 age group (Table 2 and Appendix 2). However, in 1996-7, the seroprevalence of C. trachomatis antibodies in pregnant women over 40 was 19.5% (25/128) which was the lowest of all age groups (Table 2 and Appendix 2). It is unknown what has caused this change in the seroprevalence of C. trachomatis antibodies in pregnant women over 40 because no behavioral survey for pregnant women was performed in 1987 and 1992. Nor has there been any study focusing on the fact that older pregnant women could be a possible high risk population. Therefore, there are no grounds to discuss whether pregnant women over 40 are a high risk population or not. On the other hand, the questionnaire survey showed that the length of education and experience of induced abortion had the strongest association with seropositivity for C. trachomatis (Table 5). Furthermore, although condom usage was not significantly correlated to the seropositivity of C. trachomatis, the seroprevalence in the population who reported to have never experienced condom use was higher than that of the population who reported to have experienced condom use (Table 5). In addition, the results from this questionnaire survey showed that the rate of respondents over 40 who reported to have not experienced condom use and to have experienced induced abortion were the highest of all,

and the length of education among them was the shortest, except for the age group under 19 (Table 4). Therefore, these results suggest that older pregnant women might have a high risk of C. trachomatis infection and more attention should be given to this particular age group.

The seroprevalence of C. trachomatis antibodies in each age group of the questionnaire respondents was somewhat different from that of the screening population for this study in 1996-7. In the screening population in 1996-7, the seroprevalence of C. trachomatis antibodies in pregnant women under the age of 19 was the highest (Table 2). However, as far as the respondents were concerned, the positive rate of C. trachomatis antibodies in pregnant women under 19 was at the same level as that of pregnant women aged 25-29 and lower than that of pregnant women aged 20-24 (Table 5). The positive rate of C. trachomatis in the respondents might be underestimated because the high risk population under the age of 19 may have avoided answering the questionnaires. However, the rest of the age groups had the same trend in C. trachomatis seropositivity as the screening population and the composition of the two populations was not significantly different ($p=0.143$) (Table 1). Therefore, from this questionnaire, it is reasonable to discuss

possible risks for C. trachomatis infection in pregnant women.

The risk factors for Chlamydia trachomatis infection among pregnant women.

C. trachomatis infection in pregnant women is of great concern because mother-to-child transmission of C. trachomatis happens between 30% to 70% (47, 48) and this leads to infant inclusion conjunctivitis in 18% to 50% and infantile pneumoniae in 8% to 20% (48, 49). In addition, C. trachomatis leads to late infertility and ectopic pregnancy for women with the infection(4, 5, 6, 7). Some studies on cost-effectiveness of a C. trachomatis screening program found that a screening program was cost-effective even in low prevalence area (50, 51). Furthermore, prenatal treatment for expectant mothers is much more effective than treatment for a neonate with C. trachomatis infection, in terms of ease in eradicating pathogen (52).

In Nagasaki prefecture, STD prevention study programs started in June 1996, from the mother to child health view point. The programs consisted of diagnosis, treatment and a research program. The diagnosis program included screening for C. trachomatis antibodies. The research program was to identify possible risk

factors for the seropositivity of C. trachomatis. In many countries, there has been several studies to identify the possible risk factors for C. trachomatis infection by using questionnaires and these found that major risk factors for acquiring C. trachomatis were a past history of other STDs, early sexual experience, use of oral contraceptives, multiple sexual partners, early marriage and previous induced abortion (29, 53, 54, 55, 56, 57). However, in Japan, there have not been many studies on the detailed risk factor analysis for C. trachomatis infection. The questionnaire was useful in identifying the possible risk factors. Therefore, as a research method, a self-administered questionnaire was used. In this study, lower levels of education, early pregnancy and previous induced abortion were strongly associated with the seroprevalence of C. trachomatis antibodies ($p<0.0001$). The age at first marriage and the age at first induced abortion were also associated with the seroprevalence of C. trachomatis antibodies ($p=0.043$ and $p=0.037$, respectively).

A noteworthy finding in this study was that pregnant women with lower level of education and with experience of induced abortion were possible high risk populations. In particular, in terms of the relationship between STDs and the level of education, there

has been few studies in Japan, probably due to the fear of giving rise to the stigmatization. However, from the view point of public health, it is important to make clear the relationship between the level of education and STDs because there is a survey conducted by some researchers but not published yet, showing that most Japanese have sexual intercourse only with partner with the same level of education (personal communication).

On the other hand, the proportion of pregnant women with experience of induced abortion was 14.5% and it was somewhat lower than that of previous report in Japan (58, 59). As for the association between induced abortion and C. trachomatis infection, there is a suggestion that the infection is activated at surgery and introduced to the immunologic system through damaged tissues (29). However, sexual behavior associated with induced abortion should not be neglected. Although it is unclear from this study whether pregnant women with experiences of induced abortion have a high sexual activity such as having multiple sexual partner and early sexual experience, some studies have found that the number of induced abortion experiences was associated with a high sexual activity (60, 61, 62, 63). Thus, the experience of induced abortion in pregnant women might reflect their high sexual activity in addition

to the influence of surgery. As a result, their seroprevalence for C. trachomatis antibodies might have been high.

The experience of condom use was not significantly associated with the seropositivity of C. trachomatis (p=0.344) (Table 5). Although there were several reports on the effectiveness of barrier contraceptives such as condoms in reducing transmission of HIV and other sexually transmitted diseases (64, 65, 66, 67, 68), the effectiveness of inappropriate condom use might be small. As far as oral contraceptives were concerned, there were reports that the population experienced pill use was small in Japan (58, 69). This study also showed that a small number of pregnant women reported to have used oral contraceptives (0.84%). Since in Japan, at present, a pill is not permitted as a contraceptive, but only as a medicine for the treatment of hormonal disorder in women, this small number was considered to reflect the present situation. However, recently, the stance of the government in terms of pill use as a contraceptive is shifting from forbidding it to allowing it, because women have a right to be provided with various contraceptive methods, and to choose their own contraceptive method. If oral contraceptives are allowed to be used, it is considered that the number of women who use it will increase. Therefore, more attention should also be given

to the relationship between the use of oral contraceptives and STDs.

In summary, high risk pregnant women with such factors as lower level of education, early marriage, early pregnancy, multiple induced abortions and first induced abortion at youth might be the target population for prevention program of STDs in Japan.

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Table 1. The number of participants of C. trachomatis screening and respondents of questionnaire

Age group	The No. of participants of screening (%)	The No. of respondents of questionnaire (%)
≪19	133 (1.4%)	19 (1.9%)
20-24	1,397 (14.5%)	172 (16.8%)
25-29	3,898 (40.4%)	435 (42.4%)
30-34	3,156 (32.7%)	305 (29.8%)
35-39	940 (9.7%)	84 (8.2%)
≫40	128 (1.3%)	10 (1.0%)
Total	9,652 (100%)	1,025 (100%)

The average age of participants who were screened for C. trachomatis infection was 28.9±4.3 (mean±SD). The average age of questionnaire respondents was 28.5±4.5. A t-test for the average age of the two populations was performed and the p value was 0.928. To compare the composition of the two populations, A p value was calculated by using chi square test and it was 0.143. The composition of two populations was not significantly different.

Table 2. The prevalence for Chlamydia trachomatis antibodies among pregnant women.

Age group		1987	1992	1996-97
≪19	Sample	23	17	133
	IgA (+)	9 (39.1%)	3 (17.6%)	36 (27.1%)
	IgG (+)	10 (43.5%)	7 (41.2%)	41 (30.8%)
	(+) judgment*	11 (47.8%)	7 (41.2%)	44 (33.1%)
20-24	Sample	68	59	1,397
	IgA (+)	12 (17.6%)	9 (15.3%)	351 (25.1%)
	IgG (+)	20 (29.4%)	16 (27.1%)	380 (27.2%)
	(+) judgment*	24 (35.3%)	18 (30.5%)	430 (30.8%)
25-29	Sample	54	66	3,898
	IgA (+)	7 (13.0%)	9 (13.6%)	743 (18.8%)
	IgG (+)	16 (29.6%)	15 (22.7%)	760 (19.5%)
	(+) judgment*	16 (29.6%)	17 (25.8%)	920 (23.6%)
30-34	Sample	54	62	3,156
	IgA (+)	6 (11.1%)	10 (16.1%)	524 (16.6%)
	IgG (+)	15 (27.8%)	11 (21.2%)	566 (17.9%)
	(+) judgment*	15 (27.8%)	15 (24.2%)	717 (22.7%)
35-39	Sample	52	52	940
	IgA (+)	9 (17.3%)	9 (17.3%)	157 (16.7%)
	IgG (+)	13 (25.0%)	11 (21.2%)	178 (18.9%)
	(+) judgment*	14 (26.9%)	13 (25.0%)	212 (22.6%)
≫40	Sample	24	41	128
	IgA (+)	3 (12.5%)	9 (22.0%)	18 (14.1%)
	IgG (+)	6 (25.0%)	11 (26.8%)	23 (18.0%)
	(+) judgment*	8 (33.3%)	12 (29.3%)	25 (19.5%)

* When both or one of IgA and IgG were positive, blood samples were judged as antibody-positive.

Table 3. The seroconversion of C. trachomatis antibodies among pregnant women.

Sample No.	0	1	2	3	4	5	6	7	8	9	10
1	(A/G)		(G)		(−)						
2	(G)	(−)							(−)		
3	(A/G)	(−) *				(−)		(−)			
4	(A/G)			(A/G)	(A/G)						
5	(A/G)				(−)			(−)			
6	(G)					(−)		(−)			
7	(A)	(−)			(−)						
8	(A/G)		(A/G)			(A/G)					(A/G)
9	(A/G)		(−)		(G)						
10	(A/G)			(G)					(−)		
11	(A)			(−)				(−)			
12	(A/G)			(−)		(−)					
13	(G)		(−)				(−)				
14	(A)	(−)	(−)		(−)						
15	(A/G)		(−)			(−)					
16	(A/G)		(−)	(−)			(−)				
17	(A/G)			(A/G)			(A/G)				
18	(A/G)		(A/G)					(A/G)			
19	(A)				(−)		(−)				
20	(A/G)		(−)				(−)				
21	(A)		(G)			(−)					
22	(A)		(−)						(−)		
23	(A)	(−)			(−)						
24	(A)			(−)				(−)			
25	(A/G)		(A/G)					(A/G)			
26	(A/G)		(A/G)	(A/G)		(A/G)					
27	(A)	(−)		(−)							
28	(G)	(−)	(−)					(−)			
29	(A/G)		(−)		(−)						
30	(A/G)	(A/G)					(A/G)				
31	(A/G)					(A/G)		(A/G)			
32	(G)		(G)			(−)					
33	(A/G)		(A/G)		(A/G)						

1. (A/G): both IgA and IgG were positive. (A): IgA only was positive. (G): IgG only was positive.

* Blood were collected at the same year. The duration of period collected two sample was four months.

Table 4. The demographic information related to sexual and reproductive factors in respondents of questionnaire.

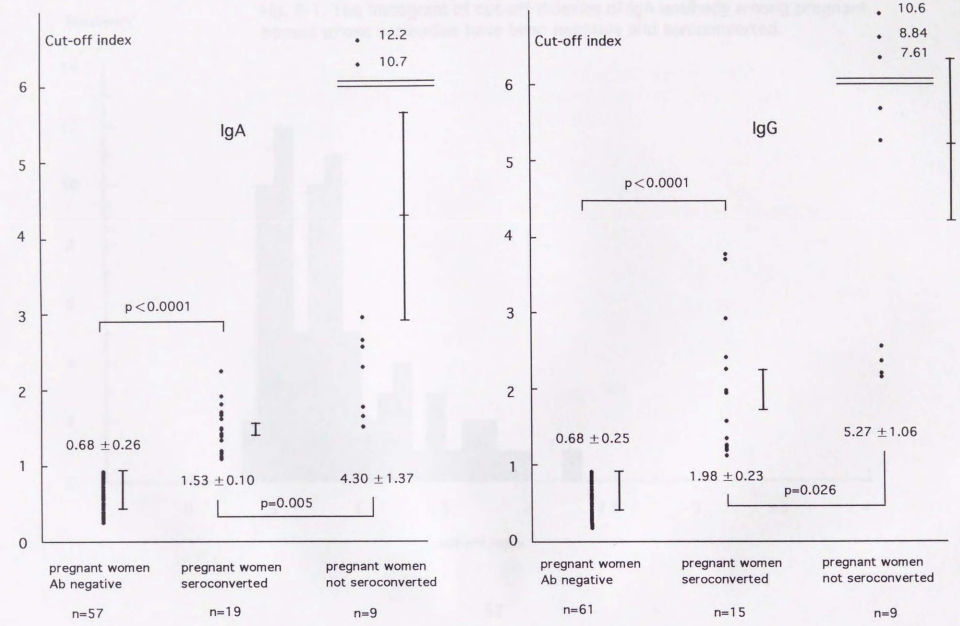
Age group	The No. of respondents	Age at first marriage	Age at first pregnancy	Pregnancy frequency	The No. of births	School carreer	Induced abortion (%) have an experience	Condom use Never use (%)
≪19	19 (1.9%)	18.5±0.25	16.5±0.50	0.28±0.14	0.11±0.08	10.9±0.36	15.8%	31.6%
20-24	172 (16.8%)	21.6±0.17	20.0±0.21	0.58±0.06	0.29±0.04	12.3±0.10	12.2%	26.5%
25-29	435 (42.4%)	25.0±0.10	23.9±0.17	0.95±0.05	0.59±0.03	13.0±0.07	15.2%	20.7%
30-34	305 (29.8%)	27.0±0.17	26.8±0.19	1.60±0.07	1.09±0.05	13.2±0.09	13.4%	21.3%
35-39	84 (8.2%)	29.4±0.50	29.6±0.50	2.00±0.14	1.46±0.10	13.3±0.15	13.1%	30.0%
≫40	10 (1.0%)	30.7±2.83	29.7±2.43	2.71±0.55	1.44±0.41	12.1±0.43	30.0%	44.4%

This result showed that mean ± standard error (SE)

Table 5. The factors associated with Chlamydia trachomatis antibodies positive rate in respondent questionnaire.

Factor	The No. of respondents	Positive rate (%)	Crude odds ratio (95% CI)	P value
Age group				
≪19	19	21.1%	1.00 –	
20-24	172	31.4%	1.72 (0.55-5.43)	
25-29	435	19.3%	0.90 (0.29-2.78)	
30-34	305	17.7%	0.81 (0.26-2.54)	p=0.043
35-39	84	14.3%	0.63 (0.18-2.22)	
≫40	10	10.0%	0.42 (0.04-4.37)	
Occupation				
Housewife	548	21.9%	1.00 –	
Part time job	115	25.2%	1.20 (0.75-1.91)	
Full time job	258	14.3%	0.60 (0.40-0.90)	p=0.821
Business on her own	48	20.8%	0.94 (0.46-1.94)	
Others	39	20.5%	0.92 (0.41-2.05)	
School carreer				
Junior high school	35	40.0%	1.00 –	
High school	530	26.0%	0.53 (0.26-1.07)	
Vocational school	170	14.7%	0.26 (0.12-0.58)	p<0.0001
College or University	270	11.1%	0.18 (0.08-0.39)	
Age at first marriage				
≪19	35	34.3%	1.00 –	
20-24	324	22.5%	0.56 (0.27-1.18)	
25-29	456	19.3%	0.46 (0.22-0.96)	
30-34	85	11.8%	0.26 (0.10-0.68)	p=0.043
35-39	13	7.7%	0.16 (0.02-1.38)	
≫40	3	0.0%	–	
Age at first pregnancy				
≪19	56	44.6%	1.00 –	
20-24	203	21.7%	0.34 (0.18-0.63)	
25-29	307	17.9%	0.27 (0.15-0.49)	
30-34	69	8.7%	0.12 (0.04-0.32)	p<0.0001
35-39	10	0.0%	–	
≫40	0	–	–	
Age at first induced abortion				
≪19	40	47.5%	1.00 –	
20-24	62	33.9%	0.57 (0.25-1.29)	
25-29	20	10.0%	0.12 (0.02-0.59)	
30-34	11	18.2%	0.25 (0.05-1.31)	p=0.037
35-39	1	0.0%	–	
≫40	0	–	–	
Pregnancy frequency				
1	336	18.5%	1.00 –	
2	360	19.7%	1.09 (0.75-1.59)	p=0.236
≫3	313	23.6%	1.37 (0.94-2.00)	
The No. of births				
0	447	21.3%	1.00 –	
1	381	19.7%	0.91 (0.65-1.28)	
2	146	19.9%	0.92 (0.58-1.47)	p=0.951
≫3	29	20.7%	0.97 (0.38-2.45)	
The No. of Induced abortions				
0	857	17.9%	1.00 –	
1	105	32.4%	2.20 (1.41-3.43)	p<0.0001
≫2	41	46.3%	3.97 (2.10-7.52)	
Condom use				
never	235	22.6%	1.00 –	
have a experience	781	19.7%	0.84 (0.59-1.20)	p=0.344

Fig. 1. The titer of cut-off index of IgA and IgG antibodies



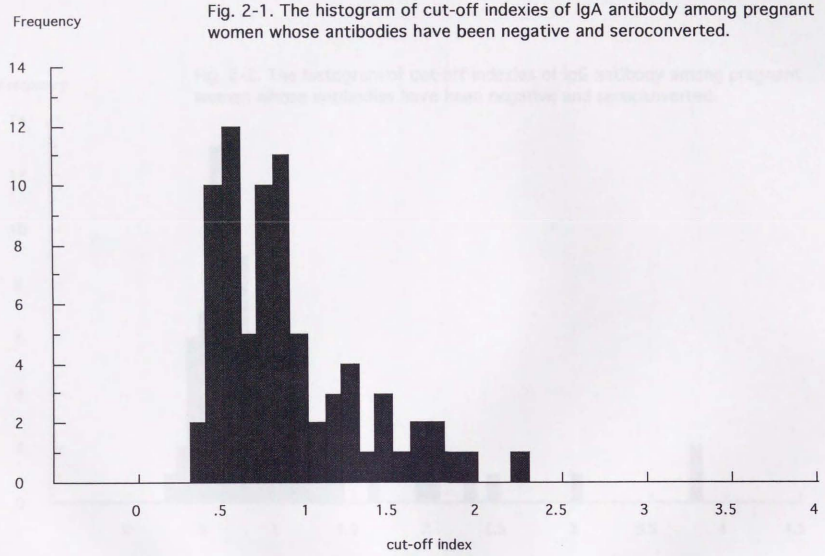


Fig. 2-1. The histogram of cut-off indexies of IgA antibody among pregnant women whose antibodies have been negative and seroconverted.

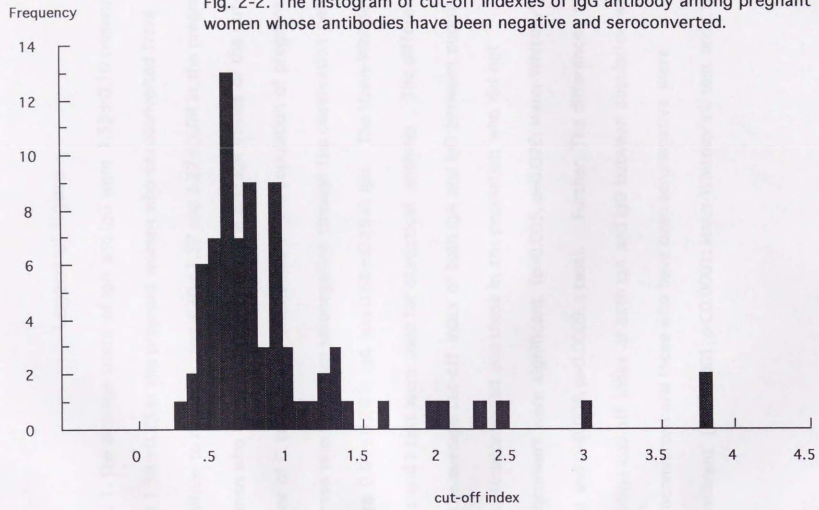


Fig. 2-2. The histogram of cut-off indexies of IgG antibody among pregnant women whose antibodies have been negative and seroconverted.

Legend of figure

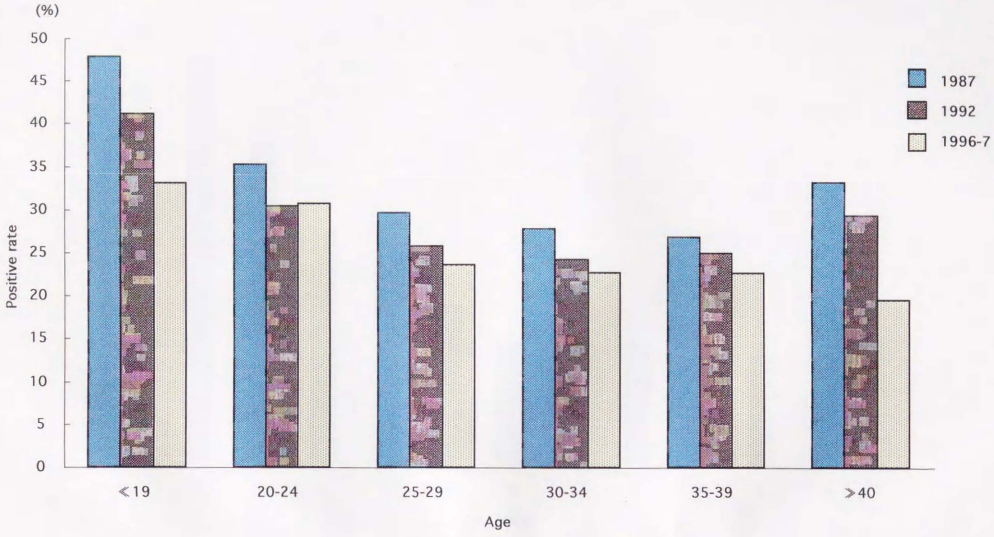
Fig. 1. The average titers of IgA and IgG were 1.53 ± 0.10 (mean $\pm$ SE) and 1.98 ± 0.23 in the pregnant women who seroconverted from positive to negative, and 4.30 ± 1.37 and 5.27 ± 1.06 in the pregnant women who did not seroconvert. The average values of the cut-off index of C. trachomatis antibodies in the population of pregnant women who have been seronegative through the observation period were 0.68 ± 0.26 in IgA and 0.68 ± 0.25 in IgG. The Mann-Whitney's U test and t test were used for statistical analysis. The differences of the average cut-off index of both IgA and IgG between population who seroconverted and those in the population who did not seroconvert were significant ($p=0.005$, $p=0.026$: Mann-Whitney's U test and $p=0.006$, $p=0.0009$: t test). Further, The differences of the average cut-off index of both IgA and IgG between population who seroconverted and those who have been seronagative were significant ($p<0.0001$, $p<0.0001$: Mann-Whitney's U test and t test).

Appendix 1. The number of births in Nagasaki prefecture from 1987 to 1995.

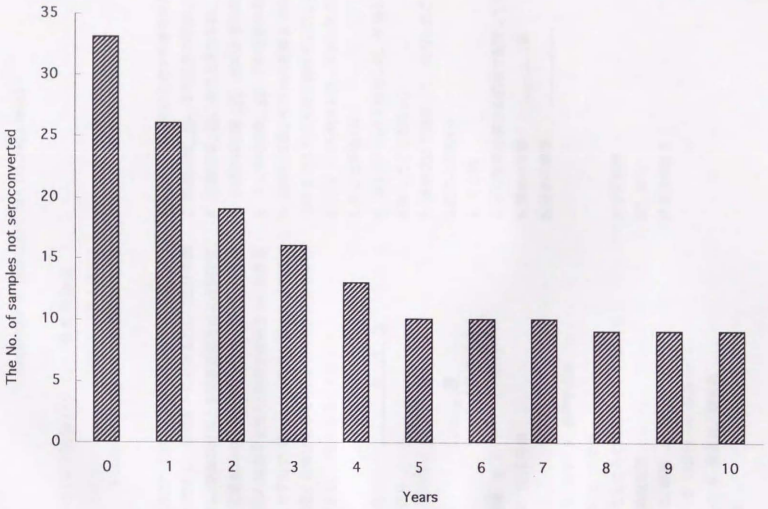
Year	1987	1988	1989	1990	1991	1992	1993	1994	1995
The No. of births	18,962	18,233	17,256	16,517	16,667	16,036	15,769	15,952	14,780

The average number of births in Nagasaki prefecture from 1993 to 1995 was 15,500±650 (mean±SD) and showing a decline in the number of births from 1987 to 1995.

Appendix 2. The seroprevalence for C. trachomatis antibodies among pregnant women.



Appendix 3. The seroconversion of C. trachomatis antibodies among pregnant women.



Appendix 4. Questionnaire (in Japanese)

妊婦ｸﾗﾐｼﾞｱ抗体ｽｸﾘｰﾆﾝｸﾞ事業問診表

長崎県STD防止研究会

日本母性保護産婦人科医協会長崎県支部

医師の先生方へ：以下の質問は妊婦ｸﾗﾐｼﾞｱ抗体ｽｸﾘｰﾆﾝｸﾞ結果を集計し、長崎県下におけるｸﾗﾐｼﾞｱ流行の現状を把握し、将来の対策と事業評価のために必要です。集団としての傾向のみを分析するためﾌﾟﾗｲﾊﾞｼｰｶが侵害される恐れはありません。回答は妊婦さんの自由意思によりますので、答えにくい質問の場合には無理に回答いただく必要はありません。その点を妊婦さんに説明した上で、回答を求めてください。

採血年月日　　年　月　日

医療機関番号　＿＿＿＿＿＿

年齢　＿＿＿歳

妊娠週数　＿＿＿週

職業

1. 専業主婦　2. ﾊﾟｰﾄﾀｲﾑ　3. ﾌﾙﾀｲﾑ

4. 自営業　5. その他

教育歴

1. 中卒　2. 高卒　3. 専門学校卒

4. 短大卒　5. 大学卒

（卒業したところに丸をつけてください）

性感染症の既往歴

1. 梅毒　2. 淋病　3. ｸﾗﾐｼﾞｱ

4. ｶﾝｼﾞﾀ　5. ﾄﾘｺﾓﾅｽ　6. 陰部ﾍﾙﾍﾟｽ

7. ﾊﾟﾋﾟﾛｰﾏ　8. 尿道炎／膀胱炎

9. その他

結婚歴

1. 未婚

2. 既婚（初婚年齢＿歳、結婚回数＿回、結婚期間＿年）

初潮年齢　＿＿＿歳

妊娠歴（回数とそのときの年齢をお答えください）

1. 妊娠回数＿回、そのときの年齢＿歳＿歳＿歳

2. 出産回数＿回、そのときの年齢＿歳＿歳＿歳

3. 自然流産回数＿回、そのときの年齢＿歳＿歳＿歳

4. 人工中絶回数＿回、そのときの年齢＿歳＿歳＿歳

5. 前回の妊娠から今回の妊娠までの期間＿年＿ヵ月

これまで以下の方法で避妊をしたことがありますか

1. ピル（よく使用した、使用したことがある、使用したことがない）

2. コンドームよく使用した、使用したことがある、使用したことがない）

3. 荻野式よく使用した、使用したことがある、使用したことがない）

4. その他

以下の質問は聞ける範囲で質問してください

配偶者の年齢　＿＿＿歳

配偶者の職業　＿＿＿＿＿

大学記載欄

通し番号：

抗体検査結果：

Appendix 5. Questionnaire (in English)

Questionnaire to Pregnant Women for C. trachoma's Screening Project

Nagasaki STD Prevention Study Group

Nagasaki blanch of Japanese gyneco-obsterical association

To Physicians: This questionnaire below, having understood the current trend of C. trachomatis from analysis on the results of the screening of pregnant women for C. trachomatis antibody, is needed to think up its future policies and to evaluate its projects. The information obtained through this questionnaire will remain confidential and respondents' privacy will be protected since the aim of its analysis is to understand the trend as a group. Answering to any questions is totally up to the respondents' will so that they may not be forced to answer whichever question they are not comfortable with. Please explain clearly that point to the respondents. After filling the questionnaire, please send it along with sample.

① Date(blood drawing) YR/MO/DY: 19_____ _____mon. _____day

② Health Facility ID#

③ Name of Pregnant Women Age

④ Weeks of Pregnancy

⑤ Occupation

1. Housewife 2. Part-time
3. Full-time 4. Self-employed 5. Other

⑥ Education

1. Junior High graduate 2. High School graduate
3. Occupational/Technical school graduate
4. Two-year college graduate
5. Four-year college/university graduate

(high school drop-out is classified as junior high graduate, etc.)

⑦ History of STD (Circle where applicable)

1. Syphilis 2. Gonorrhea 3. C. trachomatis 4. Candida
5. Trichomoniasis 6. Genital herpes 7. Papiloma
8. Uritis How many times? _____
9. other:_____

⑧ Marital status

1. Single 2. Engaged
3. Married(age at first marriage:___y.o; number of marriage:___times; duration of marriage:_____yrs)

⑨ Age at first menstruation: _____y.o

⑩ History of pregnancy(please write frequency and age of each pregnancy)

1. Frequency of pregnancy _____times Age at each pregnancy_____
2. Frequency of birth_____times Age at each birth_____
3. Frequency of natural abortion_____times
   Age at each natural abortion_____
4. Frequency of induced abortion_____times
   Age at each induced abortion_____
5. Duration between the last pregnancy and the present:
   _____YR_____MO

⑪ Have you ever tried the following contraceptive methods?(Please circle where applicable)

1. Pills(a lot, a few times, never)
2. Condoms(a lot, a few times, never)
3. Rhythmic method(a lot, a few times, never)
4. Other: Please specify_____(a lot, a few times, never)

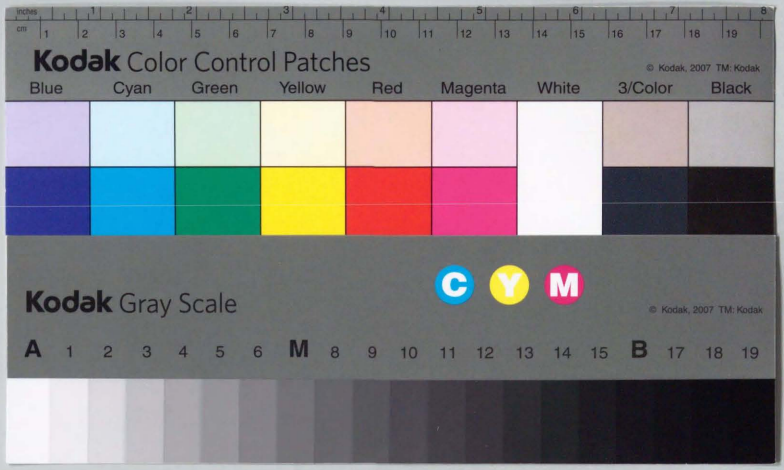
⑫ Ask the following questions as far as the respondent is willing to answer

1. Age of spouse:_____y.o.
2. Occupation of spouse:_____

Legend of appendixes

Appendix 2. The blue box, the red box and the yellow box meant the seroprevalence of C. trachomatis antibodies among pregnant women who were collected blood samples in 1987, 1992 and 1996-7, respectively.

Appendix 3. This figure showed how many antibody-positive samples remained positive during the observation periods.


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19