

RUJUKAN

PERPUSTAKAAN KAMPUS KESIHATAN
UNIVERSITI SAINS MALAYSIA



BUKU LAPORAN AKHIR GERAN USM JANGKA PENDEK

DR. SHAH REZA JOHAN NOOR

**“ A STUDY OF CHLAMYDIAL INFECTION IN
PREGNANCY IN HOSPITAL KOTA BHARU,
KELANTAN ”**



Malaysian Journal
of
OBSTETRICS
&
GYNÆCOLOGY



*Official Journal of the
Obstetrical & Gynaecological Society
of Malaysia.*

Penyelidikan
PUSAT PENGAJIAN SAINS PERUBATAN

Supplementary
VOL. 8 No. 4
June 2003

☐ Penyelidikan, PPSP
☒ Perpustakaan Perubatan, USMKK
☐ RCMO

T/Tamr : *[Signature]* Tarikh : 9/10/05

KDN PP 10619/3/2004
ISSN 0128-6013

OBSTETRIC SECTION - CLINICAL STUDY

P OBS 10

A STUDY ON CHLAMYDIA TRACHOMATIS INFECTION IN PREGNANCY IN HOSPITAL KOTA BHARU

K Seman¹, SRJ Noor¹, NAA Ghaffar¹, WZW Yusuff², ZA Hanafiah², N Maning³

¹O&G Dept, HUSM; ²O&G Dept, Hospital Kota Bharu; ³Microbiology Dept, Hospital Kota Bharu, Kelantan, Malaysia.

Objectives: (1) To determine the prevalence of genital *Chlamydia trachomatis* infection in pregnancy among the attendees of Antenatal Clinic of Hospital Kota Bharu (HKB), Kelantan; (2) To determine the clearance rate of chlamydial infection with one week course of Erythromycin Stearate 500mg six hourly.

Introduction: *Chlamydia trachomatis* infection is the most common sexually transmitted disease worldwide. In pregnancy, it has been associated with morbidity for both the mother and the newborn.

Methodology: Prospective cross sectional study. 440 pregnant women (age range 17 to 48 years old), in the first to third trimester, attending the antenatal clinic of Hospital Kota Bharu, Kelantan were tested for *Chlamydia trachomatis*. Endocervical swabs were collected for *Chlamydia trachomatis* diagnosis by Direct Fluorescence antibody (DFA) technique. The women were followed up as routine and were advised to deliver in HKB.

Results: Out of 440 pregnant women tested, 96% were Malays and four were tested positive for *Chlamydia trachomatis*, giving the prevalence rate of 0.9%. There were no statistically differences in the gravidity, parity, socioeconomic status, educational level, history of prematurity or stillbirth or abortion, between the positive and negative group. All of the four women with positive results had no symptoms and they were retested after treatment and all of them were cleared. However, small number of patients precludes further statistical analysis.

Conclusion: Due to low prevalence of genital *Chlamydia trachomatis* infection in antenatal population of HKB, universal screening is not necessary.

4) (a)

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

[illegible]

- (b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

<u>Bahasa Malaysia</u>	<u>Bahasa Inggeris</u>
WANITA MENGANDUNG	PREGNANT WOMEN
.....
UJIAN SARINGAN	SCREENING
.....
CHLAMYDIAL	CHLAMYDIAL
.....
ERYTHROMYCIN	ERYTHROMYCIN
.....
KEBERKESANAN KOS	COST EFFECTIVENESS
.....
.....
.....
.....
.....
.....

5) Output Dan Faedah Projek

- (a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbitkan).

PENYERTAAN 'PAPER PRESENTATION' DI PERJUMPAAN PERSATUAN OBSTETRIKS DAN GINEKOLOGI MALAYSIA' DI KOTA KINABALU, SABAH PADA TAHUN 2003

- (b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.**
(Jika ada dan jika perlu, sila guna kertas berasingan)

.....

.....

.....

.....

.....

.....

.....

.....

- (c) **Latihan Gunatenaga Manusia**
DR.KHAIRIAH SEMAN – pelajar ijazah klinikal

i) **Pelajar Siswazah:**
Lanjutan perubatan perbidanan dan sakit puan.
.....

ii) **Pelajar Prasiswazah:**
.....

iii) **Lain-Lain :**
.....

6. Peralatan Yang Telah Dibeli:

UJIAN PENGESAN SARINGAN UNTUK CHLAMYDIAL ie DIRECT FLUORESCENCE
ANTIGEN DETECTION TECHNIQUE

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

Pengarah Penyelidikan

T/TANGAN PENERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

27/5
PROFESSOR ABUL AZIZ BABA
Chairman of Research & Ethics Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan
USM J/P-06 - 5

Abstrak

Jangkitan *Chlamydia trachomatis* adalah jangkitan bacteria yang paling kerap dan merupakan penyakit kelamin paling tinggi kadarnya di dunia pada masa kini. Pertubuhan Kesihatan Sedunia (WHO) menjangkakan sebanyak 89 juta kes baru bagi jangkitan chlamydia berlaku pada tahun 1995 menunjukkan kadar jangkitan ini yang meningkat. Untuk wanita mengandung, jangkitan chlamydia ini memberi kesan ke atas kesihatan ibu dan bayi yang dikandung.

Objektif: Pertama; untuk mengetahui kadar jangkitan kuman *Chlamydia trachomatis* di kalangan wanita mengandung yang datang ke klinik ibu mengandung di Hospital Kota Bharu, Kelantan. Kedua; untuk mengetahui kadar keberkesanan ubat erythromycin stearate 500mg setiap enam jam yang diberi selama satu minggu dalam mengubati jangkitan ini.

Kaedah: Seramai 440 wanita mengandung (berusia di antara 17 dan 48 tahun), dari trimester satu hingga trimester tiga, yang menghadiri klinik ibu mengandung di Hospital Kota Bharu Kelantan, disaring untuk jangkitan kuman *Chlamydia trachomatis*. Sapuan dari endocervik diambil untuk diuji menggunakan teknik Direct Fluorescent Antigen.

Keputusan: Daripada 440 wanita mengandung yang diuji, seramai empat orang disahkan mengalami jangkitan ini, memberikan kadar jangkitan sebanyak 0.9%. Perbandingan statistik tidak dapat dilakukan di antara mereka yang positif dan negatif dari segi umur, bilangan kandungan, bilangan anak, status sosioekonomi, tahap persekolahan, dan juga sejarah kelahiran pramatang atau keguguran, disebabkan bilangan mereka yang positif

adalah kecil. Semua empat wanita yang dijangkiti kuman ini tidak mempunyai sebarang simptom, mereka diberi rawatan dan ujian selepas rawatan menunjukkan mereka sembuh sepenuhnya. Walaubagaimanapun, bilangan mereka yang sedikit menghalang analisis statistik seterusnya.

Konklusi: Disebabkan kadar jangkitan *C. trachomatis* di kalangan wanita mengandung di Hospital Kota Bharu rendah, maka saringan universal adalah tidak kos-efektif, dan ujian diagnostik berdasarkan simptom klinikal adalah tidak mencukupi.

ABSTRACTS

Chlamydia trachomatis infection is the most common curable bacterial infection, and is now the most common sexually transmitted disease worldwide. World Health Organisation figures estimated that 89 million new cases of genital chlamydial infections occurred in 1995, highlighting the worldwide prevalence of infections and the economic burden on healthcare delivery. For pregnant women, this infection has been associated with morbidity for both the mother and the newborn.

Objectives: This study was undertaken with the objectives to determine the prevalence of genital *Chlamydia trachomatis* infection in pregnancy among the attendees of Antenatal Clinic of Hospital Kota Bharu, Kelantan so as to ascertain whether universal screening is needed or not. The other objective is to observe the effectiveness of treatment of chlamydial infection with one-week course of erythromycin stearate 500mg qid.

Methodology: 440 pregnant women (age range 17 to 48 years old), in the first to third trimester, attending the antenatal clinic of Hospital Kota Bharu, Kelantan were tested for *Chlamydia trachomatis*. Endocervical swabs were collected for *Chlamydia trachomatis* diagnosis by Direct Fluorescence antigen detection (DFA) technique.

Results: Out of 440 tested women, four were positive, giving the prevalence rate of 0.9%. No valid statistical analysis can be made with regards to the age, gravidity, parity, socioeconomic status, education level, history of prematurity or stillbirth or abortion

between the positive and negative group because of the small number of women with positive results. All the four women with positive results were retested after treatment and all of them were cleared. However, small number of patients again, precludes further statistical analysis.

Conclusion: As the prevalence rate of genital *Chlamydia trachomatis* infection in antenatal population of Hospital Kota Bharu is very low, there is no need for universal screening to detect this infection. All of the women with positive chlamydial tests were asymptomatic. Therefore diagnostic testing based on clinical findings is unreliable.

Table Of Content

Page

1. ACKNOWLEDGEMENT	ii
2. LIST OF TABLES	v
3. LIST OF FIGURES	vi
4. LIST OF ABBREVIATION	vii
5. LIST OF APPENDICES	ix
6. ABSTRACT – MALAY VERSION	x
- ENGLISH VERSION	xii
7. INTRODUCTION	1
A. MICROBIOLOGY OF CHLAMYDIA	4
B. PREVALENCE AND CLINICAL ASPECTS OF CHLAMYDIAL INFECTION	5
C. TREATMENT	9
D. DIAGNOSTIC TESTS	11
8. OBJECTIVES	14
9. METHODOLOGY	
i. STUDY POPULATION	15
ii. SAMPLE COLLECTION	16
iii. ANTIGEN DETECTION	17
iv. STATISTICAL ANALYSIS	19
10. RESULTS	20

11. DISCUSSION

i. Prevalence of Chlamydia trachomatis infection	43
ii. Prevalence of chlamydial infection with regards to age and other demographic data	47
iii. Pregnancy outcome	49
iv. Treatment of genital chlamydial infection	51

12. CONCLUSION

13. LIMITATIONS

14. RECOMMENDATION

15. APPENDICES

i. FLOW CHART	57
---------------	----

ii. RECRUITMENT SHEET	58
-----------------------	----

iii. SAMPLE COLLECTION	59
------------------------	----

iv. LABORATORY TECHNIQUE	60
--------------------------	----

v. INFORMATION SHEET	62
----------------------	----

16. REFERENCES

List of Tables

Table 1. The age distribution of the study population	21
Table 2. The result of chlamydial test in pregnant women in HKB	22
Table 2.1. Age range and chlamydial test results	23
Table 3. Effectiveness of 1-week course of erythromycin stearate for treatment of genital chlamydial infection	24
Table 4. Ethnic group distribution	25
Table 5. Distribution of patients in relation to district of residency	27
Table 6. Distribution of patients in relation to educational level	29
Table 7. Distribution of patients in terms of gravidity and parity	31
Table 8. Distribution of patients with regards to history of abortion	35
Table 9. Distribution of patients in terms of history of premature delivery	36
Table 10. Distribution of patients with regards to history of Stillbirth	37
Table 11. Distribution of patients with regards to gestation at delivery (weeks)	39
Table 12. Distribution of patients with regards to babies' birth weight	41
Table 13. Distribution of birth complication	42

List of Figures

Figure 1: The distribution of chlamydial test results in relation to ethnic group	26
Figure 2: Distribution of patients in relation to district of residency	28
Figure 3: Distribution of patients in relation to educational level	30
Figure 4: Distribution of patients with regards to gravidity	32
Figure 5: Distribution of patients in terms of parity	33
Figure 6: Distribution of patients in terms of symptoms	34
Figure 7: Distribution of patients with regards to gestation at screening	38
Figure 8: Distribution of patients with regards to gestation at delivery	40

List of abbreviation

CDC	Centres for Disease Control
DFA	Direct Fluorescent Antibody
DNA	Deoxyribonucleic Acid
EB	Elementary Body
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked ImmunoSorbent Assay
ENND	Early Neonatal Death
IFA	Immunofluorescent Assay
LMP	Last menstrual period
LNND	Late Neonatal Death
LSCS	Lower Segment Caesarean Section
mg	milligram
MSB	Macerated stillbirth
NICU	Neonatal Intensive Care Unit
PBS	Phosphate Buffer Solution
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Diseases
PNMR	Perinatal Mortality Rate
PPROM	Premature Prelabour Rupture of Membrane
PROM	Prelabour Rupture of Membrane
RB	Reticulate Body
SPSS	Statistical Packages for Social Sciences

STDs

Sexually Transmitted Diseases

STI

Sexually Transmitted Infections

WHO

World Health Organisation

List of appendices

Appendix 1: flow chart of the study	57
Appendix 2: recruitment sheet	58
Appendix 3: sample collection	59
Appendix 4: laboratory technique	60
Appendix 5: information sheet	62

ABSTRACTS

Abstrak

Jangkitan *Chlamydia trachomatis* adalah jangkitan bacteria yang paling kerap dan merupakan penyakit kelamin paling tinggi kadarnya di dunia pada masa kini. Pertubuhan Kesihatan Sedunia (WHO) menjangkakan sebanyak 89 juta kes baru bagi jangkitan chlamydia berlaku pada tahun 1995 menunjukkan kadar jangkitan ini yang meningkat. Untuk wanita mengandung, jangkitan chlamydia ini memberi kesan ke atas kesihatan ibu dan bayi yang dikandung.

(Objektif: Pertama; untuk mengetahui kadar jangkitan kuman *Chlamydia trachomatis* di kalangan wanita mengandung yang datang ke klinik ibu mengandung di Hospital Kota Bharu, Kelantan. Kedua; untuk mengetahui kadar keberkesanan ubat erythromycin stearate 500mg setiap enam jam yang diberi selama satu minggu dalam mengubati jangkitan ini

Kaedah: Seramai 440 wanita mengandung (berusia di antara 17 dan 48 tahun), dari trimester satu hingga trimester tiga, yang menghadiri klinik ibu mengandung di Hospital Kota Bharu Kelantan, disaring untuk jangkitan kuman *Chlamydia trachomatis*. Sapuan dari endocervik diambil untuk diuji menggunakan teknik Direct Fluorescent Antigen.

Keputusan: Daripada 440 wanita mengandung yang diuji, seramai empat orang disahkan mengalami jangkitan ini, memberikan kadar jangkitan sebanyak 0.9%. Perbandingan statistik tidak dapat dilakukan di antara mereka yang positif dan negatif dari segi umur, bilangan kandungan, bilangan anak, status sosioekonomi, tahap persekolahan, dan juga sejarah kelahiran pramatang atau keguguran, disebabkan bilangan mereka yang positif

adalah kecil. Semua empat wanita yang dijangkiti kuman ini tidak mempunyai sebarang simptom, mereka diberi rawatan dan ujian selepas rawatan menunjukkan mereka sembuh sepenuhnya. Walaubagaimanapun, bilangan mereka yang sedikit menghalang analisis statistik seterusnya.

Konklusi: Disebabkan kadar jangkitan *C. trachomatis* di kalangan wanita mengandung di Hospital Kota Bharu rendah, maka saringan universal adalah tidak kos-efektif, dan ujian diagnostik berdasarkan simptom klinikal adalah tidak mencukupi.

ABSTRACTS

Chlamydia trachomatis infection is the most common curable bacterial infection, and is now the most common sexually transmitted disease worldwide. World Health Organisation figures estimated that 89 million new cases of genital chlamydial infections occurred in 1995, highlighting the worldwide prevalence of infections and the economic burden on healthcare delivery. For pregnant women, this infection has been associated with morbidity for both the mother and the newborn.

Objectives: This study was undertaken with the objectives to determine the prevalence of genital *Chlamydia trachomatis* infection in pregnancy among the attendees of Antenatal Clinic of Hospital Kota Bharu, Kelantan so as to ascertain whether universal screening is needed or not. The other objective is to observe the effectiveness of treatment of chlamydial infection with one-week course of erythromycin stearate 500mg qid.

Methodology: 440 pregnant women (age range 17 to 48 years old), in the first to third trimester, attending the antenatal clinic of Hospital Kota Bharu, Kelantan were tested for *Chlamydia trachomatis*. Endocervical swabs were collected for *Chlamydia trachomatis* diagnosis by Direct Fluorescence antigen detection (DFA) technique.

Results: Out of 440 tested women, four were positive, giving the prevalence rate of 0.9%. No valid statistical analysis can be made with regards to the age, gravidity, parity, socioeconomic status, education level, history of prematurity or stillbirth or abortion

between the positive and negative group because of the small number of women with positive results. All the four women with positive results were retested after treatment and all of them were cleared. However, small number of patients again, precludes further statistical analysis.

Conclusion: As the prevalence rate of genital *Chlamydia trachomatis* infection in antenatal population of Hospital Kota Bharu is very low, there is no need for universal screening to detect this infection. All of the women with positive chlamydial tests were asymptomatic. Therefore diagnostic testing based on clinical findings is unreliable.

INTRODUCTION

INTRODUCTION OF THE STUDY

Chlamydia trachomatis infection is the most common curable bacterial infection, and is now the most common sexually transmitted disease worldwide (Macmillan *et al*, 2000). World Health Organisation figures estimated that 89 million new cases of genital chlamydial infections occurred in 1995, highlighting the worldwide prevalence of infections and the economic burden on healthcare delivery (Williamson and Wyandt, 2000). For pregnant women, this infection has been associated with morbidity for both the mother and the newborn. In United Kingdom, the prevalence rate was about ten percent for chlamydia (Underhill *et al*, 2003). The rise in the incidence may reflect improved detection with new diagnostic techniques, but the role of changing sexual behaviour also important (Catchpole, 2001).

Antenatal and neonatal screening for chlamydial infection is part of a general campaign to prevent, or reduce the effect of maternal transmission of the microbial agents capable of infecting fetuses in utero or infants in the neonatal period. By identifying those at risks, it is possible to ensure that they received whatever remedial treatment available. The importance of screening programme in reducing the prevalence of genital chlamydial infection is stressed by the fact that majority of infected persons are more or less asymptomatic thus it is not dealt with by the traditional diagnostic medical approach. The result of the United Kingdom pilot of screening for genital chlamydial infection suggest that an opportunistic approach is acceptable to professionals and public and can achieve high population coverage (Chief Medical Officers' Advisory Group, 1998).

Evidence already exists that screening is effective in controlling genital chlamydial infection and in reducing the incidence of pelvic inflammatory disease. (Scholes *et al*, 1996; Kamwendo *et al*, 1998) Furthermore, few studies have shown that screening and intervention lead to better outcomes for some perinatal and postpartum complication (McMillan *et al* 1985; Schachter *et al* 1986; Ryan *et al* 1990; Rastogi *et al*, 2003).

By the end of year 1999, in Malaysia no detailed study or published data on the prevalence of *Chlamydia trachomatis* specifically in antenatal population has been done, hence this study. As we do not know what the real prevalence is as yet, it is hoped that this study will provide us with some raw data to help in answering questions like is there a need for a routine antenatal screening, the role of health education programme and to look for congenital infection in newborn.

Furthermore, each year in Hospital Kota Bharu, approximately ten percent of infants are born prematurely, forming up to twenty percent of Neonatal Intensive Care Unit admission and is a major contributor to perinatal mortality rate. In 1998, 80 percent of cases of Early Neonatal Death (ENND) were due to prematurity, congenital defects and sepsis, with the prematurity top in the list. In year 2000, prematurity was the cause for 26 out of fifty cases of ENND. Likewise, in 2001, with the total of 14 668 deliveries and Perinatal Mortality Rate at 25 per 1000 birth, prematurity ranked highest by causing 30 out of 70 cases of ENND and 2 out of 25 cases of Late Neonatal Death (Dr MHM Jamil, Paediatric Department, Hospital Kota Bharu; personal communication). This statistics emphasized that further evaluation as for causes of prematurity is needed.

Apart from the significant morbidity and mortality associated with prematurity, more importantly is for the humanitarian and economic aspects, it must be recognized that this long-term morbidity is potentially avoidable. Therefore, this study is also looking at the genital chlamydial infection as a possible cause of premature delivery in Hospital Kota Bharu.

A. MICROBIOLOGY OF CHLAMYDIA

In general, chlamydiae are small spherical gram-negative bacteria. They have a diameter of 0.2 to 0.4 micron and act as obligate parasites by replicating inside the cytoplasm of eukaryotic cells. Chlamydiae possess a metabolism deficient in energy production. They have a unique biphasic life cycle, with two morphologically and functionally distinct entities. The extracellular form, referred to as elementary body (EB), is the infectious but metabolically inactive form of the organism. The EB can survive outside the host cells but cannot replicate. After the EB is endocytosed by the host cells, it differentiates into a larger form called reticulate body (RB) within next six to eight hours, which undergoes replication via binary fission, followed by release of chlamydial progeny. The cell cycle is relatively slow, requiring approximately 48 hours for completion. (McGregor JA, 1999). The precise mechanisms by which EBs attaches and gain entry into the host cells are largely unknown. Chlamydia has a major outer membrane protein, which can be found in all serotypes and has been employed as antigen in the detection of the organism.

Chlamydia trachomatis is subdivided into distinct serogroups on the basis of differences in thermo stable polysaccharide antigens. Clinically, the serogroups A, B, and C primarily cause ocular diseases such as trachoma and conjunctivitis. In addition, the serogroups D, E, F, G, H, I, J and K are involved primarily in genital infections i.e. cervicitis, urethritis, salpingitis, epididymitis as well as perihepatitis, inclusion conjunctivitis and lower respiratory tract infections (McGregor JA, 1999).

B. PREVALENCE AND CLINICAL ASPECTS OF CHLAMYDIAL INFECTION

The majority of genital chlamydial infection is found in the developing world, reflecting provision and access to health care, health-seeking behaviour and the distribution of the global population. With such varied distribution, the need for a programme to identify and treat this disease is important.

Chlamydial infection present unique problems for public health programs, as it is largely clinically silent. If it is unrecognized and left untreated, not only the pathogens may remain infectious in the host for months and can be readily transmitted to sex partners, but also the long-term consequences of pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility are especially detrimental. Therefore, early detection and treatment of chlamydial infection in both women and men is critical. Most infections (60 percent to 80 percent) among women are asymptomatic, but if it is symptomatic, it has a variety of manifestation. This includes urethritis, in which patients may present with dysuria and urinary frequency; cervical inflammation where patients present with excess discharge or postcoital or intermenstrual bleeding, and clinically, there will be congested, edematous cervical ectopy with small follicles or unexpected contact bleeding from endocervical canal. However, more often than not, there will be no abnormal signs (Shaw *et al*, 1997).

Ascending infection may cause endometritis and more importantly is pelvic inflammatory disease, which may be acute, subacute or silent. Chlamydia is recognized to be associated with at least 50% of cases of acute PID in developed countries. It is also a more common cause of perihepatitis than is gonococcus (Shaw *et al*, 1997).

For pregnant women, this infection has been associated with morbidity for both the mother and the newborn. Pregnant women with cervical infection at the time of abortion or vaginal birth appear to be at an increased risk for post-abortion or postpartum endometritis or salpingitis, or both (Mardh *et al* 1981; Wein *et al* 1990). In Hospital Kota Bharu, for the year 1999, out of 5655 patients admitted to gynaecology ward, 36 cases were for endometritis, 14 for puerperal pyrexia/sepsis and 262 cases were for abortion. However, none of these cases were screened for chlamydial infection. The presence of mucopurulent cervicitis during pregnancy may indicate an increased risk of chlamydial infection and poor obstetric outcome. However, this is not a useful screening tool for chlamydial infection in pregnant women (Nugent *et al* 1992).

In men, *Chlamydia trachomatis* causes a spectrum of symptoms include urethritis, epididymitis and conjunctivitis. Up to 50 percent of reported cases of non-gonococcal urethritis and 31% of cases of acute epididymitis are caused by infection by *Chlamydia trachomatis*. An estimated 1% to 21% of all man are asymptomatic carrier of the infection and may act as reservoir for its spread (Shaw *et al*, 1997).

The prevalence of *Chlamydia trachomatis* infection varies considerably in different population. Independent risk factors that have been consistently associated with chlamydial infection include young age, having more than one sexual partner, having a new sexual partner, lack of use of barrier contraceptive devices, and concurrent gonococcal infection (Black, 1997).

Genital *Chlamydia trachomatis* infection in pregnancy is reported to be as high as fifty percent (Gyaneshwar *et al* 1987). In Thailand, the prevalence ranges between 5.7 to 6.8 % (Kilmarx 1996; Thongkrajai *et al* 1999). In Malaysia, a study by Ngeow *et al* (1990) in Kuala Lumpur using a single antigen indirect immunofluorescence test to detect chlamydial antibody showed that the pregnant women and adolescents female has higher antibody prevalence than nonpregnant and older women (34.7% for pregnant women vs 24.8% for nonpregnant women; 25.6 % for girls aged 11 to 20 vs 7.5% for women aged 21-30 years).

Therefore, there is growing concern about the association of genital chlamydial infection and poor obstetric outcomes including stillbirth, premature labour and premature delivery and low birth weight (Rastogi *et al* 1999). In The Preterm Prediction Study by Andrew and colleagues (2000), the association of second-trimester genitourinary chlamydia infection and subsequent spontaneous preterm birth was studied. The result showed that the genitourinary *C. trachomatis* infection at 24 weeks' gestation was associated with a two-fold to three-fold increased risk of subsequent spontaneous preterm birth.

High titre of Immunoglobulin G antibody to *Chlamydia trachomatis* was associated with recurrent spontaneous abortion. The mechanism may involve reactivation of latent chlamydial infection, endometrial damage from a past chlamydial infection, or an immune response to an epitope shared by a chlamydial and a fetal antigen (Witkin *et al*, 1992). Another study by Clemen *et al* (1995) showed that women with serologic evidence of *Chlamydia trachomatis* infection were significantly more likely than seronegative women to have preterm birth, an infant with lower mean birth weight and an infant with a lower mean gestational age at birth.

Another retrospective review of 530 maternal and infant records demonstrates significant morbidity in early infancy associated with perinatally acquired *Chlamydia trachomatis* infection (Guascino S, 2000). There is 50 to 70 % risk of transmission from an infected mother to the neonate during delivery (Shaw *et al*, 1997) from the exposure to chlamydiae in the birth canal. Indeed, the infants may aspirate chlamydiae-containing secretions with their first breath. In prospective cohort studies, conjunctivitis developed in 11 percent to 44 percent (Preece *et al*, 1989) of infants born to mothers with *C. trachomatis* infection, and pneumonia developed in 11 percent to 20 percent of such infants (Fromell *et al* 1979; Hammerschlag *et al* 1979; Scachter *et al* 1986) during the first year of life.

Symptoms of conjunctivitis, which include discharge and swollen eyelids, usually develop within first ten days of life. Symptoms of pneumonia, including a progressively worsening cough and congestion, most often develop within three to six weeks of birth (Williamson and Wyandt, 2000).

C. TREATMENT

As this chlamydial infection is curable, most complications associated with it in women and their infants can be avoided by appropriate treatment. However, treatment is often not initiated because the infections are frequently asymptomatic. The identification of at risk patients and treatment of these patients is a practical clinical approach in the reduction of transmission and prevention of complications. In fact, the Centre for Disease Control recommended routine screening and treatment of the high-risk obstetric patients (Centre for Disease Control and Prevention, 1998).

Chlamydiae are susceptible to antimicrobial agents that interfere with protein synthesis by blocking the ribosomal functions such as erythromycin and tetracycline.

Tetracycline has been the mainstay of treatment. The current Centers for Disease Control and Prevention (CDC) recommendation is oral doxycycline 100mg twice a day for seven days. For acute upper genital infection such as pelvic inflammatory disease, intravenous therapy is necessary.

The alternative to oral doxycycline is erythromycin, usually recommended as erythromycin base 500mg six-hourly for seven days. However, erythromycin is associated with gastrointestinal side effects resulting in unreliable compliance.

As mentioned earlier, the cell cycle of chlamydiae is relatively slow, thus relatively prolonged antimicrobial treatment is necessary to ensure clearance of the infectious

particles. However, currently, a single dose of Azithromycin is the drug of choice for chlamydial infection.

Azithromycin is a newer macrolide that was developed to overcome some of the shortcomings of erythromycin such as intolerance, pharmacokinetics, and limited antimicrobial spectrum. Azithromycin (technically an azalide) has a 15-membered ring, which is derived from the insertion of an amino group into the erythromycin ring. Azithromycin has unique pharmacokinetics that give rise to prolonged tissue levels, which allow briefer duration of therapy (3 to 5 days) for most infections and a single-dose regimen for treatment of chlamydial STDs. Numerous studies have shown no significant difference in the clinical effectiveness of a single 1-g dose of azithromycin in comparison with the standard regimen of 7 days of twice-daily orally administered doxycycline in the treatment of *C. trachomatis*-associated STD in men and women, with clinical efficacies ranging from 88 to 100%. Unfortunately, at present, there are limited data on the use of azithromycin in adolescent and in pregnancy (CDC, 1998; Miller and Martin, 2000).

Erythromycin has been recommended for pregnant women and for those whom tetracycline is contraindicated. The recommended dose is erythromycin base 500mg orally four times a day for seven days. Erythromycin crosses the placental barrier and is present in maternal milk (Philipson 1973). Treatment of the sex partners is needed so as to prevent reinfection of the index patient.

D. DIAGNOSTIC TESTS

Diagnosis of chlamydial infection must be made primarily on clinical ground because laboratory methods are imperfect.

Microscopically, there are no completely reliable features that can be used to diagnose chlamydial infection on a smear. The cells may contain cytoplasmic vacuoles of varying sizes and nuclei showing prominent margination of chromatin. Typically, *Chlamydia trachomatis* affect endocervical and squamous metaplasia cells. Features that are suggestive of chlamydial infection include cytoplasmic vacuoles which may contain minute bodies, and follicular cervicitis i.e. abundance of lymphoid cells of mixed population including tingible body macrophage (McKee 1997).

Having said that, a number of methods have been developed for detection of chlamydial infection, which varies in sensitivity and specificity. No single method has yet gained general acceptance (Beagley and Timms, 2000). The selection of a diagnostic test for detection of chlamydial genital infection depends on availability, local expertise, and prevalence of *Chlamydia trachomatis* in the test population. Cell cultures from cervical swabs are 100 percent specific and approximately 80 to 95 percent sensitive. Chlamydial culture from cervical swab specimens has an estimated sensitivity of 75 percent to 90 percent and a specificity of 100 percent, but requires 2 to 3 days for a result (Taylor-Robinson & Thomas B, 1991). Cell culture, although being the most sensitive and the gold standard, it must be emphasized that success of culture depends strongly on adequacy of sampling and laboratory technique (Beagley and Timms, 2000). It is also too expensive in

nonendemic regions, time consuming and is not widely available, so the use of non-culture techniques is very attractive. The other techniques include antigen detection, nucleic acid probes, cytology and serology (McGregor 1989).

The two common techniques for antigen detection are enzyme immunoassay (EIA) and direct fluorescent antigen detection technique (DFA), the latter is used in Hospital Kota Bharu. The time needed to obtain a result in DFA testing is about half an hour, and the time needed for an ELISA result is 3 to 5 hours. DFA employs fluorescent-labeled monoclonal antibody against major outer membrane proteins, which present in all chlamydial serovars and throughout their life cycle. The sensitivity of DFA testing is 70 percent to 95 percent, and its specificity is 85 percent to 99 percent when compared with culture of cervical and urethral specimens taken from women (Stamm *et al* 1984; Baselski *et al* 1987; Taylor-Robinson and Thomas, 1991). According to the internal evaluation of the company, the test kit used in Hospital Kota Bharu (Chlamydia-Cel IF Test, Cellabs, Australia) has sensitivity of almost 100% and specificity of 99.5%.

Polymerase chain reaction (PCR) testing of cervical specimens taken from women is 95 percent to 100 percent sensitive and almost 100 percent specific. DNA probes are about 95 percent sensitive and 98 percent to 100 percent specific when compared with culture. The results of DNA probes may be available within 2 to 4 hours, and, like ELISA, the technique can be used for large volumes of samples. However, the use of this method is currently limited because of its high cost (Starry *et al* 1997). Recently, testing the first void urine specimens with polymerase chain reaction and ligase chain reaction has shown that the

amplification tests are as sensitive as tests with endocervical swabs cultures' (Guascino, 2000).

The problem with the antigen testing is that, even after the bacteria were killed antigenic chlamydial particles may persist. Therefore, test of cure with antigen detection system, such as immunofluorescent antibody tests (IFA) may appear falsely positive even after effective treatment. Alternatively, incompletely treated chlamydia may remain viable, allowing for recurrence of infection in several days or weeks.

OBJECTIVES

OBJECTIVES

Primary objectives:

1. To determine the prevalence of genital *Chlamydia trachomatis* infection in pregnancy among the attendees of Antenatal Clinic of Hospital Kota Bharu, Kelantan.
2. To observe the effectiveness of the treatment of chlamydial infection with a week course of Erythromycin Stearate 500mg six hourly.

Secondary objectives:

1. To assess the possible association between demographic data of the women and genital chlamydial infection.
2. To assess the outcomes of pregnancy of the studied women.

METHODOLOGY

METHODOLOGY

i. Study population

This is a hospital-based cross sectional study, in which consecutive women attending the Antenatal Clinic of Hospital Kota Bharu between 1st August 2001 and 30th June 2002 were invited to participate in the study (Appendix 1: flow chart of chlamydia study). Participants volunteered by signing informed consent form (Appendix 5: Information sheet and consent form). The study population included both the symptomatic and asymptomatic women.

Patients receiving antibiotics, either systemic or local, in the preceding four weeks were excluded from the study so as to minimize the possibility of false negative results. Those with severe hypertension or other medical illnesses, those with placenta praevia or multifetal gestation were also excluded from the study in view of the possibility of early premature delivery due to the problems. In other words, we were trying to minimize the biases in the outcome of the pregnancy.

Patient's refusal is the most important exclusion criterion because patients need to sign the consent form in order to be included in the study.

ii. Sample collection

From each study subject, endocervical swab is taken using a cotton-tipped swab and smeared onto glass slide, air-dried and fixed in methanol, and are sent to the Microbiology laboratory of Hospital Kota Bharu for the diagnosis (Appendix 3: sample collection).

Once screening had taken place, the patient's name, hospital registration number, date of birth, date of screening were documented on a recruitment sheet. Each woman was interviewed according to a detailed standardized questionnaire for clinical and obstetric history. Data recorded include history of previous pregnancy, gravidity, parity and symptoms of lower genital tract infection, if any (Appendix 2: recruitment sheet).

Data on obstetric gestational age was based on the doctors' best estimation, using a combination of physical examination, ultrasonography and woman's reports of the date of her last menstrual period (LMP).

The chosen patients then will be followed up until delivery. Those with positive results were treated with oral erythromycin stearate 500mg six hourly for seven days and repeat test were done one week after completing treatment. Their sexual partners were treated with oral doxycycline 100mg daily for one week but no repeat test done.

The period of gestation at delivery, the birth weight and the birth complications were obtained from the delivery book kept in the labour ward as well as the patients' case notes.

iii. Antigen detection

The endocervical swabs that are taken will be smeared directly onto a glass slide and air-dried before being fixed in methanol for five minutes, and then air-dried again. The antigen detection is using Chlamydia-cel IF test (Cellabs, Australia). It detects the elementary bodies (extracellular form of *C. trachomatis*). The fluorescein-labelled monoclonal antibody directed against the major outer membrane protein is utilized for antigenic detection.

Most of the slides created are stored at minus 20 degree Celsius for batch analysis. Each batch consists of twenty to thirty slides. The slides are processed in batches so as to maximize the use of positive control slide.

The labeled antibodies (about 25 microLitre) are applied directly to the fixed specimen smear and then incubated at 37 degree Celsius in a moist chamber for 30 minutes in dark environment. The slides then are rinsed gently in a bath of Phosphate Buffer Solution (PBS) for about one minute and excess fluid then is drained. The entire specimen is scanned using a fluorescence microscope under oil immersion at x600-x1000 magnification (Appendix 4: Laboratory techniques).

The extracellular elementary bodies appear as bright apple-green fluorescent pinpoint, smooth-edge disc shaped bodies and can be seen against a background of counterstained cells. A positive diagnosis is made when fixed stained specimens show at least 10 chlamydial bodies. A negative diagnosis is made when fixed stained specimen is free from chlamydial bodies but cells are present, i.e. one or more of columnar endocervical or

metaplastic cells, or the presence of more than hundred erythrocytes per high power microscopic field. The presence of this many erythrocytes could not be used to assess the presence of columnar cells because the red blood cells masked the other cells present in the sample. Irregularly shaped fluorescent material that differs from chlamydial bodies described above or fluoresces white, red or yellow are regarded as negative as it represents nonspecific binding

As the collection of the endocervical swabs or specimen for *Chlamydia trachomatis* diagnosis are done by doctors manning the antenatal clinic, including the primary investigator, variation in specimen quality is expected. So, in order to overcome potential biases associated with specimen quality, every slide is assessed for the presence of columnar cells i.e. for cellular adequacy.

There are two technologists reading the slides, both are equally experienced and have been in-charge of the immunofluorescent test in Hospital Kota Bharu for past three or four years. The slides are equally distributed between the two technologists, and all of the slides are read together with the primary investigator. The slides that are read as inconclusive or positive are re-examined and confirmed by the microbiologist (Dr Nurahan Maning). As we are following the strict criteria for positivity, the final results are then decided as either positive or negative, without any inconclusive result.

iv. Statistical and data analysis

The statistical methods and tests were chosen based on recommendations from statisticians in the university.

For the calculation of sample size, the formula used was a single proportion test. With the power of the study taken as 80%, and a probability of less than 0.05 as level of statistically significant, this study needs a total of 427 participants to make it statistically significant, but considering the possibility of those that failed to be follow-up, we aimed for 500 participants.

For the data, it will be analyzed by Fisher exact test and Student t-test where appropriate, while the computer programme chosen was Statistical Package for Social Sciences (SPSS) version 11.0.

RESULTS

RESULTS

A total of 440 pregnant women with age ranging from 17 to 48 years old, in their first to third trimester, attending the antenatal clinic of Hospital Kota Bharu, Kelantan between 1st August 2001 and 31st July 2002 were recruited into the study (Table 1). The parameters in the recruitment sheet were analyzed in terms of normal distribution, frequency and percentage.

As for the quality of the specimen, all of the slides were judged to be adequate, that is, all of the slides have at least one columnar or metaplastic cell. Because the criteria for positivity are followed strictly, (i.e. positive diagnosis is made when fixed stained specimens show at least 10 chlamydial bodies which appear as bright apple-green fluorescent pinpoint, smooth-edge disc shaped bodies), the final results are decided as either positive or negative, no inconclusive result.

Table 1: The age distribution of the study population

Mean	29.63
Std. Error of Mean	0.308
Std. Deviation	6.452
Median	30.00
Mode	30
Minimum	17
Maximum	48

The age of the women in the study population ranges from 17 to 48 years old. The mean age is 29.63 with standard deviation of 6.452. The mode of age is 30, which constitutes 6.4% (28 patients) of the studied population. The age is normally distributed, that is consistent with the normal population.

Table 2: The result of Chlamydial test in pregnant women in Hospital Kota Bharu

	Frequency	Percent
Negative	436	99.1
Positive	4	0.9
Total	440	100.0

Of the 440 women tested, four had positive DFA test, giving the sample prevalence rate of 0.9 percent. In the other words, we are 95% sure that the prevalence of the genital *Chlamydia trachomatis* in antenatal population will be between 0.2 to 2.3 percent. The 95% confidence interval of prevalence was calculated based on the binomial distribution (Stata 7.0).

In view of the small number of women with positive chlamydial test, no valid comparison in terms of demographic data and pregnancy outcome between the positive and negative group of women can be made, thus no statistical analysis attempted. Subsequently, the secondary objectives are not achieved. Therefore, these subsequent results are presented in terms of frequency and percentage only.

Table 2.1: Age range and chlamydial test results

		Result		Total (%)
		Negative	Positive	
Age in years	25 or less	133	1	134 (30.5%)
	More than 25	303	3	306 (69.5%)
Total		436	4	440 (100%)

The women at 25 years old or less represent 30.5 % of the study population. The age cut off at 25 years old is taken as previous studies have shown that those under 25 were at higher risk of contracting *Chlamydia trachomatis* (Black 1997; CDC 1998). However, in this study, 75% of women with positive chlamydial test are more than 25 years of age.

Table 3: Effectiveness of 1-week course of erythromycin stearate for treatment of genital chlamydial infection

Patient	Pretreatment result	Post treatment result
1	Positive	Negative
2	Positive	Negative
3	Positive	Negative
4	Positive	Negative

All of the women with positive results had been cleared of the infection with one-week course of erythromycin stearate 500 mg 6-hourly. However, the very small sample size precludes further statistical analysis.