A STUDY OF CHLAMYDIAL INFECTION IN PREGNANCY IN HOSPITAL KOTA BHARU

By

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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

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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 *Chlamydial 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-abortal 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-gonoccocal 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 immunoflourescence 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; Scahchter *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.

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 immunoflourescent 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.

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 flourescein-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

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.

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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.

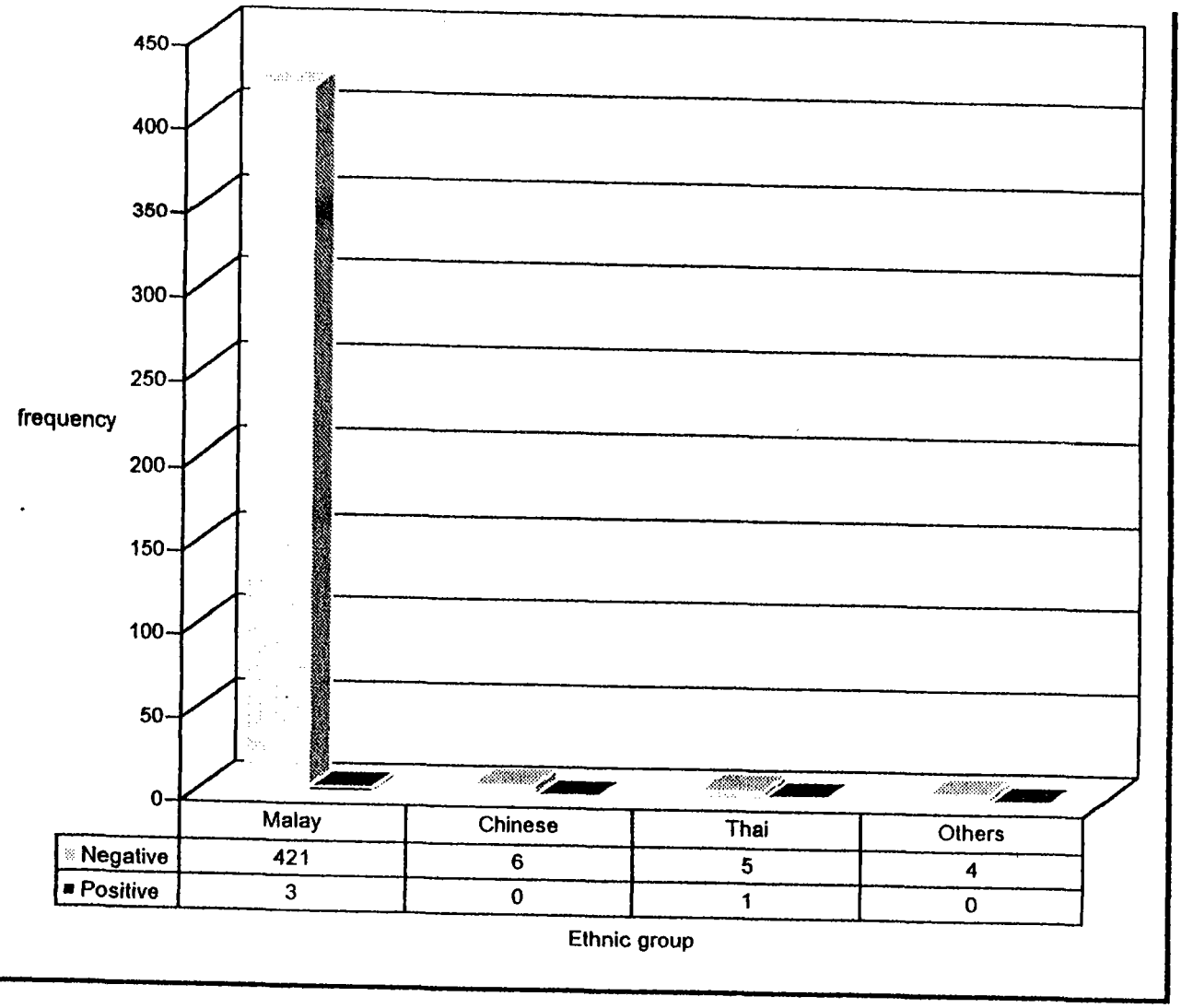


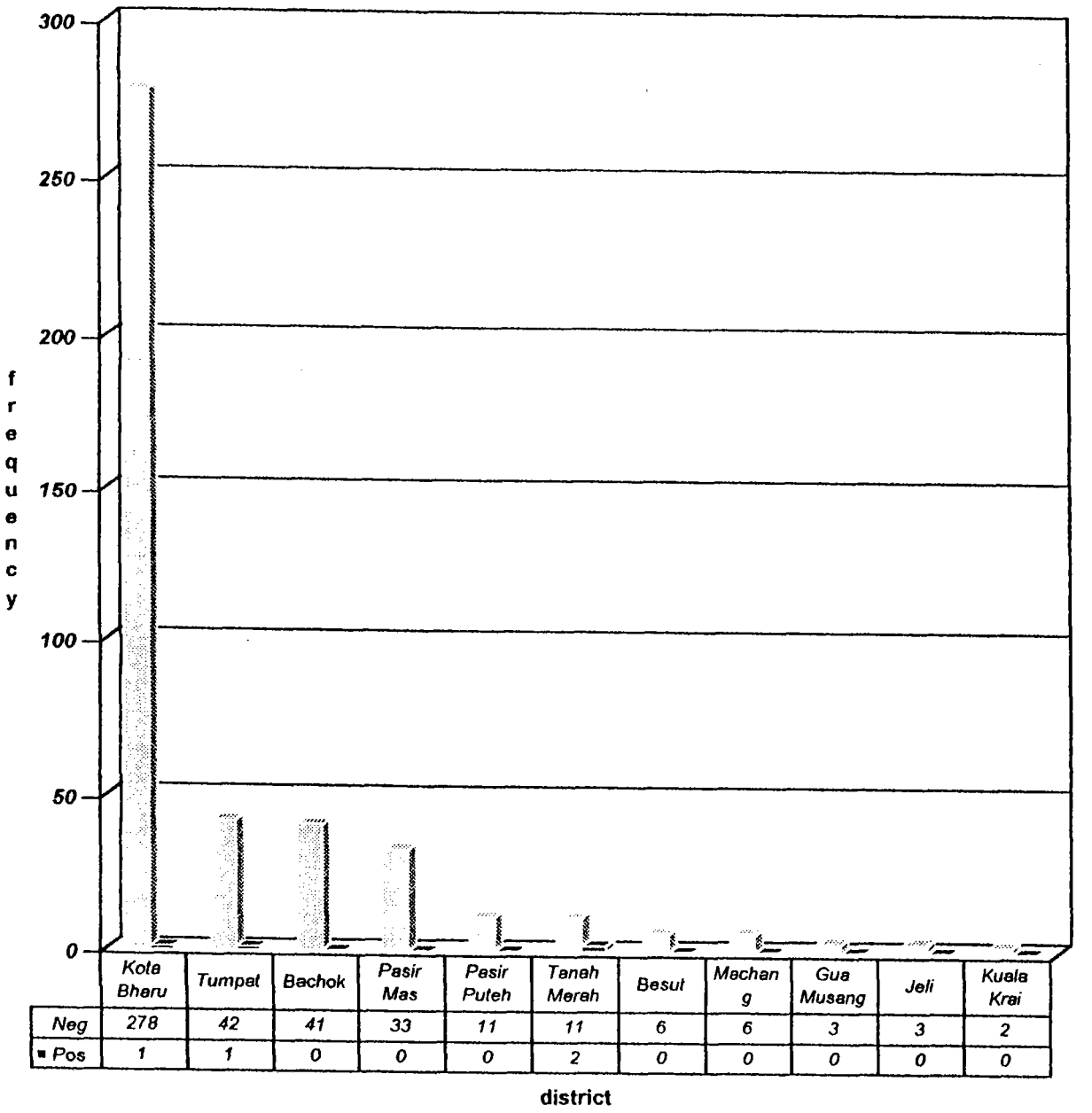
Figure 1: the distribution of chlamydial test results in relation to ethnic group

Out of six studied women living in Kelantan who were of Thailand descendants, one was positive for *Chlamydia trachomatis*. The other three women with positive results were Malays.

Table 5: Distribution of patients in relation to district of residency

	Frequency	Percent
Kota Bharu	279	63.4
Tumpat	43	9.8
Bachok	41	9.3
Pasir Mas	33	7.5
Tanah Merah	13	3.0
Pasir Putch	11	2.5
Besut	6	1.4
Machang	6	1.4
Gua Musang	3	0.7
Jeli	3	0.7
Kuala Krai	2	0.5
Total	440	100.0

As the study is conducted in Hospital Kota Bharu, more than 60% of the tested pregnant women were from Kota Bharu area, followed by Tumpat, Bachok and Pasir Mas (9.8%, 9.3% and 7.5% each). Other districts in Kelantan contribute to less than 10% of the studied population.



	Kota Bharu	Tumpat	Bachok	Pasir Mas	Pasir Puteh	Tanah Merah	Besut	Machang	Gua Musang	Jeli	Kuala Krai
Neg	278	42	41	33	11	11	6	6	3	3	2
Pos	1	1	0	0	0	2	0	0	0	0	0

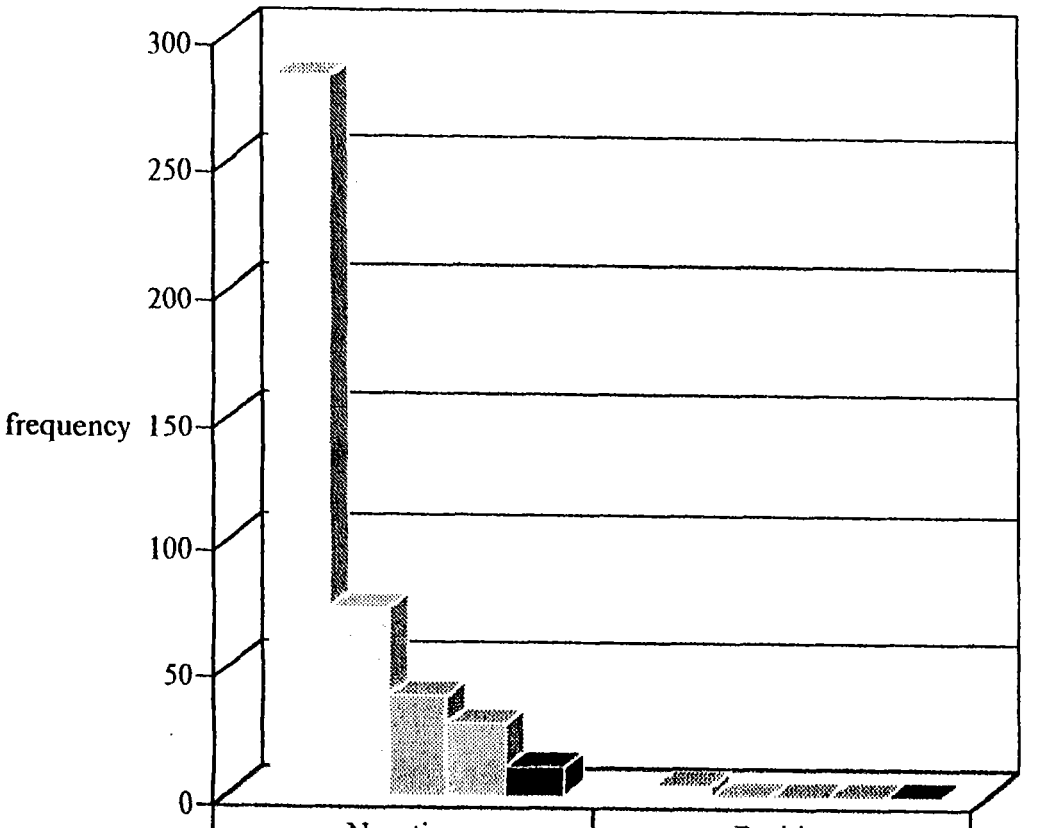
Figure 2: Distribution of patients in relation to district of residency

Only one of the 279 patients from Kota Bharu district tested positive for *C. trachomatis* but two out of twelve women from Tanah Merah district were positive chlamydial testing.

Table 6: Distribution of patients in relation to educational level

Level of education	Frequency	Percentage (%)
No formal education	11	2.5
Primary level	28	6.4
Lower secondary	74	16.8
High school	288	65.5
Tertiary	39	8.9
Total	440	100.0

Nearly ninety eight percent of this study group has received their formal education and majority of them had undergone education training up to high school level. Only 2.5 percent did not have any formal educational, whereas another 8.9 percent had education up to tertiary level.



	Negative	Positive
High school	284	4
Lower secondary	74	0
Tertiary level	39	0
Primary school	28	0
No formal education	11	0

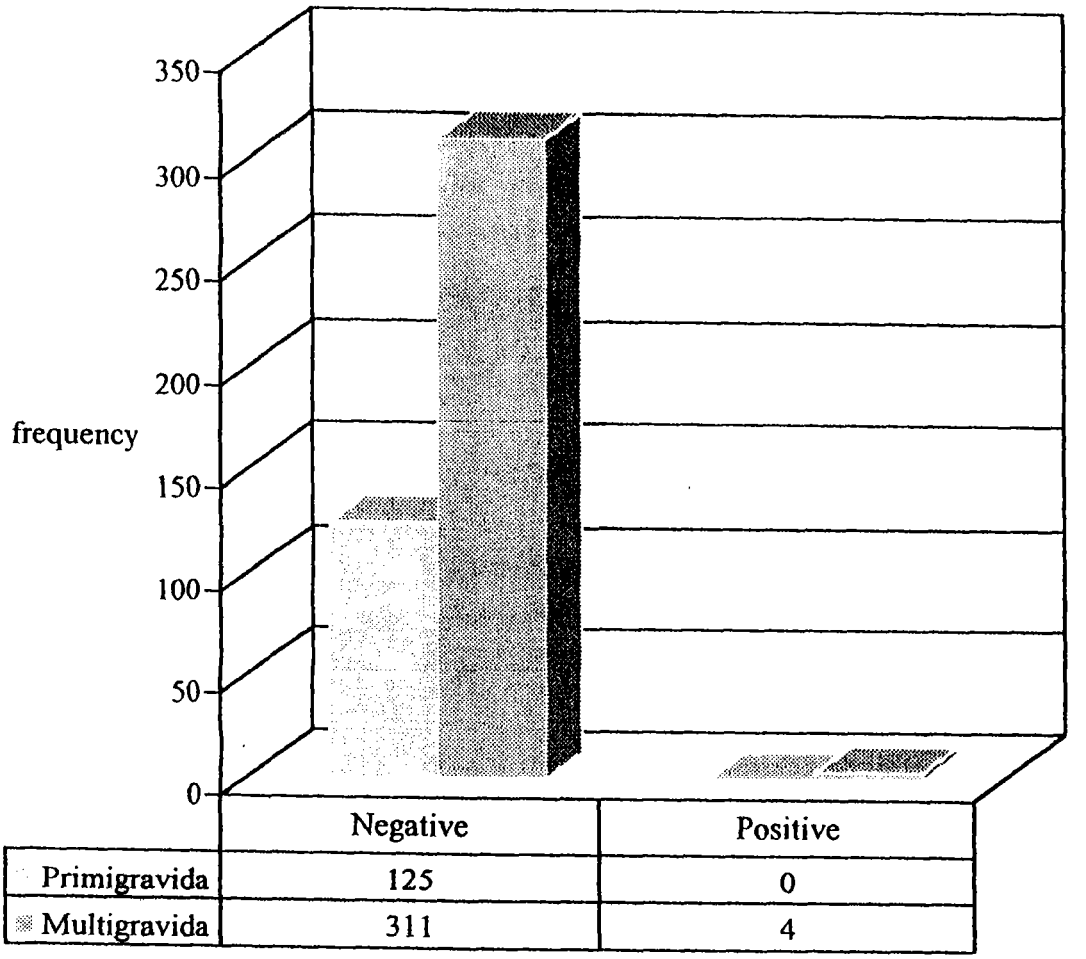
Figure 3: Distribution of patients in relation to educational level

All of the four women who were tested positive for *Chlamydia trachomatis* had formal education up to high school level.

Table 7: Distribution of patients in terms of gravidity and parity

		Frequency	Percentage (%)
Gravidity	Primigravida	125	28.4
	Multigravida	315	71.6
Parity	Nulliparous	144	32.7
	Multiparous	296	67.3

In this sample of 440 women studied, 125 (28.4 percent) were primigravida and the rest were multigravida. There are more women who were multiparous (67.3%) compared to those who were nulliparous (32.7%).



	Negative	Positive
Primigravida	125	0
Multigravida	311	4

Figure 4: Distribution of patients with regards to gravidity

Four women who were multigravida tested positive for the *Chlamydia trachomatis*. However, none of the primigravid women tested positive.

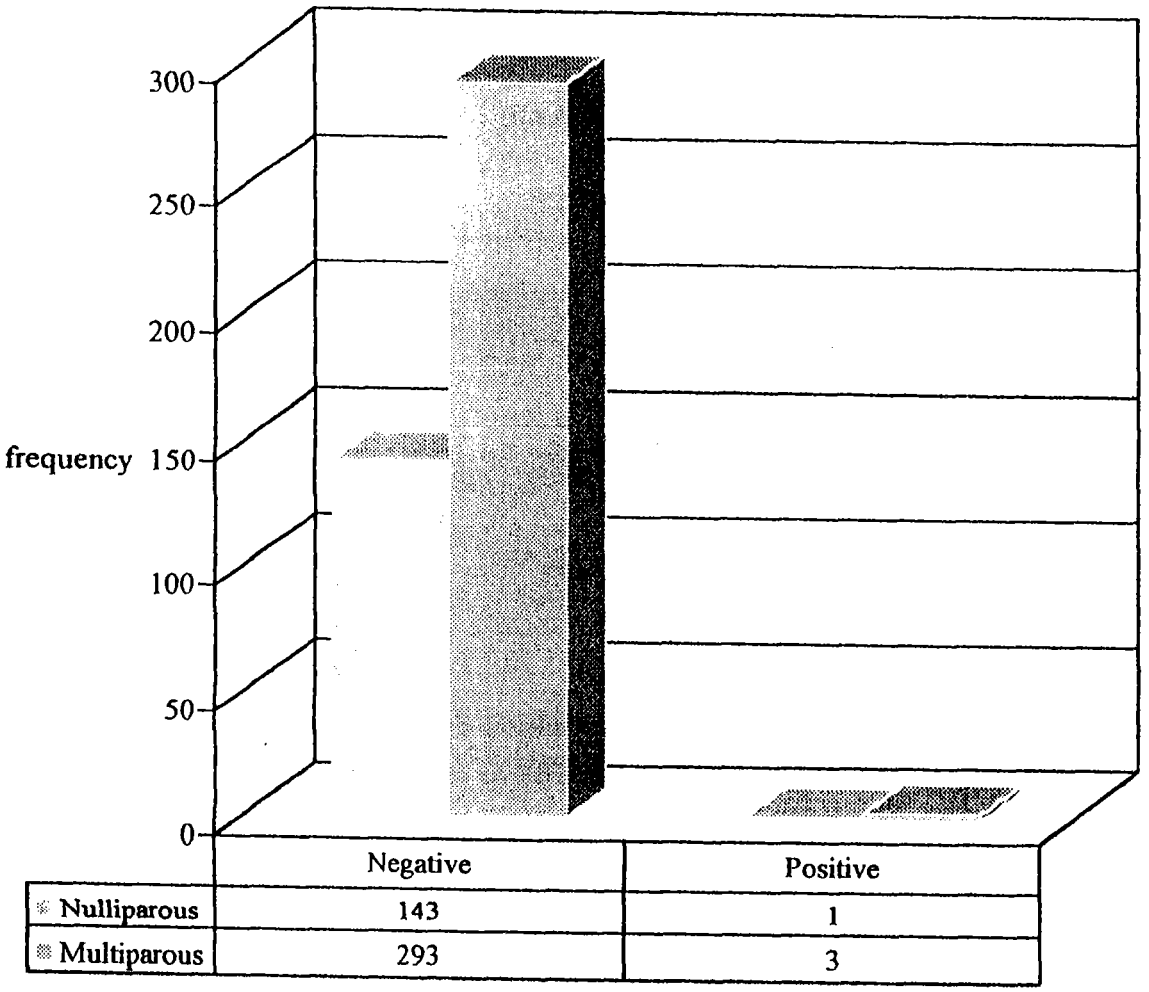


Figure 5: Distribution of patients in terms of parity

Among the one hundred forty four nulliparous women, one individual tested positive for *Chlamydia trachomatis*, whereas, three women from the two hundred and ninety six multiparous women were positive.

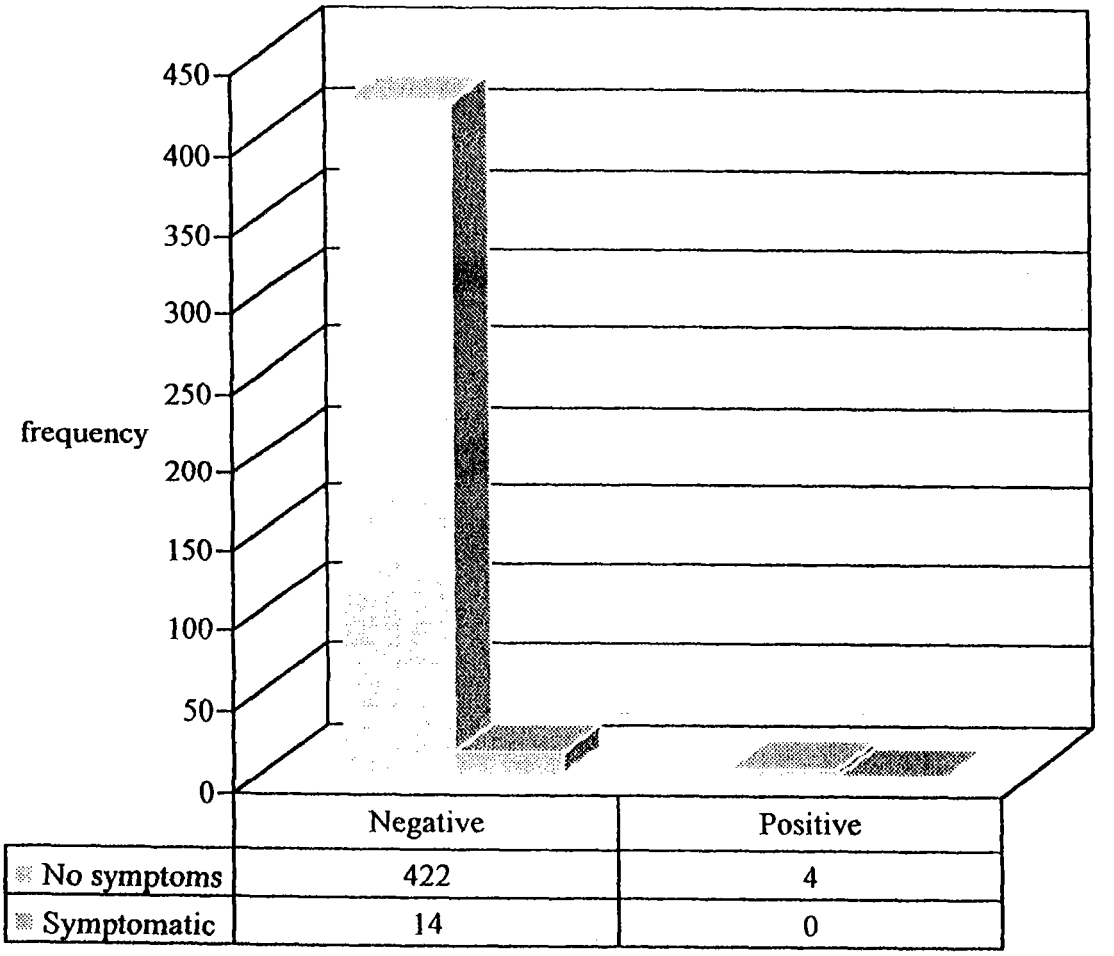


Figure 6: Distribution of patients in terms of symptoms

Majority of the women tested were asymptomatic (426). The fourteen women who had symptoms of vaginal infection had negative chlamydial testing and those four women tested positive were asymptomatic of any vaginal symptoms.

Table 8: Distribution of patients with regards to history of abortion

	Result		Total (%)
	Negative	Positive	
No abortion	343	3	346 (78.6)
History of abortion	93	1	94 (21.4)
Total	436	4	440 (100)

There were more women with no history of abortion (78.6%) compared to those with history of abortion (21.4%). One of the women with positive chlamydial testing had history of one abortion as compared to three women without any history of abortion.

Table 9: Distribution of patients in terms of history of premature delivery

	Result		Total (%)
	Negative	Positive	
No premature delivery	426	4	430 (97.7%)
History of premature delivery	10	0	10 (2.3%)
Total	436	4	440

The majority of the women studied (97.7%) had no history of premature deliveries when compared to those with history of premature deliveries. Those with positive chlamydial testing have no history of premature delivery.

Table 10: Distribution of patients with regards to history of Stillbirth

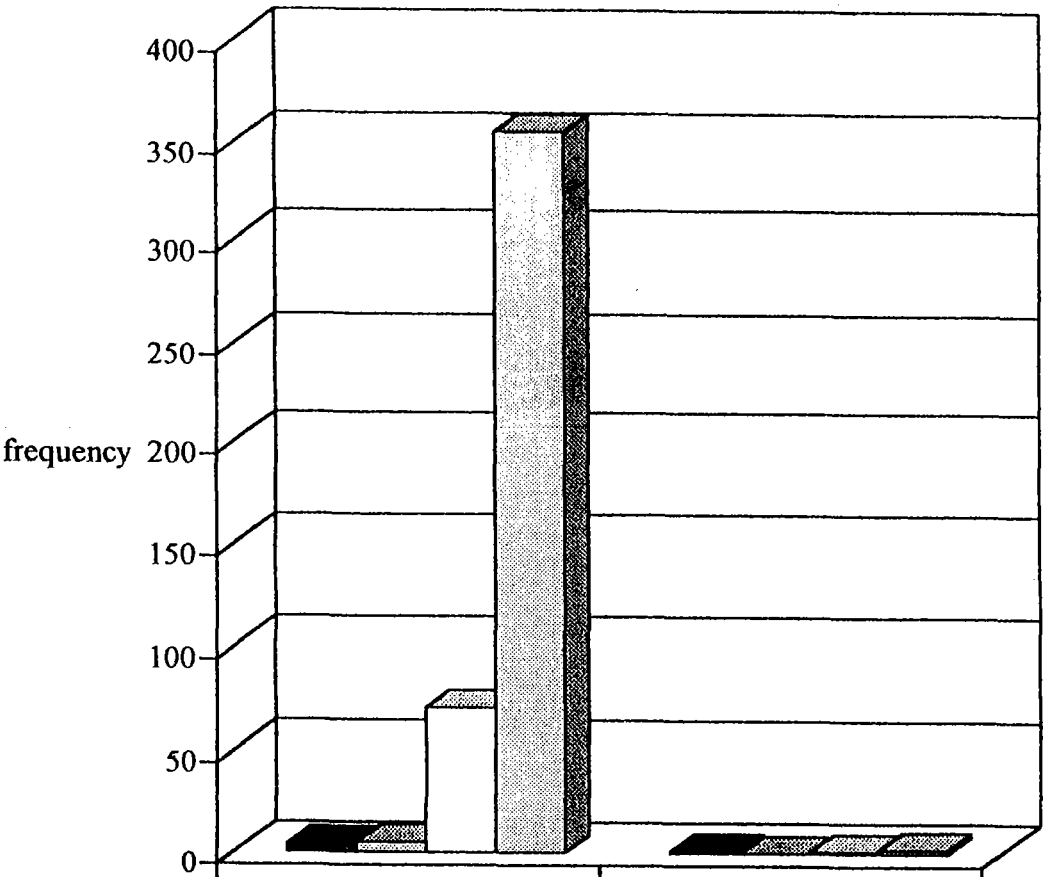
	Result		Total (%)
	Negative	Positive	
No stillbirth	424	4	428 (97.3%)
History of stillbirth	12	0	12 (2.7%)
Total	436	4	440 (100%)

None of the women with positive chlamydial tests had history of stillbirth. Out the 440 women studied, twelve women had history of at least one stillbirth.

Table 11: Distribution of patients with regards to gestation at delivery (weeks)

Mean	37.76
Median	38.0
Mode	39
Std. Deviation	3.308
Variance	10.945

Majority of the women gave birth at term (37 weeks or more). The mean gestation at delivery is 37.76 weeks and the mode is 39 weeks of gestation with a standard deviation of 3.308.



	Negative	Positive
Less than 24 weeks	4	1
24 to 32 weeks	5	0
33 to 36 weeks	71	1
37 weeks or more	356	2

Figure 8: Distribution of patients with regards to gestation at delivery

Out of four patients who were positive for Chlamydia trachomatis, one had abortion at 16 weeks POA, one had a premature delivery at 34 weeks of gestation and the other two patients had term deliveries.

Table 12: Distribution of patients with regards to babies' birth weight

BIRTHWEIGHT	Result		Total
	Negative	Positive	
Less than 1.5kg	2	0	2
1.5 to 2.5kg	48	1	49
2.6 to 4 kg	378	2	380
More than 4 kg	4	0	4
Total	432	3	435

Five women had abortion in first or early second trimester, therefore, the birth weight of the babies available for only 435 women. Two women gave birth to very low birth weight babies and another 49 women gave birth to low birth weight babies. Only one of them tested positive for *Chlamydia trachomatis*.

Table 13: Distribution of birth complication

Birth Complications	Result		Total (%)
	Negative	Positive	
No complication	355	0	355 (80.7)
Silent or complete miscarriage	4	1	5 (1.1)
Threatened abortion	1	0	1 (0.2)
LSCS	14	2	16 (3.6)
MSB	2	0	2 (0.5)
PPROM/premature delivery	41	1	42 (9.6)
Premature contraction	8	0	8 (1.8)
PROM	8	0	8 (1.8)
PROM-admitted to NICU	1	0	1 (0.2)
Term – admitted to NICU	1	0	1 (0.2)
Oligohydramnios	1	0	1 (0.2)
Total	436	4	440 (100)

(Legend: LSCS= Lower Segment Casearean Section, MSB= Macerated Stillbirth, PPROM= premature prelabour rupture of membrane, PROM= prelabour rupture of membrane, NICU= neonatal intensive care unit).

Majority of the women (80.7%) had no obstetric complication. Only one out of 41 cases of premature delivery (including those with or without history of premature prelabour rupture of membrane-PPROM) had positive chlamydial test.

DISCUSSION

ISCUSSION

Prevalence of *Chlamydia trachomatis* infection

Studies based on screening are the most reliable method of measuring the prevalence of infection within population group because they are not affected by trends in case selection and laboratory testing. They are also the most reliable means of detecting the asymptomatic cases and the greatest potential benefits of screening asymptomatic patients for *Chlamydia trachomatis* infection are the prevention of complications especially infertility and perinatal complications, and prevention of disease spread. It is difficult, however, to extrapolate the findings of this study to the general population since it has been of limited size and undertaken among health services attendees (Catchpole, 2001).

Screening persons at risk, who lack clinical evidence of infection, for sexually transmitted diseases such as *Chlamydia trachomatis*, is a central strategy for prevention of treatable bacterial STDs. Reducing the incidence of the personal suffering and economic burdens brought on by *C. trachomatis* infections requires establishment and maintenance of effective epidemiologically based prevention and control programs. How to mount such programs most effectively is receiving increasing interest in screening the asymptomatic chlamydial infections.

The cost effectiveness of screening for *C. trachomatis* depends on its prevalence in the population being screened. Moreover, it is affected by the cost and accuracy (sensitivity and specificity) of the screening test. Phillips and colleagues employed a decision analysis

model comparing the strategy of no routine testing with strategies of using routine cultures rather than less expensive rapid antigen detection tests (direct immunoflourescent or enzyme immunoassay). They concluded that screening was cost-effective and that the choice of test depended on expected local prevalence, cost considerations, and availability of laboratory expertise. They found that use of rapid antigen test reduced overall costs if the prevalence of infection was 7 percent or greater (Phillips *et al*, 1987).

In our study, the prevalence of genital Chlamydial infection in pregnant women attending the antenatal clinic in Hospital Kota Bharu is 0.9% (Table 2). This prevalence is too low for the practice of universal screening to be cost effective. Having said that, women deemed to be at high risk such as having new sexual partners should be tested whenever they have pelvic examination done.

The prevalence of *Chlamydia trachomatis* infection in pregnant women obtained in this study is low (0.9%) compare to the other studies mentioned earlier (Gyaneshwar *et al* 1987; Kilmarx 1996; Thongkrajai *et al* 1999; Underhill *et al*, 2003). The result of this study was also in contrast to the study done by Ngeow in Kuala Lumpur in 1990 for which they found that pregnant mothers had higher prevalence of anti-chlamydial Immunoglobulin M, reflecting higher prevalence of acute infection in pregnancy, that is 34.7% for pregnant women vs 24.8% for nonpregnant women (Ngeow *et al*, 1990).

However, based on the sample prevalence rate of 0.9 percent, 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). Therefore, the result of this study is in agreement with the latest sexually transmitted infection survey conducted by the Ministry of Health of Malaysia in 1999-2000. It was a cross-sectional Sexually Transmitted Infections (STI) prevalence survey conducted by the Division of Disease Control, Ministry of Health, Malaysia, in conjunction with the University Hospital, Ministry of Education, Malaysia. They recruited a total of 1070 antenatal mothers from August to October 1999, and 208 sex workers enrolled from April to November 2000. Urine was tested using PCR technique for *Chlamydia trachomatis, Neisseria gonorrhoea* and *Trichomonas vaginalis*. Blood was tested using RPR and TPHA for treponemal antibodies, and two different ELISA for HIV. Among women attending antenatal clinics, the most prevalent STI was chlamydiosis with the prevalence rate obtained was 1.6%. This is followed by trichomoniasis (0.5%) and treponemal seropositivity (0.3%) and gonorrhoea (0.2%)- WHO Surveillance Report, 2002.

This low incidence of chlamydial infection obtained in this study can be due to the fact that majority of the participants were of Malay ethnic group, and majority of them had monogamous marriage with single sexual partner. Furthermore, the subjects in this study were of heterogenous group, i.e. including both low and high-risk women. It is expected that if the study population were a group of women deemed to be high risk for this sexually transmitted disease, such as those with recurrent miscarriage or history of ectopic pregnancy, or those with multiple sexual partners, the result would be different.

In addition, many methods were applied to diagnosis of *Chlamydia trachomatis* infection. The different studies on prevalence of *Chlamydia trachomatis* infection used different screening test, with their different sensitivity and specificity, and this may have influenced

the different prevalence rate obtained in the studies. 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). In this study, antigen testing was used. Therefore, inconsistent sampling method and relatively low sensitivity of direct antigen testing as compared to antibody testing, the one that was used in the study by Ngeow and colleagues in 1990, could be a cause of low prevalence rate obtained in this study.

ii. Prevalence of genital chlamydial infection with regards to age and other demographic data

Stratification of the data on the detection of *Chlamydia trachomatis* as a function of the subjects' age showed that the detection rate increased as the age of the subjects decreased. Age was identified as a major risk factor for *Chlamydia trachomatis* (Black 1997; CDC, 1998; Catchpole 2001). The highest prevalence of chlamydial infection was observed among the 15-19 and the 19-24 age groups (WHO STI report, 2002). MacMillan and colleagues found a high prevalence of chlamydial infection in teenagers and virtually no infection in women age more than 30 years old regardless of clinical presentation (MacMillan et al, 2000). Age was an even stronger predictor for a repeat positive test than it was for a single positive test, and older women seem to have relative resistance to infection, which can be a consequence of either mucosal immunity, or a more mature protective cervical epithelium (Norman J, 2002). From our study, however, no valid conclusion can be made between positive and negative group with regards to age despite 30% of our study population aged less than 25 (Table 2.1). This is because of the very low number of women with positive chlamydial test (Table 2).

Likewise, the association between *Chlamydia trachomatis* infection in pregnancy and other demographic data such as place of residency, educational level, gravidity and parity also cannot be ascertained due to limited number of women with positive results.

In this study, those women with high risk for sexually transmitted diseases, such as those with multiple marriages and with Human Immunodeficiency Virus infection, were

surprisingly not noted to have higher rate of *C.trachomatis* infection. This is most likely due to the fact that majority of HIV cases in this population is infection through intravenous drug abuse, rather than promiscuity thus less exposure.

The presence of symptoms such as per vaginal discharge, vaginal discomfort and pain is not significantly associated with genital chlamydial infection. However, majority of the women tested were asymptomatic (426 out of 440 women). The 14 women who had symptoms of vaginal infection had negative chlamydial testing. All of the four women tested positive were asymptomatic of any vaginal symptoms (Figure 6).

i. Pregnancy outcomes

Untreated *Chlamydia trachomatis* infection in pregnancy has been shown to be associated with adverse outcomes as well as deleterious effects to neonates (Gravett *et al*, 1986; Hammersclag MR, 1999; Nyari *et al* 2001). Rastogi and colleague studied a cohort of 122 pregnant women attending antenatal clinic in Northern India and found the prevalence rate of 21.3%, with increased incidence of stillbirth, prematurity and low birth weight in the C.trachomatis women (Rastogi *et al*, 1998). Premature delivery is the most important perinatal problem in Hospital Kota Bharu, so *Chlamydia trachomatis* was thought to have a causative role in the origin of the premature delivery. However, this study showed that the prevalence of chlamydial infection was too low to have any significant effect on the rate of premature delivery.

Chlamydia trachomatis infection has also been associated with recurrent abortion. Witkins and colleague had shown that high titre of ImunoglobulinG 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). In this study, 346 women (more than 75%) had no history of miscarriage, but one out 75 women with history of one miscarriage had positive chlamydial test. Unfortunately, this current pregnancy also ended up in silent miscarriage. The chlamydial infection could have been one of the actiological factors for her recurrent miscarriage.

Out of the four women with positive result, one was 20 years old clerk, in her second pregnancy and had history of an abortion. Unfortunately, the current pregnancy also ended up in silent miscarriage at 16 weeks gestation. The second woman was a 33 years old housewife, in her eighth pregnancy, had premature delivery following premature rupture of membrane at 34 weeks of gestation. Her baby weighed 2 kg with good Apgar score but was admitted to NICU for observation in view of her PPROM. The third woman was a 33 years old housewife, in her third pregnancy and delivered at term via Lower Segment Caesarean Section for two previous LSCS. Lastly, the fourth woman was a 33 years old teacher, in her third pregnancy had undergone Lower Segment Caesarean Section for history of two previous Caesarean Sections at term.

iv. Treatment of genital *Chlamydia trachomatis* infection

The efficacy of topical antibiotic prophylaxis has not been proven to prevent nasopharyngeal colonisation and possible subsequent sequelae. Thus, antenatal screening and eradication of chlamydial infection is appropriate. Sweet and colleague has demonstrated that oral erythromycin given to women at approximately 36 weeks of gestation is adequately tolerated and effectively reduces the risk of neonatal chlamydial infection (Sweet *et al*, 1987). Screening and treatment of maternal infections may also be performed early in pregnancy, but incomplete treatment of partners and reinfection may make this approach less effective (Scholes *et al*, 1996).

Treatment with oral erythromycin appears to be save and effective, and if given at a recommended dose for a short duration, the side effects are well tolerated (Schachter *et al* 1986; Blank-Payne *et al* 1990). Patients who were positive for *Chlamydia trachomatis* were treated with erythromycin as soon as the diagnosis of the infection was confirmed. All of them were found to be *C. trachomatis* negative in subsequent follow-up (Table 3). Sexual partners of the positive patients were also simultaneously treated with doxycycline but no confirmation test was done for them. However, the neonates in this study were not followed-up beyond delivery, and unfortunately, the small number of patients precludes further statistical analysis.

CONCLUSION

Conclusion

1. The prevalence rate of genital *Chlamydia trachomatis* infection in pregnant women attending the antenatal clinic of Hospital Kota Bharu was 0.9%. This suggests that universal screening for *Chlamydia trachomatis* in pregnant women in this hospital is not needed.

2. The women with positive chlamydial tests were asymptomatic; suggesting that diagnostic testing based on clinical findings is unreliable.

3. Repeat chlamydial testing in the women with the positive results showed a 100% clearance with one-week course of erythromycin stearate 500mg six hourly. However, further studies are required to see the effectiveness of this antibiotic regime in eradicating the genital chlamydial infection in pregnancy.

4. No conclusion as to the association between the genital chlamydial infection and age, gravidity and parity, district of residency and prior history of abortion or stillbirth or premature delivery can be made due to small number of positive patients. However, it would be helpful to screen those with recurrent abortion or other adverse pregnancy outcomes.

5 Finally, this study has produced some raw data, which can be the basis for future studies. It is my hope that this study will instill enough enthusiasm for other people

to help answering the questions of screening which will go a long way in saving the unborn child.

LIMITATIONS

LIMITATIONS

Our study has some potential limitations. First, a limitation of antigen detection tests in that they require invasive specimen such as cervical or urethral swabs taken as part of genital examination in a specialist clinic setting. This invasive procedure which are uncomfortable and time consuming, was not really welcomed by the pregnant women attending the antenatal clinic unless they have other reasons for pelvic examination, thus the rate of recruitment of patients into this study was relatively slow. Out of over 1500 suitable patients attending antenatal clinic in Hospital Kota Bharu during the study period, only 440 agreed to take part in the study. It seems that the piece of information that needed to be stressed to the patients was the fact that genital chlamydial infection could only be controlled through screening for asymptomatic infection. Unless they understand it, they would be reluctant to have pelvic examination for screening purposes.

Secondly, the level of experience of laboratory personnel also affects performance of the study using the direct fluorescence test method, and the labour and skills needed to perform the test limit its use to low volumes of specimen.

Another limitation in this study is the possible source of error from inadequate sampling of cells. As the collection of the endocervical swabs or specimen for *Chlamydia trachomatis* diagnosis were done by doctors manning the antenatal clinic, including the primary investigator, who were of different degree of training in sample collection, it is expected that the quality of the specimen would be varied. The variation in specimen quality had been shown to have a significant impact on determining the prevalence of *C. trachomatis* in

a population, thus training and periodic retraining of clinicians who obtain cervical specimen for chlamydial testing are necessary (Welsch *et al*, 1997). To overcome these potential biases, every slide was assessed for the presence of columnar cells by the two technologists responsible for the tests, and all of the slides were judged to be adequate.

Another limitation is associated with the technique used in the study for testing the chlamydial infection. As has been mentioned earlier, direct fluorescent antigen (DFA) testing with the use of fluorescein-conjugated monoclonal anti-bodies and enzyme-linked immunoassay (ELISA) are the nonculture techniques to diagnose cervical infections most widely used in clinical practice. The time needed to obtain a result in DFA testing ranges from 15 minutes to 1 hour, and the time needed for an ELISA result is 3 to 5 hours. The sensitivity of DFA testing is 70 percent to 100 percent, and its specificity is 85 percent to 98 percent when compared with culture of cervical and urethral specimens taken from women (Stamm *et al* 1984; Forbes *et al* 1986). Having said that, most of the slide specimens in this study were stored at -20 degree Celsius for a period of time until the number of the slides reached at least 20. This was done in order to maximize the use of positive control slide. The maximum time the slides were stored was sixty-six days. This storage may have some effect on the sensitivity or specificity of the test even though, according to manufacturer, the storage would affect neither the sensitivity nor the specificity of the test kit.

RECOMMENDATION

Recommendation for future research

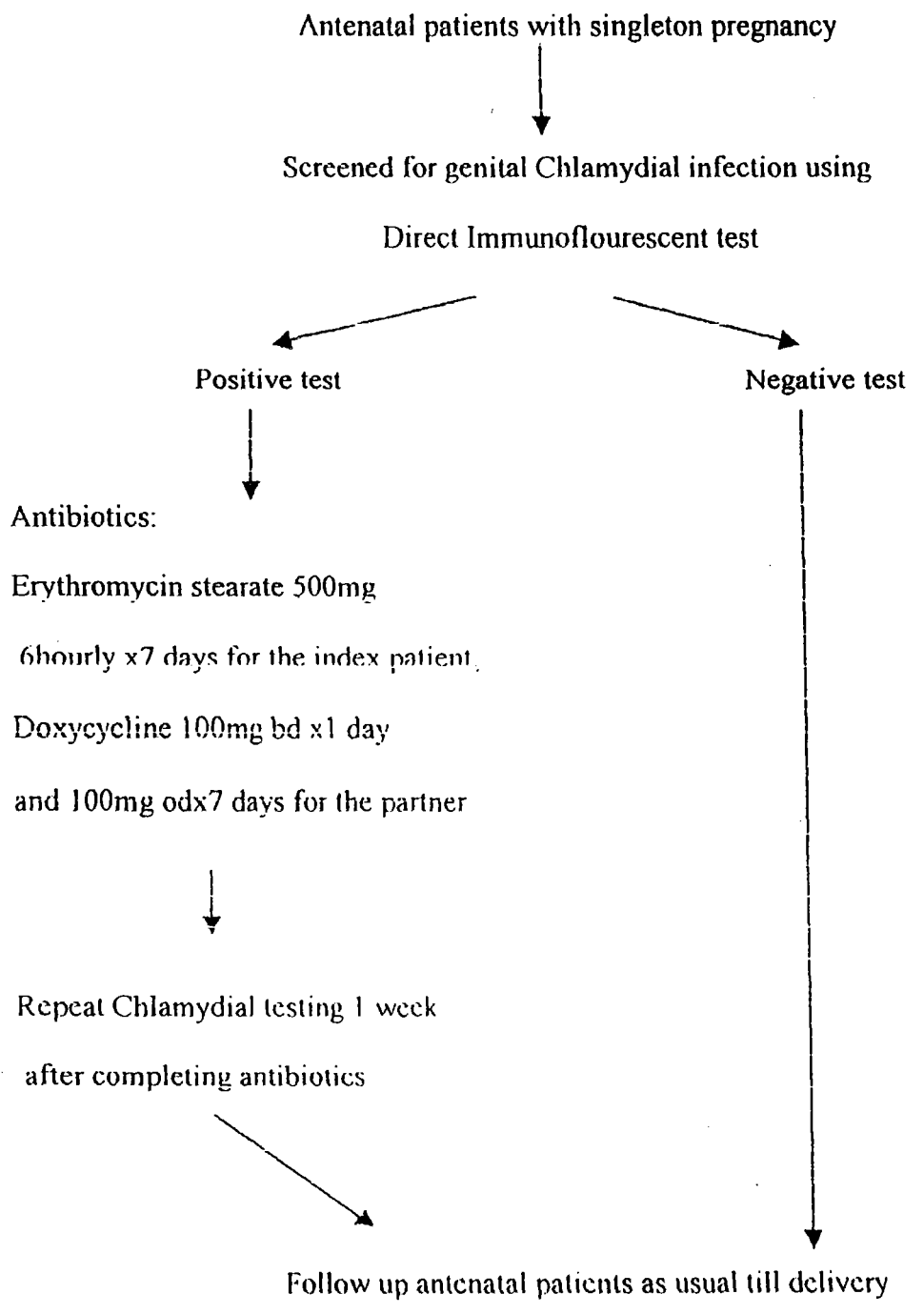
1. Future studies examining the prevalence of *Chlamydia trachomatis* in pregnancy should have a bigger sample size and the sampling should be done in a way such that the study population would be comprises of equal number of women from each ethnic group in Malaysia. The study therefore would be more reflective of the Malaysian population.

2. The study should also include the follow up of the neonates for longer duration so as to ascertain the risks and association of poor obstetric outcome and genital chlamydial infection.

APPENDICES

Appendix I

FLOW CHART of CHLAMYDIA STUDY



Appendix 2: Recruitment sheet

This form is part of a study to gather data on chlamydial infection in pregnant mothers.
Consent is required and their information below is confidential
Kindly provide the participant's details in the spaces and options below.

Name _____

NRIC _____ **Age** _____

Race Malay/ Chinese/ Indian/ Siamese **District** _____

Education Primary/ lower secondary/ high school/ tertiary

Occupation _____ **Husband's occupation** _____

Gravida _____ **Para** _____ **Abortion** _____

LMP _____ **EDD** _____ **REDD** _____

No. of previous premature delivery _____ **Stillbirth** _____

OUTCOME

Date of delivery _____ **Gestational age (weeks)** _____

Birthweight _____

Complications Neonatal infection/ LBW/MSB/IUGR/PPROM

CHLAMYDIAL INFECTION

Vaginal symptoms Nil/present (specify) _____

Date of HVS taken _____ **Batch no.** _____

Result Positive/ negative/ inconclusive

If positive, antibiotic given _____ **Compliance (days)** _____

"A child is nature's way of giving the world another chance"
For further information, please contact Dr Khairiah Seman – ext 2214/2145.

SAMPLE COLLECTION

Dorsal position

↓

Using Cusco's speculum

↓

Clean the cervix with cotton swab (if necessary)

↓

Take endocervical swab using cotton-tip swab
(Rotate the orange stick a few times in the cervical canal to get the endocervical cells)

↓

Roll back and forth on glass slide

↓

Air-dried

↓

Fix in methanol for 5 minutes

↓

Air-dried

↓

To microbiology Lab on the same day (together with usual pathology lab form)

Appendix 4

LABORATORY TECHNIQUE

The antigen detection is using Chlamydia-cel IF test (Cellabs, Australia). It detects the Elementary bodies (extracellular form of *C.trachomatis).* The flourescein-labelled monoclonal antibody directed against this outer membrane protein is utilized for antigenic detection.

Fixed specimen smear

↓

Labeled antibodies (about 25 microL) were applied directly

↓

Incubated at 37 degree Celcius in a moist chamber for 30 minutes in the dark.

↓

Rinsed gently in a bath of Phosphate Buffer Solution (PBS) for about one minute

↓

Excess fluid was drained.

↓

The entire specimen was scanned using a flourescence microscope under oil immersion at 600x to1000x magnification.

↓

Positive slides were confirmed by the microbiologist

Kelayakan penyertaan

Doktor yang bertangggungjawab dalam kajian ini telah membincangkan kelayakan untuk menyertai kajian ini dengan dengan anda. Adalah penting anda berterus terang dengan doktor tentang sejarah kesihatan dan kehamilan anda.

Kriteria yang membolehkan anda meyertai kajian ini adalah anda sedang hamil dengan satu bayi sahaja, iaitu tidak mengandung kembar.

Anda tidak boleh meyertai kajian ini sekiranya anda:

1. mengandung kembar
2. ada masalah kedudukan uri yang di bahagian bawah
3. anda mengidap penyakit-penyakit yang merbahaya seperti masalah jantung, buah pinggang atau hati, barah dan kencing manis atau darah tinggi yang teruk.
4. Anda pernah menerima ubat antibiotik dalam masa 4 minggu sebelum anda meyertai kajian ini.

Prosedur kajian

Pada lawatan di mana anda dipelawa untuk menyertai kajian ini, anda akan ditanya mengenai kandungan anda sekarang dan juga sejarah kandungan yang lalu. Semuanya akan direkodkan di dalam satu borang yang akan disimpan oleh doktor yang bertanggungjawab dalam kajian ini.

Kemudian pemeriksaan dalaman akan dilakukan di mana sedikit contoh sel dari dalam pangkal rahim anda akan diambil dan disapukan keatas slid kaca sebelum dihantar ke makmal untuk diuji untuk kuman *Chlamydia trachomatis* ini.

Anda kemudian akan diminta untuk datang kembali ke klinik dalam masa satu minggu. Pada lawatan anda yang kedua, keputusan pemeriksaan kuman yang dilakukan pada minggu sebelumnya akan dilihat. Sekiranya didapati anda positif untuk jangkitan

kuman ini, anda akan diberi ubat antibiotik Erythromycin 500 mg, 4 kali sehari untuk selama satu minggu. Oleh kerana jangkitan kuman ini boleh merebak melalui hubungan kelamin, suami anda juga akan diberikan rawatan dengan Tetracycline untuk selama satu minggu.

Pemeriksaan dalaman kali kedua akan dilakukan dua minggu selepas anda memakan ubat yang diberikan.

Rawatan susulan anda akan diteruskan seperti biasa sehingga anda bersalin.

Risiko

Tidak ada apa-apa risiko yang terlibat dalam kajian ini. Apa yang mungkin anda alami adalah rasa tidak selesa buat seketika semasa pemeriksaaan dalaman dilakukan.

Penyertaan dalam kajian

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda boleh menolak penyertaan dalam kajian ini atau anda boleh menamatkan penyertaan anda dalam kajian ini pada bila-bila masa, tanpa sebarang hukuman atau kehilangan sebarang manafaat yang sepatutnya diperolehi oleh anda.

Soalan

Sekiranya anda mempunyai sebarang soalan mengenai kajian ini atau hak-hak anda, sila hubungi **Dr Khairiah Seman** di Klinik Pakar Obstetrik dan Ginekologi, Hospital Kota Bharu, nombor telefon 7502214.

Kerahsiaan

Maklumat perubatan anda akan dirahsiakan oleh doktor dan kakitangan kajian dan tidak akan didedahkan secara umum melainkan jika ia dikehendaki oleh undang-undang

Rekod perubatan anda yang asal akan dilihat dan diteliti oleh doktor yang bertanggungjawab dalam kajian ini dan maklumat perubatan anda yang diperolehi akan disimpan dalam komputer dan diproses dengannya. Data dan keputusan hasil kajian ini tidak akan mengenalpasti anda secara perseorangan.

Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemindahan data seperti yang dihuraikan di atas.

Tandatangan

Untuk dimasukkan ke dalam kajian ini, anda mesti menandatangani serta menarikhkan halaman tandatangan.

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Untuk menyertai kajian ini, anda mesti menandatangani mukasurat ini

Dengan menandatangani mukasurat ini, anda mengesahkan yang berikut:

- Anda telah membaca maklumat dalam Borang Maklumat dan Keizinan Pesakit ini, dan anda telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Semua soalan anda telah dijawab dengan memuaskan.
- Anda, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor apabila diminta.
- Anda boleh menamatkan penyertaan anda dalam kajian ini pada bila-bila masa.
- Anda telah pun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi anda.

Nama pesakit RN

Tandatangan Tarikh

Tandatangan doktor Tarikh

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