PREVALENCE OF CHLAMYDIA INFECTION AMONG PATIENTS WITH ECTOPIC PREGNANCY AND INCOMPLETE ABORTION AT THE KENYATTA NATIONAL HOSPITAL

A dissertation submitted as part fulfilment for award of Master of Medicine degree in Obstetrics and Gynaecology of University of Nairobi

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DECLARATION

I declare that this dissertation is my original work and has not been submitted at any other university for the award of a degree.

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CERTIFICATE OF AUTHENTICITY

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DEDICATION

I dedicate this dissertation to my late father Silas who always encouraged me to get involved in research work; Baba this is my maiden paper.

To my dear family, Emmy, Eugene, Elaine and Wilfrida; for your prayers and encouragement and the long days away from you.

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Finally to God Almighty for the strength to face each day.

Table of contents **page**

ABBREVIATIONS

βhCG	**beta human Chorionic Gonadotrophin**
A & E	**Accident and emergency**
C. trachomatis	***Chlamydia trachomatis***
D & C	**Dilatation and curettage**
DNA	**Deoxyribonucleic Acid**
ELISAs	**Enzyme-linked Immunosorbent Assays**
Ig	**Immunoglobulin**
KNH	**Kenyatta National Hospital**
mg	**Milligrammes**
mIU/ml	**Milli-international units per millilitre**
MVA	**Manual vaccum aspiration**
N. gonorrhoea	***Neisseria gonorrhoea***
Ob/Gyn	**Obstetrics and Gynaecology**
PCR	**Polymerase Chain Reaction**
PID	**Pelvic Inflammatory Disease**
SHO	**Senior House Officer**
STD	**Sexually transmitted disease**
STIs	**Sexually transmitted infections**
UoN	**University of Nairobi**
WHO	**World Health Organisation**

ABSTRACT

Background: Ectopic pregnancy is the leading acute gynaecological emergency in Kenya. There has been an association between Chlamydia infection and ectopic pregnancy.

Objective: To describe and compare the prevalence of Chlamydia infection in patients with ectopic and incomplete abortion at the Kenyatta National Hospital

Design: A case control study. The cases were patients with ectopic pregnancy while the controls were those with incomplete abortion.

Setting: Acute gynaecological ward at the Kenyatta National Hospital.

Subjects: Patients with ectopic pregnancy and incomplete abortion.

Methods: A cervical swab was taken from a patient with an ectopic pregnancy post-operatively. Another swab was taken from an age matched client presenting at the acute gynaecological ward with an incomplete abortion. Detection of Chlamydia antigens in the samples was done through rapid immunoassay using Diaspot^{TM} Chlamydia Rapid Test Device. Social demographic profiles of the participants were also extracted using a structured questionnaire.

Data management: Data was collected using a structured questionnaire by the principal investigator. Data entry was done using Access database while data analysis was done using the SPSS 12 for Windows programme.

Results: 40 participants were recruited with ectopic pregnancies and 40 with incomplete abortions. The prevalence of *Chlamydia* was 8% (3/40) among

those with ectopic pregnancy and 3% (1/40) in those with incomplete abortion. The difference was not statistically significant.

Discussion: Prevalence of Chlamydia infection was found to be lower than expected in both cases and controls.

Conclusions and recommendations: Although not statistically significant, prevalence of *Chlamydia* was 3 times greater among patients with ectopic pregnancy than among those with incomplete abortion. Studies on other causes of tubal damage leading to ectopic pregnancy should be done.

INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacterium that commonly attaches to columnar or transitional epithelium. *C. trachomatis* infections are an enormous public health problem throughout the world, accounting for most bacterial sexually transmitted diseases. WHO estimated that there were sixteen million new cases of chlamydia in sub-Saharan Africa in 1999.[1] Although there are no recent global *Chlamydia* prevalence estimates country data from US and UK suggest that the prevalence may have increased.[2]

A study in women undergoing tubal ligation found chlamydia prevalence to be 14.9%,[3] while 9 % of women aged 18 – 40 years attending an STD clinic in Nairobi, were found to have Chlamydia.[4] Lagarde, et al in a multicenter study in Kisumu, reported the prevalence of chlamydia to be 4.5% in women selected from the general population.[5]

Most pregnant women have asymptomatic infection but some present with urethral syndrome, urethritis or Bartholins gland infection.[6] Chlamydia

prevalence was 29% after screening 1090 women in labour and at 7 and 14 days postpartum in a study in Nairobi,[7] while in Durban South Africa, cervical infections were diagnosed microbiologically in 8.2% of asymptomatic pregnant black women. These were *N. gonorrhoea* in 4.1% and *C. trachomatis* in 4.7%.[8] Romoren, et al in a study among 703 antenatal care attendees in Botswana found that the prevalence of chlamydia and gonorrhoea was 8% and 3% respectively.[9] Braddick and colleagues found chlamydia in 8 % of pregnant women attending antenatal clinic in Nairobi, Kenya.[10]

Ectopic pregnancy is implantation of the blastocyst anywhere else other than in the endometrial lining of the uterine cavity. 95% of these involve the fallopian tubes. Other sites of implantation are cervix, ovary, broad ligament and intra-abdominal. Racheal, et al detected anti-Chlamydia immunoglobulin in 51% and 67% of patients with ectopic pregnancy in the United Kingdom and Trinidad respectively.[11]

Abortion refers to the termination of pregnancy from whatever cause before the fetus is capable of extrauterine life. Spontaneous abortion refers to those terminated pregnancies that occur without deliberate measures, whereas induced abortion refers to termination of pregnancy through a deliberate intervention intended to end the pregnancy. Another widely used term for spontaneous abortion is *miscarriage*. By convention, abortion is usually defined as pregnancy termination prior to 20 weeks' gestation or less than 500-g birth weight. Several studies have found inconclusive or no evidence that *Chlamydia* infection causes abortion.[12-15]

Qualitative lateral flow immunoassay is a method of determining the presence of an antigen (or antibody) in blood sample when the specimen is exposed to the specific antibody (or antigen) labelled with a fluorochrome and observing the antigen-antibody reaction of precipitation. During the test, a solution extracted from the cervical swab was applied to the test device. If Chlamydia antigen was present in the solution, it reacted with antibodies at the test region of the device and generated a coloured line (the test line). Another coloured line appeared at the control region of the device indicating adequate volume of specimen (the control line). Chlamydia antigen may persist even after appropriate treatment; therefore the test is positive in cases of past infection.

The Diaspot^{TM} Chlamydia Rapid Test Device is a qualitative lateral flow immunoassay from Apon biotechnology, United States. When compared to the gold standard for detection of Chlamydia antigens on cervical swabs, Polymerase Chain Reaction, it has a sensitivity of 88.5 % and specificity of 96.7 %. Its relative accuracy is 93.7 %.[16]

LITERATURE REVIEW

In 1907, Halberstaedter and von Prowazek, working in Java, described the transmission of trachoma from man to orangutans by inoculating their eyes with conjunctival scrapings.[17] The trachoma "virus" was first isolated in the chick-embryo yolk sac in China in 1957 by Wang and colleagues and reported in the Chinese Medical Journal.[18] The aetiological relationship of this organism with trachoma was proved in 1958 by the inoculation of human volunteers.

The term '*Chlamydia*' appeared in the literature in 1945. That chlamydiae were not viruses became evident in 1965 with the advent of tissue culture techniques and of electron microscopy, when evidence for bacterial rRNA, ribosomes and cell wall structures in chlamydiae finally became overwhelming. For years, *Chlamydiales* was the only bacterial order that had just one family and one genus (*Chlamydiaceae* and *Chlamydia*, respectively).[19]

C. trachomatis is an exclusively human pathogen has been recognized as a major cause of sexually transmitted and perinatal infection. Worldwide incidence has been estimated at 92 million annually, with about 16 million occurring in sub-Saharan Africa. This is second to South and South-east Asia with 43 million annual cases.[1] Studies among male and female adults between 15 and 49 years of age in a low to middle class suburb and unmarried female plantation workers in Kenya found prevalence of Chlamydia to be 1.5 to 3.2 %[20,21]

Pathophysiology: *C. trachomatis* preferentially infects the columnar epithelium of the eye and the respiratory and genital tracts. The infection induces an immune response but often persists for months or years in the absence of antimicrobial therapy. Serious sequelae often occur in association with repeated or persistent infections. The precise mechanism through which repeated or persistent infection elicits an inflammatory response that leads to tubal scarring and damage in the female upper genital tract is not yet clear.[22] Natural human infection results in strong serological responses to the major

outer membrane protein OmpA, but antibodies to outer membrane protein PorB are low or absent.[23]

Risk factors: In women include age younger than 25 years, presence or history of other sexually transmitted diseases and multiple or a new sexual partner within the last 3 months. Use of oral contraceptive pills and the presence of cervical ectopy also confer an increased risk of Chlamydia infection.[22]

Complications of Chlamydia infection include pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility and chronic pelvic pain. Simms, et al found chlamydia in 27% of PID cases and none in women undergoing bilateral tubal ligation.[24] Chlamydia infection also increases the risk of transmission of human immunodeficiency virus.[25] Feist, et al found no association between miscarriages and infection with *C. trachomatis.*[26] Other studies by Oakeshott, Ostaszewska-Puchalska, Sozio and Osser and their colleagues found no conclusive evidence that Chlamydia causes abortion.[12-15] However, Cohen, Sinei, Bukusi, et al in a study at KNH found that more frequent histories of spontaneous abortions reported by infertile women were also consistent with atypical upper genital tract infection including Chlamydia. They found antichlamydia antibodies in 34% of women with abortions.[27]

Symptoms: In adults, the clinical spectrum of sexually transmitted *C. trachomatis* infections parallels that of gonococcal infections. Both infections have been associated with urethritis, proctitis, and conjunctivitis in both sexes; with epididymitis in men; and with mucopurulent cervicitis, acute salpingitis, bartholinitis, and the Fitz-Hugh–Curtis syndrome (perihepatitis) in

women. Both infections can be associated with septic arthritis. Generally Chlamydia infections produce fewer symptoms and signs than gonococcal infections at the same anatomic site. Increasing evidence suggests that many Chlamydia infections of the genital tract, especially in women, persist for months without producing symptoms. Simultaneous infection with *C. trachomatis* often occurs in women with cervical gonococcal infection and in heterosexual men with gonococcal urethritis.[22]

Diagnosis: This is based solely on laboratory tests. *N. gonorrhoea* and *C. trachomatis* are commonly asymptomatic; among men in a rural African population in Tanzania only 24 of 158 (15%) infected subjects complained of urethral discharge at the time of interview.[28] Cell culture isolation has specificity of 100 % and sensitivity of 70 to 90% but is expensive and highly specialized and hence not widely available. Giemsa stain of exudates in adults with genital infection is only 40% accurate. Serological methods either complement fixation or microimmunofluorescence test are positive in 20 to 40% of sexually active women. However, most do not have a current infection. Moss and colleagues, in a study among women attending a genitourinary clinic, found that up to 50% of all chlamydia Ig G-positive cases were due to nongenital chlamydia (*C. pneumoniae* and *C. psittaci*).[29]

Other laboratory tests include direct smear fluorescent antibody testing that has a sensitivity of 90% and specificity of 98%. Polymerase chain reaction, ligase chain reaction and DNA probe assays for detection of *C. trachomatis* are more rapid and less expensive.

Treatment: Doxycycline 100 mg orally twice daily for 7 days for non-pregnant patients or azithromycin 1 g orally as a single dose can eradicate Chlamydia from the cervix. An alternate regimen is erythromycin ethylsuccinate 800 mg orally 4 times daily given for a minimum of 7 days. Non-pregnant patients who cannot tolerate erythromycin should consider ofloxacin 300 mg twice daily or levofloxacin 500 mg orally once daily for 7 days. Post-treatment cultures are not usually advised if doxycycline, azithromycin, or ofloxacin is taken as described above and symptoms are not present; cure rates should be higher than 95%.[30]

Several regimens are used to treat Chlamydia infection in pregnancy. Drugs of choice are either oral erythromycin 500 mg four times a day or oral amoxicillin 500 mg three times a day both for seven days. Alternative therapies are oral erythromycin 250 mg four times a day for fourteen days or oral azithromycin 1 g single dose. Jacobson, Kacmar, Wehbeh and all their co-workers demonstrated the efficacy of azithromycin for Chlamydia infection in pregnancy.[31-33] Adair and associates treated 106 women with erythromycin (93-percent cure) or azithromycin (88-percent cure).[34] Tetracyclines are avoided because of concerns regarding fetal dental discoloration.

Follow-Up: Except in pregnant women, test-of-cure (i.e., repeat testing 3–4 weeks after completing therapy) is not advised for persons treated with the recommended or alterative regimens, unless therapeutic compliance is in question, symptoms persist, or reinfection is suspected. Moreover, the validity of chlamydial diagnostic testing at less than 3 weeks after completion of

therapy (to identify patients who did not respond to therapy) has not been established. False-negative results might occur in the presence of persistent infections involving limited numbers of chlamydial organisms. Testing conducted at less than 3 weeks after completion of therapy in persons who were treated successfully could yield false-positive results because of the continued presence of nonviable organisms.[35]

Vaccination against human Chlamydia infections is currently unavailable. However, animal trials on candidate vaccines specifically for adolescent women are ongoing. The current challenge is to develop an effective delivery vehicle for induction of a high level of immunological response. When developed, vaccines could be delivered trans-dermally via a patch, by nasal spray or vaginal cream.[36]

Ectopic pregnancy

Pathophysiology: The fallopian tube lacks a submucosal layer, hence the fertilized ovum promptly burrows through the epithelium, and the zygote comes to lie within the muscular wall. At the periphery of the zygote is a capsule of rapidly proliferating trophoblast that invades and erodes the subjacent muscularis. At the same time, maternal blood vessels are opened, and blood pours into the spaces lying within the trophoblast or between it and the adjacent tissue. The tubal wall in contact with the zygote offers only slight resistance to invasion by the trophoblast, which soon burrows through it. The embryo or fetus in an ectopic pregnancy is often absent or stunted. The invading, expanding products of conception may rupture the oviduct at any of

several sites. Tubal rupture in the first few weeks, the pregnancy is situated in the isthmic portion of the tube. When the fertilized ovum is implanted well within the interstitial portion, rupture usually occurs later. Rupture is usually spontaneous, but it may be caused by trauma associated with coitus or bimanual examination.[37]

With intraperitoneal rupture, the entire conceptus may be extruded from the tube, or if the rent is small, profuse hemorrhage may occur without extrusion. If an early conceptus is expelled essentially undamaged into the peritoneal cavity, rarely it may reimplant almost anywhere, establish adequate circulation, survive, and grow. Usually small conceptuses are resorbed. Occasionally, if larger, they may remain in the cul-de-sac for years as an encapsulated mass, or even become calcified to form a *lithopedion*.[37]

Risk factors for tubal damage and dysfunction are associated with the occurrence of ectopic pregnancy. They include tubal corrective surgery, tubal sterilization and previous genital infection[38]. The absolute risk of ectopic pregnancy is extremely low with use of intra-uterine contraceptive device. However, when a woman becomes pregnant during IUD use, the relative likelihood of ectopic pregnancy is greatly increased.[39] After one previous ectopic pregnancy, the chance of recurrence is 7 to 15%.[38,40] There is strong evidence linking Chlamydia infection and infertility; chlamydia antibody titers have been associated with tubal occlusion and prior ectopic pregnancy and chlamydia trachomatis seropositive serology has been found to be higher in women with ectopic pregnancy compared to controls (81% vs 63%).[41,42]

Frost and colleagues found that Chlamydia infections play a major part in salpingitis and infertility in central Africa.[43] Other risk factors are multiple sexual partners, infertility and previous pelvic or abdominal surgery.[44]

Symptoms: These are pelvic and abdominal pain (95%) and amenorrhea with some degree of vaginal spotting or bleeding (60 to 80%). With rupture, the woman suddenly is stricken with severe lower abdominal pain, frequently described as sharp, stabbing, or tearing in character. Vasomotor disturbances develop, ranging from vertigo to syncope. Tenderness on abdominal and vaginal examination, especially on motion of the cervix, is demonstrable in over 75 % of women with ruptured or rupturing tubal pregnancies. This may be absent prior to rupture. Identification of non-clotting blood in the peritoneal cavity by paracentesis or culdocentesis is suggestive of a bleeding ectopic pregnancy. Absence however, does not exclude an ectopic pregnancy.[45]

Current serum and urine pregnancy tests that use ELISAs are sensitive to levels of chorionic gonadotrophin of 10 to 20 mIU/mL, and are positive in over 99% of ectopic pregnancies.[46]

In abdominal sonography, absence of a uterine pregnancy, fluid in the cul-de-sac, and an abnormal pelvic mass, ectopic pregnancy is almost diagnostic if a pregnancy test is positive.[47] There has been much improvement in the early diagnosis of ectopic pregnancy using vaginal sonography which allows ultrasonic detection of a uterine gestation as early as 1 week after missed

menses. When serum βhCG levels exceed 1000 mIU/mL, a gestational sac is seen half the time.[48]

Treatment: This is either surgical or medical. Surgical therapy is by laparoscopy or laparotomy. Laparoscopy is more cost-effective and has a shorter recovery time—1.3 versus 3.1 days.[49] Procedures include salpingotomy, salpingostomy, salpingectomy and segmental resection and anastomoses. Medical therapy is with single or multiple dose systemic methotrexate at a dose of 50 mg/m^2. The patient must be hemodynamically stable, have a pregnancy less than 6 weeks gestation and a tubal mass smaller than 4 cm in diameter. She must avoid sexual intercourse, alcohol and folic acid supplements until the ectopic pregnancy has resolved.[37]

Spontaneous abortion or miscarriage: This is the most common complication of pregnancy and is defined as the passing of a pregnancy prior to completion of the 20th gestational week. It implies delivery of all or any part of the products of conception, with or without a foetus weighing less than 500 g. Threatened abortion is bleeding of intrauterine origin occurring before the 20th completed week, with or without uterine contractions, without dilatation of the cervix, and without expulsion of the products of conception. Complete abortion is the expulsion of all of the products of conception before the 20th completed week of gestation, whereas incomplete abortion is the expulsion of some, but not all, of the products of conception. Inevitable abortion refers to bleeding of intrauterine origin before the 20th

completed week, with dilatation of the cervix without expulsion of the products of conception. In missed abortion, the embryo or foetus dies, but the products of conception are retained in utero. In septic abortion, infection of the uterus and sometimes surrounding structures occur.[50]

Hemorrhage into the decidua basalis, followed by necrosis adjacent tissues causes the ovum to detach, stimulating uterine contractions that result in its expulsion. In later abortions, the retained fetus may undergo *maceration* - the skull bones collapse, the abdomen distends with fluid, the internal organs degenerate and skin softens and peels. Alternatively, amniotic fluid is absorbed, the fetus becomes compressed and desiccated (*fetus compressus)* or resembles parchment *(fetus papyraceous).*[51]

More than 80 percent of abortions occur in the first 12 weeks of pregnancy, and at least half result from chromosomal anomalies resulting in abnormal zygote development. Various infections are uncommon causes of abortion in humans. In 2002, Oakeshott and associates reported an association between second- but not first-trimester spontaneous abortion and bacterial vaginosis.[12]

Iodine deficiency, antiphospholipid and antithyroid antibodies and poor glucose control in diabetes mellitus have been associated with miscarriages. Tobacco, alcohol and caffeine have been linked to increased risk of abortion and fetal anomalies. Uterine anatomical defects resulting from abnormal müllerian duct formation or fusion and cervical incompetence result in second trimester abortions.[51]

The clinical aspects of spontaneous abortion separate into five subgroups: threatened, inevitable, complete or incomplete, missed, and recurrent abortion.

Laboratory tests: These include complete blood count and pregnancy tests. If significant bleeding has occurred, the patient will be anemic. Both the white blood cell count and the sedimentation rate *may* be elevated even without infection. Falling or abnormally rising plasma levels of βhCG are diagnostic of an abnormal pregnancy, either a blighted ovum, spontaneous abortion or ectopic pregnancy.[50]

Transvaginal ultrasound: This is helpful in documenting intrauterine pregnancies as early as 4–5 weeks' gestation. Fetal heart motion should be seen in embryos at least 5–6 weeks gestation. [50]

Management of abortion: In threatened abortion, bed rest and pelvic rest are recommended while for inevitable or incomplete abortion, evacuation of the uterus by MVA or administration of oral prostaglandins should be considered. Grouping and cross-match for possible blood transfusion and determination of Rhesus status should be obtained. For complete abortion , the patient should be reassured and discharged.

Treatment of septic abortion involves hospitalization and intravenous antibiotic therapy. An MVA should be performed, and a hysterectomy may be necessary if the infection does not respond to treatment.[50]

Hypothesis

The prevalence of Chlamydia infection is higher among patients of ectopic pregnancy than those with incomplete abortion.

Study justification

Current local guidelines on the management of ectopic pregnancy do not include treatment of Chlamydia infection[52]. It is not common practice to treat patients with ectopic pregnancy for Chlamydia. Previous studies on STIs among patients presenting with ectopic pregnancy have not focused on Chlamydia and none have used rapid immunoassays such as Diaspot™. Previous studies have compared patients with ectopic pregnancies to those with on going pregnancies. However this may cause bias as STIs are likely to lead to miscarriages of intrauterine pregnancies. Therefore patients seeking post-abortion care services are an ideal comparison group. This study will be the first done in the Kenyan setting where prevalence of undiagnosed STIs is high.

Ectopic pregnancy is a rare outcome compared to intrauterine pregnancy therefore a case control study design is best suited to study the association with Chlamydia infection.

A study by Kaaria showed that patients seen at KNH with abortion were in the same socio-economic class as those with ectopic pregnancy unlike those attending antenatal clinic who are in a higher class.[53] Hence women with incomplete abortion are a better control group for patients with ectopic pregnancy because they belong to the same socio-economic class.

CONCEPTUAL FRAMEWORK

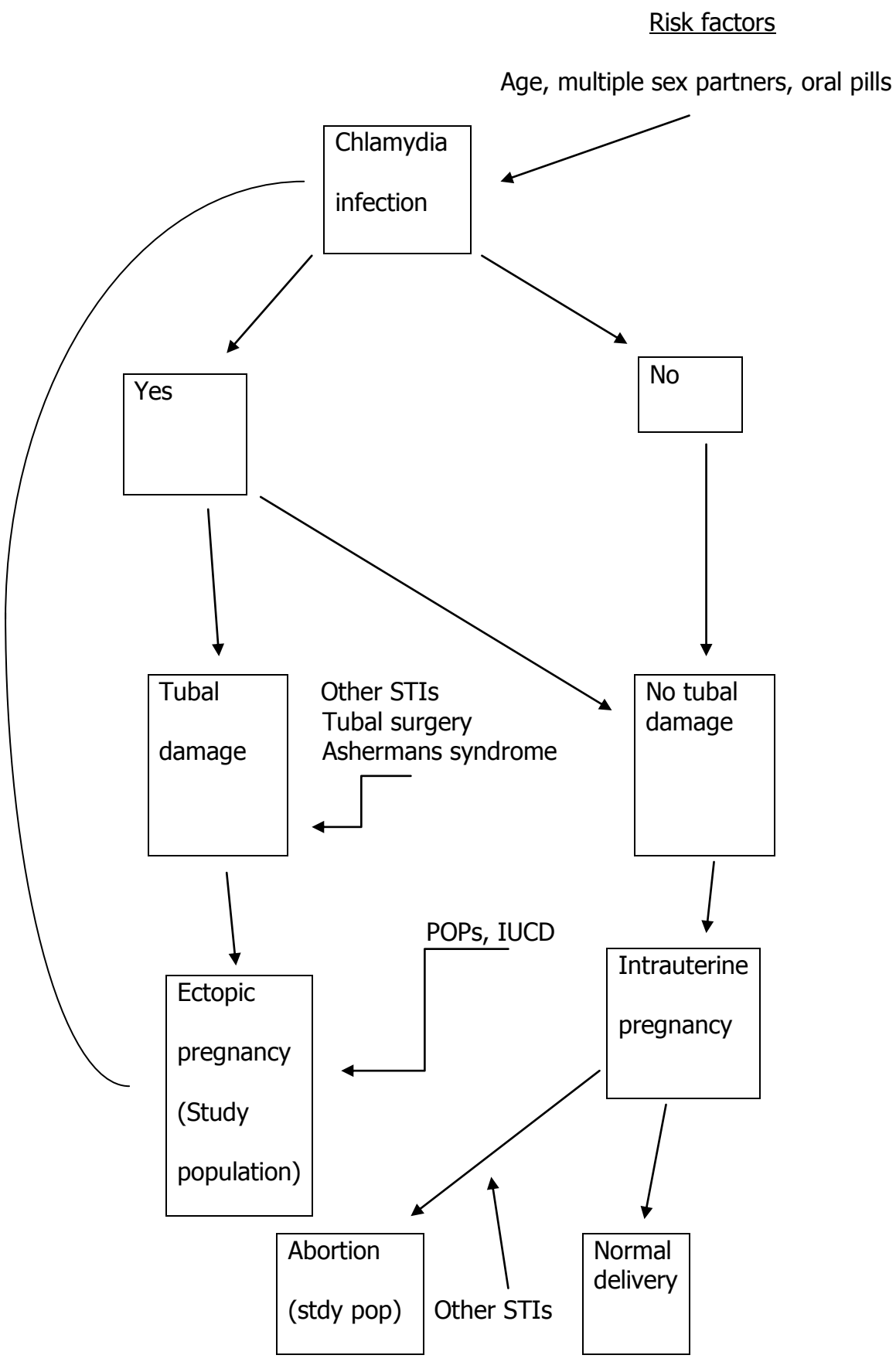
NARRATIVE

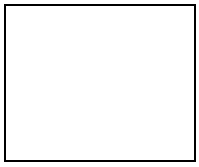
In our study, we set out to relate the presence of *Chlamydia trachomatis* antibodies to ectopic pregnancy by demonstrating increased prevalence compared to patients with incomplete abortion.

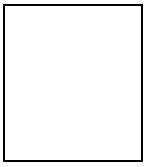
Risk factors for Chlamydia infection include age, multiple sexual partners and oral contraceptive pills. This infection when present may lead to tubal damage and subsequent tubal dysfunction. This ultimately leads to ectopic pregnancy whose patients comprised the cases in our study population. Other causes of tubal damage are other STIs like bacterial vaginosis, tubal surgery and Asherman's syndrome. Ectopic pregnancy may also result from use of progestin only pills and intrauterine contraceptive device.[39]

Tubal damage may be absent even after Chlamydia infection leading to intrauterine pregnancy. This results in either delivery or abortion. Patients with abortion made up the control group of our study population. Abortion has been associated with other STIs e.g. syphilis.

DIAGRAMMATIC







OBJECTIVES

Broad objective:

To describe and compare the prevalence of Chlamydia infection in patients with ectopic pregnancy and incomplete abortion at the Kenyatta National Hospital.

Specific objectives:

1. To determine obstetric, sexuality, clinical and socio-demographic characteristics of patients presenting ectopic pregnancy and incomplete abortion at KNH.
2. To determine and compare the prevalence of *Chlamydia* infection among patients with ectopic pregnancy and incomplete abortion at KNH.
3. To determine obstetric and socio-demographic factors associated with *Chlamydia* infection.

METHODOLOGY

Study Design

This was a case control study. The cases were patients with ectopic pregnancies and the controls age-matched patients with incomplete abortions. The exposure was *Chlamydia* infection.

Study site

The study was done at the KNH acute gynaecological ward. KNH serves the population within and around the city of Nairobi and is also a National Teaching and Referral Hospital. It also serves as the teaching hospital for the College of Health Sciences of the University of Nairobi and the Kenya Medical Training College. The Department of Obstetrics and Gynaecology is one of the medical specialist departments at KNH.

The obstetrics unit consists of four antenatal/postnatal wards, a labour ward, maternity operating theatre, antenatal and post natal clinics while the gynaecology unit consists of a screening room (number 7) in the A & E department, gynaecology outpatient clinic (number 18) and acute and cold gynaecological wards – 1 D and 1 B respectively. Most evacuations for abortion are done in the Procedure Room in ward 1D.

The acute gynaecological ward admits patients from the A & E department and the gynaecology clinic. Each day at least one patient with ectopic pregnancy is admitted while six with incomplete abortion are treated by MVA. The department also has special outpatient clinics which include infertility, colposcopy, oncology and urogynaecology (fistula).

Study population/Study period

The study population included patients presenting with ectopic pregnancies and incomplete abortions at KNH, in April and May 2011. The principal investigator recruited patients with ectopic pregnancy from Sunday to Saturday and those with incomplete abortion on the same days. With an average of one ectopic per day, the sample size was achieved in two months.

Inclusion Criteria

- Patients operated at KNH for ectopic pregnancy
- Women presenting with incomplete abortion at KNH.
- Those who consented.
- Women aged between 14 and 40 years both ages inclusive.

Exclusion Criteria

- Women with other causes of bleeding in early pregnancy e.g. blighted ovum.
- Those who decline to give consent.

Sample size estimation

The sample size was calculated using the following formula [Fleiss JL Statistical Methods for Rates and Proportions (2nd edition). Wiley:New York, 1981.]. This formula was selected as we were comparing the proportions of Chlamydia infection between two groups.

For unequal groups of size n_1 and n_2, where $r = n_2/n_1$, is

$$n_1' = \frac{\left\{z_{\alpha/2}\sqrt{(r+1)\bar{p}\,\bar{q}} + z_{\beta}\sqrt{rp_1q_1 + p_2q_2}\right\}^2}{rd^2}$$

where $\bar{p} = \frac{p_1 + rp_2}{r+1}$ and $n_2 = rn_1$.

For small samples, employ a "continuity correction"

$$n_1 = \frac{n_1'}{4}\left(1 + \sqrt{1 + \frac{2(r+1)}{n_1' r|d|}}\right)^2.$$

Factor under consideration "prevalence of chlamydia"

1ST GROUP "Ectopic Pregnancies"

2ND GROUP "Abortions"

Assuming a Chlamydia infection rate of 67% among women with ectopic pregnancies (p_1) and the rate among women with abortion to be 50% less (p_2), and that we enroll an equal number of cases and controls (r = 1). To have 80% power to detect this difference with 95% confidence, we need to enroll 40 cases (n_1) and 40 controls (n_2) after applying the continuity correction for small samples.

Sample size **80**

Study instruments

A pre-coded questionnaire was used. We collected data on socio-demographic characteristics, history of STI, clinical presentation, history of contraceptive use, knowledge of *Chlamydia* infection and chlamydia test results.

Sampling technique

Convenient sampling of consecutive patients between 3rd April and 27th May, 2011 until desired sample size achieved.

Consenting procedure

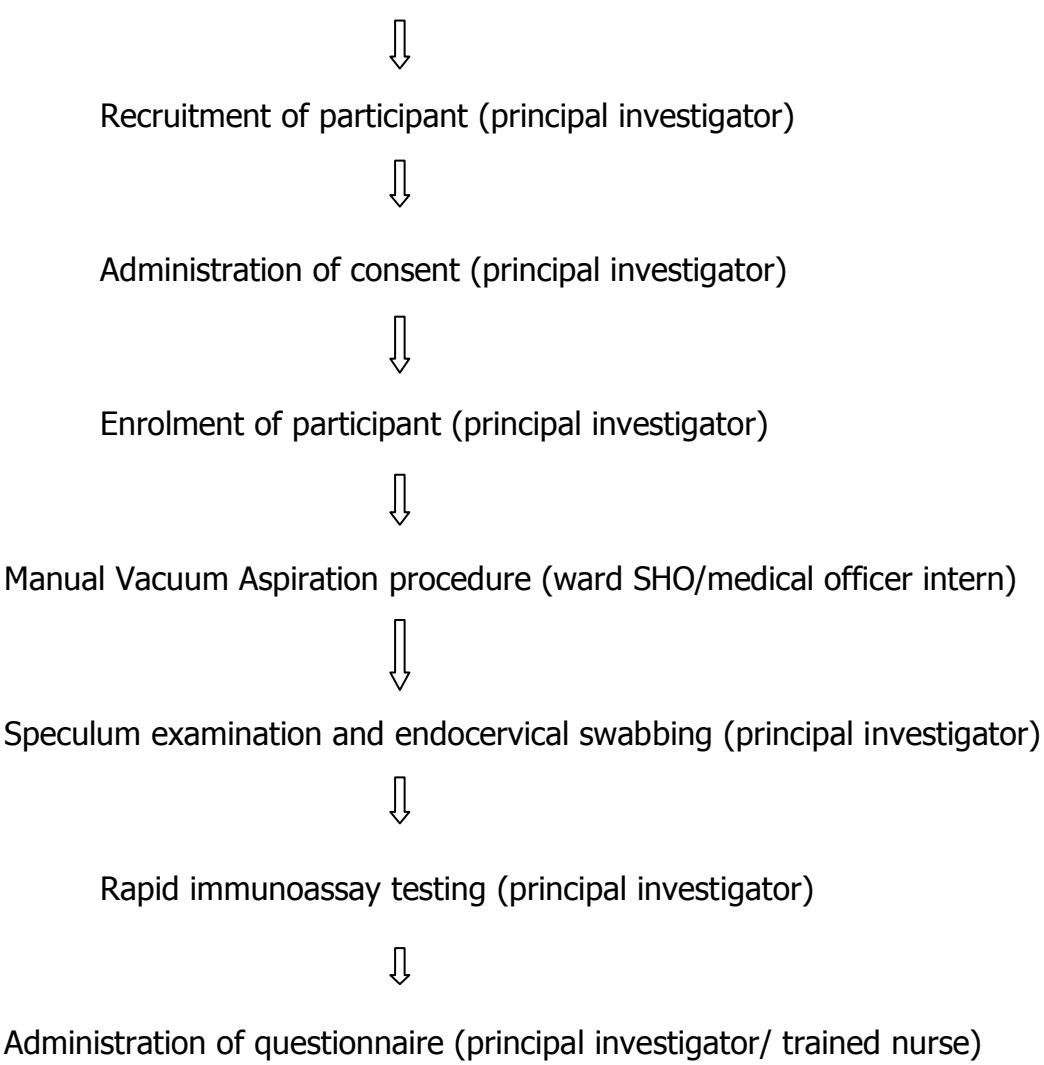
On recruitment of the selected participant, the principal investigator provided the consent form to the participant or read to those who could not. The participant then signed the form. For those below 18 years of age, consent was given by their guardians who had accompanied them to hospital. Patients who declined to consent were treated just as those who did without discrimination. Those who participated in the study were able to know if they had *Chlamydia* infection and if so were treated.

Recruitment of cases

Patients with gynaecologic emergencies including ectopic pregnancies are admitted through the A & E department. After surgery, they are admitted to the acute gynaecological ward (1 D). Every morning, the principal investigator identified patients who had surgery for ectopic pregnancy the previous day. He explained to the patient the purpose of the study and obtained consent. The participant so recruited underwent a speculum exam during which endocervical swabbing was done. Rapid immunoassay testing using Diaspot™ test kit was done to the specimen on the swab after which the tests results were communicated to the participant. If positive, treatment was given with oral doxycycline 100 mg twice daily for a week. The questionnaire was

administered by the principal investigator or trained nurse (research assistant).

Fig 1: Flow chart of screening, recruitment and enrolment of cases (Ward 1 D)



Recruitment of controls

On the same or following day, a patient with an incomplete abortion of the same age plus or minus 5 years as the ectopic pregnancy patient was recruited by the principal investigator prior to MVA. Upon explanation of the purpose of the study, consent was administered. The SHO on duty in ward 1 D then proceeded with the MVA procedure as follows. The participant was put in semi-lithotomy position and vulval toilet was done using chlorhexidine. A sterile speculum was then lubricated with normal saline and inserted to visualize the cervix.

A sterile swab (provided in the Diaspot^{TM} test kit) was introduced into the cervical canal, care being taken not to touch any surface of the speculum or genital area of the patient. The swab was then rotated 360^{o} in one direction, left to stand for 15 seconds then withdrawn.

The endocervical swab was put in an extraction tube that had 5 drops of **reagent A** of Diaspot^{TM}. The bottom of the tube was compressed and the swab rotated 15 times. After 2 minutes, 220 microliters of **reagent B** of Diaspot^{TM} was added into the tube using a pipette (provided in the Diaspot^{TM} test kit), the bottom compressed and the swab rotated 15 times. The solution turned clear with a light green or blue tint. After 1 minute, the swab was withdrawn while squeezing the tube and a dropper tip fitted to the top of the extraction tube. Then 3 full drops of the solution were then added to the Diaspot^{TM} Chlamydia Rapid Test Device and the result read after 10 minutes. The appearance of 2 distinct coloured lines indicated a positive result while 1 coloured line in the control line region denoted a negative result. If the

control line failed to appear, that was an invalid test and may have been due to an inadequate specimen volume or incorrect procedure technique.

On completion, test results were given to the participant and if positive, treatment provided with doxycycline 100 mg twice daily for one week. A questionnaire was then administered.

The files/outpatient cards of study participants were labelled with a coloured sticker with the initials "CPS" (Chlamydia prevalence study). This was to prevent double participant recruitment.

Quality control measures

Positive and negative controls were tested to confirm the test procedure and to verify proper test performance. In addition, the Diaspot^{TM} test device had an internal procedural control. A coloured line appearing in the control line region confirmed sufficient volume and correct procedural technique.

Data collection and management

Data collection was conducted by the principal investigator and one trained nurse in the acute gynaecological ward using a coded questionnaire. A biostatistician carried out data entry and analysis using Statistical Package for Social Sciences (SPSS 12) for Windows. Data analysis involved descriptive statistics like cross tabulation, frequency ranges and mean. Chi-square was used for proportions and p-value for significance.

Ethical considerations

- Approval for the study was sought and obtained from the Ethical and Research Committee of KNH.
- Voluntary informed consent was obtained from every participant prior to collection of the cervical swab and administration of the chlamydia rapid strip test and of the questionnaire.
- Refusal to participate did not affect the care given
- Results of chlamydia rapid test were availed to participants. Participants with positive results were treated immediately.
- Data and information were treated with confidentiality. The participant's names and in-/outpatient number did not appear on the questionnaire. A serial number was used.

Study limitations

This study had several limitations. Twenty patients with incomplete abortion could not be included as controls as they were admitted at night and discharged immediately after the MVA. However, we were able to achieve the required number of controls.

Although excess blood on the swab might cause a false positive result, the risk of this was minimised by doing endocervical swabbing at the end of the MVA procedure in patients with incomplete abortion. At this time, bleeding was minimal.

The Test used to detect *Chlamydia* antigens was not the gold standard, therefore the results may not be genralizable.

RESULTS

Between 3rd April and 29th May 2011, we recruited a total of 80 female patients presenting at KNH, 40 with ectopic pregnancy and 40 with incomplete abortions.

Table 1: Socio-demographic data of participants

Parameter	Abortion N=40 (*)	Ectopic N=40 (*)	All participants N=80 (*)	p value
Age				
Mean (95% CI)	26.5 (24.7-28.3)	28 (26.7-29.5)	28 (26.1-28.4)	0.16
Median (range)	26 (14-40)	28 (18-37)	27 (14-40)	
Marital status				
Married	32 (80%)	36 (90%)	68 (85%)	
Single	6 (15%)	3 (8%)	9 (11%)	0.543
Divorced/ Seperated	2 (5%)	1 (2%)	3 (4%)	
Education level				
Primary	25 (63%)	23 (58%)	48 (60%)	
Secondary	13 (33%)	15 (38%)	28 (35%)	0.893
University/ College	2 (4%)	2 (4%)	4 (5%)	
Occupation				
Unemployed	6 (15%)	5 (12%)	11 (14%)	
Self-employed	25 (63%)	17 (43%)	42 (53%)	0.183
Casual	8 (20%)	17 (43%)	25 (31%)	
Skilled	1 (2%)	1 (2%)	2 (2%)	

* Are percentages unless indicated

The table above is a presentation of the socio-demographic data of the study participants. The participant's ages ranged from 14 to 40 years with a mean age of 28 years. The participants with ectopic pregnancies ranged in age from

18 to 37 years with a mean of 28 years while those with abortions were between 14 and 40 years with a mean of 26.5 years. Most of the participants were married (68 / 80) while none was a widow. Most of the study participants were either self employed or casual employees (67/80) while only 2 were in skilled labour. Among those with abortion, 63% were self-employed. There was no difference in marital status, education, age or occupation between those with ectopic pregnancy and those with incomplete abortion. Therefore the two populations were similar.

Table 2: Obstetric and gynaecologic characteristics

Parameter	Abortion N=40 (*)	Ectopic N=40 (*)	All participants N=80 (*)	p value
Number of births				
0	3 (8%)	3 (8%)	6 (8%)	
1	11 (28%)	10 (25%)	21 (25%)	
2	17 (43%)	13 (33%)	30 (38%)	0.661
3	6 (15%)	11 (28%)	17 (21%)	
>3	3 (6%)	3 (6%)	6 (8%)	
Previous ectopic	0	1 (100%)	1	
Previous abortions				
0	33 (83%)	32 (80%)	65 (81%)	
1	5 (13%)	7 (18%)	12 (15%)	0.711
2	2 (4%)	1 (2%)	3 (4%)	
Age at first pregnancy				
Mean (95% CI)	18.7 (18-19.3)	18.3 (17.6-19)	18.5 (14-28)	0.514
Median (range)	19 (16-24)	19 (14-28)	19 (14-28)	

* Are percentages unless indicated

Obstetric and gynaecologic characteristics of the study participants are presented in table 2 above. An equal number of participants with ectopic pregnancy and incomplete abortion were recruited, 40 in each group. An equal number in these two groups were nulliparous while 59% (47/80) had 2-3 previous births. Only 1 participant had a prior ectopic pregnancy and her current admission had been for ectopic pregnancy. In our study, 81% (65/80) of the participants had on history of previous abortions. Mean age at first pregnancy was 19 years. No statistically significant differences were noted between those with ectopic pregnancy and incomplete abortion in obstetric and gynaecologic characteristics data collected.

Table 3: Sexual Characteristics

Parameter	Abortion N=40 (*)	Ectopic N=40 (*)	All participants N=80 (*)	p value
Age at first sexual intercourse				
Mean (95% CI)	19.2 (18.5-20)	19.2 (18.3-20)	19.2 (18.6-19.7)	0.935
Median (range)	19 (16-24)	18 (14-29)	18 (14-29)	
History of STI				
Yes	4 (10%)	2 (5%)	6 (8%)	0.396
No	36 (90%)	38 (95%)	74 (92%)	
Type of STI				
Discharge	3 (43%)	1 (33%)	4 (40%)	0.778
Lower abdominal pain	4 (57%)	2 (67%)	6 (60%)	
Treatment for STI				
Yes	4 (10%)	2 (5%)	6 (8%)	0.396
No	36 (90%)	38 (95%)	74 (92%)	
HIV status				

Positive Negative	1 (3%) 39 (97%)	6 (8%) 34 (92%)	7 (9%) 73 (91%)	0.048
Number of lifetime sexual partners 1 2 3 >3	 24 (60%) 12 (30%) 3 (8%) 1 (2%)	 16 (40%) 14 (35%) 7 (17%) 3 (8%)	 40 (50%) 26 (33%) 10 (12%) 4 (5%)	 0.236
Duration with current partner Mean (95% CI) Median (range)	 8 7	 7 6	 7 7	

* Are percentages unless indicated

Data on sexual characteristics is presented in the table (table 3) above. Mean age at first intercourse was similar in both groups, 19.2 years. Only 6 (8%) participants had a history of STIs, 60% (6) of these presenting with lower abdominal pain. Among our study participants, 1/40 (3%) of the abortion group was positive for HIV serology, while 6/40 (8%) of the ectopic group was HIV positive. This difference between the two groups was statistically significant (p = 0.048). The average duration of stay with the current partner was 7 years in those incomplete abortion and 8 years in those with ectopic pregnancy. While 5% (4/80) of participants had more than 3 lifetime sexual partners, 75% (3/4) of these had ectopic pregnancies.

Table 4: Knowledge on Chlamydia

	Abortion N=40	Ectopic N=40	All participants N=80	p value
Yes No	5 (13%) 35 (87%)	3 (8%) 37 (92%)	8 (10%) 72 (90%)	0.456

In the table 4 on knowledge on Chlamydia, only 10% (8) of participants were aware of Chlamydia. There was no significant difference between those with incomplete abortion and those with ectopic pregnancy.

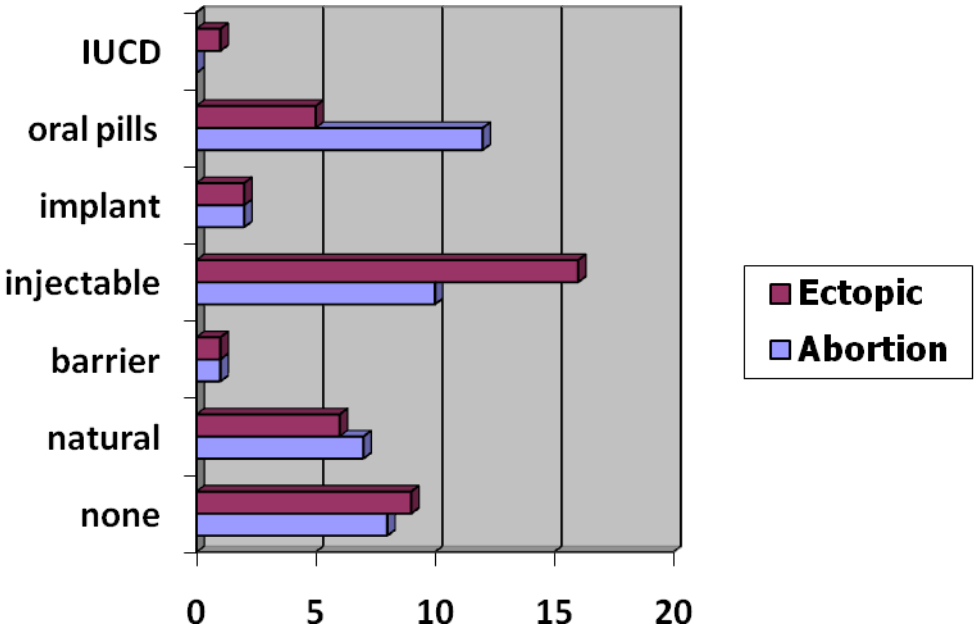
Table 5: Clinical characteristics

Symptom	Abortion N=40	Ectopic N=40	All participants	p value
Vaginal bleeding				
Yes	40 (100%)	29 (73%)	69	0.0004
No	0	11 (27%)	11	
Abdominal pain				
Yes	37 (93%)	39 (98%)	76 (95%)	0.305
No	3 (7%)	1 (2%)	4 (5%)	
Amenorrhea				
Yes	39 (98%)	39 (98%)	78 (98%)	0.320
No	1 (2%)	1 (2%)	2 (2%)	
Discharge				
Yes	15 (38%)	11 (27%)	26 (33%)	0.521
No	25 (62%)	29 (73%)	54 (67%)	
Dizziness				
Yes	13 (33%)	16 (40%)	29 (36%)	0.485
No	27 (67%)	24 (60%)	51 (64%)	

The presenting complaints of participants are presented in the table above. Among patients with ectopic pregnancy, 86% (29/40) had vaginal bleeding. Almost all of the participants in both groups had amenorrhea (39/40), while abdominal pain was more common in those with ectopic pregnancy (39/40) than with incomplete abortion (37/40). More participants with abortion than ectopic reported vaginal discharge (38% and 27% respectively) while dizziness was more common those with ectopic pregnancy (40%) than abortion (33%).

The graph below (graph 1) represents the type of contraceptive used by the participants prior to current pregnancy. Injectable contraceptives were the most common type of contraceptive used as they were used by 33% of participants, followed by oral pills (22%). The least common type of contraceptive used was IUCD; only one participant with ectopic pregnancy had used this method prior to current pregnancy.

Graph 1: Contraceptive use prior to current pregnancy



Graph 2 below represents of the *Chlamydia* antibody test, Three patients in the ectopic group and 1 patients in the abortion group were positive. This difference was not statistically significant. Due to the small number of positive tests we had inadequate power to detect a difference.

Graph 2: Results of Chlamydia antibody test

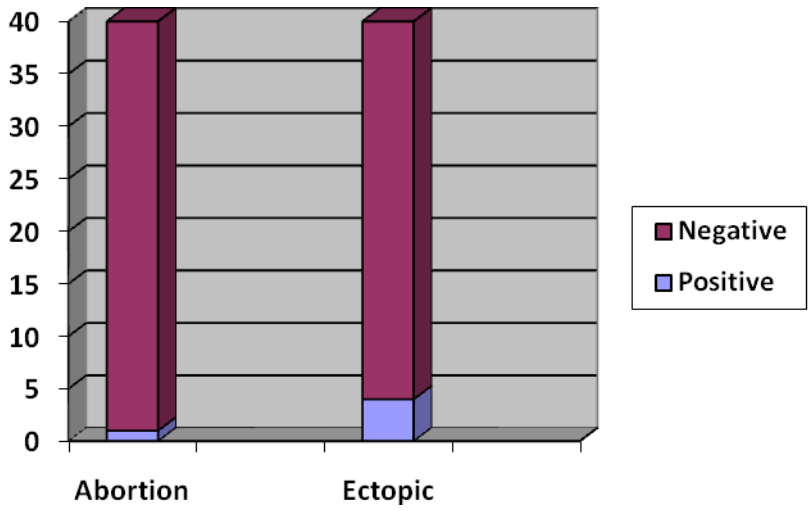


Table 6: Association of Chlamydia results with other characteristics

Characteristic	Chlamydia test positive	Chlamydia test Negative	p value
STI history			
Yes	0	6 (8%)	0.562
No	4 (100%)	70 (92%)	
Marital status			
Single	0	10 (13.2%)	
Married	4 (100%)	64 (84.2%)	0.690
Divorced/separated	0	2 (2.6%)	
Education level			
Primary	2 (50%)	46 (61%)	
Secondary	2 (50%)	25 (33%)	0.737
Tertiary	0	5 (7%)	

Occupation Unemployed Self-employed Casual Skilled	 0 4 (100%) 0 0	 11 (14%) 38 (50%) 24 (32%) 3 (4%)	 0.272
HIV status Positive Negative	 0 4 (100%)	 7 (9%) 69 (91%)	 0.528

All the participants who tested positive for *Chlamydia* (4/80) did not give a history of STI. All 4 who were positive for *Chlamydia* were married as were a vast majority of those who tested negative (64/76; 84.2%). All the women recruited for our study had formal education with 7% of those who tested negative for Chlamydia having college/university education. One half of those with negative test results were self-employed (38/76) as were all those with positive results (4/4). On HIV status, 8% of participants with positive Chlamydia test results were HIV seropositive.

DISCUSSION

This case control study is unique in the patients recruited as all other studies compare *chlamydia* prevalence between women with ectopic and intrauterine pregnancy. We compared prevalence of *chlamydia* in patients with ectopic pregnancy and incomplete abortion and this explains the younger age group compared to that by Lagarde and colleagues. Most of our study participants

were in the 15 to 29 years age range (78%) unlike 48% in the study by Lagarde and colleagues.[5] Our study was among women presenting in the acute gynaecologic ward while Lagarde, et al recruited women from among the general population.[5] Romeron et al in their study among ANC attendants had 32% of participants in this age group. These included those with gonorrhoea and *Chlamydia*.[9]

In this study, history of STI was found to be low at 8%. WHO estimates of the prevalence of STIs to be 11.9%.[1] In our study the prevalence of *Chlamydia* among those with ectopic pregnancy was 8% which is much lower than that found by Racheal, et al in the United Kingdom and Trinidad (51% and 67%).[11] This is due the lower overall prevalence of STIs in our study (8%) unlike in the West Indies where this was found to be 25%.[54] This is in keeping with current perceptions that with the advent of HIV the prevalence of treatable STIs maybe reducing.[58] This is attributed to increased condom use and extensive STI treatment programmes

Only 20% (8/40) of participants had heard of *Chlamydia* infection. This is very low when compared with a study by Macmillan and colleagues who found that 50% of their study participants were aware of this infection.[55] Norkels and Oakeshott found that 44% of women attending an inner city clinic knew about *Chlamydia.*[56] This marked difference may be due to the presence of screening programmes for chlamydia in the setting of the latter two studies. No studies have reported on knowledge in settings where there are no screening programs.

Data from the 2008-09 Kenya Demographic and Health Survey showed that HIV prevalence among women aged 15 – 49 was 8% which is similar to what we found in our study.[57] Nine percent (7/80) of our participants were positive for HIV serology. Due to the well established HIV serology screening programme at KNH, all the women who participated in our study were aware of their HIV status. Ninety three percent of all women admitted to ward 1 D are screened for HIV.

Although *Chlamydia* infection increases the risk of HIV transmission, none of the patients who tested positive for chlamydia antibodies had HIV seropositivity, however our numbers are too small.[50]

Prevalence of *Chlamydia* infection among participants with ectopic pregnancy was 3 times that among those with abortion. However this difference was not statistically significant due to low prevalence. This is however lower than the reported 8 fold increase in Chlamydia prevalence among patients with ectopic compared to term pregnancy. The fact that we used patients with abortion may explain the reduced difference.[9,11]

CONCLUSIONS

Overall Chlamydia prevalence in the population studied was lower than expected. Although prevalence was higher among patients with ectopic pregnancy, the difference was not statistically significant due to small numbers.

RECOMMENDATIONS

Routine treatment of Chlamydia among patients with ectopic pregnancy and abortions is not indicated.

In view of the low prevalence in our setting, there is need to look for other causes of tubal damage and ectopic pregnancy e.g. Bacterial Vaginosis.

There is need for larger studies to determine the prevalence of chlamydia among patients accessing services at KNH using rapid tests. DNA PCR tests are very expensive and inaccessible to the majority of patients and clinicians.

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PATIENT INFORMATION AND CONSENT FORM

Study to determine the prevalence of Chlamydia infection among patients with ectopic pregnancy and abortion at the Kenyatta National Hospital

Investigator: Dr. Eric Wanjala, Master of Medicine student, Department of Obstetrics and Gynaecology, University of Nairobi.

Supervisors:

Professor Shadrack B. O. Ojwang, Consultant Gynaecologic Oncologist, Professor, Department of Obstetrics and Gynaecology, University of Nairobi.

Dr. James N. Kiarie, Consultant Obstetrician and Gynaecologist, PMTCT Programme Advisor, University of Nairobi.

KHN Ethical Review committee Chairperson, Professor K. M. Bhatt, Telephone number 0202726300

Researcher's statement

We are asking you to participate in a research study on presence of antibodies to chlamydia infection among women with ectopic pregnancies and abortion at the Kenyatta National Hospital. The purpose of this form is to give you information about the study.

Please read the form carefully or listen carefully as it is read to you. You may ask questions about the purpose of the research, the possible risks and benefits, your rights as a volunteer and anything else about the research.

When all your questions have been answered, you can decide to participate in the study or not. If you wish we can give you a copy of this form for your records.

Purpose and benefits

The purpose of this study is to measure the prevalence of Chlamydia infection among women with pregnancy in the tube or uterus at Kenyatta National Hospital. Chlamydia infection often has no symptoms in many patients and is associated with pregnancy in the tube, infertility and infection in the reproductive and urinary system. This study will be useful in detecting the presence of this infection and preventing some of the above illnesses.

Procedure

If you consent to participate in the study, I will take a specimen from your cervix and place it on a test strip. The test result will be available in about 10 minutes and you will interpret it. If the result is positive, treatment will be given on the spot. I will also obtain information about you using a questionnaire.

Risks, stress or discomfort

Collection of the specimen, carrying out the test and completing the questionnaire will take about 15 minutes of your time.

Collection of specimen will cause some discomfort as a metallic or plastic speculum will be used.

If the test is positive, treatment will be given on the spot.

Confidentiality

All information obtained from you will be treated with utmost confidentiality. Your name will not appear on the questionnaire; a study number will be used instead.

You may choose to withdraw from the study, refuse to answer questions or decline the Chlamydia antibody test. Your decision will not affect health care service delivery to you at Kenyatta National Hospital.

Investigators signature..... Date.....

Participant's statement

I voluntarily agree to participate in the study on prevalence of Chlamydia infection in women with ectopic pregnancy and abortion at Kenyatta National Hospital. I understand that participation in the study does not entail financial benefit. I have been informed that information obtained will be treated with utmost confidentiality and my treatment will not be compromised if I decline participation or withdraw from the study.

I have had a chance to ask questions and if I have questions later, I will ask the researcher. If I have questions later about my rights as a research subject, I can call the ethical review committee at Kenyatta National Hospital on phone number 020726300.

Participant's signature or left thumb

print.....

Date.....

Participant's number.....

Questionnaire

1. Age of participant.....(years).
2. Type of pregnancy.....Ectopic / abortion
3. Last menstrual period.....
4. Marital status
    a. Single
    b. Married
    c. Divorced/separated
    d. Widowed
5. Level of education
    a. No formal education
    b. Primary
    c. Secondary
    d. University/college
6. Occupation
    a. Unemployed
    b. Self employed
    c. Employed (casual)
    d. Skilled employment (professional)
7. Number of births.....
8. Number of previous ectopic pregnancies.....

9. Number of previous abortions.....

10. Age at first sexual intercourse..... (years).

11. Number of lifetime sexual partners.....

12. Age at first pregnancy.....(years).

13. Contraceptive method used prior to the pregnancy

    a) None

    b) Natural methods

    c) Barrier methods

    d) Injectable progestin

    e) Progestin implant

    f) Combined oral pills

    g) Intrauterine device

    h) Tubal ligation

    i) Others.....

14. History of sexually transmitted infections

    a) Yes

    b) No

15. If yes what

    Ulcer, discharge, lower abdominal pain

16. Treatment for STI.....Yes/No/Don't know

17. HIV status

    a) Positive

    b) Negative

    c) Unknown

15. Have you ever heard of chlamydia……..Yes / No

16. Result of chlamydial antibody test……Negative/Positive

17. Any history of tubal surgery……..Yes / No

18. Clinical presentation

a) Vaginal bleeding……Yes/No

b) Abdominal pain….Yes/No

c) Amenorrhea……Yes/No……..If Yes, weeks……

d) Per-vaginal discharge

e) Dizziness……Yes/No

19. Abortion ……induced / spontaneous

20. Type of ectopic……tubal / abdominal /other……..

21. Duration with current partner……..