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STUDY ON THE ETIOLOGY OF ACUTE PELVIC INFLAMMATORY DISEASE IN WOMEN WITHIN THE AGES 16 - 40 YEARS AS SEEN AT THE SPECIAL TREATMENT CLINIC (STC), NAIROBI CITY COMMISSION

A dissertation presented in part fulfillment for the degree of Master of Medicine (Pathology and Microbiology) of the University of Nairobi.

By

Dr. Margaret Osolo Oduor

1991

1.1

DECLARATION

I certify that this dissertation is my original work and has not been presented for a degree in any other University.

..... Date..14/5/1991.....

Dr. Margaret Osolo Oduor

MB.ChB (Nairobi)

This dissertation has been submitted for examination with our approval as University supervisors.

..... Date..16.5.91.....

Dr. F. A. Plummer, MD, FRCP (C)

Assistant Professor, Department of Microbiology and Medicine, University of Manitoba, visiting lecturer, Department of Medical Microbiology, University of Nairobi.

..... Date..16th. May 1991.

Dr. J. O. Ndinya-Achola,

MB.ChB, MSc.

Chairman, Department of Medical Microbiology, University of Nairobi.

1.2

TABLE OF CONTENTS

1.3

LIST OF ABBREVIATIONS

STC	Special Treatment Clinic
PID	Pelvic Inflammatory Disease
IUCD	Intrauterine Contraceptive Device
STD	Sexually Transmitted Disease
LGT	Lower Genital Tract
UGT	Upper Genital Tract
SBA	Sheep Blood Agar
TM	Thayer Martin
KNH	Kenyatta National Hospital
HPF	High Power Field
PPNG	Penicillinase Producing *Neisseriae gonorrhoeae*
Ml	Millimetres
RPR	Rapid Plasma Reagin
Mg	Milligrammes
CAMP	Christie, Atkins and Munch Peterson
SD	Standard Deviation
Gc	Gonococcal
Gm+ve	Gram Positive
Gm-ve	Gram Negative
CT	*C. trachomatis*
TL	Tubal Ligation
GNDC	Gram Negative Diplococci
NS	Not Significant
X^2	Chi-Square

1.4

LIST OF TABLES AND FIGURES

PAGES

1.5

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2.0

SUMMARY

Acute Pelvic Inflammatory disease is a common gynecological problem in many parts of Africa. It is one of the major problems seen at the Sexually Transmitted Disease Clinic at Nairobi City Commission. In this study, 100 women within the ages 16-40 years with clinical signs and symptoms of acute pelvic inflammatory disease and another 100 women within the same age group but without clinical signs and symptoms of acute PID were enrolled for the study.

The main objective of the study was to study the prevalence of *Neisseria gonorrhoeae, Chlamydia trachomatis* and Group B *Streptococcus (Streptococcus agalactiae)* in women with clinical signs and symptoms of acute pelvic inflammatory disease. Also to study the relation of acute PID in terms of age, parity, marital status and contraception method used by the patient. These findings were then to be compared to those seen in the control women.

These women were screened for *N. gonorrhoeae, C. trachomatis, Group B. Streptococcus* using endocervical cultures on Thayer Martin, blood agar and irradiated McCoy cell lines, and also by Gramstain of endocervical smears. They were also screened for *Treponema pallidum* antibodies using rapid plasma reagin card test.

Acute pelvic inflammatory disease was found to be more prevalent in the younger age group below 25 years of age with a peak at 16-20 years.

Oral contraceptive use was correlated with a significantly decreased risk of acute PID (odds ratio 0.78, p-value<0.05) and IUCD use correlated with a significantly increased risk of acute PID (odds ratio 1.27, p-value<0.05).

Gonococcal acute PID was found to be commoner in a younger age group than non-gonococcal acute PID. Risk of gonococcal acute PID was also found to be significantly increased in the single, separated (odds ratio 3.12; p-value<0.05) and the divorced women (odds ratio 1.67; p-value<0.05).

Chlamydial acute PID was found to be commoner in a younger age group as compared to non-chlamydial acute PID.

Group B streptococcus was isolated from only 1% of acute PID cases.

2.1 INTRODUCTION AND LITERATURE REVIEW

Pelvic inflammatory disease (PID) is defined as an ascending infection of the uterus, fallopian tubes and broad ligaments (1). It has also been defined as a non-specific inflammatory response of the upper genital tract of women (2, 3); and by some as a term which poorly describes a number of infectious conditions, namely, cervicitis, endometritis, salpingitis, salpingo-orphoritis, peritonitis or perihepatitis (4). In this text, PID and salpingitis are used synonymously.

PID is a major health problem in many parts of the developing world (5). It is a common gynecologic problem in many parts of Africa. About 40% of acute admissions to the gynecology wards in two Nairobi hospitals are due to PID (6) and 44% of all gynecological admissions in Rhodesia was also found to be due to PID (7). PID has also been found to be the most significant of all the complications of sexually transmitted diseases in American medicine (8).

Pathophysiology

Infections of the upper female genital tract can be divided into two main groups namely:

a. Those that are primarily genital and
b. Those that are primarily non-genital.

Infections that are primarily genital ascend from the lower genital tract (LGT) upwards to involve the upper genital tract. In women in the fertile years the majority of the tubal infections are primarily genital. Only about 1% of tubal infections in women within the fertile years are caused by

direct spread of infections from other organs of the pelvic cavity the most frequent being from the appendix (9).

The factors that determine the spread of an infection in the lower genital tract upward to the fallopian tubes are poorly understood but some observations have been made that might be of importance in this context. These include:

a. Cervical factors

In health, the cervix offers a functional barrier for the ascent of micro-organisms. The cervical plug is present during major part of the menstrual cycle and this mechanically hinders the penetration of the cervical mucosa by the pathogens. Cervical secretions of antibodies and enzymes (for example lysozymes) as well as slow downward flow of secretions has been suggested to confer resistance against invading micro-organisms (10).

The cyclical variations in the sexual hormones cause changes in all the cervical protective properties mentioned above. It has been found that these protective properties of the functional barrier are at their lowest at the time of ovulation and when the mucous plug is absent, that is during menstrual bleeding and after abortion or parturation. Hence PID onset occurs more commonly during these periods (10, 11, 12).

b. Hormonal factors

Hormonal treatment causes changes in the properties of the functional barrier. Administration of progesterone which induces a mechanically resistant cervical plug seems to protect from ascending infection (13). The use of combined (estrogen) oral contraceptives inhibit ovulation as

well as periovulatory changes in the cervix. Women using this method of contraception are less likely to develop salpingitis than women using other contraceptives (14).

Estrogens on the other hand causes squamocuboidal junction between epithelia of the portio and cervix (transitional zone) to move further out on the surface of the portio thus offering a larger area of ectopic cervical cuboidal epithelium to the invading organisms. This may facilitate the establishment of infections for example by gonococci, chlamydia and mycoplasma (14).

c. Uterine myometrium activity

The muscular activity of the myometrium might be of importance for the mechanical transport of particles from the internal cervical oriffice upwards in the genital tract (2). The micro-organism make its way up the female genital tract either by adherence and an accelerator organismic (coital) uterine contractions or by being carried along by spermatozoa (4). It has also been observed that the presence of intrauterine contraceptive device (IUCD) in the uterine cavity increase myometrial activity while administration of progesterone decreases the myometrial activity (13). This observation might explain in part why women using IUCD have an increased risk of developing PID while those on progesterone drugs have a decreased risk as compared to non-contraceptive users.

Risk factors

a. Contraceptive use

A large number of studies have demonstrated an increased rate of acute salpingitis in women who use IUCD as compared to non-users (14, 15, 16).

Risks increases for salpingitis among women who use IUCD as compared to non-users has been calculated to be 3 to 5 fold. In women who have not been pregnant at any one time, the risk increased 7 to 9 fold (14-15).

Most studies, however, agree on a decreased risk of salpingitis in women using oral contraceptives (14, 15, 17, 18). Further among women with clinical signs and symptoms suggestive of PID, those who use oral contraceptives have laparascopic evidence of acute PID less often than other women (19, 20).

b. Sexual activity

There is increased risk of salpingitis in the sexually active. The risk of acquiring sexually transmitted disease and hence STD related salpingitis is correlated with the number of sexual partners (18). Those women with multiple sexual partners stand a greater risk of acquiring STD and hence related PID.

In women who are not sexually active, for example nuns, acute salpingitis is extremely rare (13).

c. Iatrogenic factors

In medical practice, some procedures are performed that open up the cervical canal and can bring micro-organisms into the upper genital tract. These procedures include legal abortion, IUCD insertions, curretage and histero-salpingography. In a series of salpingitis patients, such procedures preceded onset of the disease within four weeks in up to 12% of the cases (21).

d. Age and parity

Some studies have found PID to be more prevalent among women of younger age (14). In most series one out of four women afflicted with salpingitis has been below the age of 25 years (13). Of the total number of women with salpingitis, one third have their first episode of the disease before the age of 20 years (22). Approximately three out of four women afflicted with salpingitis are nulliparous, and half of the salpingitis patients have never been pregnant at any one time (22).

Sexually transmitted disease related salpingitis is proportionally more common in women less than 25 years of age than in those over 30 years of age. On the other hand infections caused by anaerobic bacteria are more prevalent in the somewhat older salpingitis patients and also in women who have repeated infections (22, 23).

Aetiological agents

Pelvic inflammatory disease can be initiated by numerous organisms, the majority of cases are probably attributable to gonorrhoea and chlamydia.

a. *Neisseria gonorrhoeae*

This is the classic sexually transmissible agent accepted as a cause of acute salpingitis. It is the major pathogen in PID in many areas of Africa (6, 7, 24). Although the reported ratio of gonococcal to non-gonococcal cases seen varied in different areas and also with time in the same setting, it has been suggested that between 10 and 15% of women with cervical gonorrhoea are complicated by salpingitis. On the other hand, the reported incidence of cervical gonorrhoea in patients with acute salpingitis varied from 0-65% (6, 7, 23, 24, 25, 26, 27, 28, 29).

b. *Chlamydia trachomatis*

This is the most common cause of sexually transmitted disease in many western societies (30, 31). Some data from United States suggest that chlamydia is at least as prevalent as gonorrhoea. In United States STD clinics, endocervical chlamydia isolation rates range from 12-22% (32). Several African studies have also found a high prevalence of chlamydia. A study in Gambia of women attending for antenatal care 6.9% had endocervical chlamydia (33). A Kenyan survey found chlamydia antibodies in 91% of women attending STD, antenatal and family planning clinics (34).

Gonococcal and chlamydial infections often coexist. In several European and American studies 25-60% of women with gonorrhoea also had

chlamydia (35). In one Gambian study, of the 65 men with gonorrhoea, 14% also had chlamydial infections (32).

c. *Mycoplasma hominis*

This pathogen is also believed to play a prominent role in the pathophysiology of PID (35). There have been several reports on isolation of *Mycoplasma hominis* from the cul-de-sac or the internal female genital tract in women with PID (25). The percentages of *Mycoplasma hominis* isolates from varied studies in the western world ranged between 4-72% (23, 27, 37).

d. Anaerobic bacteria

Facultative as well as strictly anaerobic bacteria as well as aerobic bacteria species normally found in the endogenous flora of the lower genital tract and the bowel have been isolated from the upper genital tract of women with PID (23, 29, 36, 38). The anaerobic organisms so isolated include *Bacteroides Clostridium* and *Peptostreptococcus*.

Anaerobes are found more commonly in cul-de-sac specimens of women who have had repeated episodes of salpingitis than in those having their first attack of the disease (25).

e. Uncommon etiologic agents

These include primary respiratory tract pathogens like Group A streptococcus (39), *Haemophilus influenzae* (37), *Camplylobacter fetus* (40), Actinomyces (41), *Enterobius vercularis* (42) and *Ureaplasma urealyticum* (37).

Clinical manifestations and diagnosis

Due to the limited availability of the diagnostic facilities to the majority of the physicians especially those working in developing countries, the diagnosis of PID is based on a series of signs and symptoms. Signs and symptoms usually seen in PID cases include low abdominal or pelvic pain which is subacute in onset and dull in character, purulent vaginal discharge, metrorrhagia, signs and symptoms of urethritis, symptoms of proctitis, dyspareunia pain or discomfort in the upper right abdomen and nausea and vomiting (13, 21, 22).

Objective findings include: rectal temperature of 38° C or more, palpable adnexial mass and increased number of leukocytes in microscopy of wet mounts of vaginal contents.

Laboratory findings

a. Erythrocyte sedimentation rate (ESR) of more than 15mm per hour.

b. White blood cell count of more than 10,000/mm^3.

c. Antichymotrypsin, oromucoid and C-reactive protein levels in serum of greater than 80%.

d. Isoamylase specific for genital tract, the levels in serum are either decreased or absent (43, 44).

Clinical diagnosis

This is based on a combination of signs and symptoms. A minimum criteria of the following signs and symptoms is clinically accepted as signifying acute PID (2, 21).

a. Low abdominal pain.

b. Signs of lower genital tract infection (purulent vaginal discharge on microscopy or observed).

c. Pelvic tenderness.

Based on the presence of the above three signs and symptoms only (minimum criteria) PID is confirmed in 50-61% of cases (2, 21).

If in addition to the minimum criteria, a patient has the following symptoms and/or signs.

a. Temperature of $\geq 38^{o}$ C
b. Palpable adnexial mass and
c. Irregular menstrual bleeding;

the probability of the disease increases to 68-96% (2, 21). The probability increases even further if one finds any of the following:

a. ESR > 15mm/hour

b. WBC > 10,000/mm^3

c. Increased levels of C-reactive protein in serum

d. Cervical cultures positive for Gonococci or Chlamydia.

Prognosis

Prognosis of acute salpingitis is good. Death from acute PID is rare and if it does occur, it is mainly due to rupture from a tubo-ovarian abscesses with generalized peritonitis. In such cases the mortality has remained at 8-9% over the years (45, 46).

Sequelae

The late sequelae of salpingitis are : chronic abdominal pain, infertility and an increased risk of ectopic pregnancy.

a. Chronic abdominal pain

The cause of chronic abdominal pain is thought to be due to increased intra-ovarian pressure induced by cycle-related volume changes, which are made painful by adhesions surrounding the ovaries. Such pain was recorded in 18.1% of those women in a follow-up study who had had acute salpingitis (47). This kind of pain was correlated with a number of infections and was more common in women who are infertile.

b. Infertility

Acute PID is the major cause of infertility in women within the reproductive age (47, 48, 49). It has been observed that it is a major cause of tubal occlusion and hence infertility. After one episode of acute PID the corresponding incidence of tubal occlusion is 12.8%, this increases three times after two episodes and six times after three or more episodes of the disease (47).

c. Ectopic pregnancy

Post infection tubal damage is one of the most common etiologic factors in ectopic (tubal) pregnancy. In a series of tubal pregnancies about 50% of them had a previous history of earlier salpingitis (50). Women in the post-salpingitis state were found to have a 7-10 fold increased risk for ectopic pregnancy as compared to women who have never had the disease (50).

The importance of understanding the pathogen(s) responsible for acute PID therefore lies in the need to institute appropriate and adequate therapeutic measures to reduce the complications that would follow inadequate treatment. Unrecognized or inadequately treated PID has a high risk of causing subsequent infertility or pregnancy wastage (ectopic pregnancy).

3.0

AIMS AND OBJECTIVES

The general objective was to study the aetiology of acute PID and its relation to age, parity, marital status and method of contraception used by the affected individual as compared to the controls as seen at special treatment clinic, Nairobi City Commission.

The specific objectives were:

1. To evaluate the objective criteria for clinical and laboratory diagnosis of acute PID.

2. To determine the prevalence of *Neisseria gonorrhoeae, Chlamydia trachomatis* and Group B Streptococcus (*Streptococcus agalactiae*) in patients with acute PID as compared to the controls.

3. To study the pattern of presenting signs and symptoms of acute PID in relation to the pathogens isolated.

4. To study the distribution pattern of the isolated pathogens in terms of age, parity, marital status and contraception method being used.

5. To compare the distribution pattern of gonococcal related acute PID and non-gonococcal related acute PID.

6. To compare the distribution pattern of Chlamidial related acute PID and non-Chlamidial related acute PID.

7. To determine the prevalence of *Treponema pallidum* (syphilis) antibodies in sera of acute PID cases as compared to the control.

4.0

MATERIALS AND METHODS

This study was conducted at STC Nairobi City Commission which serves as a referral clinic for sexually transmitted diseases and skin diseases. The referrals are made from City Commission health centres and dispensaries all over Nairobi city.

This study was carried out between the months of July and December 1989. One hundred women between the ages of 16-40 years with clinically verified acute PID and a similar number of controls without signs and symptoms of acute PID (control group) were enrolled for the study. Selections of these cases was done by the investigator to avoid discrepancy.

Signs and symptoms of acute PID which were used as inclusion criteria in the study were:

a. Persistent lower abdominal or pelvic pain not exceeding 7 days.

b. Pelvic tenderness on palpation

c. Purulent vaginal discharge

d. Fever $\geq 38^{o}$ C

e. Palpable adnexial mass

f, metrorrhagia

g. Nausea and vomiting

h. Symptoms of urethritis.

Patients were excluded if they had taken antibiotics within the last seven days, when signs and symptoms have lasted for more than 7 days and if they refused to give consent.

Patients with a combination of symptoms a, b and c or a, b and any two of the other symptoms d-h were enrolled as the study 'cases' after obtaining their consent. Control group was drawn from those women who had come to the clinic with other complaints but had been clinically verified as not having acute PID. Controls were also enrolled after obtaining their consent to be included in the study.

At enrollment, demographic data, method of contraception, parity, marital status, presenting symptoms and physical findings on examination were entered in a questionaire (See Appendix I). It was then specified on the questionaire whether the patient enrolled belonged to the study cases or to the control group as enrollment of both the two groups was done concurrently each day.

After the general physical examination and palpation of the abdomen to elicit tenderness and any palpable masses, a vaginal speculum examination was then performed. It was noted whether there was purulent vaginal discharge or not. Endocervical swabs were then obtained using Calcium alignate on plastic shaft swabs. From each patient two swabs were obtained:

For the first swab, sheep blood agar (SBA) and Thayer Martin (TM) media were inoculated in that order, and a smear was made on a clean

microscopy glass slide for direct Gram stain. The tip of the second swab was immersed in the Chlamydia transport medium.

Smears for microscopy on glass slides were air dried in an upright position in a rack at room temperature. At the end of the clinic day they were transported back to the laboratory in the department of medical microbiology, University of Nairobi, Kenyatta National Hospital (KNH) where Gram staining was done (See Appendix III) and analysis under the light microscope performed. The slides were reported as shown below and results entered in Appendix II.

Number of pus cells per high power field (HPF):

No pus cells	= 0 (-)
Pus cells < 5/HPF	= + (1+)
Pus cells 5-10/HPF	= ++ (2+)
Pus cells 11-20/HPF	= +++ (3+)
Pus cells 21-30/HPF	= ++++ (4+)
Pus cells >30/HPF	= +++++ (5+)

Micro-organisms present were also reported on Appendix II. Gram negative diplococci were specifically looked for.

Smears on the TM plates for isolation of *N. gonorrhoeae* were held at room temperature (22° C).after inoculation until the end of the clinic day. They were then transported back to the laboratory in humidified candle extinction jars. Once in the laboratory, streaking was done and plates inoculated in humidified candle extinction jars at 37° C and examined at 48 hours. *N. gonorrhoeae* was identified by colonial morphology, oxidase reactivity and its Gram stain characteristics.

Penicillinase producing *N. gonorrhoeae* (PPNG) strains were identified by the chromogenic cephalosporin method. This method tests for the production of the enzyme Beta-lactamase by PPNG strains. This procedure is as follows: A solution of the chromogenic cephalosporin in dimethyl sulfoxide (DMSD) is made and diluted in phosphate buffered water to a pH of 7.0 and cephalosporin concentration of 500 mg/ml. This solution is yellow. Few drops of this solution is placed in a microtiter plate well and to this is immersed a single colony of *N. gonorrhoeae* from culture plate. This is then mixed and left to stand for 2-3 minutes. If the strain being tested is PPNG positive, the colour of the solution changes from yellow to red purple. But if the stain is PPNG negative, then no colour change of the solution is observed.

Smears made on the SBA plates were for the isolation of Group B streptococcus. After primary inoculation, these plates were held at room temperature until transportation to the laboratory. Once in the laboratory, streaking was done and plates incubated aerobically at 37° C and examined at 48 hours. Group B streptococcus was identified by their colonial morphology, Gram stain characteristics, catalase test and confirmed by CAMP test.

Specimens for isolation of *Chlamydia trachomatis* were placed in Chlamydia transport medium and held at room temperature until transportation to the laboratory in ice jar. Specimens were then stored at -70° C until cultured for *Chlamydia trachomatis*. Cultures were done on cyclohexamide treated McCoy cells and examined at 72 hours, using a monoclonal antibody fluorescent stain. At 72 hours coverslips were removed and fixed with methanol then stained with fluorescent dye. The coverslips were then examined under fluorescent microscope.

Under fluorescent microscope, cells stain reddish and *Chlamydia trachomatis* lemon green, which fluoresce. Grading of the positive results were as follows:

>50 particles in all the fields scanned	= 4+
25-50 " " " " " "	= 3+
10-24 " " " " " "	=2+
<10 " " " " " "	∓1+

From each patient, 5ml of blood was obtained by venepuncture for syphilis screening. These were collected in sterile tubes and taken back to the laboratory where they were screened for syphilis using RPR. Results were reported as reactive or non-reactive.

Patients were given feedback of both the Gram stain, cultures and RPR results. Those found positive were treated for respective pathogens isolated using the current treatment methods at the clinic.

These regimes included:

For **gonorrhoea**: - 3.5 Ampicillin + 250 mg Augmentin
+ 1 g Probenecid given as a single dose at the pharmacy under supervision.

For **Syphilis:** - Intramascular injection of 2.4 mega units of Benzathine penicillin given as a single dose for those who have no symptoms and signs but positive on screening for syphilis. Those patients found to be positive and also have signs and symptoms of syphilis were given 2.4 mega units of Benzathine penicillin given on day one then on every fourth day for a total of four doses.

Data analysis

Odds ratios were used to estimate the associations of acute PID with marital status, and also with contraceptives' use and also to estimate associations between gonococcal related acute PID with marital status and with contraceptives' use. The significance of these associations were measured by a Chi-square (X^2) test. Significant P value was taken at 5% level and degree of freedom as one.

RESULTS

The total number of patients studied were 200, 100 study cases and another 100 controls.

Age

The age ranged between 16-40 years. The study cases group was found to have a mean age of 23± 5 while the control group had a mean age of 25±6 years. Frequency distribution of ages for both groups are shown in Table 1.

Parity

The range was found to be between 0-8 for both study cases and control groups. Nulliparous women were found to form 29% of the study cases group and 23% of the controls. Most patients from both groups were found to be of low parity; 69% of the study cases group and 73% of the controls had between 0-2 children. These findings are shown in Table 2.

Marital Status

Majority of the cases were married; 65% of the study cases and 56% of the controls were married. Only 4% of the study cases and 11% of the controls were either separated or divorced (Table 3).

CONTRACEPTION

Most of the study patients used no contraceptives 78% of the study cases and 72% of the controls used no contraceptives. Only 10% of the study cases compared to 13% of the controls used oral pills while 5% of the study cases compared to 3% of the controls wore IUCD (Table 3).

GRAM STAIN

Gram staining was done on all the 200 specimens. Quantity of pus cells as seen under x 100 high power field was noted. All the study cases had pus cells in their smears ranging from 1+ to 5+ while only 26% of the controls had pus cells in their smear ranging from 1+ to 4+. Gram negative diplococci were detected in 42 of study cases and from 4 of the controls (Table 4). Of the 42 positive study cases, 25 were confirmed by cultures as N. gonorrhoeae giving a sensitivity of 70.0%. And of the 58 negative study cases on Gram Stain 52 were also negative by cultures for N. gonorrhoeae. This gave a specificity of Gram Stain for detection of N. gonorrhoeae as 83.3% . Other studies give sensitivity and specificity of single Gram Stain on cervical secretions to range between 50-70% and 95-100% respectively (13) (Figure 2).

ENDOCERVICAL CULTURES FOR N, GONORRHOEAE

Out of the 100 study cases, 96 were analysed for N. gonorrhoeae by cultures. Culture plates of 4 cases got contaminated hence could not be interpreted. Of these 96 cases, 29(32.2%) grew N. gonorrhoeae of which 27 (93.8%) were non-penicillinase producing strains while 2 (6.2%) were penicillinase producing strains. Low isolation rate of PPNG strains could be due to (i) Bias in selection of study cases . (ii) Small size of the study group.

Of the 100 controls, only 98 specimens were examined after cultures for N. gonorrhoeae. Two plates got contaminated. Only one control grew non PPNG strains.

Comparing the gonococcal acute PID and non gonococcal acute PID, it was found that the mean age for gonococcal acute PID was 22±4 years while that for nongonococcal acute PID was 24±5. Mean parity for both groups was 2±1.

Out of the 29 gonococcal acute PID, 18 (61.8%) were married while 11 (38.2%) were not married. Twenty four (82.6%) of these gonococcal acute PID cases used no contraceptives while only 2 (6.9%) used oral contraceptives and only 1 (6.9%) wore IUCD.

From the non-gonococcal acute PID, 68.4% were married while only 1.5% were either separated or divorced. 76% used no contraceptives while 10.4% used oral contraceptives and 6% wore IUCD. These findings are summarized in Table 5.

Endocervical cultures for C. trachomatis

Speciment from 94 study cases and from 93 controls were cultured for Chlamydia trachomatis. Specimens from 6 study cases and from 7 controls could not be traced from the storage packs where they had been kept while awaiting cultures. Of the 94 cultured specimens from the study group 4(4.2%) were positive for C. trachomatis (Figure 1). There was no detection of C. trachomatis from any of the controls. Mean age for the chlamydia related acute PID was 21±5 as compared to the mean age of 24±5 for the non-chlamydia related acute PID. Mean parity for the Chlamydia related acute PID and non-chlamydia related acute PID were

1±1 and 2±2 respectively. Of the four *C. trachomatis* positive cases, 3 (75%) were married while 1 (25%) was single. From the non-chlamydia related acute PID, 27.7% were single while 67.7% were married. Again 3 (75%) of these 4 positive cases used no contraceptives while only 1 (25%) used oral contraceptives (Table 6).

Endocervical cultures for Group B Streptococcus (*Streptococcus agalactiae*)

From all the 100 study cases as well as 100 controls cultures for Group B streptococcus were done. Only 1(1%) from each respective group grew Group B streptococcus (Figure 1).

Screening for syphilis

Sera from all the 100 study cases and 100 controls were screened for Syphilis. From the study cases group, 5 (5%) were found positive while 1(1%) from the control group was found positive. Of the 5 positive cases from the study group, 3(60%) were found also to be positive for *N. gonorrhoeae* on cultures while 1(20%) was also positive for *C. trachomatis* on cultures.

Presenting signs and symptoms

All the 100 study patients presented with lower abdominal or pelvic pain, purulent vaginal discharge and pelvic tenderness on palpation. Additional signs and symptoms encountered were: symptoms of urethritis in 10%, metrorrhagia in 4% and palpable adnexial mass in 1%.

Table 1: Frequency distribution of the ages of study cases and of controls.

Age in Years	Number of Subjects	
	Study cases (n=100)	Controls (n=100)
Mean ± SD	23 ± 5	25 ± 5
Age groups		
16 - 20	41	24
21 - 25	33	41
26 - 30	15	25
31 - 35	9	4
36 - 40	2	2
Total	100	100

This table shows that acute PID is commoner in a younger age group with peak at 16-20 years as compared to the general population attending the clinic.

Table 2: Frequency distribution of the parity of both study cases and controls

Parity (Number of children)	Number of subjects	
	Study cases (n = 100)	Controls (n = 100)
Mean ± SD	2 ± 2	2 ± 2
0	29	23
1	30	29
2	10	23
3	9	10
4	7	5
5	4	5
6	1	3
7	1	2
8	1	1
Total	100	100

This table shows that acute PID is commoner in the nulliparous and in the low parous women (para 4 or less) than in the multiparous women (para 5 or more).

Table 3: Frequency distributions of marital status and contraceptive use amongst study cases and controls

	Number of subjects (100)	(100)	Odds Ratio	X^2	P value
Marital status:					
Single	32	33	0.96	0.06	<0.05
Married	65	56	1.29	6.69	>0.01(NS)
Separated	2	7	0.45	2.78	<0.05
Divorced	2	4	0.69	0.67	<0.05
Total	100	100			
Contraceptive method:					
None	78	72	2.69	0.24	<0.05
Oral Contraceptive	10	13	0.81	0.39	<0.05
IUCD	5	3	1.22	0.50	<0.05
Barrier	2	0	2.02	2.50	<0.05
TL	2	5	0.56	1.29	<0.05
Depoprovera	3	7	0.84	1.14	<0.05
Total	100	100			

This table shows that the separated and the divorced are at significantly increased risk of getting acute PID. It shows that oral contraceptive use, tubal ligation and depoprovera use significantly protect against acute PID while IUCD use and non-contraceptive use are significantly associated with increased risk of acute PID.

Table 4: Gram stain results in terms of pus cells per high power field and gram negative diplococci detected

Number of pus cells per HPF.	Number of Subjects	
	Study Cases (n = 100)	Controls (n = 100)
0	0	73
1+	25	15
2+	43	2
3+	16	7
4+	9	3
5+	7	0
Total	100	100
N. gonorrhoeae Positive	42	4
negative	58	96
Total	100	100

This table shows that all the study cases had pus cells ranging from 1+ - 5+ in their cervical secretions. From the control group only 27% had pus cells in their cervical secretions and 73% of them did not have any pus cells.

Table 5: Comparison between gonococcal related acute PID and non-gonococcal related acute PID

	Number of Subjects (%)		Odds ratio	X^2	P Value
	GC (n=29)	non GC (n=67)			
Age mean ± SD	22 ± 4	24 ± 5			
Parity (mean ± SD)	2 ± 1	2 ± 1			
Marital status:					
Single	8 (27.2)	20 (29.8)	0.92	2.86	<0.05
Married	18 (61.8)	46 (68.4)	0.81	12.25	>0.001(NS)
Separated	2 (6.9)	0 (10)	3.10	0.5	<0.05
Divorced	1 (3.4)	1 (1.5)	1.67	0	<0.05
Total	29 (99.3)	67 (99.7)			
Contraceptive method					
None	24 (82.6)	51(76.0)	1.34	6.98	>0.01 (NS)
Oral contraceptives	2 (6.9)	7 (10.4)	0.96	2.78	<0.05
IUCD	1 (3.4)	4 (6.0)	0.67	1.80	<0.05
Barrier	1 (3.4)	1 (1.5)	1.67	0.0	<0.05
TL	0 (0)	2 (3.0)	0.0	2.0	<0.05
Depoprovera	1 (3.4)	2 (3.0)	1.10	0.33	<0.05
Total	29 (99.7)	67 (99.7)			

This table shows that (1) Acute PID had a significantly increased risk in the separated and the divorced (2) IUCD and barrier use are significantly correlated with increased risk of acute PID while oral contraceptives use, tubal ligation and depoprovera are significantly correlated with a decreased risk of acute PID.

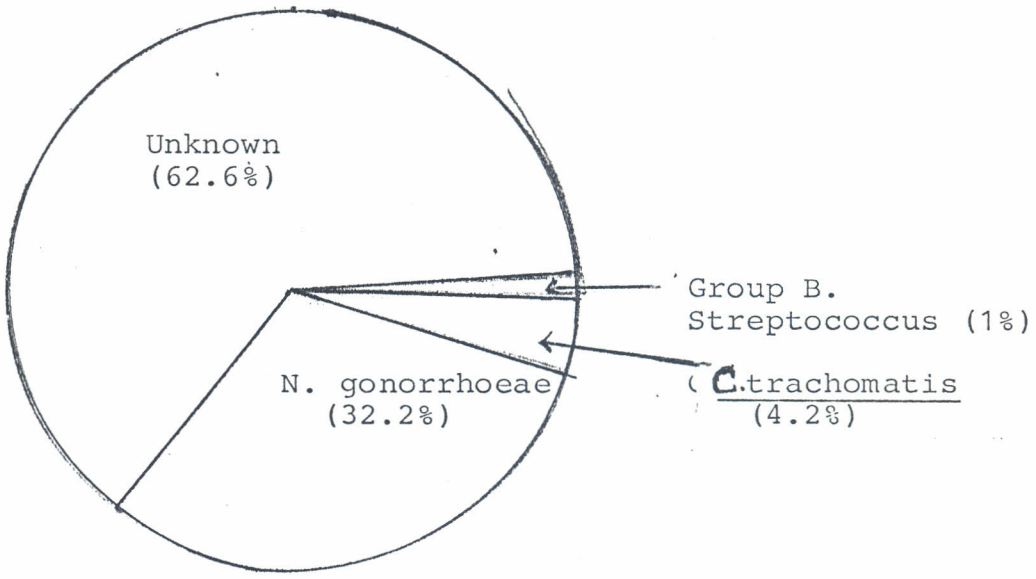
Table 6: Comparison between *C. trachomatis* related acute PID and non-*C. rachomatis* related acute PID.

		Number of Subjects (%)	
		CT Positive (n = 4)	CT Negative (n = 90)
Age in years	Mean ± SD	21 ± 5	24 ± 5
Parity	Mean ± SD	1 ± 1	24 ± 5
Marital status	Single	1 (25)	25 (27.7)
	Married	3 (75)	61 (67.7)
	Separated	0	2 (2.2)
	Divorced	0	2 (2.2)
Total		4 (100)	90 (99.8)
Contraception	None	3 (75)	70 (77.7)
	Oral Contraceptive	1 (25)	8 (8.8)
	IUCD	- -	5 (55.0)
	Depoprovera	- -	3 (3.3)
	TL	- -	2 (2.2)
	Barrier	- -	2 (2.2)
Total		4 (100)	90 (99.7)

This table shows that Chlamydia acute PID is more prevalent in a younger age group as compared to non-Chlamydia related acute PID.

NB - These figures were too small to be subjected to any statistical analysis.

Figure Ia. Percentages of different isolated pathogens in acute PID cases by cultures.



Footnote: One case was positive for both N. gonorrhoeae and C. trachomatis.

Figure Ib. Percentages of different isolated pathogens in the controls by cultures.

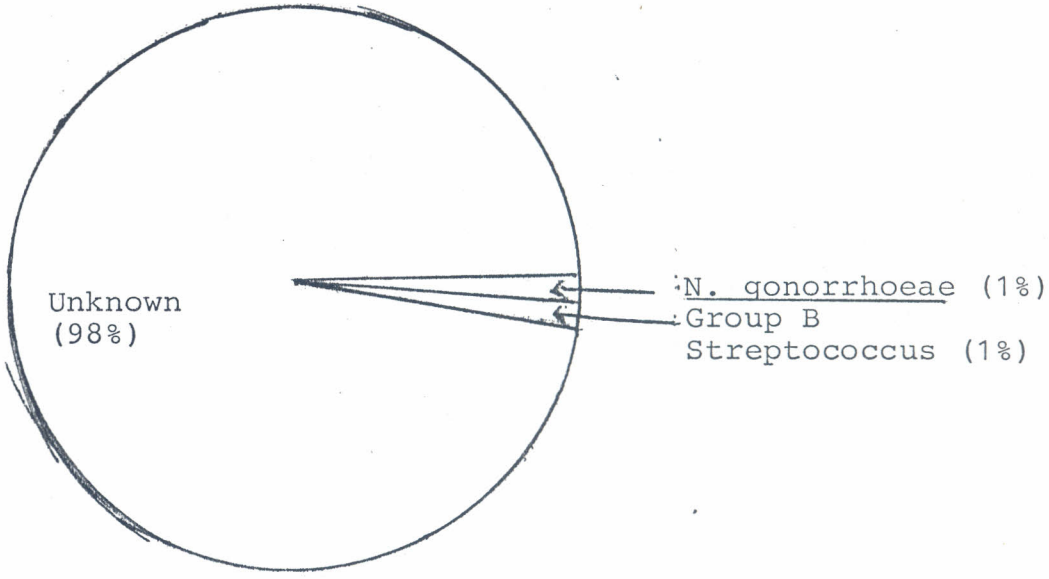
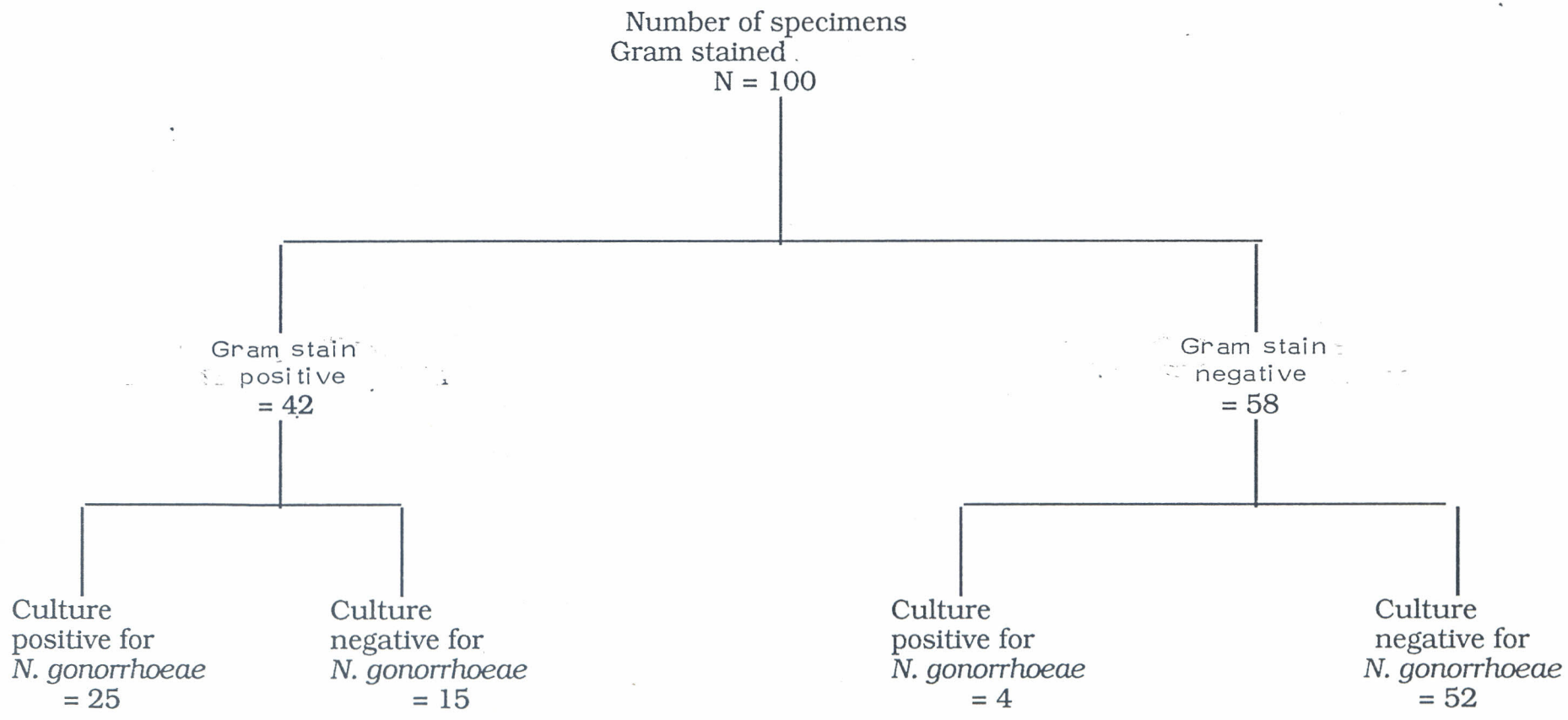


Figure 2: Comparison between Gram stain results and culture results for *N. gonorrhoeae* identification from the study cases



Four specimens (2 initially positive on Gram stain and 2 negative on Gram stain) got contaminated during culturing hence could not be reported on from cultures.
Specificity of Gram stain for detection of *N. gonorroheaea* was found to be 83.8% and its sensitivity was 70.0%

6.0

DISCUSSION

Pelvic inflammatory disease constitutes a serious health problem in developing countries (5). In this study, carried out at STC Nairobi City Commission, acute PID was found to be more prevalent in a younger age group (mean age 23±5 years) as compared to the control population (mean age 25±5 years) attending the clinic.

Most of the acute PID cases (74% as compared to 65% of the controls) were 25 years of age or below with a peak at the age group 16 - 20 years (41% as compared to 14% from the controls.) Only 26% of patients compared to 35% of the controls were 26 years of age or above. These figures compare well with those of studies done elsewhere. Norman, Cooperman and Gastro (51) in their study in Cook Country Hospital, Chicago found that 51% of all the acute PID admissions were 20 years of age or younger. Only 28% were 26 years of age or older. Eugene et al (52) in their study in the United States found the highest figures of PID to be at 15 - 19 and 20 - 24 years of age groups. Adelusi et al (53) in their study at a special treatment clinic in Nigeria, however, found most PID patients to be below 30 years of age (70.7%) with a peak at 25 - 29 age group (43.9%).

Amongst the study cases group as well as from the control group, the mean parity was 2±2 and 29% of the study group as compared to 23% of the control group were nulliparous. Figures from Sweden report that approximately 3 to 4 women with salpingitis are nulliparous (2,13) while figures from Norman et al, in Chicago (51), report that 46% of the patients were nulliparous. In this study it was found out that 65% of the study cases and 56% of the controls were married while only 4% of the study patients and 11% of the control group were either separated or divorced.

Contraceptive usage has been documented to influence the prevalence of acute PID (54). In this study most of the patients used no contraceptives, 78% from the study cases group and 72% from the control group. Only 10% of the study group compared to 13% of the control group used oral pills while 5% of the study group compared to 3% of the control group wore IUCD. It can be appreciated that oral contraceptive use was reported less often by acute PID cases than by the controls while IUCD use was reported more frequently by acute PID cases than by the controls.

From these figures, it was found that IUCD users have a significantly increased risk of having acute PID and oral contraceptive users a significantly decreased risk of acute PID as compared to the non use of these methods. Same findings have been documented in studies done elsewhere (14, 15, 16, 17, 18).

Agents of sexually transmitted diseases have been found to be the major pathogens of acute PID in Africa. From this study 30.2% of the study cases compared to 1% of the controls were gonococcal positive on cultures while an additional 15% as compared to 3% from the controls were positive on Gram stain but not confirmed by cultures. This gave a probable isolation rate of *N. gonorrhoeae* from acute PID cases as 45.2%. Isolation rate of *N gonorrhoeae* from acute PID patients vary from place to place and from time to time. Other studies found percentages ranging between 0 - 65% (6, 7, 23-29, 55). The sensitivity of a single endocervical culture on selective media is only 80%. Hence the actual incidence of gonorrhoea among these study patients could have been higher than observed.

Gonococcal acute PID was found to be more common amongst younger women (mean age is 22±4 years) as compared to non-gonococcal acute PID. Gonococcal acute PID is significantly more common in the divorced and the separated women. IUCD use and barrier use are correlated significantly with an increased risk of acute PID while oral contraceptives, TL and Depoprovera are significantly correlated with a decreased risk of acute PID. Correlation of increased risk of acute PID in barrier method users has not been documented, instead barrier method has been shown to reduce the risk of acute PID in its users. The observation in this study can be explained on the assumption that may be these women do not use these barrier methods all the time they are having intercourse.

Studies done elsewhere also found gonococcal acute PID to be commoner in a younger age group (51, 56). The women with gonococcal acute PID are also said to have a more severe illness of recent onset (51). In this study this was not observed.

Chlamydia trachomatis is another important pathogen in the etiology of STD-related acute PID. In this study it was isolated only from 4.2% of the study cases and none from the control group. It was found to occur in a younger age group (mean age 21±5) as compared to non chlamydia related acute PID (mean age 24±5 years).

This isolation rate was, however, very low compared to those obtained in other studies both locally and in other parts of the world. Isolation rates in these studies range between 12-38% (32, 57). The low isolation rate of *C. trachomatis* in this study could be due to several factors amongst which two major ones are:

i. Storage of specimens at -72° C over some period of time. This has been documented to decrease isolation rates by 20% (58).

ii. Diagnostic criteria.

The cut point for patients to be included in the study was duration of signs and symptoms not lasting more than 7 days. *C. trachomatis* is known to cause a more insidious indolent disease, so most likely many of these patients presented (or sought medical attention) late that is after seven days and hence were not enrolled for the study. Of the 4 cases with *C. trachomatis*, 3 of them used no contraceptives while one was on oral contraceptives and none wore IUCD. Some studies have suggested in the past that oral contraceptive use may enhance chlamydial acute PID and one control study found oral pills to significantly protect against symptomatic acute PID (59, 60, 61). The isolation rate of *C. trachomatis* in this study was too low to allow for any reasonable conclusions to be made.

Concomitant infection with both *N. gonorrhoea* and *C. trachomatis* in the same patient with acute PID was only seen in one of the study cases. Some studies done in the past had observed 25-60% (35). The low rates in this study cannot be explained. It could be due to the difference in populations studied. Though it is documented that gonococcal acute PID presents with more severe signs and symptoms and are more likely to have a purulent endocervical discharge as compared to other pathogens of acute PID, this was not observed in this study (13).

Group B Streptococcus was isolated from one of the study cases as well as one of the controls. The significance of this pathogen in the etiology of acute PID was doubted.

Mycoplasma hominis though also another important agent of STD - related acute PID, was not looked for in this study due to limited finances.

Anaerobes though have also been implicated with PID, their isolation as well as that of other non-STD related PID micro-organisms from The UGT of women with salpingitis has been proportionally more common in patients with clinically severe often suppurative infection than in women with clinically benign disease (13). Since this study was carried out in an outpatient clinic where most of the patients seen had clinically benign disease, cultures for anaerobes were not done.

one

6.1

CONCLUSIONS

1. Acute pelvic inflammatory disease patients as seen at the special treatment clinic, NCC, are younger and of lower parity than the general clinic population.

2. Acute PID is less commoner in the separated and the divorced as compared to married or single women.

3. *Neisseria gonorrhoea* is a commoner pathogen in association with acute PID cases compared to either *Chlamydia trachomatis* or Group B Streptococcus.

4. Gonococcal acute PID is commoner in a younger age group, in the separated and divorced women than non-gonococcal acute PID.

5. Chlamydial acute PID is also commoner in a younger age group compared to non chlamydial acute PID.

6. There is no notable difference in the presenting signs and symptoms in relation to micro-organisms isolated.

6.2

RECOMMENDATIONS

1. Since Neisseriae gonorrhoeae remains the major pathogen of acute PID, all patients presenting with clinical signs of acute PID should be screened for the pathogen.

2. Control measures should also be employed: Sex education should be given in high schools and colleges so that the students are made aware of the disease and its consequences. People should be discouraged from self treatment with locally purchases antibiotics, instead they should be encouraged to seek prompt medical attention as soon as they suspect they have sexually transmitted disease.

3. Chlamydia trachomatis is a more lethal pathogen in terms of tubal damage. Emphasis should be laid on its detection where facilities allow in patients with acute PID so that early treatment can be given to decrease the risk of its complications.

7.0

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8.0

APPENDIX I

Clinical history:

a. Date b. Code number.....

c. Name of Patients.....

d. Age..... e. Parity.....

f. Marital status - Single Married Separated

Divorced Widowed

g. Method of Contraception - None Oral pills

IUCD Barrier

Others (specify) - Tubal ligation,

Depoprovera

Clinical Diagnosis

i. Symptoms

- Low abdominal or pelvic pain Yes/No
- Duration in days.....
- Purulent vaginal discharge Yes/No
- Metrorrhagia Yes/No
- Nausea and vomiting Yes/No
- Symptoms of urethritis Yes/No

ii. Signs - Pelvic tenderness on palpation

iii. Any other important physical findings (Specify)

APPENDIX II

Laboratory Data

Findings on:

a. Direct Gram stain

- Pus cells:
  - absent - 0
  - <5/HPF - 1+
  - 5-10/HPF - 2+
  - 11-20/HPF - 3+
  - 21-30/HPF - 4+
  - >30/HPF - 5+
- Micro-organisms present/absent
- If present describe -

b. Culture growths obtained:

Neisseria gonorrhoeae	Present/Absent
Chlamydia trachomatis	Present/Absent
Group B Streptococcus	Present/Absent

c. Screening for syphilis results:

Rapid plasma reagin (RPR) card test

Reactive/Non-reactive.

APPENDIX III

Gram Stain Method:

1. Fix the dried smear (methanol fixation for two minutes is used)

2. Cover the fixed smear with crystal violet stain for 30-60 seconds

3. Cover the smear with Lugol's iodine for 30-60 seconds

4. Wash off the iodine with clean water.

5. Decolorize rapidly (few seconds) with acetone-alcohol. Wash immediately with clean water

6. Cover the smear with neutral red stain for 2 minutes

7. Wash off the stain with clean water

8. Wipe the back of the slide clean, and place in a draining rack for smear to air-dry

9. Examine the smear microbiologically first with 40x objective to check the staining and to see the distribution of material and then the oil immersion objective (100x power) to look for bacteria and cells.