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Prevalence of Pelvic Inflammatory Disease among Women Attending the Gynecology Clinic at Kampala International University Teaching Hospital, Uganda

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ABSTRACT

Pelvic inflammatory disease (PID) is major health problem in developed and developing country involving more young women. It is associated with high rate of female reproductive health morbidity; it can complicate with ectopic pregnancy, infertility and chronic pelvic pain. A poor response therapy increases the likelihood of these complications; this could be due to an increase in antimicrobial resistant pathogens. This was a cross-sectional study conducted among women who attended gynecology clinic at Kampala International University Teaching Hospital. Consecutive enrolment of 324 participants who consented to participate was done daily until a required sample size was realized from November 2019 to January 2020. Structured questionnaires were used to collect data on associated factors; endocervical swab was taken from patient clinically diagnosed with PID. Culturing for colony characteristics followed by Gram stain was used for provisional identity of pathogenic bacteria. Further identification was done by a set of biochemical tests. Antibacterial susceptibility pattern of isolated bacterial pathogens was determined by Kirby Bauer disc diffusion method, a rapid diagnostic test to detect Chlamydia antibody in the endocervical swab sample was also used to identify the Chlamydia trachomatis carriers among the patients. Data was analyzed using STATA VERSION 14.2. The prevalence of pelvic inflammatory disease was 19.1%. Not being educated, having two or more sexual partners, previous history of PID and induced abortion, also the previous use of contraceptives specifically the use of IUD ,were all significantly associated with Pelvic inflammatory disease (P value <0.05). The prevalence of PID is HIGH at KIU-TH**.**

Keywords: prevalence, PID, women, pathogens

INTRODUCTION

Pelvic inflammatory disease is an infection which affects the upper female reproductive tract from the internal cervical opening, the uterus, fallopian tube, ovaries and pelvic peritoneum [1]. The oldest and clear description of pelvic inflammatory disease was done by Mauriceau in 1693 when he described puerperal infection with abscess in both sides of the uterus [2]. While the first description of the bacterial agent that causes pelvic inflammatory disease was after Neisser discovered *Neisseria gonorrhea* in 1879. This description was done by Westermark in 1886 when he demonstrated the presence of *Neisseria gonorrhea* in tubal pus and Wertheim in 1894 the first to demonstrate these organisms invading the tube. Chronic pelvic inflammatory tumors unconnected with the puerperal state were described by Simpson in 1843; which described the condition as pelvic cellulitis. *Neisseria Gonorrhea* was since then known as the leading cause of pelvic inflammatory disease (PID) and its history is strongly linked to the one of pelvic inflammatory disease. It was thought to be caused solely by an inflammation occurring in the cellular tissue of the pelvis [3].

Virchow in 1862 referred to the term cellulitis, introduced parametritis or perimetritis. Parametritis was thought to result from a variety of causes such as a blow or fall, excessive excitement, the use of aphrodisiacs, and use of abortifacents.

The treatment of gonorrhea towards 1892 was then done by quack doctors by the introduction of potassium permanganate solutions for urethral irrigations, followed by the use of silver salt [4]. In 1930 a group of specialists in Massachusetts decided that it was time to make the management of gonorrhea a proper function of respectable medicine and disclosed that sulphonamide drugs would cure gonorrhea. This was then used for a short span of five years as gonococcus proceeded to develop overwhelming resistance to every new member of this group of drugs and later penicillin was used as single or multiple dose in the treatment of gonorrhea which led to a new assessment of this disease as a cause of serious morbidity in both males and females [4]. According to [5] in South Africa, who did a study on antimicrobial resistance among women with pelvic infection, he reported that PID continues to manifest resistance to many classes of antibiotics such as tetracycline, macrolide, and quinolone. In order to overcome this, different treatment regimens have been developed and different studies have shown the different clinical and microbiological cure rate concerning *Neisseria gonorrhea* and other associated microorganism as the cause of PID is known to be in 70% of cases of the polymicrobial condition [6].

Purpose of the study

To determine the prevalence of pelvic inflammatory disease among women attending gynecology clinic at Kampala International University Teaching Hospital.

RESEARCH METHODOLOGY

Study design

This was a cross sectional study. Laboratory investigations were done to achieve the prevalence, the common bacteria isolates and antibacterial susceptibility pattern in women with pelvic inflammatory disease attending gynecology clinic at Kampala international university teaching hospital. Association between PID and different factors was established.

Study area

The study was conducted at Kampala International University Teaching Hospital found in Ishaka Bushenyi Municipality at approximately 60km from Mbarara town along Mbarara Kasese highway.

Study population

The study populations were all women of reproductive age in the catchment area

Target population

All women of reproductive age attending gynecology clinic at Kampala international university teaching hospital shall be considered for inclusion in this study.

Inclusion criteria

All the women at the reproductive age attending gynecology clinic of Kampala international university teaching hospital as well as emancipated minors.

Exclusion criteria

Women on antibiotics, pregnant women, unconscious patients who cannot consent and minors were excluded from the study.

Sample size determination

Objective number 1, the sample size was determined using [7] formula with the estimated prevalence of 50%, because the of lack of current prevalence of PID in Uganda

$$n = \frac{z^2 pq}{d^2}$$

Where;
n = Desired sample size
z = Standard normal deviation at 95% level of confidence;
z= 1.96
p = Prevalence of pelvic inflammatory disease in Uganda, assumed at 50%, and d= Level of precision=0.05

$$n = \frac{(Z_\alpha)^2 x\, p(1-p)}{(d)^2}$$

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2}$$

N_1= 384 this is considered as an assumption of sample size.

Objective number 2:

Musa *et.al* [8] in the study done in Mbarara Regional Referral Hospital on screening for *Chlamydia Trachomatis* among women of reproductive, found that 26% of women were carriers of *Chlamydia Trachomatis*. [8]

$$n = \frac{z^2 pq}{d^2} \qquad n = \frac{(Z_\alpha)^2 x\, p(1-p)}{(d)^2}$$

Where;
n = Desired sample size
z = Standard normal deviate at 95% level of confidence; z= 1.96
p = prevalence of Chlamydia trachomatis in reproductive aged women, using a study done in Mbarara by Musa and colleagues [8]
d=Level of precision= 0.05

n= $\frac{(1.96)^2\text{-}0.2(1\text{-}0.2)}{(0.05)^2}$

N_2= 295

Specific objective 3: Mark *et al [9]* reported that women with a previous history of PID have a greatest risk of having subsequent PID, OR = 5.9**,** the proportion (p) PID among woman with previous history of PID 13.1% and among women without previous history of PID was 3.6%
r=ratio of previous history of PID to those without previous history of PID, r=13.1:3.6, r=3.6:1=3.6
Where; =z-statistic at α=0.05, = z-statistic at β=0.84 hence statistical power = 80%.
Using the formula [10]: where

$$n = \frac{(1+r)^2\,[Z_\alpha + Z_\beta]^2}{r\,(lnOR)^2 x\, p(1-p)}$$

$$\frac{(1+3.6)2 \times (1.96+0.84)2}{3.6(\ln 5.9)2 \times 0.131(1-0.131)}$$

n_3=118

Decision on the sample size

Sample size for objective number 2 was considered since this was a research done in Uganda (Mbarara Regional Referral hospital) and reflects the true Ugandan reality**.** 10% of this sample size was added to minimize non response or loss of some laboratory request form, therefore the sample size was:**N_2=324**

Sampling technique

Consecutive sampling method was used to select participants who consented to be part of the study. All the women of reproductive age who met the inclusion criteria was invited to participate in the study, the participants was enrolled according to their order of arrival in gynecology clinic and this was carried out on a daily basis until the required sample size was achieved.

Data collection instruments

A pretested questionnaire was administered to each participant who consented to participate to the study in order to collect information on socio-demographic, gynecological and sexual behavior factors that related to the development of pelvic inflammatory disease in. A detailed history was taken in English, translated in local language where necessary for women who could not understand English physical examination was carried out and the endocervical sample was taken from patient with symptoms and of PID in order to achieve all the objectives.

Sample processing and analysis

Isolation

Samples collected using a sterile procedure with the endocervical swab stick was inoculated on blood agar, chocolate agar, Mac Conkey agar, Thayer Martin medium, and different biochemical tests were used. After, they were incubated both aerobically and anaerobically at 37^{o}C for 24-48hrs. Colony morphology were observed according to shape, size, elevation, margin and surface characteristics.

Rapid diagnostic test was used in order to identify the *Chlamydia trachomatis* antibody careers within the endocervical sample of the participants, the isolation of Chlamydia which uses living cells (McCoy cell) was not done due lack of this specific media to culture *Chlamydia trachomatis,* this rapid Chlamydia test was used to determine the percentage of Chlamydia carriers among the patient with pelvic inflammatory disease.

Direct gram microscopy

A direct smear was made for Gram stain according to [11]; a drop of sterile normal saline was added on the center of a clean dried glass slide and the swab containing the sample rolled in the drop of normal saline spreading it on the glass slide in a circular motion to make a thin smear. The smear was then allowed to air dry and then heat fixed by passing it at least three times over a Bunsen flame. The slide was placed on the staining rack and flood with crystal violet solution for 60 seconds, wash with clean water and cover with lugol's Iodine and then it was allowed to act for a minute. The slide was washed in clean water and then decolorized with 50% acetone alcohol under slow running tap water until a faint pink color is observed or no more color tend to flow from the smear. The process of discoloration was not exceeding 30 seconds.

After decolorizing, it was washed in clean water and counter stain with neutral red solution. The slide was again washed in clean water; air dried and observed under the microscope with X100 objective lens (oil immersion lens).

Gram positive bacteria were observed as blue or purple color and Gram negative as red or pink color. Also, the morphology and shape of the bacteria were identified whether they are cocci, diplococcic, cocci in chains, clusters, and whether they are rods in appearance [11].

Identification of bacterial isolates

Biochemical tests

The isolates were identified using the conventional biochemical tests such as catalase, optochin, coagulase, indole, citrate utilization, urea utilization, triple sugar iron agar fermentation, MR-VP test and oxidase as described below;

Catalase test

Catalase test was carried out according to the method described by [11], to determine the ability of the isolate to produce the enzyme, catalase. A drop of 3% hydrogen peroxide was added to a loop full of the test organisms. Presence of bubbles indicated catalase activity.

Indole test

Indole test was carried out according to the method described by [11] to determine the ability of the isolate to degrade amino acid tryptophan and produce tryptophanase, enzyme were tested. 1% tryptophan broth in a test tube were inoculated with isolate and incubated at 37°C for 48 hours. After 48 hours, 1milliliter of chloroform was added to the broth. The test tube was shaken gently, and 2.1 ml of Kovac's reagent were added and again shaken gently, this was allowed to stand for 20 minutes. The formation of red coloration at the top layer, indicated positive while yellow coloration indicated negative.

Urease test

Urease test was done according to the method described by [11] to determine the ability to hydrolyze urea to produce ammonia and carbon dioxide. Test organism was inoculated into urease broth and incubated at 30°C for 72 hours. Purplish pink coloration of the medium indicates positive reaction.

Citrate utilization

This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and incubated for 24 to 72 hours. The development of deep blue color after incubation indicated a positive result [11].

Triple sugar iron test

Triple sugar iron test was carried out according to the method described by [11]; the test determines the ability of the organism to ferment the three sugar component of the medium: glucose, lactose and sucrose. The medium contains a pH indicator (phenol red) and a detection system (thiosulphate and ferrous sulphate) for hydrogen sulphide (H2S).

The medium was prepared as an agar slant. The test organism was inoculated by stabbing the medium using sterilized straight wire loop and the surface of the

slope were also streaked with the test organism. The tests were incubated at 37°C for 72 hours. After incubation, gas production was determined by observing the cracking of the medium, and production of H2S was observed by the blackening of the bottom of the medium.

Coagulase test

This test was used to identify *Staphylococcus Auraus* which produces the enzyme coagulase. The rapid slide test was done by placing a drop of distilled water on each end of slide. Then a colony of the test organism (previously checked by Gram staining) was emulsified in each of the drops to make two thick suspensions.

A loopful of plasma was added to one of the suspensions and mixed gently. Formation of clumps of the organisms within 10 seconds was suggestive of a positive test while absence of these clumps indicated negative results. For suspected Staphylococcus auraus isolates which turn negative for the rapid slide test, tube test was done by emulsifying several isolated colonies of test organism in 1 ml of diluted rabbit plasma (1:5) dilution to give a milky suspension. The tubes were then incubated at 35°C in water bath for 4 hours. These were examined at intervals of 1, 2 and 4 hours for clot formation by tilting the tube through 90°**.** If the test is still negative, the tube was left at room temperature overnight and examined again for Staphylococcus auraus that produce a delayed clot [11].

Oxidase test

The test was used in identification of organisms which produce the enzyme cytochrome oxidase. A filter paper soaked with the substrate tetra methyl-p-phenylenediamine Dihydrochloride was moistened with sterile distilled water. Using a piece of stick or glass rod, a colony of the test organism was smeared on the filter paper. The development of a blue-purple color within a 10 seconds indicated positive test while absence or formation of a blue-purple color after 10 seconds was considered negative [11].

Data analysis plan

Data from questionnaires were entered in Microsoft Excel 2010, and thereafter exported to STATA 14.1. Socio-demographic, sexual behaviors and gynecologic factors were summarized as means and medians, standard deviations and interquartile range (for continuous variables) were determined. Proportions, percentages and frequencies were used for categorical variables using STATA 14.1.

Ethical considerations

Informed consent

Informed consent and respect for participant's voluntary recruitment was observed. Informed consent for participants were obtained and signed after fully explaining the details of the study to them in English and local languages where necessary (copy attached at Appendix). Participants were not forced to enroll themselves if they don't want to, they were free to withdraw from the study any time they wish without coercion or compromise of care they are entitled to.

Risks and adverse events to study participants

Patients may undergo pain during swabbing and speculum examination, however, the process of obtaining a swab was done gently and professionally to minimize risk of pain and minimize re-infection as far as possible. Additionally, culture and sensitivity are the recommended guidelines prior to antibiotic therapy to minimize the risk of antibiotic resistance.

Approval procedure

Approval to carry out the study shall be sought from the department of obstetrics and gynecology, the faculty and post graduate directorate and finally, Research Ethics Committee of Kampala International University. This approval letter shall be presented to the hospital administration of KIU-TH. Permission shall be sought from the administration of the hospital before the study is conducted. The study will be registered with Uganda National Council for Science and Technology.

RESULTS

Table 1: Socio demographic factors

Characteristics	Frequency	%
Age (years)		
<20	31	9.6
20-29	205	63.3
30-39	71	22.0
40-49	17	5.1
Education		
None	11	3.4
Primary	99	30.6
Secondary	111	34.4
tertiary	103	31.6
Occupation		
None	127	39.2
Farmer	85	26.2
Professionals	51	15.7
Business	31	9.6
Manual laborer	30	2.3
Monthly income (UGX)		
none	10	3.1
<300000	230	71.8
300000-600000	66	26.5
>600.000	18	5.6
Marital status		
Single	86	26.5
Married	238	73.5

The above table illustrates that 63.3% of participants are aged of 20-29 years, 34.4% have secondary education, 39.2% have no occupation, 71.8% of participants have a monthly income of less than 300.000 Uganda Shillings and 73.5% are married.

Table 2: Gynecological factors

Characteristics	Frequency	%
Parity		
Zero	98	30.3
1-3	153	47.2
>3	73	22.5
Had PID before		
No	224	69.1
Yes	100	30.9
Had miscarriage before		
No	264	81.5
Yes	60	18.5
Use Contraceptive		
No	132	40.7
Yes	192	59.3
Intra Uterine Procedure		
No	281	86.7
Yes	43	13.3
Type contraception		
Condoms	38	19.8
Pills	61	31.8
Injectables	65	33.8
IUD	28	14.6
Type of miscarriage		
Spontaneous	41	68.3
induced	19	31.7

From the above table, 47.2% of the study participants had delivered at least one to three times, 69.1% had had miscarriage of which 68.3% were spontaneous, 59.3% of the study participants had ever used contraceptive methods of which 33.6% had

used injectable contraceptive methods and 86.7% had not had intrauterine procedures

Table 3: Sexual behavior factors

Characteristics	Frequency	Percent
Number of of sexual partners		
None	20	6.2
One	253	78.0
More than one	51	15.8
Age of initiation sexual activity(year)		
< 15	25	7.7
16-20	242	74.7
>20	57	17.6
Condom Use		
Sometimes	84	25.9
Every time	38	11.8
Never	202	62.3
Smoking		
Never smoke	316	97.5
Ever smoke	8	2.5

The above table shows that, the age of initiation of sexual activity for the majority of participants was 16-20years in 74.7%, most of the study participants denied the use of condoms with 62.3% and 97.5% were nonsmokers.

Table 4: Overall prevalence of PID

prevalence	Fr	%(95% CI)	P-Value
Non PID	262	80.9	NA
PID	62	19.1(15.2-23.8)	

From the above table, the overall prevalence of PID is 19.1%

DISCUSSION

In this study we determined the prevalence of pelvic inflammatory disease among women of reproductive age attending gynecology clinic at KIU-TH and found that of the 324 women enrolled 81 presented symptoms and signs of PID they underwent endocervical swabs for culture and sensitivity with also a rapid Chlamydia test, 62 were positive for infection giving a prevalence of 19.1%. This is High compare to 4.4% reported by [12] in USA, it is also high to [13] in Yaounde who found 5.1%, this could be explain by the setting where these studies were conducted in urban areas compare to our study which was conducted in rural setting.

This result fall in the range reported by [14] who reported PID as a cause of gynecological admissions in 17-37%, Therefore, there is a disproportionate prevalence of PID This could be attributed to the different levels of coverage health services and reporting across countries.

CONCLUSION

The prevalence of pelvic inflammatory disease is high and significant risk factors were not being educated, having previous history of PID, have ever use IUD as a family planning method and undergoing any intrauterine procedure.

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