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RESEARCH ARTICLE

SCREENING OF INFERTILE FEMALES FOR LOWER GENITAL TRACT INFECTIONS

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Abstract

Introduction: Infertility has been known to cause serious social and emotional problems worldwide. Besides other causes of female infertility, the role of female reproductive tract infection is well recognized. Lower genital tract infection, be it symptomatic or asymptomatic, need to be diagnosed and treated properly. In view of this our study was done.

Aim & Objectives: To evaluate the bacteriological profile of lower genital tract in infertile females.

Methodology: It was a cross sectional type of study. After taking consent, three swabs (high vaginal swab, endocervical swab and swab from lateral vaginal wall) were taken from 100 infertile women. A questionnaire covering demographic data, menstrual history, medical history, history of infertility, etc. was completed for each of the participants. Isolation and identification of the isolates were done as per conventional techniques. Antibiotic Susceptibility Testing was done for the aerobic isolates as per CLSI guidelines.

Results: In our study, 47% of females were asymptomatic and majority them showed positive microbiological growth. E.coli and S.aureus were the most common aerobic isolates and Prevotella spp. was the most common anaerobic isolate. Majority of the anaerobes were associated with bacterial vaginosis. Majority of our isolates were susceptible to Gentamicin.

Conclusion: The absence of clinical symptoms does not rule out the possibility of an ongoing acute inflammatory state due infective agents. Hence, both asymptomatic and symptomatic females should be screened for lower genital tract infections as the consequences may lead to infertility.

Introduction:-

Infertility is a major public health problem across worldwide and in India. World Health Organization (WHO) defines infertility as "the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year"[i] [1]. As per WHO, 60 to 80 million couples worldwide currently suffer from infertility[ii] [3]. Total infertility is divided into primary and secondary infertility. Definitions of primary infertility vary between studies, but the operational definition, put forth by the WHO, defines primary infertility as the "Inability to conceive within two years of exposure to pregnancy (i.e. sexually active, non-contracepting, and non-lactating) among women 15 to 49

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year old"[iii] [5]. Secondary infertility refers to the inability to conceive following a previous pregnancy. As per WHO operational definition, primary infertility is defined as "the inability to conceive within two years of exposure to pregnancy (i.e. sexually active, non-contracepting and non-lactating) among women 15-49 year old" [ref 123] while Secondary infertility refers to inability to conceive following a previous pregnancy. WHO estimates the overall prevalence of primary infertility in India to be between 3.9 and 16.8 % [ii] [3].

Besides other causes of female infertility, the role of female reproductive tract infection is well recognized. Infections may be in the form of PID or lower genital tract infection, either of which can be asymptomatic or symptomatic. These infections need to be diagnosed and treated properly to set the patient free from the grasp of infertility.

In view of this, our study was carried out to evaluate lower genital tract infection in infertile females.

Material and Methods:-

It was a cross sectional study carried out for a period of one year (2014-2015) in a Tertiary care teaching hospital in Western India. The study was approved by the institutional ethics committee. A total of 100 married reproductive age group females (18 – 49 years) attending infertility clinic, with the exception of those receiving antibiotic therapy in previous one month, were included in the study. A questionnaire covering demographic data, menstrual history, contraceptive use, recent antibiotic use, medical history & history of vaginitis and infertility was completed for each of the participants. After taking consent, 3 swabs (endocervical swab, High Vaginal swab & a swab from outer third of vaginal wall) were collected under speculum examination with sterile swab and were transported in Amie's transport media/ Robertson Cooked Meat Media.

Saline wet mount was prepared to detect Trichomonal infection. The presence of Trichomonas with characteristic motility in wet mount was considered diagnostic of Trichomonal infection. A gram stained smear was examined for the composition of bacterial morphology. For the diagnosis of bacterial vaginosis, the swab from outer third of vagina was analyzed as per Nugent scoring system (Table 1)[iv] [74].

High Vaginal Swab was processed for aerobic culture as per conventional methods. The endocervical swab was processed for detecting Mycoplasma and Gonococcal infection. In case of growth, discrete colonies were picked and processed further for identification by conventional methods. Antibiotic susceptibility reporting was interpreted as per CLSI 2014 guidelines[v] [143].

The high vaginal swab transported in RCM was processed anaerobically by conventional methods. In case of growth, discrete colonies were picked from the growth and processed further for identification up to genus level using Special Disc Potency test as mentioned in Wadsworth anaerobic manual [vi] [144].

Results:-

The various findings of our study are analysed as follows. 69% of our study population belonged to the category of Primary infertility and 20% of patients belonged to the category of secondary infertility. The mean age of our study population was 26.18 years. Mean and median BMI of our study was 26.14 kg/m^2 and 26.93 kg/m^2 respectively. Mean age of menarche in our study was 14.13 years. Among our study population, 47% of females were asymptomatic. Among the symptomatic patients, majority of them complained about per-vaginal discharge followed by menstrual irregularities, itching and burning micturition. 35% of patients were having Bacterial Vaginosis as per Nugent's scoring criteria. BV was higher in the second week (i.e., Day 7 to Day 13) of the menstrual cycle.

Out of 100 samples, positive microbiological growth was seen in 79% of the samples. 61.7% of asymptomatic females showed positive microbiological growth. 47% of our patients showed polymicrobial growth.

Out of total population, in 79 patients who had positive microbiological growth, aerobes were isolated from all of them and anaerobes were isolated from 10 patients.

Most predominating aerobic isolate was E.coli (29%) followed by S.aureus (27%) while Prevotella spp. was the most predominant anaerobic isolate. Distributions of the aerobic and anaerobic isolates are shown in Table (1) and in Table (2) respectively. In our study, 80% of the anaerobes were associated with Bacterial vaginosis.

Table 1:- Distribution of aerobic isolates in relation to sample size.

Organism	**Percentage (%)**
Gram negative bacilli(n=73)	
Escherichia coli	29
Klebsiella spp.	18
Acinetobacter spp.	9
Pseudomonas spp.	6
Enterobacter spp.	5
Citrobacter spp.	3
Serratia marcescens	2
Burkholderia cepacia	1
Mycoplasma spp.	0
Gram Positive cocci (n=44)	
Staphylococcus aureus	27
Enterococcus spp.	16
Streptococcus agalactiae	1
Others(n=0)	
Neisseria gonorrhoea	0
Trichomonas vaginalis	0
Note: n= No. of isolates	

Table 2:- Distribution of Anaerobic isolates in relation to sample size.

Anaerobic organism	**Total [n (%)]**
Prevotella spp.	4 (40)
Veilonella spp.	3 (30)
Bacteroides spp.	2 (20)
Peptostreptococci spp.	1 (10)
Total	10 (100)
Note: n=number of isolates; No. in parenthesis ( ) depicts % of isolates	

Antibiotic susceptibility pattern of the aerobic isolates are given in Table (3) and Table (4).

Table 3:- Resistance pattern of the Gram Positive Isolates (%).

	Pen	**Clin**	**Tra**	**Rif**	**Ctri**	**Gen**	**Cip**	**Lev**	**Van**	**Tec**	**Lnz**
S. aureus	100	44	15	11	32	20	67	41	0	0	0
Enterococcus spp.	100	69	56	44	100	20	47	25	0	0	0
Streptococcus agalactiae	100	0	0	0	0	0	0	0	0	0	0
Note: Pen: Penicillin, Gen: Gentamicin, Rif: Rifampicin, Cip: Ciprofloxacin, Lev: Levofloxacin, Ctri: Co-Trimoxazole, Clin: Clindamycin, Lnz: Linezolid, Van: Vancomycin, Tec: Teicoplanin, Tra: Tetracycline											

Table 4:- Resistance pattern of the Gram Negative Isolates (%).

	Pip	**Amc**	**Ptz**	**Cep**	**Cef**	**Cftz**	**Ctr**	**Ctxm**	**Cfp**	**Ime**	**Amk**	**Gen**	**Cip**	**Ctri**	**Col**
E.coli	89.7	65.5	17.9	86.2	69	44.8	44.8	48.3	44.8	0	10.3	6.9	31	58.6	0
Klebsiella spp.	100	80	30	100	75	45	35	53	22	0	7.5	0	20	25	0
Enterobacter spp.	100	75	0	75	75	20	20	20	0	0	0	0	0	34	0
Citrobacter spp.	100	100	50	100	100	50	50	100	50	0	50	0	25	50	0
Acinetobacter spp.	100	55	17	100	100	44	44	44	6	0	6	11	6	6	0

Pseudomonas spp	100	30	10	90	90	10	10	10	10	0	0	0	50	90	0
Serratia marcesens	100	100	0	100	100	0	0	0	0	0	0	0	0	0	0
B. cepacia	100	0	0	100	100	100	100	100	100	0	0	0	0	0	100
Note: Rx: Antibiotic name, Pip: Piperacillin, Amc: Amoxycillin-Calvulanic acid, Ptz: Piperacillin-Tazobactam, Cep: Cephalothin, Cef: Cefuroxime, Cftz: Ceftazidime, Ctr: Ceftriaxone, Ctxm: Cefotaxime, Cfp: Cefepime, Ime: Imepenem, Amk: Amikacin, Gen: Gentamicin, Cip: Ciprofloxacin, Ctri: Co-trimoxazole, Col: Colistin															

Discussion:-

Worldwide, about 60 to 80 million couples currently suffer from infertility while the overall prevalence of primary infertility in India is between 3.9 and 16.8 % [3].

When the definition of Primary and Secondary infertility as in Parvez et al. [24], was adopted in our study, 69% of patients were belonging to the category of Primary infertility and 20% of patients were belonging to the category of secondary infertility while, 11% patients were such who could not be assigned to either of the categories which was due to the reason that their duration of infertility was less than 2 years but more than one year.

Female age plays an important role in infertility. After age 35, there is a significant decrease in fecundity [36]. The mean age of our study population was 26.18 years which is similar to that of Paul et al. (mean age 25.9 +/- 3.12 years) [86] but higher than Seddigheh et al. (19.9 +/- 4.5 years) [42]. The median age of our study population was 26 years which is similar with Paul et al. (26 years) [86] and Gupta et al. (29 years) [85]. Extremes of BMI, i.e. both high and low BMI can affect fertility of the individual. Mean and median BMI of our study was 26.14 kg/m2 and 26.93 kg/m2 (Table 3), which is similar with that of Seddigheh et al. [42], where the mean and median BMI of their study population were 27.6 ±4.8 kg/m2 and 27.3 kg/m2, respectively.

As per the clinical history provided by our study population, there was not much variation in frequency between symptomatic and asymptomatic population which is unlike the findings of Paul et al. [86] that suggest infertile women are less likely to have abnormal vaginal discharge, or report burning during urination and vaginal itching. The mean age of menarche (in years) in our study was 14.13 years, which is bit higher than the mean age of menarche of the population studied by Paul et al. (13.4 +/- 1.5) [22].

Various studies like Anna et al. [87], Mania et al. [134], Noortje et al. [135] and Salah et al.

[136] suggest that bacterial vaginosis is significantly associated with infertility. 35% of our study population were diagnosed of having bacterial vaginosis. Prevalence of BV can be as high as 70.3% [109] and as low as 5% [84]. In a cross-sectional study by Claudia et al., the prevalence of BV ranged between 15-30% [26]. In our study, BV was higher in the second week of the menstrual cycle relative to the later days of the cycle (Table 7), which is in concordance with Morison et al [27]. While study by Claudia et al. shows higher prevalence of BV during 14+ days [26]. The probable cause behind variation in the prevalence of BV in relation to the phase of menstrual cycle may be due to the influence of ovarian hormones on urogenital infection.

In our study 79% of samples showed positive microbiological growth which is higher than the prevalence of growth as shown by Gupta et al. (55.8%) [85] and Rodriguez et al. (47.3%) [84]. When the high vaginal sample was inoculated on a culture media, 61.7 % of asymptomatic infertile women showed positive microbial growth in our study which is in concordance with Adewale et al. where 69.8% of asymptomatic yielded positive lower genital tract infection [123].

Out of our total study population, in 79 patients who had positive microbiological growth, aerobes were isolated from all of them while anaerobic growth was there in 10 samples. The most prevalent aerobic organism (Table 12) in our study was E.coli (29%) and S.aureus (27%) which in similar to the finding of Ghiasi et al [109]. S.aureus was the most common organism isolated by Momoh et al. (38.7%) followed by Klebsiella spp. (22.6%) [14].

The presence of high rate of isolation of E.coli and S.aureus in our study should be given importance because as per the findings of Siftjit et al. [129] , Kaur et al. [120] and Vijay et al. [130] they cause agglutination of human spermatozoa and thus, leading to infertility. Findings of Sushila et al. [121] suggests that E. coli may be carrying some adhesion sites through which it binds to human spermatozoa and causes agglutination. Vijay et al. [130] showed that incubation of spermatozoa with S. aureus results in reduced sperm motility.

In our study, isolation rate of Acinetobacter spp., Citrobacter spp. and Burkholderia cepacia was 9%, 3% and 1% respectively. Even though studies showing similar results were not found, their isolation should not be overlooked as all these patients gave a history of prior instrumentation. As per Novy et al., endometritis may follow these procedures due to introduction of contaminated cervical mucus into the uterus [50].

Broadly speaking, the Gram negative isolates in our study showed high level resistance to Piperacillin, Cephalothin and Cefuroxime followed by Co-trimoxazole while they showed least resistance to Imipenem and Colistin followed by Gentamicin and Piperacillin- Tazobactam. In Spencer et al., Gentamicin, Cefotaxime and Ciprofloxacin had mean bactericidal effects of 80%, 76% and 54% respectively against all the Gram-negative bacteria encountered [59]. As per Sangeetha et al., when a study was conducted on patients with clinical suspicion of vaginitis, Gram negative isolates were more sensitive towards β–lactams/ β–lactamase inhibitor combination, Aminoglycosides and Meropenem [126]. Similar to our study, results of Anchana et al. reveal that Imipenem showed excellent activity (100%) against all gram negative groups of bacteria and Aminoglycosides were highly active against most clinically significant aerobic Gram-negative bacilli, including P. aeruginosa and members of the family Enterobacteriaceae [125]. As per Sandhiya et al., which comprised of females of reproductive age group complaining of vaginitis, the most effective chemotherapeutic agents against fermenters group (E. coli and Klebsiella species) were Amikacin, Levofloxacin, Imipenem, Tigecycline, Cefoperazone –Sulbactum and higher generation Cephalosporins while the most effective antibiotics against nonfermenters (Pseudomonas species and Acinetobacter species) in our study were Amikacin, Levofloxacin, Imipenem, Cefoperazone – Sulbactum and Tigecycline [127].

Gram positive isolates in our study showed high level resistance to Penicillin, Ciprofloxacin and Clindamycin followed by Tetracycline, Levofloxacin and Rifampicin while they showed least resistance to Linezolid, Vancomycin and Teicoplanin followed by Gentamicin. In Spencer et al., Cefotaxime, Ciprofloxacin and Streptomycin had mean bactericidal effects of 77%, 57% and 51% respectively against all the Gram-positive bacteria encountered [59]. As per Sangeetha et al., when a study was conducted on patients with clinical suspicion of vaginitis, antibiotics like β–lactams/ β–lactamase inhibitor combinations, Vancomycin and Linezolid were found to be more effective against all Gram positive isolates [126].

Conclusion:-

From our study, we can draw out the following conclusions:

1. Both asymptomatic and symptomatic females should be screened for lower genital tract infections.
2. Emphasis should be laid on regular screening of infertile females for BV.
3. All our aerobic isolates showed less resistance to Gentamicin.

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