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RESEARCH ARTICLE

MICROBIOLOGICAL STUDY OF LEUCORRHOEA WITH SPECIAL REFERENCE TO GARDNERELLA VAGINALIS

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Abstract

Leucorrhoea is one of the commonest complaint among women of reproductive age group. A total number of 130 symptomatic study group, 20 controls and 10 antenatals with bad obstetric history in the age group between 15-45 years attending the Department of Gynaecology & Obstetrics, Government General Hospital, Vijayawada were taken up for Microbiological study. A detailed history was taken and vaginal swabs were collected and processed by standard microbiological techniques to isolate and identify different agents causing Leukorrhoea. Among the study group, women belonging to urban population 98(75.38%), low socio-economic status 82(63.07%) and illiterates 70(53.85%) formed the major group and leucorrhoea was common in the age group 21-30 years. Bacterial isolates were predominant with *Gardnerella vaginalis* forming major group with 42(32.30%). *Candida species* isolated was 32(24.61%) and *Trichomonas vaginalis* 24(18.46%). Other bacterial isolates like *Diphtheroids* 23%, *Coagulase negative Staphylococci* 19.23%, *Other Streptococci* 13.07%, *Staph aureus* 11.53%, *Acenitobacter* 6.15%, *Escherichia coli* and *Klebsiella* 5.38%, *Pseudomonas* and *Micrococci* 3.07% and *β hemolytic Streptococci* in 1.53%. Antimicrobial susceptibility testing revealed that most of the other bacterial isolates were sensitive to Ciprofloxacin followed by Gentamycin and Erythromycin. *Gardnerella vaginalis* was more sensitive to Ampicillin(73.33%) followed by Metronidazole (66.66%) Erythromycin (53.33%) and Gentamycin (40%). Microbial infections of lower genital tract which are common in sexually active women in the reproductive age group may lead to adverse birth outcomes during pregnancy. Early and proper laboratory diagnosis and timely treatment of infected women along with sexual partners is essential in everting obstetric emergencies and improves pregnancy outcome.

INTRODUCTION

Leucorrhoea is one of the commonest complaint among reproductive age group and major sign of vaginitis for which women seek advice of a Gynaecologist. About 80% of women in the age group 15-45 years attending the Gynaecology outpatient department are suffering from infection of vagina with common presenting complaints such as vaginal discharge, foul smell and itching. Leucorrhea means 'A running of which substance' and the term should be restricted to mean an excessive amount of normal discharge, dries to leave a brownish yellow stain on clothing – Jeffcoate (1975). Literally leucorrhoea means white discharge which includes all excessive discharge which is purulent, yellow or watery but not blood stained – Shaw (1999). David A. Eschenbach studies (2000AD) on vaginal

infection and discharge, graded as normal with 1-2ml and copious more than 3ml with malodor. In the present study of 130 individuals, 93(71.54%) were in the reproductive age group having profuse vaginal discharge and malodour.

Chen et al 1979 & 82 in his two studies found putrescine and cadaverine were the two most abundant amines in vaginal fluid of women with untreated non specific vaginitis as the cause for malodour of vaginal discharge. Vaginitis which cause leucorrhoea is a clinical condition with pathological invasion of the vaginal epithelium by the microorganisms. Women in reproductive age group are at an increased risk for vaginitis. Vaginal discharge may be physiological and pathological. Various microbial agents which cause infection of vagina leads to leucorrhoea. The three main forms of infectious vaginitis are Trichomonial vaginitis, Candidial vaginitis and Bacterial vaginosis. There is considerable decrease in number of lactobacilli in the vagina and the pH is altered and results in increased growth of other bacteria. Common agents of bacterial vaginosis include *Gardnerella vaginalis, mobiluncus, bacteroides, saprophytes, mycoplasma hominis,* and others. Vulvovaginal candidiasis is characterized by pruritis and cotton cheese like discharge caused by *candida* species. Vaginal Trichomoniasis is associated with a copious, yellow or green and sometimes frothy discharge caused by *Trichomonas vaginalis.*

It is an established fact that reproductive tract infections are sexually transmitted infections which increase the woman's susceptibility to HIV infections and Carcinoma cervix. The available literature on this subject by various authors in Indian subcontinent reveals wide variations in the incidence of these microorganisms. This study is undertaken to assess the role of micro-organisms as a cause of vaginal discharge and an attempt is made to know the antibiotic susceptibility pattern of the bacterial isolates in leucorrhoea patients which help in proper patient care in this era of antibiotics and immunodeficiencies.

MATERIALS AND METHODS :

Study was conducted on 130 women attending outpatient department of Gynaecology & Obstetrics, Government General Hospital, Vijayawada, with complaints of vaginal discharge (Leucorrhoea) of one week duration with or without associated vaginal discomfort, pruritus and dysurea. 10 antenatal women with bad obstetric history (H/o abortions, preterm labour) along with a control group of 20 women with complaints other than leucorrhoea were included in the study. A detailed Obstetric and Gynaec history along with general history of diabetes, hypertension, contraception was noted. Local examination of genitalia without any antiseptic lubricant was carried out. Patients on antibiotic drug treatment or within 2 weeks or genital prolapse or malignancy of genital tract were excluded from the study.

Method of sample collection : Vaginal pH was noted using pH strips (pH 2.0-10.5) from the mid lateral vaginal wall using sim's speculum and forceps. The nature and colour of vaginal discharge, cervix and vaginal walls were inspected. 3 high vaginal swabs were collected with sterile cotton swabs from the posterior fornix and transported to microbiology laboratory in Amie's transport medium for processing. Whiff test (Amine test) was performed by addition of 2 drops of 10% KOH to vaginal fluid collected on the speculum and enhanced fishy odor was noticed in a positive test. The samples were processed in the Department of Microbiology, Siddhartha Medical College, Vijayawada within one hour of collection by conventional microbiological procedures like wet mount, gram staining and inoculated on basal culture media, both bacterial and fungal. All the samples were subjected to Saline wet mount and 10% KOH mount in which motile pear shaped trichomonads and Budding yeast cells were observed.

Staining : Smears were prepared from 2nd vaginal swab for Grams stain and Giemsa and observed for presence of clue cells, pus cells, gram negative coccobacilli, gram negative curved rods (mobiluncus) and budding yeast cells and were graded according to Nugent's scoring system. Score 1-3 is normal, 4-6 is intermediate and 7 or more indicates bacterial vaginosis. **Culture:** The third vaginal swab was inoculated on 5% sheep blood agar, chocolate agar, MacConkey's agar, Sabourauds dextrose agar and incubated in 5-10% Co_2 at 35^{0}C for 48 hrs and the isolates were identified by standard microbiological laboratory procedures including Antibiotic sensitivity pattern.

RESULTS

In the present study 130 women with vaginal discharge and malodor were diagnosed as having Bacterial vaginosis, Candidial vaginitis and Trichomonial vaginitis. This study also includes 20 control group and 10 antenatal women. 75% of women in the study and control group belonged to urban areas (Fig.I) and 82(63.07%) in study and 14(70%) in control group belonged to low socioeconomic status (Fig.II). Women in study group 70 (53.85%) and 14 (70%) in control group were illiterate (Fig.IV).

Complaints of leucorrhoea were common in the age group of 21-30 years. Highest incidence was seen in the age group 21-25 years(29.23%) and 26-30 (26.15%) followed by 31-35(13.08%), 36-40(11.54%) and 16-20(10.77%) years. The number of women in the age group of 41-45 years were 11(8.46%). In the control group of 20, women were in the age group of 31-35 years (35%) followed by 36-40 (10%) and 41-45 (5%) (Table II).

A total of 93 were having profuse vaginal discharge (71.54%) and women with minimal discharge were 37(28.46%). Polymicrobial infection in study group with X^2 Value 4.5 at df. and Test of significancy = P value < 0.05 in vaginal discharge when compared to controls(Table I). Profuse greyish watery discharge was seen in 46(35.38%) followed by purulent frothy discharge in 21.54%, curdy white discharge in 20% and mucoid discharge in 23.07%. In control group minimal mucoid discharge was present in 75% followed by curdy white in 25%. According to Nugent's scoring system by Gram's staining, smears 70 (53.85%) belonged to category II followed by category III 37 (28.46%) and 23 (17.69%) category I.

Among the 130 study group, correlation with vaginal pH, Amine test, Clue cells, pH>5 is seen in 96, positive amine test in 44, presence of clue cells in 42 cases and *Gardnerella vaginalis* was isolated in culture in 14 cases.

Among the leucorrhoea cases in the study Bacterial isolates were more in number than *candida species* and *Trichomonas vaginalis*. *Gardnerella vaginalis* with positive amine test, clue cells and pH > 5, with greyish white discharge, formed major group with 32.30%(42), Candida sp 24.61%(32) (*Candida albicans* 12.30%, *Candida tropicalis* 10.76%, *Candida krusei* 0.76%, *Candida pseudotropicalis* 0.76%) and *Trichomonas vaginalis* 18.46%(24)(Table III) -Other bacterial isolates were *Diphtheroids* 23%, *Coagulase negative Staphylococci* 19.23%, other *Streptococci* 13.07%, *Staph aureus* 11.53%, *Acinetobacter* 6.15%, *Escherichia coli & Klebsiella* 5.38%, *Pseudomonas & Micrococci* 3.07%, *Beta hemolytic Streptococci* 1.53% and *Aspergillus flavus* in one case were isolated and No growth was observed in 5 (Table IV). *Trichomonas viginalis* was not observed in control group. *Gardenerella vaginalis* was isolated in 2 cases (10%), *Diphtheroids* 35%, other *Candida sp* 30%, other *Streptococci* 13.07%, *Micrococci* 15%, *Acinetobacter* 10%, *Candida albicans, Enterococci, Staphylococcus aureus & coagulase negative Staphylococci* 5%, and no growth in 5% among the control group.

Antenatal women with Bad obstetric history of previous abortions and perinatal mortality were studied to find the microorganisms which leads to unsuccessful pregnancy out come. Among 10 cases 3 cases were positive for Amine test and clue cells with pH>5 and one was culture positive for *Gardnerella vaginalis*. *βhemolytic streptococci* was isolated in 2, Staph *aureus* in 3, *Candida sp* in 3 cases and *Acinetobacter* in 1.

Antimicrobial susceptibility test showed most of the other bacterial isolates were sensitive to Ciprofloxacin followed by Gentamycin 10 mcg and Erythromycin 15 mcg. *Coagulase positive Staphylococci* was more sensitive to Ciprofloxacin 13 (72.22%), Erythromycin 11 (61.11 %) followed by Piperacillin, Teicoplanin and Ampicillin 9 (50%). *B hemolytic Streptococci* were 100% (4) sensitive to Ampicillin 10 mcg, Erythromycin 15 mcg, Gentamycin 10 mcg and Ciprofloxacin. *Klebsiella species* showed same sensitivity to Tetracycline 30 mcg, Gentamycin, Ciprofloxacin and Amikacin 1 (14.28%) and it was more sensitive to Meropenem 4 (45.14%). *Escherichia coli* was sensitive to Ciprofloxacin 4 (57.14%) followed by Meropenem 10 mcg disc 3(42.85%). *Pseudomonas* was resistant to all drugs except ciprofloxacin 1 (25%). *Acinetobacter* was sensitive to Gentamycin 5 (55.55%) followed by Erythromycin 3 (33.33%). It was observed most of the isolates in *Gardnerella vaginalis* were more sensitive to Ampicillin 10 µgm 11(73.33%) followed by Metronidazole 50 µgm 10 (66.66%) and Metronidazole 25 µgm 6 (40%), Erythromycin 15µgm 8 (53.33%), Gentamycin 10µgm 6 (40%) followed by Ciprofloxacin 1gm and Penicillin 10units 5 (33.33%). .

TABLE – I : POSITIVITY ON CULTURE

Study groups	Number studied	No isolation		Mono microbial		Poly microbial	
		No	%	No	%	No	%
Control group	20	1	5	6	30	13	65
Leucorrhoea cases (Gy OP)	130	5	3.8	16	12.3	109	83.5
Antenatal group	10	0	0	10	100	0	0
Total	160	6	3.75%	32	20%	122	76.25%

TABLE - II : AGE – WISE DISTRIBUTION OF LEUCORRHOEA CASES

Age in group	No. of cases	%	Controls No.	%
16-20	14	10.77 %	2	10
21-25	38	29.23 %	6	30
26-30	34	26.15 %	2	10
31-35	17	13.08 %	7	35
36-40	15	11.54 %	2	10
41-45	11	8.46 %	1	5
Total	**130**	**100**	**20**	**100**

TABLE - III : PATHOGENIC MICROBIAL FLORA IN LEUCORRHOEA CASES

	Organism	Study group		Control group		A.N cases	
		No	%	No	%	No	%
Bacterial	Bacterial vaginosis (Clue cell +ve in Gramstain) Gardneralla vaginalis culture +_(ve)	42 14	32.30	2	10	1	10
Fungal 25.38%	Candida albicans	*16*	*12.30*	*1*	*5*	*1*	*10*
	Candida tropicalis	14	10.76	6	30	1	10
	Candida cruezi	1	0.76	0	0	0	0
	Candida pseudotropicalis	1	0.76	0	0	1	10
	Aspergillus flavus	1	0.76	0	0	0	0
Protozoal	Trichomonas vaginalis (in wet film)	24	18.46	0	0	0	0

TABLE -IV : OTHER BACTERIAL ISOLATES IN LEUCHORRHOEA CASES

Organism isolated	Study group		Control group	
	No	%	No	%
Diphtheroids	30	23.07	7	35
C N Staphylococci	25	19.23	1	5
Staph. Aureus	15	11.53	1	5
Enterococcus faecalis	8	6.15	1	5
Acinetobacter	8	6.15	2	10
Escherichia Coli	7	5.38	0	0
Klebsiella sp	7	5.38	0	0
No growth	5	3.85	1	5
Pseudomonas sp	4	3.07	0	0
Micrococci	4	3.07	3	15
Group B Streptococci	2	1.53	0	0

FIGURE : I DEMOGRAPHIC DISTRIBUTION OF CASES :

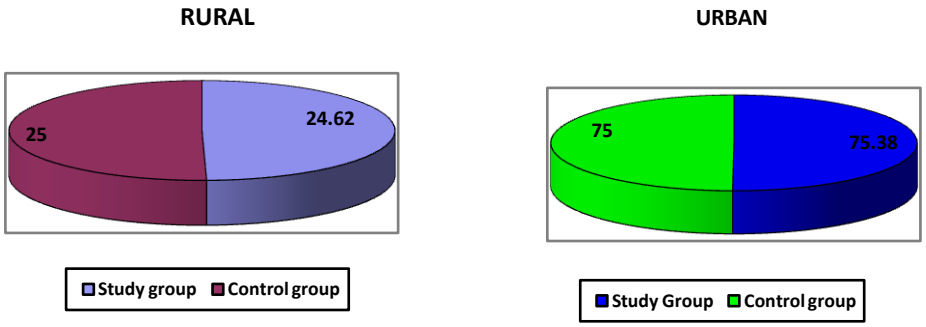


FIGURE : II DISTRIBUTION OF CASES ACCORDING TO SOCIO-ECONOMIC STATUS.

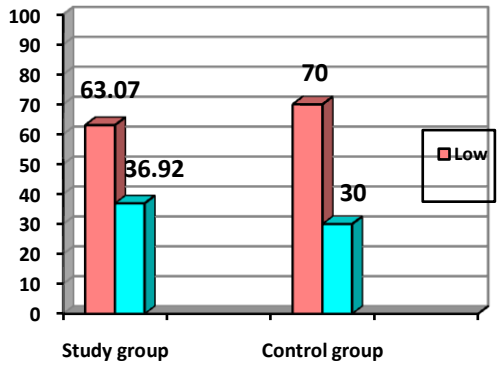


FIGURE : III

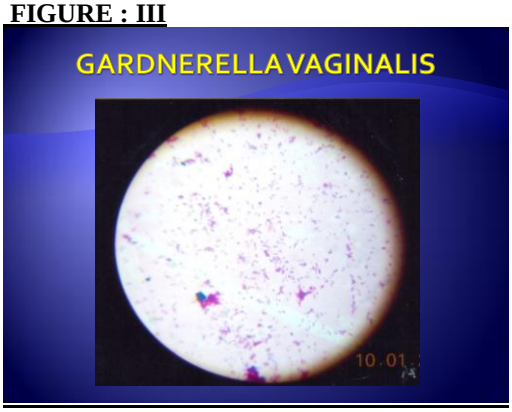


FIGURE : IV DISTRIBUTION OF CASES ACCORDING TO LITERACY STATUS

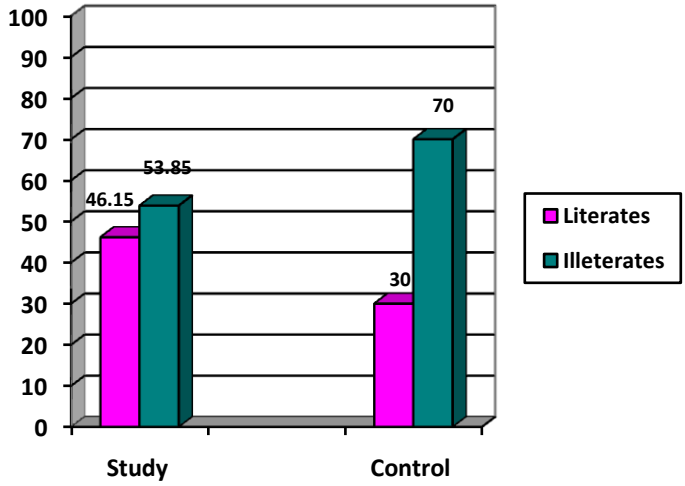


FIGURE : V

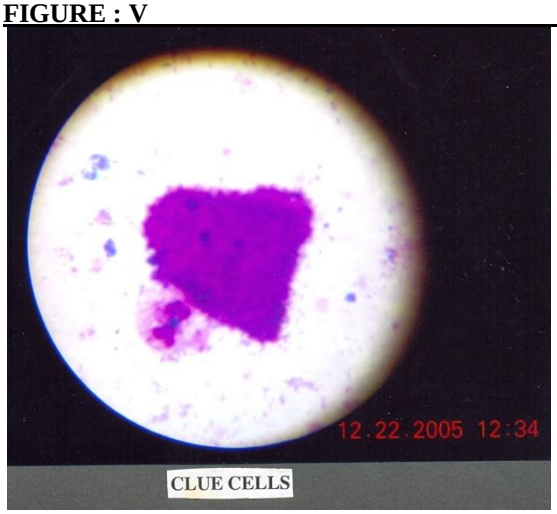
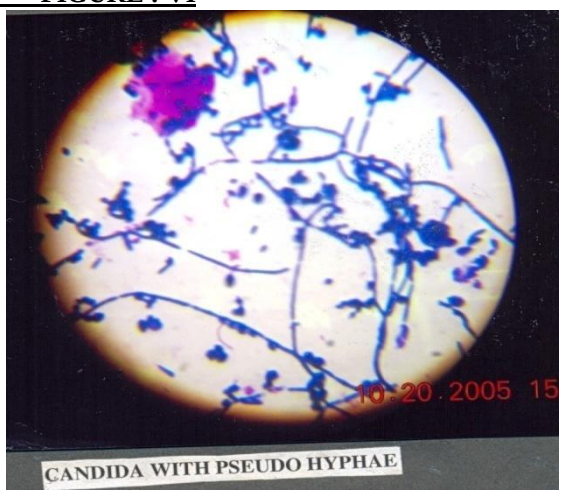


FIGURE : VI



DISCUSSION :

Studies on microbiological evaluation by various workers on Leucorrhoea suggests that vaginal symptoms such as discharge, malodor and pruritus are the most common complaints of women attending the Gynaecology outpatient department. A number of 130 women in the reproductive age group with complaints of leucorrhoea, 10 antenatal women with bad obstetric history and a control group of 20 with complaints other than leucorrhoea were considered for the study.

Women belonging to urban population 98(75.38%), low socio economic status 82(63.7%) and illiterates 70(53.85%) formed the major group and Leucorrhea was common in the age group of 21-30 years and these findings coincides with studies by Bansal KM (2001) reporting the highest number of RTI in the age group of 21-30 years. Bacterial vaginosis was the predominant (32.30%) cause of leucorrhoea in the study group followed by candidiasis (25.38%) and Trichomoniasis (18.46%).

Diagnosis of Bacterial vaginosis was based on Amsel criteria which was accepted as "gold standard". It was found that 3 of the 4 criteria were positive in 42 of 130 study group and *Gardnerella vaginalis* was isolated in 14. P.S. Rao et.al, (2004) reported prevalence of Bacterial vaginosis by smear positive in 104 cases and culture was positive in 88 out of 505 women in reproductive age group 15-44 years. Eschenbach et.al,(1988) observed that Gram's staining was more specific and better than culture of *Gardnerella vaginalis* in Bacterial vaginosis.

The incidence of bacterial vaginosis in Asymptomatic antenatal women was 10%(1) and this correlates with studies by Jack D Sobel (1997) reporting an incidence of bacterial vaginosis in pregnant women as 10–29% and Shoron L Hiller & RP Nugent et.al, (1995) as 6%. Amount of discharge was profuse in 93{71.74%) which was closer to the study of Srivastava (2004) who reported (78.26%).

Incidence of *Gardnerella vaginalis* was 32.3% which correlates with the study by J.W. Mahadani (1998) with an incidence of 32.90%, Vijay D et.al, (1999) 43.39% and Gupta V et.al, (2003) 44.6%. *Gardnerella vaginalis* was frequently associated with *Staphylococcus* which was also reported by Vijay D et.al, (1999). It was observed *Gardnerella* was more susceptible to Ampicillin followed by Metonidazole, Gentamycin, Ciprofloxacin and Erythromycin. Pheifer T.A et.al, (1978) reported that Metronidazole therapy was effective in treatment of Bacterial vaginosis and Jack D. Sobel (1997) reported multiple divided dose regimens.

Of the 10 antenatal cases, 3 were met with Amsel criteria and diagnosed as Bacterial vaginosis and *Gardnerella* isolated in one case. Among these 3 cases one case had preterm labour previously. *Staphylococcus aureus* was isolated in 3, *Candida species* was isolated in 3, *β hemolytic streptococci* was isolated in 2 and *Acinetobacter* in 1 case.

Mendiratta D.K. (1992) reported 24.33% of vaginal Candidiasis in 300 symptomatic women, Jack D.Sobel (1997) 25% and Snehalatha et.al, (2000) reported an incidence of 25.4%. A higher incidence 31 % was reported by Puri Kips (2003) and Sharma A.K. (2004) 26.3%. The incidence in our study was 25.38%. It was observed that culture of vaginal swabs for isolation of Candida proved to be a superior method in detecting vaginal Candidiasis as compared to Gram's staining and wet mount examination and the same conclusion was made by Mendiratta D.K. (1992).

Jack D. Sobel (1997) reported the incidence of *Trichomonas vaginalis* as 20%, J. W. Mahadani (1998) 16.45%. The incidence of *Trichomonas vaginalis* was 18.46% in our study. Sara M. Mariani (2003) reported, that 50% of cases may be asymptomatic and mixed infections were noticed in 25% and the prevalence of infection has been estimated 9% to 22% in pregnant women and most importantly 2% to 17% of children born from infected mothers are infected. Hence the necessity of appropriate prevention and early diagnostic strategies in high risk pregnant women.

It was observed that *Trichomonas vaginalis* was associated with bacterial vaginosis in 9 cases among 24 cases. It was also observed by Jane R.Schwebke (2003) in a study of 88 women who attended the family planning clinics with complaint of vaginal discharge, 37 were found with *trichomonas vaginalis* and among them 86% also met with Bacterial vaginosis. Sobel D (1997) reported the incidence of 20% and *trichomonas vaginalis* was identified in 30-40% of the male sexual partners of infected women. If untreated in pregnancy it leads to pre mature rupture of membranes, pre term birth. In non pregnant women it causes post hysterectomy cellulitis. The cure rate increases more than 90% when sexual partners are treated simultaneously with 500 mg of Metronidazole twice/day for 7 days. Metronidazole is the drug of choice in pregnancy.

CONCLUSION:

Leucorrhoea due to specific or non specific vaginitis is common in females especially in reproductive age group. Present study revealed that vaginitis is mostly polymicrobial in nature and Laboratory confirmation is essential to know the etiology. Bacterial Vaginosis in Antenatal cases is associated with attendant possibility of

Amnionitis leading to premature rupture of membranes. Early diagnosis and appropriate treatment will evert obstetric emergency and improves the pregnancy outcome.

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