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RESEARCH ARTICLE

PREVALENCE OF ANAEROBIC ORGANISMS IN BACTERIAL VAGINOSIS IN INFERTILITY CASES

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ABSTRACT

Three hundred female patients attending infertility clinic of obstetrics and gynaecology, RMMCH were enrolled in this study for a period of two years. Exclusion criteria included vaginal bleeding and pregnancy. Two high vaginal swaps were taken from each group of patients in three groups, Group1 comprising of 100 primary infertility patients, Group 2 comprising of 100 secondary infertility cases and Group 3 of 100 idiopathic infertility patients. Specimens were processed anaerobically and the results were analyzed. *Bacteroides, peptococci, peptostreptococci, fusobacterium, prevotella, veilonella and mobiluncus* were the anaerobic organisms found in bacterial vaginosis. A significant raise of anaerobic organisms (70%) were found in idiopathic infertility cases against lesser positive anaerobic organisms (56%) in secondary infertility. Bacteroides were found predominantly in 30% of idiopathic infertility cases as against 22% of positive cases in secondary infertility.

INTRODUCTION

The unhealthy vaginal environment in the Bacterial vaginosis can be described as an imbalance in the vaginal bacterial ecosystems, where the hydrogen peroxide producing lactobacilli are diminished and anaerobes and mycoplasmas are abundant (Barton et al, 2003). Several factors including poor hygiene, disturbed gastrointestinal flora, antibiotics, spermicides, infected partner, use of contraceptives, surgical procedures, catheter etc could lead to disturbance in the vaginal flora resulting in reduction of lactobacilli count and overgrowth of potentially pathogenic microorganisms.

Clinical diagnosis was first proposed by Dukes (1955) and formalized by Amsel's (1983) criteria which include changes in discharge, increase (>4.5) in pH, presence of clue cells, (epithelial cells coated with bacteria) in microscopic wet mount, and a fishy odour when discharge is mixed with 10% potassium hydroxide in affected female infertility patients. Diagnosis of Bacterial vaginosis includes gram stain of vaginal secretions, culture for isolation of organism, biochemical tests for metabolic byproducts by gas liquid chromatography and proline amino peptidase tests etc. (Mathew et al, 2001). Primary infertility is clinically considered when the couple has never conceived after one year of uninterrupted intercourse. Secondary infertility is considered when the couple achieved conception once or more and thereafter became infertile. Idiopathic infertility or unexplained infertility means there has been no definite cause of infertility is identified (Pratt, 1990) As the etiology and pathogenesis of Bacterial vaginosis are still unclear, more studies are needed particularly in various types of infertility cases to detect the prevalence of anaerobic organisms, this study was chosen.

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MATERIALS AND METHODS

A prospective blind study which involved 300 patients, divided into 3 groups, Igroup-100 women of primary infertility cases, II group-100 women of secondary infertility cases, III group-100 women of idiopathic infertility cases attending the department of obstetrics and Gynaecology, Raja Muthiah Medical College and Hospital, Annamalai nagar, Chidambaram, were selected. A detailed clinical history was taken from each patient and after getting consent from them, speculum examination was done aseptically. Two high vaginal swabs were taken from each patient. Colour, consistency, odour and other characteristics of vaginal discharge along with the complaints of itching were noted. pH of the vaginal discharge was detected directly using the indicator paper for pH on the vaginal wall. Amine odour test was also performed (Phiefer, 1978).

One swab was used for wet mount smears for gram staining, for the presence of pus cells, epithelial cells, clue cells and yeasts. A positive diagnosis of Bacterial vaginosis is made if 3 out of 4 Amsel's criteria were present. Second swab was inoculated in to appropriated

culture media, in to liquid medium like Robertson cooked meat medium and thioglycollate medium and into Columbia base lake horse blood kanamycin agar plates which were incubated anaerobically in Gaspak system at 36 degree C for 48-72 hours. Subcultures were made and colonies were identified based on gram staining and biochemical tests. Gram stain smears were read for morphing and scoring patterns according to Amsel's criteria. Each smear was examined for gram negative/positive/variable rods and gram positive cocci. Gram negative anaerobic bacilli were tested and the isolates which grew on the bile medium and hydrolysed aesculin were identified as Bacteriodes species. Isolates which failed to grow on bile or hydrolyse aesculin were identified as Prevotella species (Koneman, 1992).

RESULTS

Out of the total 300 infertility patients included in the study, 52 of secondary infertility cases, 68 of idiopathic infertility cases belonged to 36-40 years of age and this shows that as the age of marriage increases the infertility rate also increases in women (Table 1). A total of 144 patients were found to be positive by Amcel's criteria where as 165 patients were found to be culture positive for anaerobic organisms causing Bacterial vaginosis (Table 2).

Table 1. Age-wise distribution of women in each group

Age of the patient	Primary Infertility	Secondary Infertility	Idiopathic Infertility
20-25 yrs	12	3	2
26-30 yrs	15	13	10
31-35 yrs	40	32	20
36-40 yrs	33	52	68
Total	100	100	100

Table 2. Comparison of patients on the basis of Amsel's criteria and culture positive cases

Group	No of patients	Malodorous homogenous vaginal discharge	pH>4.5	Amine test	Clue cells	Cases showing 3 out of 4 AMCEL'S criteria positive	No of cases culture positive for anaerobes
Primary Infertility	100	24	18	14	16	31	39
Secondary Infertility	100	56	38	18	20	52	56
Idiopathic Infertility	100	68	56	46	24	61	70

Seventy cases were positive for anaerobes in idiopathic infertility which is significant against 56 positive cases in secondary infertility. Among the anaerobic organisms *bacteroides* were predominantly found positive (30 in idiopathic infertility cases, 22 in secondary infertility cases and 18 in primary infertility cases) contributing to a larger extent of various damages produced by Bacterial vaginosis (Table 3).

Table 3. Number of anaerobic organisms found positive by culture

Organisms	Primary Infertility	Secondary Infertility	Idiopathic Infertility
Bacteroides	18	22	30
Peptococci	9	12	14
Peptostreptococci	4	6	8
Fuso bacterium	3	8	8
Prevotella	2	6	5
Veillonella	2	1	3
Mobiluncus	1	1	2
Total	39	56	70

DISCUSSION

The prevalence of Bacterial vaginosis is generally higher among sexually active women, 52% of secondary infertility cases were of 36-40 years of age which could be compared to a study by Brown (1996) who showed that 60.11% of secondary infertility cases belonged to 36-40 years in his study. Bacterial vaginosis is the commonest cause of abnormal white discharge in women of child bearing age though it is not regarded as a sexually transmitted disease. The most common form of vaginosis in the U.S is proved to be bacterial vaginosis (Yens Shafer, 2003).

Spiegel (1991) suggested that organisms like *peptostreptococci, bacteroides, fusobacterium, peptococci and mobiluncus* organisms may be associated with Bacterial vaginosis, similarly all these organisms were detected even in our study. In a study by Easrnon et al., (1992) anaerobic *bacteroides* and other gram negative rods were isolated in 60% of infertility patients, where as in our study *bacteriodes* are the predominating organisms, 22 of them were found in secondary infertility cases and 30 were positive in idiopathic infertility patients. Bacterial vaginosis appears to be particularly common in sub-Saharan Africa where several studies reported high prevalence rates, ranging from 20-49% among women presenting to Sexually Transmitted Disease clinics with vaginal discharge, from 21-52% among pregnant women attending antenatal clinics (Mayaud et al, 1998) and from 37-51% in community based studies which stated that lifestyle practices such as vaginal douching have also been associated with an increased prevalence of Bacterial vaginosis (Walraven et al., 2001). Women with tubal infertility and menstrual problems like anovulation were three times more likely to have BV than other women with endometriosis. As per the study of Hotzman (2001) patients with idiopathic infertility were more likely to have bacterial vaginosis than with other causes of infertility, which could be proved even in our study, as 70 of idiopathic infertility cases with Bacterial vaginosis were culture positive for anaerobic organisms.

Bacterial vaginosis has been associated with an increased incidence of vaginal cuff cellulitis and abscess

formation. It is also associated with many obstetric and gynaecological complications including preterm labour, premature rupture of membranes, chorio amnionitis, puerperal endometritis, urinary tract infection, post operative cellulitis and cervical dysplacia (Thomason et al., 1991). The present report stresses that Bacterial vaginosis was proved to put patients at increased risk of upper-genital tract infections with severe consequences that would affect fertility and the outcome of pregnancy. As only very few statistics are available about Bacterial vaginosis in primary and secondary infertility cases in India, we suggest that for early diagnosis of Bacterial vaginosis, use of DNA probes and other newer techniques are mandatory in future, especially in women with any kind of infertility causes in reproductive age groups.

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