PREVALENCE OF G. VAGINALIS AMONG FEMALE PATIENTS ATTENDING THE KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA.

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ABSTRACT

One thousand, seven hundred and seventy-one (1771) female patients with offensive vaginal discharge, 49 of whom were between the ages of 5 months and 15 years as against 1722 who were above the age of 15 years were examined for infection with Gardnerella vaginalis, Trichomonas vaginalis, Candida albicans, Neisseria gonorrhoeae and Lactobacillus species. Of the 1722 women above 15 years of age, 1016 were pregnant whilst 706 were not. Forty-two (42%) per cent of the above 15 years age-group as against 36.7% of the under 16 years age-group were infected with G. vaginalis alone. Amongst the under 16 years age-group, G. vaginalis was never detected in association with T. vaginalis or with C. albicans. On the other hand, however, amongst the above 15 years age-group, G. vaginalis could be identified co-existing with T. vaginalis, C. albicans and N. gonorrhoeae.

From the results G. vaginalis-associated vaginosis may be considered as a non-sexually transmitted disease. The reason for this consideration is discussed and advantage is also taken of other published works on "Bacterial vaginosis" to attempt to unravel the possible pathogenetic processes involved in the genesis of Bacterial vaginosis.

A simple, rapid and reliable diagnostic method for G. vaginalis has been suggested.

Keywords: Gardnerella vaginalis, sexually transmitted disease (STD), vaginosis, pathogenesis.

MEDICINE

INTRODUCTION

Long before some small Gram-negative bacilli (Haemophilus vaginalis) were observed in "flour paste-like" vaginal discharges of sexually active women with non-specific vaginitis (a misnomer) in 1966(1), similar organisms had been observed in clinically similar conditions (2). However, the lack of adequate knowledge of the growth requirements and biochemical characteristics of this organism resulted in its being variously named as Haemophilus vaginalis or Corynebacterium vaginale (1, 3).

In 1980, thanks to thorough and well executed taxonomic and biochemical studies of the organism, it was accorded a genus of its own and renamed Gardnerella vaginalis (4,5,6,7,8) and the disease associated with it is today generally known as "Bacterial vaginosis".

With the controversy over the nomenclature and the taxonomic position of the organism now resolved, other areas of controversy have emerged, namely, the role of G. vaginalis in the pathogenesis of bacterial vaginosis.

While some researchers assert that G. vaginalis is the causal organism of bacterial vaginosis, previously known as non-specific vaginitis (1,9), others consider G. vaginalis and other organisms together, namely, anaerobes such as Bacteroides species, Fusobacterium species, peptococci, vibrio-like organisms or Mobilluncus species and Mycoplasma among other vaginal microbiota, to be responsible for the pathogenesis of bacterial vaginosis (12, 13, 14, 15).

Furthermore, the mode of transmission of G. vaginalis - associated vaginosis is also still debatable, although many are those who accept the thesis that bacterial vaginosis is transmitted through sexual means.

This preliminary communication aims at contributing to the understanding of the role of G. vaginalis in the pathogenesis of bacterial vaginosis and at elucidating

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the mode of transmission of G. vaginalis, taking advantage of other published works on the subject and to suggest the adoption of a simple, rapid and reliable diagnostic method for G. vaginalis. It is also the aim of this paper to stimulate further debate and discussions on the controversial issues raised here.

It is planned that future work will concentrate on answering some of the issues raised in this paper.

PATIENTS AND METHODS

Cervical swabs were collected in the case of females aged 16 years and above (1722), under direct vision during examination with a Cusco speculum, from a total of 1771 females attending the Komfo Anokye Teaching Hospital, Kumasi. Included in this survey were 1722 samples taken from women aged 16 years and above and comprising 1016 (59.0%) pregnant and 706 (41.0%) non-pregnant women, whilst 49 samples were from girls aged between 5 months and 15 years.

The major complaints of both adults and children with vaginal discharge in our study population, varied from vaginal fluid of unspecified coloration and consistency, to offensive vaginal discharge in our study population, varied from vaginal discharge or both of these.

As controls, 125 asymptomatic females of various ages were included in the survey.

Definition of Cases

In this survey cases were considered as G. vaginalis - associated vaginosis, when vaginal discharge from such patients fulfilled the criteria originally suggested by Gardner and Dukes (1) and recently by Blackwell and Barlow (16), but which we have slightly modified and which include the following physico-chemical and microscopic findings:

(i) Physical

Malodourous vaginal fluid which has pH value of between 4.5 and 5.5, average 5.0, i.e. less acidic than in normal vagina.

(ii) Chemical

Emmission of "fishy" amine or ammoniacal odour when 1 - 2 drops of 10% KOH are added to dried-up or saline-emulsified vaginal discharge.

(iii) Microscopic

Identification of "clue cells" (vaginal epithelial cells with Gram-negative and Gram-variable microorganisms), noting a leukocyte cell-count of 0 - 3 per HPF and detecting no Lactobacilus species, T. vaginalis, Candida species nor N. gonorrhoeae, in Gram-stained vaginal smears.

RESULTS

The results (Table 1) show that of a total of 1771 malodourous specimens examined, 741 (41.3%) thereof contained G. vaginalis alone (723 of 1722 or 42.0% for the age-group above 15 years and 18 of 49 or 36.7% for the under 16 years old age-group) or in combination with other micro-organisms. From this table it is discernible that of 1771 samples examined, 22.4% represented cases of moniliasis while 7.5% and 3.0%, respectively, represented cases of trichnomoniasis and gonorrhoea.

Table 1: Age Distribution pattern of female patients with offensive vaginal discharges in relation to individual microorganisms identified

Age Group	No. Examined	G. vaginalis		C. albicans		N. gonorrhoeae		T. vaginalis	
		No.	% age	No.	% age	No.	% age	No.	% age
0 - 15	49	18	36.7	9	18.37	12	24.49	4	8.33
16 - 35	1533	654	42.66	357	23.29	39	2.54	118	7.70
36 - 50	159	69	36.50	31	16.40	3	1.58	12	6.35
Total	1771	741*	41.8	397	22.42	54	3.05	134	7.56

* 537 of which contained only G. vaginalis whilst 204 contained G. vaginalis and other microorganisms in various combinations (Table 2).

Of this figure (741) were five hundred and thirty-seven samples (537) or 72.5% which contained only G. vaginalis; the remaining 204 (11.5%) contained G. vaginalis in combination with other microorganisms, the various combinations of which are shown in Table 2. Specimens showing other microbial combinations but without G. vaginalis numbered 27 and are depicted in Table 3. It can be seen that there were 19 specimens with T. vaginalis and C. albicans, 4 containing T. vaginalis and N. gonorrhoeae and two (2) each containing, respectively, C. albicans and N. gonorrhoeae and T. vaginalis, C. albicans and N. gonorrhoeae.

In this study, there were 1016 pregnant women as against 755 non-pregnant women which included 49 children aged below 16 years. Of the 1016 pregnant women, 541 (53.2%) had G. vaginalis - associated bacterial vaginosis as against 200 of 755 or 26.49% non-pregnant females (Fig.1)

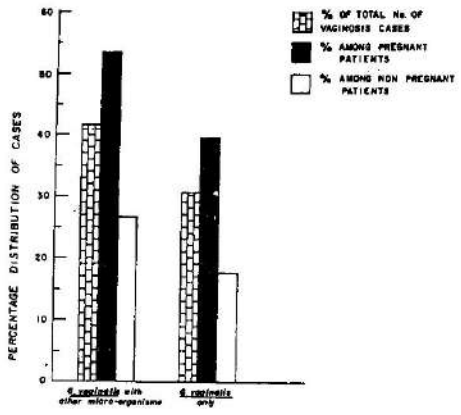

Fig. 1: Pregnancy and Gardnerella Vaginalis Associated Vaginosis

It is interesting to note that amongst the asymptomatic control group of 125 women of varying ages and sexual experiences, G. vaginalis was detected in 25 of them, giving a prevalence rate of 20%.

It was observed that no specimen obtained from the under 16 year-old girls yielded G. vaginalis in combination with T. vaginalis, Candida albicans or Lactobacillus species. The results further revealed, however, that amongst the above 15 years age-group, G. vaginalis existed in combination with T. vaginalis on the one hand and with Candida albicans on the other in 1.6% and 8.8% of cases, respectively.

Table 2: Pattern of G. vaginalis in combination with other microorganism

MICROBIAL COMBINATIONS WITH G. vaginalis					
	IN COMBINATION WITH G. vaginalis				
G. vaginalis	C. albicans	N. gonorrhoeae	T. vaginalis	Lactobacillus Species	No. of Cases encountered n = 204
+	+	+	-	-	3
+	+	-	+	-	9
+	-	-	+	-	27
+	+	-	-	-	153
+	-	+	-	-	12

Furthermore, G. vaginalis and Candida species were observed together in vaginal discharges from both pregnant and non-pregnant women, albeit in the ratio of 2:1 in favour of the pregnant women. The frequency of occurrence of G. vaginalis and Neisseria gonorrhoeae together in the same specimen was found to be 4.3 times lower among the under 16 years age-group (0.17%) than amongst the above 15 years age-group (2.87%). Interestingly, of the total number of 1771 vaginal smears examined no Lactobacillus species in association with G. vaginalis was encountered.

Analysis of leukocyte counts on all cases of G. vaginalis - associated vaginosis indicated that counts were between 0 and 3 cells per high power field (HPF). However, much higher but variable counts (5-50/HPF) were recorded on specimens from cases with moniliasis, trichomoniasis and gonorrhoea. The presence of G. vaginalis in association with one or the other of the causal organisms of the three conditions mentioned, vide supra, neither increased nor decreased the leukocyte counts. On the other hand, however, asymptomatic cases yielded no leukocytes or an occasional leukocyte.

Finally, all vaginal smears subjected to the 10% KOH test emitted foul smelling ammoniacal or "fishy" amine odour and were shown to contain "clue cells". The pH measurements were consistent, lying between 4.5 and 5.5 in virtually all specimens with "clue cells" and "fishy" amine odour.

DISCUSSION

Of a female study population of 1771 all of who had abnormal vaginal discharge, 933 had malodorous thin vaginal discharge, characteristic of G. vaginalis - associated vaginosis. Seven hundred and fourty-one (741) of the vaginal discharges yielded G. vaginalis alone or in combination with other microorganisms. However, of this number, 537 (75.47%) vaginal specimens yielded only G. vaginalis, thus making these the true cases of bacterial vaginosis. The ratio of these cases between those women below the age of 16 years and those above age 15 years was 1:1 suggesting that G. vaginalis - associated vaginosis affects all females irrespective of age and pubertal status. This may mean that bacterial vaginosis may not be entirely sexually transmitted, since all the children under 16 years of age in this study were virgins. Furthermore, this may mean that the G. vaginalis demonstrated in the vaginal discharges of these children may have been endogenous in origin and, therefore, may constitute part of the normal vaginal microbiota. These deductions are compatible with suggestions by other researchers (10,11). The virtual constancy of the prevalence rates of G. vaginalis, lying between 36.70% and 42.66% for the age-groups looked at (Table 1) in our study further buttresses our suggestion that G. vaginalis may be part of the normal vaginal bacterial flora.

Attempts at recovering G. vaginalis from male patients with NSU and from sexual partners of females with G. vaginalis infection, have yielded varying results, with some investigators (1, 13, 15, 17, 18) achieving isolation rates between 7.2% and 57%. Moss (19), however, failed to isolate G. vaginalis from male patients with NSU. It is to be realized that even when G. vaginalis was isolated from the male urethra, no association between symptoms and urethral carriage of the organisms could be established, suggesting that G. vaginalis is not a primary pathogen in males with NSU (17, 18). All the same, G. vaginalis has been shown to persist in the male urethra for many months (1). This finding may mean that G. vaginalis may be sexually transmissible. These findings notwithstanding, further prospective studies on both males and females are needed to ascertain whether or not G. vaginalis is truly sexually transmitted.

In our study, G. vaginalis was detected from among 933 malodorous vaginal specimens, either alone or in combination with other microorganisms which, on their own, are aetiologic agents of STD's, namely, T. vaginalis, Candida albicans and N. gonorrhoeae. The distribution of identified causal agents of trichomoniasis, moniliasis and gonorrhoea, in the absence of G. vaginalis were 7.56%, 22.41% and 3.05%, respectively. No Lactobacillus species was detected in any of the specimens examined.

Table 3: Pattern of Microbial combinations without G. vaginalis

MICROBIAL COMBINATIONS MINUS G. vaginalis			Number of Specimens Examined n = 27
C. albicans	N. gonorrhoeae	T. vaginalis	
+	-	+	19
-	+	+	4
+	-	+	2
+	+	+	2

It can be deduced from the above results that candidiasis/moniliasis is the commonest proven STD encountered in our study. This finding is consonant with findings from other African countries (20,21,22). This condition (candidiasis) is followed by trichomoniasis, with gonorrhoea taking the rear. It must be stressed, however, that these rates may be found to vary from country to country and even in the same country and that they are also dependent on the study population investigated.

It is to be noted further that of the 3 causal agents of STDs studied, namely, gonorrhoea, moniliasis, trichomoniasis, only Candida albicans has the propensity to thrive in low acid pH in the vagina occasioned by pregnancy, diabetes, antibiotic therapy or progesteron. It is not surprising, therefore, that of a total of 1016 pregnant as against 755 non-pregnant women in the study, 541 (53.25%) and 200 (26.49%), respectively, should be infected by G. vaginalis, ie. twice as many pregnant women were infected with G. vaginalis, suggesting that pregnancy may play a role in bacterial vaginosis. The ratio between pregnant as against non-pregnant patients with only G. vaginalis infection is also 2:1 (Table 4).

Table 4: Pregnancy and Gardnerella vaginalis - Associated vaginosis

Pregnancy	No. examined	G. vaginalis with other Microbes		Only G. vaginalis	
		No.	%	No.	%
Pregnant	1016	541	53.25	403	39.67
Non-pregnant	755	200	26.49	134	17.75
Total	1771	741	41.84	537	30.32

Over the years, it has been found that cultures of vaginal discharges from clinical cases of bacterial vaginosis have virtually always yielded G. vaginalis in association with anaerobic bacterial pathogens (12, 13, 14, 16) which latter organisms convert amino acids produced by G. vaginalis into amines (12) and ammonia (22). These products of bacterial metabolism raise the vaginal pH with concomitant reduction in vaginal redox potential (Eh). This condition favours rapid growth and multiplication of G. vaginalis in the vagina.

It, therefore, stands to reason to consider the relationship between G. vaginalis and anaerobic bacteria to be synergistic. This synergistic relationship is curtailed in the presence of metronidazole, rendering G. vaginalis more vulnerable to innate host defence mechanisms (9,12,13,23). The association between G. vaginalis and anaerobic bacteria in the vagina has been suggested to be necessary for the pathogenesis of bacterial vaginosis (12, 13).

It may be added that vaginal lactobacilli exhibit antibiosis in the vagina by virtue of their ability to produce lactic acid which lowers the vaginal pH and thus prevents the colonization of the vagina by Gram-positive and Gram-negative as well as facultative anaerobic organisms. In our study no lactobacilli could be detected so that the antagonistic effect expected against the vaginal anaerobes is negated so that there is a tilt in the balance between the activities of the vaginal lactobacili and those of anaerobic bacteria in favour of the latter resulting in raised vaginal pH which favours the genesis of bacterial vaginosis. The mechanisms and triggering factor(s) responsible for the tilting of the scale in favour of a rise in vaginal pH resulting in bacterial vaginosis may include pregnancy. With the decrease in the vaginal acidity G. vaginalis most effectively adheres to vaginal epithelial cells to form "clue cells" whose presence is suggestive of bacterial vaginosis.

With our finding that the calculated prevalence rate for the pregnant women was twice as high as that for not only the non-pregnant women but also for all the non-pregnant females (children as well as adults) together in the study, we are of the opinion that aside of vaginal pH and Eh variations due to vaginal microbiota interactions, pubertal status as well may constitute patent factors responsible for the pathogenesis of bacterial vaginosis. We also consider hormonal changes in the female vagina, following pregnancy to play a significant role in the genesis of bacterial vaginosis. The whole condition or pathogenetic process may, therefore, be considered a consequence of a disturbance in the vaginal bacterial ecosystem, occasioned by hormonal changes in the vagina, with non-sporing anaerobes acting as potentiating organisms.

Finally, we suggest that the detection of "clue cells" per se in a vaginal smear from a suspected patient with bacterial vaginosis, in our experience, should aid but not be the sine qua non for the diagnosis of bacterial vaginosis.

It has been realised that G. vaginalis outside the vaginal environment is so labile that its isolation by culture methods has often been lower than by microscopy (24), hence our failure to attempt bacterial isolation.

For this reason we make bold to suggest the employment of a combination of clinical as well as physicochemical and microscopic ("clue cells") parameters, vide supra, for rapid, simple and fairly reliable tool for the diagnosis of G. vaginalis - associated vaginosis in busy, all-purpose but often ill-equipped hospital laboratories in developing countries.

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