

## CLINICAL AND BACTERIOLOGICAL STUDY OF VAGINAL DISCHARGE DURING PREGNANCY

by

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Vaginal discharge is the most distressing symptom and the commonest complaint during the child-bearing period, though its treatment, notwithstanding modern advances, remains a challenge to the practising obstetrician. In the past it was considered a normal accompaniment of pregnancy and was borne stoically as the pregnancy, but more and more women are now-a-days seeking relief for vaginal discharge. It is for the attending obstetrician to determine the precise aetiological agent and institute the requisite therapy. Gonococcal and syphilitic infections of the vagina as the two commonest causes of vaginal discharge have been relegated to the background with the introduction of antibiotics, having been replaced by infestation with candida group and trichomonas. Further, the advent of antibiotics has resulted in the reversal of the ratio of trichomonas to candida group infestation from the previous 4:1 to the present 1:3 (Lee and

Kiefer, 1954). The incidence of trichomonas infection during pregnancy has been reported to vary from 8% (Shah, 1958) to 38% (Satyavati, 1959), as against the reported frequency of candida infection varying from 5.7% (Gardener *et al*, 1957) to 82.6% (Pickhardt and Breen, 1957). Velayudhan and Kurup (1963) found mixed infection by both these offending agents; Pace and Schantz (1956), however, could not find any mixed infection in their cases. Keeping in view the varying incidence of vulvovaginal candidiasis and trichomoniasis as reported by different workers, it was considered worthwhile to investigate the incidence of candida group and trichomonas infestation in women of Patiala area during pregnancy and to determine their relationship, if any, with the gestational period, parity, vaginal flora and pH.

### Material and Methods

Two hundred pregnant women attending the out-patients, the antenatal clinics and maternity wards of the Lady Dufferin Hospital, Patiala, comprised the subject material for the present investigation. After inserting a dry sterilized Cusco's speculum in the vagina, the discharge was collected from the upper part of the

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posterior fornix and the lateral vaginal wall with two sterilized cotton-tipped applicators. In case the discharge was scanty, scrapings were collected from the vaginal rugae with two swabs. The two were then treated as follows:

(i) Two tubes of Sabourauds' medium containing chloromycetin were inoculated with the material on one of the swabs and then cultured at 37°C for the candida group of organisms. Growth of candida group of organisms was identified by their smooth, white or cream coloured buttery colonies. Further confirmation of the diagnosis was obtained by making a wet smear in a drop of lactophenol blue. The yeast cells were seen as round or oval, highly refractile, doubly contoured cells, staining slightly blue. Cultures examined after 48-72 hours often showed the presence of hyphae also. The culture tubes not showing any growth were incubated for one week before declaring them negative. The confirmation of *Candida albicans* was based on its ability to form chlamydo spores on corn meal agar containing 1% Tween 80.

(ii) With the same swab as used for inoculation of the culture media, two smears were made on clean glass slides; one of them was stained by Gram's method and the other with Giemsa's stain. The Gram's stained smear was graded for vaginal flora according to the techniques outlined below (Doderlein, 1892).

(a) Grade I: When Doderlein's bacilli alone were present, unaccompanied by other micro-organisms.

(b) Grade II: When in addition to Doderlein's bacilli, other Gram positive or negative bacilli, cocci or coccobacillary forms were also seen.

(c) Grade III: When various other micro-organisms were present, but no Doderlein's bacilli.

Giemsa's stained smears were searched for trichomonas. In the patients yielding negative smears for trichomonas at the first instance, the examination was repeated twice with fresh smears during the next 3-4 days, before declaring these patients as finally negative for trichomonas infestation.

(iii) One ml. of sterilized normal saline was poured into the tube containing the second swab and shaken for obtaining an emulsion. One drop of this emulsion was mixed with 10% KOH on a clean glass slide and examined for the presence of monilia; a cover slip preparation for trichomonas was made with another drop.

(iv) The pH of the discharge on the speculum was determined by using a BDH universal indicator paper.

#### *Observations and Discussion*

##### *Overall Incidence of Vaginal Candidiasis and Trichomoniasis*

Out of the 200 pregnant women investigated in the present study for the incidence of vaginal candidiasis and trichomoniasis, 104 i.e., 52% were positive for *Candida* group and/or trichomonas, while the remaining 96, i.e., 48% were negative for either of these. Candidal infection was more frequent than trichomonas infestation as shown by the fact that whereas 67 cases were positive for *Candida* group, only 27 showed trichomonas; the infestation being mixed in the remaining 10. In other words, the incidence of *Candida* infection was 2.5 times more than trichomonas infestation. These findings are in line with the view of the majority of the modern workers that candida infection has now-a-days become more common than trichomonas infestation. (Lee and Kiefer, 1954; Pace and Schantz, 1956; Clark and Solomons,

1959; and Menon and Jehan, 1962). However, Amonkar (1959) and Satyavati (1959) found a higher incidence of trichomoniasis in pregnant women. The reason for the increased incidence of candida group infection is probably indiscriminate local use of antibiotics, killing the normal flora, thereby allowing an uninhibited growth of candida group (Purandare *et al*, 1962) and the increased use of contraceptive tablets and jellies making the vagina favourable for fungal growth (Whittington, 1951).

In 10, i.e., 5% of our cases, vaginal discharge examination showed mixed infection with candida and trichomonas. These findings are in conformity with those of Satyavati (1959), Clark and

pregnant women in our study, the incidence being 45.5%. Next in order was grade II (36.5%); only 18% showed grade III bacterial flora. Pregnancy being a hyperoestrogenic state is specially conducive to the maintenance of grade I flora and the increased oestrogenic concentration is often sufficient to improve the vaginal flora from grade III to grade II and from grade II to grade I (Bernstine and Rakoff, 1953). This point is brought home more startlingly by the study of vaginal flora in 96 subjects who were negative both for trichomonas and candida infections because infestation by either of these organisms indicates vitiation of the bacterial flora. These findings are set in Table 1.

TABLE I

*Bacterial Flora in Cases Positive for Candida Group and/or Trichomonas and in Those in Whom Candida or Trichomonas Were not Found*

Grade	Cases positive for candida and/or trichomonas		Cases in whom neither candida nor trichomonas was isolated	
	Number	%	Number	%
Grade I	30	28.8	61	63.5
Grade II	49	47.1	24	25.0
Grade III	25	24.1	11*	11.5

\* Six of these cases had cervical erosion.

Solomons (1959), Mehrotra *et al* (1960), Duttachaudhary *et al* (1962), Menon and Jehan (1962) and Velayudhan and Kurup (1963), in whose series incidence of mixed infestation varied from 4.3 to 6.8%. Correct diagnosis of the mixed infection by these organisms is important from therapeutic point of view.

#### *Bacterial Flora, Vaginal pH and Pregnancy*

Grading of the bacterial flora in the vaginal smears according to the criteria of Doderlein (1892) showed that grade 1 flora was the commonest finding in the

It is, therefore, apparent that pregnancy facilitates the maintenance of grade I bacterial flora in the absence of infection, the figure in our series being 63.5% as against 28.8% in cases positive for Candida and/or Trichomonas. Further, even in the presence of infection, grade III bacterial flora was seen in 24.1% of cases only, the remaining having grades I and II, thereby proving that the increased levels of oestrogens during pregnancy helped maintain grade I and grade II flora, even in spite of infection.

No statistically significant relationship

could be determined between the grading of vaginal flora and pH of the vaginal secretions, a finding in agreement with that of Weinstein and Wickerham (1938).

#### *Candidal/Monilial Vaginitis*

Out of the 200 cases studied in our

and the stained smears, respectively. Similar have been the observations of Satyavati (1962).

The distribution of 77 *Candida* positive cases in relation to the age, parity and the period of pregnancy is given in

Table II.

TABLE II  
Distribution of 77 *Candida* Positive Cases in Relation to Age, Parity and Period of Pregnancy

Total No. of subjects	Age			Parity		Period of gestation (in trimesters)		
	16-20	21-30	31-40	Primi.	Multi.	1st	2nd	3rd
77	14 (34.1%)	51 (40.8%)	12 (35.3%)	16 (30.1%)	61 (40.9%)	2 (14.2%)	13 (36.1%)	62 (41.3%)

Note:— Figures in parentheses indicate the percentage of positive cases in relation to the total number investigated in each sub-group.

series 77, i.e., 38% (including 10 with mixed infection) were positive for monilial infection. Conflicting reports have appeared in the literature in regard to the incidence of *Candida* infection during pregnancy; figures varying from 5.7% (Cardener *et al*, 1957) to 82.6% (Pickhardt and Breen, 1957) have been reported. The incidence of 38.5% *Candida* infection in our study corresponds to the 37% reported by Johnson and Mayne (1948), 40% by Mehrotra *et al* (1960), 39.6% by Menon and Jehan (1962), 33.3% by Daftary *et al* (1962) and 34.8% by Velayudhan and Kurup (1963).

Culture of the vaginal swabs for the isolation of *Candida* group of organisms proved to be superior method in detecting this infection, as compared to examination of the wet and/or the stained smears. Whereas 38.5% of the cases turned out to be positive for fungal infection on culture, only 14.5% and 21.5% were proved to be positive in the wet

A perusal of Table II shows that candidiasis during pregnancy bears direct relationship to the parity and the period of gestation, the highest incidence being seen in multipara during the third trimester. Greater frequency of *Candida* infection in multiparae in comparison to the primiparae has also been reported by Bose and Rana (1953), Pandya *et al* (1958), Duttachaudhary *et al* (1962) and Gupta and Sachan (1963). Fungal infections are more common in parous women than in the non-parous (Pickhardt and Breen, 1957; Amonkar, 1959; Narvekar *et al*, 1959; and Gupta and Sachan, 1968). May be that repeated pregnancies with trauma of parturition somehow make the genital tract more susceptible to fungal infection. The peak of the acidic pH attained during the last trimester as a result of maximal glycogen concentration could explain the higher incidence of *Candida* which thrive in an acidic medium. It may be pertinent to

add that the vaginal pH range in our cases of candidiasis during pregnancy varied from 4.2-5.5 as against the overall pH of 4.0-6.5 for all the subjects.

Majority of the pregnant women suffering from candidiasis had associated grade I and grade II bacterial flora, the respective figures being 31.8% and 47% as against 19.3% in association with grade III. Similar have been the observations of Carter *et al* (1940), Bose and Rana (1953) and Amonkar (1959).

#### *Candida Albicans Vs. Other Candida*

Out of 77 cases suffering from candidiasis, candida albicans as the causative agent could be demonstrated in 41, i.e., 53.2%. An incidence varying from 54 to 72% of candida albicans infection in cases of vaginal candidiasis has been reported by other workers (Dawkins *et al*, 1953; Narvekar *et al*, 1959; Dutta-chaudhary *et al*, 1962; and Singh and Sharma, 1962). As *Candida albicans* is the main pathogen in the candida group, it would naturally be expected that the vaginitis due to this species would more likely be symptomatic than that due to infection by other candida. A comparison of the symptoms produced by *Candida albicans* with those by candida other than *Candida albicans* showed that while 84.3% of cases positive for *Candida albicans* had symptoms, only 33.8% of candida other than *Candida albicans* were responsible for symptoms, thus stressing that *Candida albicans* is the most pathogenic organism of the candida group. In 15.7% of cases in our series, *Candida albicans* was asymptotically present in the vagina. Dawkins *et al* (1953) and Mehrotra *et al* (1960) have reported that *Candida albicans* may exist saprophytically, while others (Jillson and Lyle, 1956) doubt the presence of this organism in the normal vagina.

#### *Trichomoniasis*

*Trichomonas vaginalis* infestation was discovered in 37, i.e., 18.5% of the pregnant women investigated. This figure includes 10 cases in whom trichomonas was associated with monilial infection. In the literature, wide variations (8 to 38.1%) have been reported regarding the incidence of trichomonas infestation during pregnancy (Johnson and Mayne, 1948; Bose and Rana, 1953; Kean and Day, 1954; Shah, 1958; Satyavati, 1959; Daftary *et al*, 1961; and Velayudhan and Kurup, 1963). Our findings are in line with 15% incidence reported by Kean and Day (1954), 24% by Bose and Rana (1953), 23.8% by Daftary *et al* (1961), 16.9% by Menon and Jehan (1962) and 20.4% by Velayudhan and Kurup (1963).

Because of the common belief that a pH below 5 is inimical to trichomonas, the incidence of this infestation can, therefore, be expected to be low during pregnancy, the vaginal pH values being between 4 and 5. But the findings in the present study and of those of other workers cited earlier, clearly show that trichomonas vaginalis infestation during pregnancy is not uncommon, an incidence as high as 38.1% having been reported by Satyavati (1959). The probable explanation for this may lie in the fact that hypertrophy of the cervical glands pours out a secretion of alkaline nature into the vagina, thereby making its secretion less acidic.

An investigation of the pH in cases of vaginal trichomoniasis demonstrated the range of 5.5-6.5, the mean value being 5.7, while in cases negative for trichomonas and candida it ranged from 4.0 to 4.8 with a mean of 4.2. This shows that trichomonas vaginalis prefers a sub-acidic medium, i.e., more on the alkaline

side. Support to our results is lent by the observations of Amonkar (1959), Menon and Jehan (1962) and Velayudhan and Kurup (1963), in whose series also the vaginal pH in trichomonas infestation during pregnancy was above 5.5.

In Table III has been set the distribution of pregnant subjects suffering from trichomoniasis in relation to age, parity and period of pregnancy.

twice (Table III). Conflicting views on the subject of vaginal trichomoniasis in relation to the gravid state have been expressed by different workers. Shah (1958) and Velayudhan and Kurup (1963) found a higher incidence of trichomonas infestation in multiparae, but according to Schmid and Kamnikar (1926) and Greenhill (1928), on the other hand, it is more in the primiparae their results being comparable to those

TABLE III  
Distribution of 37 Cases of Trichomoniasis in Relation to Age, Parity and Period of Pregnancy

Total No. of subjects	Age			Parity		Period of gestation (in trimesters)		
	16-20	21-30	31-40	Primi.	Multi.	1st	2nd	3rd
37	9 (21.9%)	24 (19.2%)	4 (11.7%)	14 (27.4%)	23 (15.4%)	—	8 (22.2%)	29 (19.3%)

Note:— Figures in parentheses indicate the percentage of positive cases in relation to the total number investigated in each sub-group.

In the present study, the incidence of vaginal trichomoniasis worked out to be higher in the earlier period of reproductive life, i.e., up to 30 years as compared to the later period, i.e., 31-40 years. According to most of the workers, the commonest age for trichomonas vaginalis infestation is in the age group 20-40, i.e., in the prime of the reproductive period. (Angelucci, 1936; Daftary *et al*, 1962; and Menon and Willmott, 1962). However, according to Gwelessiancy and Papitachvili (1935), the incidence of this infestation is higher in earlier age group, i.e., between 20-25 years. Furnis (1930) has, however, reported that trichomonas infestation of the vagina bears no relationship to age.

Our findings show that primigravidae are more prone to vaginal trechomoneasis in comparison to the multigravidae, the

incidence in the former being almost in the present study. Further, trichomonas was found more frequently in multiparae than in nulliparae by Amonkar (1959) and Narvekar *et al* (1959). It would, therefore, appear logical to assume that trauma of repeated pregnancies and deliveries might predispose a woman to trichomonas vaginalis infestation, but the findings of many workers, cited above, do not favour this contention.

A perusal of Table III shows that the incidence of vaginal trichomoniasis was almost identical during the second and the third trimesters of pregnancy (22.2% and 19.3%, respectively), there being none in the first. Daftary *et al*, (1961) also did not find any difference in the incidence of trichomoniasis during the second and the third trimesters, although

it was much less in the first. Feo (1953) had maximum cases positive for trichomonas vaginalis in the last trimester.

To determine the relationship, if any, between the vaginal trichomonas infestation and the local bacterial flora our results have been set in Table IV.

mixed infestation by candida and trichomonas were seen out of a total of 73 pregnant women with grade II flora investigated by us. As against this, the remaining 5 cases of mixed infestation having grade III bacterial flora were discovered out of a total of 36 pregnant

TABLE IV

*Relationship of Trichomonas Infestation and Bacterial Flora of the Vagina*

Bacterial flora	Cases investigated	Positive for trichomonas	pH range
Grade I	91	1 ( 1.1%)	5.5
Grade II	73	17 (23.2%)	5.5-6.0
Grade III	36	19 (52.7%)	5.5-6.5

It is apparent from the Table that trichomonas vaginalis infestation is commonly associated with grade III bacterial flora, the incidence being 52.7% as compared to 23.2% with grade II and only 1.1% with grade I bacterial flora. That trichomonas infestation is more frequently in association with grade III bacterial flora has been the finding of most of the workers. (Bernstine and Rakoff, 1953; Satyavati, 1959; and Menon and Wilmott, 1962); our figures are in agreement with theirs. This association is also to be expected because trichomonas lives in happy symbiosis with other bacteria, specially non-haemolytic streptococcus (Bernstine and Rakoff, 1953).

*Mixed Infestation with Candida Group and Trichomonas Vaginalis*

Mixed infestation with candida group and trichomonas vaginalis was demonstrable in 10, i.e., 5% of our cases. Five of these pregnant women were primigravidae, the remaining 5 multiparae. Grade II vaginal bacterial flora was seen in 5 of these pregnant women, the remaining 5 having grade III. These 5 cases of grade II bacterial flora having

women with grade III bacterial flora. It is, therefore, obvious that mixed infestation with trichomonas and candida is twice more frequently associated with grade III bacterial flora than with grade II. The vaginal pH in these 10 cases ranged between 4.5-5.8; the mean being 5.5. No significant difference was found in the incidence of mixed infestation in relation to age or period of gestation.

*Symptoms and Signs in Vaginal Candidiasis and/or Trichomoniasis*

Whereas 70.3% of cases with trichomonas vaginalis infestation presented with one symptom or another, the corresponding figure for candida cases having symptoms was 62.5%. A comparison of the signs and symptoms in cases positive for candida and/or trichomonas showed that the symptom were more severe when infestation with both these microorganisms was present together (Table V).

Vaginal discharge was a common symptom in both the trichomonas and candida infestations, the percentage positive being 55.5% and 53.7% respectively. Pruritus was more common with monilia

TABLE V

*Incidence of symptoms and signs in Candida Positive, Trichomonas Positive and Mixed Infection Cases*

Symptoms and signs	Candida positive		Trichomonas positive		Mixed infection	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
<b>Symptoms:</b>						
Discharge	36	53.7	15	55.5	7	70.0
Pruritus	20	29.8	5	18.5	4	40.0
Burning micturition	8	11.9	7	25.1	3	30.0
Frequency of micturition	8	11.9	4	14.8	2	20.0
Dysuria	3	4.4	2	7.4	2	20.0
Dyspareunia	3	4.4	2	7.4	1	10.0
<b>Signs:</b>						
Vulvitis	10	19.9	3	11.1	2	20.0
Vaginitis	7	10.4	4	14.8	2	20.0
Cervicitis and erosion cervix	3	4.4	4	14.8	2	20.0

infection (29.8%) as compared to trichomonas positive cases (18.5%). Urinary symptoms, on the other hand, were more frequent in trichomonas infestation as compared to candida infection. Bernstine and Rakoff (1953) and Narvekar *et al* (1959), also made similar observations.

Vaginitis was present in both types of infestations, but was more common with trichomonas, giving an incidence of 14.8% as compared to the 10.4% in candida infection. Cervicitis and cervical erosion were also more common in trichomoniasis, an incidence of 14.8% as compared to the 4.4% in candida positive cases. As against this, vulvitis was more frequent in candida, an incidence of 19.9% as compared to 11.1% in trichomonas positive cases. Three cases out of ten of vulvitis showed severe vulval oedema in candida infection, while none of them with trichomonas infestation did. Similar findings have been reported by

Bernstine and Rakoff (1953) and Narvekar *et al* (1959).

#### *Summary*

Vaginal discharge in 200 pregnant women was investigated for the incidence of candida group and trichomonas vaginalis infestation and its relationship, if any, to vaginal pH, bacterial flora, age, parity, period of pregnancy and symptomatology. Candidal infection was 2.5 times more frequent than trichomonas vaginalis infestation. From candidiasis cases, *Candida albicans* as the pathogen was isolated in 53.2%. Fungal infection of the vagina was more common in multipara and more frequently associated with grade I and II vaginal bacterial flora and acidic pH. *Trichomonas vaginalis*, on the other hand, was more often seen in primigravida along with grade III bacterial flora; the vaginal pH in these cases being alkaline or slightly acidic. A



mixed infestation by trichomonas vaginalis and candida group was responsible for severer symptoms than infection by either of the organisms alone.

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