THE FORMATION OF GERMTUBES BY CANDIDA ALBICANS IN HUMAN AMNIOTIC FLUID

by

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Introduction

Vagina, particularly during pregnancy, harbours a number of microorganisms, some of which are pathogens or potential pathogens. The high glucose content and low pH of the vagina during pregnancy favour the growth of yeast and yeast like fungi, that is Candida. Vaginal Candidiasis is not uncommon during pregnancy. Inspite of the free access of pathogenic microorganisms of vagina to the foetus following rupture of membranes during delivery, foetal infection with these organisms is not frequent. Amniotic fluid has been considered to possess antimicrobial activity which is thought to be responsible for transmission of these organisms to placents and foetus. (Galask and Snyder, 1968; Bergman, *et al* 1972; Kitzmiller, *et al* 1973; Schlievert, *et al* 1975; Miller, *et al* 1976; Evans, *et al* 1977 and Jankowski, *et al* 1977). Inhibition of the growth of C. albicans (the most pathogenic species of Candida) of amniotic fluid has been reported by Auger (1980).

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Accepted for publication on 21-5-81.

Since germtube formation is considered to be the invasive form of C. albicans, the present study was conducted to find out the formation of germtube by C. albicans in amniotic fluid and was compared with that occurring in the human serum. In both these media multiplication of the same organism was also studied at 25°C when no germtube formation can occur.

Material and Methods

The formation of germtubes by a single strain of C. albicans, obtained from the Department of Microbiology, Dr. Sampurnanand Medical College, Jodhpur was studied in 50 amniotic fluids, collected aseptically during delivery at full term. The amniotic fluids so collected were kept at 4°C till used on the same day or a day or 2 days later. For comparison formation of the germtubes by the same strain of C. albicans was also studied in 10 samples of serum obtained from apparently healthy individuals.

Routine examination of urine and blood was undertaken for each individual to exclude diabetes mellitus. One of the patients was found to be diabetic.

Four of the amniotic fluids, 2 each which showed no germtube formation and high percentage of germtube formation respectively (vide infra) were also subjected to total protein estimation and

dialysis in normal saline. Then they were evaluated for their ability to induce the formation of germtube by the same strain of C. albicans. The technique employed for the study of formation of germtube was that of Joshi *et al* (1979). A suspension of organisms was made by dissolving a couple of colonies in 1 millilitre of normal saline. Then the number of the organisms was counted by haemocytometer and the suspension was diluted with normal saline to make a concentration of 10^7 organisms/ml. 0.1 ml. of this suspension was added to 0.9 ml of amniotic fluid or serum to make a final concentration of 10^6 per millilitre. It was then incubated at 37°C in a water bath for 3 hours. A drop of the fluid from bottom of the tube was taken on a clean glass slide, covered with coverglass and examined under microscope to count the germtube containing cells per 100 cells. A smear was also prepared and stained with Giemssa stain then examined under microscope to exclude any gross contamination of bacteria whose presence may interfere in the formation of germtube of C. albicans (Purohit *et al* 1977). Contaminated samples were not included in the study.

The pH of all the amniotic fluids was also recorded before testing them for induction of germtube formation.

During the present study multiplication of the yeast forms of C. albicans was also found out in randomly selected 10 samples of amniotic fluids and 2 samples of serum. For this a synchronized culture of C. albicans obtained as per Dabrowa (1967) was used in a final concentration of 10^6 organisms/ml in the fluid to be tested. After achieving this concentration the test tubes containing organisms and media were kept at 25°C for 6 hours. During 0 hour, 3 hour and 6 hour, a count of blastospores was made by haemocytometer and per cent increase in cells was recorded.

Results

I—Germtube formation in amniotic fluid and serum:

The results of germtube formation in amniotic fluid of full term pregnant women and serum of healthy individuals is shown in Table I. Specimen photographs of the germtube formation for Candida albicans of different cases examined by us are included herewith and these photos belong to the percentage range specified. The mean percentage of germtube formation in sera of healthy individual was 71.80 (Fig. 1), whereas in amniotic fluid it was 6.19 (Fig. 2). In 39 samples of amniotic fluid, the mean germtube formation ranged from 0-10% (Fig. 3). Four samples showed a range of 11-20%, whereas in remaining 7 samples

TABLE I

Germtube Formation in Amniotic Fluid of Full Term Pregnant Women

S.N. for groups	Mean % of G.T. formation arranged in 10 groups	No. of amniotic fluid samples	No. of sera
1	0 - 10	39	0
2	11 - 20	4	0
3	21 - 30	3	0
4	31 - 40	1	0
5	41 - 50	1	1
6	51 - 60	0	0
7	61 - 70	1	3
8	71 - 80	0	4
9	81 - 90	1	1
10	91 - 100	0	1
	Total	50	10

of amniotic fluid the germtube formation ranged from 21-80.18%. Only 1 sample of the amniotic fluid was having a very high percentage of germtube formation (80.18%). The patient was a case of frank diabetes, blood sugar level was 250 mg.% by Folin-Wu method.

II—Influence of various factors on germtube in amniotic fluid:

The pH of all the amniotic fluid ranged from 8 to 10.5 which was within the range in which C. albicans formed germtubes. Total protein content estimation which was estimated in the 4 samples of amniotic fluid ranged from 0.625 gm to 1.250 gms. It did not show any relation to the ability of amniotic fluid to form germtubes. Dialysis of these fluids in saline did not improve the germtube forming ability of the amniotic fluid rather in the two samples in which the germtube formation was 64.8% and 80.18%, after dialysis the ability of formation of germtubes diminished markedly to 10.5% and 14.5% respectively.

III—Multiplication of yeast forms of C. albicans:

The study on the growth of C. albicans in amniotic fluids and serum samples incubated at 25°C revealed that in serum mean rise in blastospores was 304.78% within 3 hours of incubation, after 6 hours mean rise was 431.72%. In amniotic fluids the rise in the number of cells was very low, except in 3 samples in which it was significantly high comparable to serum samples. The per cent rise of blastospores in amniotic fluid and serum is shown in Table II. One of the samples of amniotic fluids in which significant rise in blastospores formation was observed from the patient of diabetes mellitus.

Discussion

In this study formation of germtube by a single strain of C. albicans was studied in 50 samples of amniotic fluid and 10 samples of human sera. The rate of multiplication of this organism at 25°C was also studied in randomly selected 10

TABLE II

Percentage Rise of Blastospores in Amniotic Fluid

In 10 samples of amniotic fluid				In 2 samples of serum			
% rise after 3 Hrs.	% rise after 6 Hrs.	Mean % rise after 3 Hrs.	Mean % rise after 6 Hrs.	% rise after 3 Hrs.	% rise after 6 Hrs.	Mean % rise after 3 Hrs.	Mean % rise after 6 Hrs.
23.8	50.0	26.17	45.17	295.63	393.5	304.78	431.72
26.32	51.1			—	—		
18.25	41.1			—	—		
17.5	34.8			—	—		
32.5	42.5			—	—		
35.51	45.15			313.94	469.95		
20.13	38.33			—	—		
21.0	40.31			—	—		
55.40	65.5			—	—		
21.20	43.0			—	—		

samples of amniotic fluids and 2 samples of normal sera. The results of germtube formation in human serum are caparable to that reported earlier by Taschdjian *et al* (1960) and Joshi *et al* (1979). A significant diminution of germtube formation was observed in amniotic fluids, revealing thereby the presence of inhibitors of germtube formation in amniotic fluids. Earlier reports have revealed the inhibition of growth of C. albicans in amniotic fluid (Auger, 1980), which was also confirmed in the present study. In this study such inhibition was observed in 7 out of 10 samples, compared to the growth which occurred in human serum. However, in all the samples there was a definite rise in the number of cells from the original number. The present study indicates that probably the inhibition of formation of germtubes by the amniotic fluid is responsible for relatively infrequent intrauterine ascending infection inspite of the presence of the C. albicans in vagina of majority of pregnant women. Whether the factor(s) which inhibit the formation of germtubes is/are the same which inhibit multiplication of C. albicans needs/need to be investigated. However, it is clear from the present study that neither the protein contents nor the dialysable substances are responsible for this, dialysable substances rather help in the formation of germtubes by C. albicans because there was a significant reduction in the formation of germtubes following dialysis of the two amniotic fluid in which it was fairly high before dialysis. It is also clear from the present study that the pH of the amniotic fluids has nothing to do with the inhibition of formation of germtubes of C. albicans as the pH range was within the limits in which this organism can form germtubes (Joshi *et al* 1979).

Conclusion

This study has revealed that amniotic fluid inhibits formation of germtubes by C. albicans even more than it inhibits the growth of this organism, the inhibiting factor is neither related to the protein content nor to the dialysable substances present in the amniotic fluid. Further studies are required to identify the factor/factors which inhibit the formation of germtubes by C. albicans. Whatever this or these may be they appear to play a great role in prevention of ascending infection of Candida albicans.

Acknowledgement

Authors are highly grateful to the Principal, Dr. K. P. Singh, who has provided us facilities for this study. We thank Mr. S. L. Purohit for the technical assistance needed in the study.

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See Figs. on Art Paper V