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Title : Use of Cervical Cytology, Vaginal pH and Colposcopy as Adjuncts to Clinical Evaluation of Ayurvedic Vaginal Douche, Panchavalkal, in Leucorrhoea.

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Short Title : Panchavalkal Vaginal Douche for Leucorrhoea.

Key words: Leucorrhoea, Ayurveda, Vaginal pH, Panchavalkal douche, Papanicolaou smear, Colposcopy.

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Abstract

Objective : To use cytology, vaginal pH and colposcopy as adjuncts to clinical assessment of efficacy of traditional Ayurvedic douche alone in treatment of uncomplicated leucorrhoea.

Subjects : Forty two cases of uncomplicated leucorrhoea ie. without Pelvic Inflammatory Disease or associated gynaecological or systemic pathology

Methods : History, clinical examination, Papanicolaou (Pap) smear, Gram smear, Vaginal pH, and colposcopy were carried out before treatment, at 2 weeks and post treatment at 1 monthly examination. Daily douches with modified Panchavalkal (decoction from barks of *F. bengalensis*, *F. religiosa*, *F. infectoria*, *F. glomerata* and *A. lebbek*, 25gms each), were given for 14 days. Symptoms were evaluated by a diagrammatic visual grading score.

Results: Thirty six cases (85.7%) had clinical relief or cure ($p < 0.001$; Chi square test). The symptom score was reduced from 5.4 ± 1.9 to 2.2 ± 2.2 (Mean \pm SD) at 2 weeks and 2.3 ± 2.2 (Mean \pm SD) at 1 month ($p < 0.001$; paired 't' test) i.e. at 2 weeks post treatment . out of 29 cases who had cervical erosion, 20 cases showed reduction in area of erosion whilst no change was seen in 9 cases. Colposcopy (n=21) confirmed reduced vascularity, inflammation, discharge and epithelialisation of TZ in 19 cases. Vaginal pH

(n =28) was reduced in 13 and remained normal in 7 and remained same in 4 cases. In 4 cases there was a nonsignificant rise. Pap smears (n=37) showed improvement in severity of inflammatory changes in 25 cases. Specific infections were seen in 24 cases initially, and in 15 cases at 1 month.

Conclusions : Apart from symptoms and signs, cervical cytology, colposcopy and vaginal pH were very useful in evaluation of the traditional Panchavalkal douche treatment of uncomplicated leucorrhoea. Multiple infections and resistant cases were identified. Panchavalkal provided symptomatic relief in 85.7% of cases.

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Introduction

Leucorrhoea is a common symptom and afflicts upto 75% of women at least once in their lifetime ¹. It may be innocuous and physiological as during ovulation or in pregnancy, or it may be a forerunner of serious complications like pelvic inflammatory disease (PID), ectopic gestation or cervical cancer. All over the world more than 300 million cases of sexually transmitted diseases (STDs) in women are reported annually and leucorrhoea is a common symptom ¹⁻⁴. As in other developing countries, in India, Reproductive Tract Infections (RTIs), which include STDs, are common ⁵⁻⁷. Our earlier work has shown that the prevalence rates are high, not only in the high risk population but also in the relatively

low risk population like Copper-T users and those attending family welfare clinics and antenatal clinics⁶⁻⁸. Recent community studies in other parts of India have confirmed this⁹. It becomes extremely important therefore to evaluate all available methods of treatment and prevention of complications.

In India, traditional systems of medicine, like Ayurveda, are used by large sections of society. Many current practitioners of Ayurveda use Panchavalkal (PVK) douche for treatment of leucorrhoea. PVK is a decoction prepared from the mixture of barks of five medicinal plants and has been described for the treatment of women's diseases in Ayurvedic texts¹⁰. Laboratory studies over the decades by Bhatt and coworkers have shown 'in vitro' and in vivo antimicrobial activity against several aerobic and anaerobic organisms, some of which are also known to cause cervico-vaginal infections¹¹. They had also shown interesting results in patients with infected burns using modified Panchavalkal i.e. Parish substituted by Shirisha.

Despite extensive clinical use of PVK for vaginal infections or cervical erosions by Ayurvedic physicians there is no published data which shows its efficacy or otherwise. Some thesis work by Ayurvedic postgraduates shows clinical relief only but objective criteria or laboratory tests have not been used. One clinical study has shown some beneficial effect of oral PVK capsules in prevention of postoperative complications of gynecological surgery¹². The present study was undertaken to objectively evaluate the effect of PVK douche in the treatment of leucorrhoea. The parameters used for assessment were a semiquantitative assessment of symptom relief, per speculum and per

vaginal examination, cervical cytology (Papanicolaou smear), endocervical gram smear, vaginal pH and colposcopy done before and after treatment. The project was approved by the Ethics Committee and was carried out by Bhavan's SPARC in collaboration with Sion Ayurved Mahavidyalaya (SAM).

Material & Methods

Subject enrollment : A total of 129 consecutive ambulant cases referred to the joint leucorrhoea clinic of Bhavan's SPARC and SAM were evaluated between June 1999 and September 2001. Out of these 42 cases with uncomplicated leucorrhoea ie. without pelvic inflammation or associated local or systemic pathology, were admitted to the study group as per the following criteria:

Inclusion criteria : 1) Willingness to use PVK douches as the only method of treatment during the study period of 2-4 weeks 2) Willingness to come to the hospital for daily douches for 14 days 3) History of tubal ligation or condom use for contraception 4) Abstinence from sexual intercourse during study period 5) Age between 20 and 50 years 6) Informed written consent after reading the information about the study.

Exclusion criteria : 1) Pelvic Inflammatory Disease (PID) using standard criteria 2) Abnormal Papanicolaou smear with intraepithelial or invasive cancer 3) Unexplained vaginal bleeding 4) Abnormal findings on pelvic sonography 5) Pregnancy 6) Fibromyoma, polyp, ovarian tumour or cyst, prolapse 7) Systemic disease like hypertension, diabetes, heart disease, tuberculosis, 8) Severe anaemia i.e. Hemoglobin < 8

gms%, 9) Use of antibiotics or local vaginal formulation within the previous month, 10) Endocervical smear positive for gonococci 11) Positive VDRL or HIV test.

Preparation of douche

The barks of 5 medicinal plants (*Vata or Ficus bengalensis*, *Udumbar or Ficus glomerata*, *Plaksha or Ficus infectoria*, *Ashwatta or Ficus religiosa* and *Shirisha or Albizzia lebbek*) were purchased from a standard Ayurved pharmacy (Arya Vaidya Rasashala, Mumbai). Packets of 25 gms of mixture with equal proportions of each type of bark by weight (5 gms each) were prepared and stored at room temperature, away from sunlight, with silica gel bags to avoid absorbance of moisture. Fresh decoction (Kwath) was prepared for daily use by boiling 25 gms of PVK in 2 litres of water in an open vessel till it was reduced to 500 ml^{10,13}. The mixture was filtered and when tepid warm, was used for douching under hygienic conditions with disposable plastic nozzles used separately for each patient.

Dose : PVK douche was given daily in the gynecological ward between 10.00 am and 12.30 am for 14 days by Ayurvedic physicians.

Follow up: All cases were advised to come for daily douching and were assessed initially and at 1 week (symptom score only), 2 weeks and 4 weeks ie. 2 weeks post treatment. They were advised abstinence from sexual intercourse for 2 to 4 weeks . The initial and 1 monthly examinations were carried out in the same phase of the menstrual cycle to avoid the confounding effect of cyclic changes in pH , and colposcopic findings¹⁴. Women with persistent specific infections at the end of 4 weeks, or those who worsened earlier were given appropriate modern drugs¹⁵⁻¹⁷. Their male partners were prescribed modern

drugs whenever indicated. All anemic women were prescribed oral iron therapy at the end of 2 weeks of treatment.

Pretherapy investigations

Symptom score: A specially designed case record form with symptom scoring was used at each visit. The amount of discharge was scored by the patient on demonstration of a schematic diagram ie. Visual grading (Fig 1). The criteria used for overall assessment are given in Table I.

Gram stain smear : Endocervical smear was Gram-stained and examined for pus cells, bacteria, clue cells, candidiasis and intracellular gram negative diplococci.

Papanicolaou smears : Two smears (ectocervical and endocervical) were collected at each examination using a presterilised (gamma radiated) disposable wooden spatula and endocervical brush (Contech devices). The severity of inflammation was assessed by cytoplasmic and nuclear changes, polymorphonuclear leucocytes, lymphocytes, histiocytes, transformed lymphocytes and cocci or bacteria in the background. Inflammatory changes like abnormal eosinophilia, cytoplasmic granules, vacuoles, phagocytosis, perinuclear halo, cytolysis, coarse chromatin, nuclear haziness, crenation, karyorrhexis and karyolysis were noted in all smears.

Specific cytological changes typical of *Bacterial Vaginitis* (clue cells, cocci, bacilli), *Chlamydia trachomatis* (intracytoplasmic vacuoles with eosinophilic granules and presence of transformed lymphocytes), *Trichomonas vaginalis* (pear shaped or

pleomorphic bluish grey bodies, 8-15 μ in diameter, if well preserved with eccentric nuclei and flagella), *Candida* (budding yeast or branching hyphae or spores), *Herpes Simplex Virus* infection (ground glass appearance of nuclei, multinucleation, intranuclear inclusions, megalocytes), or *Human Papilloma Virus* infection (koilocytes, megalocytes, multinucleation, dyskaryosis, hyperkeratosis, parakeratosis) were recorded as per literature criteria and as reported by us earlier^{18,19}.

Vaginal pH : Vaginal pH was measured in 28 cases by a separate sterile swab placed in anterior vaginal fornix and using laboratory grade pH paper (Qualigens) before the application of acetic acid and after ascertaining abstinence.

Colposcopy : The ASCON colposcope was available after November 1999. the amount of discharge, vascularity, acetowhite areas and extent of transitional zone (TZ) were noted in 21 cases before and after application of 5% acetic acid.

Results

The overall clinical response was good and thirty six cases (85.7%) showed clinical cure or improvement (Table II). However investigations revealed a worsening of Pap smears (increased severity of inflammatory changes in epithelial cells, increased cocci and short rods, and increased leucocytes and histiocytes) in 7 cases and lack of improvement or no change in another 5 cases.

Tolerability : Panchavalkal douches were tolerated very well. There were no major side effects of the treatment in 41 cases. Only one case complained of increased discharge .

One case had mild transient local itching for 2 days and another case had mild tolerable dryness of vagina for 2 days.

Three cases reported significant relief of backache following douche treatment.

Symptom score : Thirty six cases (85.7%) had clinical partial or total relief of symptom of leucorrhoea ($p < 0.001$, Chi square test). The mean symptom score for the amount of discharge was 5.4 ± 1.9 (Mean \pm SD) before treatment. This was reduced to 2.2 ± 2.2 at 2 weeks and 2.3 ± 2.2 at 1 month. The reduction in amount of discharge was statistically highly significant ($p < 0.001$, Student's paired 't' test).

Vaginal pH : Vaginal pH before and after treatment was measured in 28 cases (Fig 2). The mean vaginal pH before treatment was 5.3 ± 1.2 (mean \pm S.D). This was reduced to 4.8 ± 0.7 (mean \pm S.D) at 1 month ($p < 0.001$; Student's paired 't' test). In 13 cases the pH was reduced and in another 7 it remained normal (≤ 4.5). In 4 cases the pH which was higher than normal, remained same, and in 4 cases there was a nonsignificant rise in pH.

Cytology :

Papanicolaou smears : The results of analysis of Pap smears are given in Table III. In 5 cases Pap smears and Gram smears could not be done at 2 weekly, and in another 5 cases at 1 monthly check up either because they were in periods, or because of nonmedical reasons. Cytological manifestations specific to Bacterial vaginitis, Chlamydia, Trichomoniasis, Candidiasis and HSV or HPV infections as mentioned in methodology section were observed in 24/42 (57%) cases initially, in 7 (19%) cases at 2 weekly examination and in 15 (40%) cases at 1 monthly examination. Multiple infections were

seen in 8 cases. Smears from 6 cases showed marked inflammatory changes but no specific organisms or cytopathology could be identified.

Gram smears : These did not give any additional information in the study cases as none of the smears showed Gram-negative intracellular diplococci. Clue cells and yeast were seen in the smears as in Pap smears also. However one case excluded from study for PID did show Gram negative diplococci.

Colposcopy : Colposcopy was carried out in 21 cases before and after treatment (Table IV). Except for 1 case (No 20) all showed reduced vascularity at follow up. In 1 case (No.4) cervicitis (congestion and discharge) reduced after treatment whilst in two cases (No. 16,17) only congestion and redness were seen which were reduced.

In 13/21 cases there was a significant reduction in the area of TZ after application of acetic acid. In 6 cases (No. 2,3,5,8,13,14) there was almost complete healing of erosion and corresponded with reduction in size of TZ. One case (No.20) showed increased vascularity, edema and discharge discontinued douches on 8th day.

Non responders

Though 86% of cases were relieved symptomatically an analysis of cases according to laboratory parameters revealed that those with specific infections on cytological examination, specially Chlamydia trachomatis or HPV infection, and those with moderate or large erosions responded less or poorly to PVK douche treatment.

Discussion

Panchavalkal douche is routinely prescribed by Ayurvedic physicians for a subset leucorrhoea with vitiation of pitta dosha^{10,20}. However there is no published clinical and laboratory data on the use of the PVK vaginal douche. Apart from the antimicrobial effect of alcoholic extracts 'in vitro'^{11,12} there is evidence that the barks of Panchavalkal and many other medicinal plants of the genus ficus have constituents like anthrocyanins, terpenoids, bioflavonoids which may be responsible for antimicrobial and anti inflammatory activity^{11,21}.

In the present study objective methods for clinical and laboratory assessment were used to determine the effectiveness of PVK douche when it is used as the only method of treatment of uncomplicated leucorrhoea. Symptom scoring, pH measurement and assessment of inflammatory changes in Pap smears and colposcopy were used in addition to the clinical assessment of symptoms and per speculum and bimanual gynecological examination. The Pap smear is now increasingly used in the diagnosis of some cervicovaginal infections and the Bethesda system of reporting now makes it mandatory to report associated infections²². In addition to screening for precancerous and cancerous conditions of the cervix we have made use of Pap smear for cytological diagnosis of seven reproductive tract infections since 1985. We have compared it with other diagnostic tests like anaerobic culture, immunofluorescence and peroxidase antiperoxidase test and have reported on its usefulness^{6-8,18,19}. Recently we have reported a sensitivity of 58% and specificity of 90% for Pap smear diagnosis of Chlamydia trachomatis by comparing 4 non-culture diagnostic methods^{23,24}. This has also been the

observation of Panuco et al in a recent report and they reported higher sensitivity and specificity²⁵. Confounding variables which may change the outcome of treatment were taken care of in the present study. Cases with PID, HIV seropositivity, Diabetes mellitus, severe anemia and systemic diseases were excluded. Care was taken to ensure abstinence during the study period. Partner treatment, health education and further management of the couple were included in this study.

The results show good symptomatic relief ($p < 0.001$) in 85.7% of cases. The persistence of symptom relief and improved laboratory parameters at 1 monthly examination ie 2 weeks after completion of the treatment rules out a placebo effect due to mere douching.

In spite of symptomatic relief as many as 11 cases showed persistent ($n = 5$) or severe ($n = 7$) inflammatory smears. These were mainly in those cases who had specific infections in Pap smears or large or moderate erosion of the cervix at initial examination confirmed by colposcopy. Only 1 case had worsening of symptoms and discontinued treatment. She also failed to respond to conventional antibiotics given subsequently. The healing effect seen in 13 cases of erosion, and significant in 6 cases, indicates the usefulness of PVK for local treatment. Modification of formulation and dose searching may improve the response to PVK douche treatment. Adjuvant therapy with systemic drugs (Ayurvedic or Modern) may be necessary for cure.

Although the Papanicolaou smear is well established as method, for diagnosis of leucorrhoea it is not commonly used for assessing efficacy of treatment of leucorrhoea. It has been used more often to determine the efficacy of treatment of HPV related lesions or

intraepithelial neoplasias. Wet smears, cultures, PCR or cytochemistry, fluorescence based methods have been used more often for follow up of infections. In this group of low risk behavior the incidence of gonorrhoea was low. Only 1 case had gram negative diplococci and she was excluded from the study as she had PID. In our earlier study where we had used endocervical cultures as well as swabs from a similar group of women the incidence of gonorrhoea was 1.7 % ⁷.

The analysis of response of specific infections to PVK douche indicated that those with Chlamydia or viral infections had persistent inflammatory or worsened smears. Those with Trichomoniasis or Candidiasis or Bacterial vaginitis responded better, however the numbers are too small in each group to generalize. The use of multiple plants with several active principles may be an in built mechanism in Ayurvedic methods of treatment whereby bacterial resistance is prevented. Thus this may be an added advantage in the modern era when resistance to several organisms causing STDs is an increasing cause of concern ^{26,27}.

Conclusions

Daily panchavalkal douches for 14 days provided symptomatic relief in 85.7% of cases of uncomplicated leucorrhoea ($p < 0.001$). Cervical cytology, vaginal pH and colposcopy were utilized in confirming its antimicrobial and anti-inflammatory effects. Cytology was very useful in assessing the severity of infection and in identifying some specific infections.

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Legend to Figure 3

Figure 3 a,b,c, Papanicolaou smear (x 400) a of patient a) before starting PVK douche treatment b) at 2 weeks c) at 1 month

Legend to Figure 1.

Figure 1. Visual grading of symptom of leucorrhoea for amount of discharge.

Legend to Figure 2.

Fig 2. Vaginal pH in leucorrhoea cases before and after PVK douche treatment.

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Table I
Assessment Criteria

Parameter	Cured	Improved	No Change	Worse
Symptom Score*	Decreased to ≤ 2	Decreased by 2	No change	Increased by 2
Signs	Cervix clean	Mild infection	No change	Worse
pH	≤ 4.5	Decreased	No change	Increased
Pap smear **	Negative or mild inflammation few wbc Doderlein's	Inflammation less, normal morphology wbc less	No change	Increased inflammation, cytolysis karyorrhexis karyopyknosis

**bacilli, No
specific
infection.**

bacteria less

**perinuclear
halos
phagocytosis**

*** Refer Visual Grade Figure 1.**
**** Details given in methodology**

Table II
Overall Response To PVK Douche Treatment For 14 Days
(N = 42)*

Response	Cured	Improved	No Change	Worse
Symptoms (N=42)	17	19	3	3
Signs (N=42)	21	9	6	6
Cytology (N=37)	3	22	5	7
Colposcopy	8	9	3	1

(N=21)

pH (N=28)	5	8	11 #	4
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* As per criteria in Table I and details in methodology section

7 remained within normal rangem; 4 remained high

Table III
Cytological Manifestations Of Genital Infections In Papanicolaou Smears Of
Women Treated With PVK Douche.
(N=42)*

Pap smear report	Before PVK treatment (N=42)	2 weeks after treatment (N=37)	Post treatment (N=37)
Bacterial vaginitis	10	6	5
Candidiasis	6	1	6
Chlamydiasis	13	5	9
Trichomonas vaginitis	6	1	2
HPV infection	5	1	4
HSV infection	3	7	1

Specific infections	24	7	15
Non specific infection	18	30	22

*** 5 cases did not come for 2 weeks follow up; another 5 cases did not come for post treatment follow up.**

**Table IV
Colposcopic Findings Before And After PVK Douche Treatment**

S.No.	TZ Initial	TZ 2 weeks	TZ 1 month	Inflammation*
1.	>75%	50%	50%	Decreased
2.	>75%	ND	<25%	Decreased
3.	75%	50%	<25%	Decreased
4.	Cervicitis	Mild	Mild	Decreased
5.	75%	<25%	<25%	Decreased
6.	25%	20%	25%	Decreased
7.	>75%	75%	50%	Decreased
8.	<25%	<25%	Healed	Decreased
9.	<25%	ND	<25%	Decreased
10.	50%	<25%	ND	Decreased
11.	50%	30%	ND	Decreased
12.	75%	50%	60%	No change
13.	70%	Healed	Healed	Decreased
14.	70%	30%	30%	Decreased
15.	50%	25%	ND	Decreased
16.	Absent	Absent	Absent	Decreased
17.	Absent	Absent	Absent	Decreased

18.	50%	ND	30%	Decreased
19.	10%	Absent	Absent	Decreased
20.	90%	100%	ND	Increased
21.	30%	ND	25%	Decreased

* cervicitis, vaginitis, vascularity, edema, discharge.

TZ=Transformation zone as % area of ectocervix (Reduction due to epithelialisation)

ND = Not Done

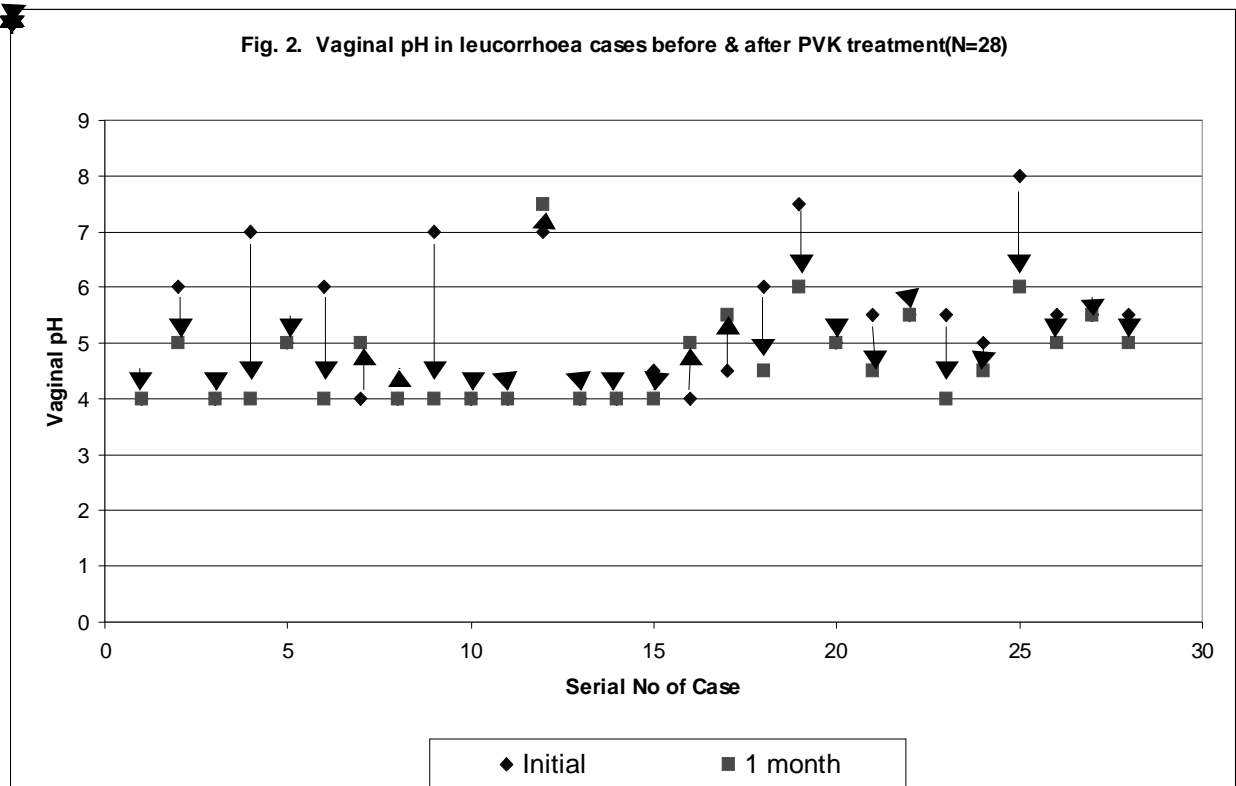


Table 5. Cytological Manifestations Of Genital Infections In Papanicolaou Smears of Women Treated With PVK Douche (N=42)*

Pap smear report	Before PVK treatment (N=42)	2 weeks after treatment (N=37)	Post treatment (N=37)
Bacterial vaginitis	10	6	5
Candidiasis	6	1	3#
Chlamydiasis	13	5	9
Trichomonas vaginitis	6	1	2

HPV infection (Koilocytosis)	5	1	4
HSV infection(nuclear changes)	3	7	1
<hr/>			
Specific infections	24	7	15
Non specific infection	18	30	22
<hr/>			

- **5 cases did not come for 2 weeks follow up; another 5 cases did not come for post treatment 4 weeks follow up.**
- **However 6 were positive with culture**