Detection of *Trichomonas vaginalis* among Females Attending Private Gynaecological Clinics in Kirkuk Province Using Different Laboratory Methods.

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Abstract:

Background: *Trichomonas vaginalis* infection is a common sexually transmitted protozoal infection and is associated with several adverse health outcomes, such as preterm birth, delivery of a low–birth weight infant, and facilitation of sexual transmission of human immunodeficiency virus. The annual incidence rate in Iraq has been estimated range between 1.33% to 34%. However; there are no data on the prevalence of trichomoniasis among all reproductive-age women.

Patients and methods: Total of 161 women from 15- 61 years who participated in the study form 1st October 2011 to 30th September 2012 with collection of vaginal swab specimens after taking consent from each patient. The vaginal fluids extracted from these swabs were evaluated for the presence of *Trichomonas vaginalis* using direct wet mount preparations, staining, and cultivation on special culture media.

Results: Over all, 33 (20.49%) of 161 women were positive for trichomoniasis with 98.12 % of specificity and 100% of accuracy of wet mount preparations technique for parasite detection. Compared to culture methods that show low sensitivity and specificity $P< 0.05$ .Women aging from 50 years and above and those aging from 20 to 30 years revealed high rate of trichomoniais than other age groups $P<0.05$. Relationships between *Trichomonas vaginalis* distribution and color of vaginal discharges, pH, signs, symptoms, residency of women, occupation, height and body weight were statistically significant, greenish vaginal discharge with pH ranging from 4 to 6 reveal more trichomoniaisis mostly associated with high dominancy of burning sensation and vaginal itches, $P < 0.05$. While thin and short women from urban area and officers, their vaginal discharges examination show high frequency of trichomoniasis in contrast to other women, $P<0.05$. Trichomonas coexists were recognized with high rates of *Gardnerella vaginalis* and *Candida albicans* in vaginal discharges especially among women aging from 20 to 30 years, $P < 0.05$.

Conclusions: Under clinical examination women with abnormal vaginal discharges, burning sensation and genital itches should be checked for trichomoniasis using high sensitive and specific tests: direct wet amount preparations and fixed slide stained with giemsa stain, in addition to measuring the pH of the vaginal fluid and performing germ tube and whiff test for detecting vaginal thrush and bacterial vaginosis caused by *Candida albicans* and *Gardnerella vaginal* respectively.

Key words: Trichomonas, Gardnerella, Candida, Discharge, Sensitivity.

Introduction:

Trichomonas vaginalis is a flagellated protozoan parasite that causes vaginal infections in women, including vaginitis, cervicitis and urethritis in male [(1)]. It is predominantly spread via unprotected intercourse with an infected partner.

Potential outcome of this STD in females include pelvic inflammatory disease, ectopic pregnancy, preterm labor, and predisposing agent for acquitting human immunodeficiency virus (HIV) [(2)]. Investigators have reported epidemiologic associations between trichomonas infection and subsequent cervical neoplasia and carcinoma [(3)]. Between (10-50%) of women are asymptomatic, amongst the reminder were the commonest symptoms involving vaginal discharge, vulval itching, dysuria and offensive odor, a strawberry cervix is visible by naked eye in (2%) of cases and in more women on colposcopy [(4)]. Direct observation of a wet smear from posterior cervix reveal (40-80%) of cases whereas culture of organism will correctly diagnose 95% of cases. *Trichomonas vaginalis* trophozoites are sometimes reported on cervical cytology, the sensitivity being (60-80%) but false positive rate is about (30%), for this reason if cervical smear is suggestive of trichomonal infection, it is worth to confirm the diagnosis by the above two methods [(5)]. Other options for detecting the parasite include serological tests such as. ELISA, IFA and non-serological test involving parasite genome amplification by using polymerase chain reaction PCR [(6)] .This study aims are to assess the relationship between trichomonal infection in women and age, laboratory methods usage, body indices, color of vaginal discharge, pH of discharge, women occupations and residency in addition to coexisting with other microorganisms.

Patients and Methods:

From 1st of October 2011 to 30th of September 2012, cross sectional study was carried on 161 women attending private gynecological clinics of Kirkuk province suffering from abnormal vaginal discharge, itching, dysuria and dyspareunia, their age ranges from 15 years to above than 61 years, for each woman complete information were arranged in a special questionnaire. Body indices such as height and weight were determined by using metric scale and sensitive electrical balance respectively. After clinical examination, two high vaginal swabs were taken from each woman, by passing bivalve vaginal speculum without using antiseptic lotions. Directly each swab was soaked inside the second cover of swab containing transport medium, then the swabs were transported using ice pack box as it was recommended for sample collection and preservation till processing. Then the first swab was preceded for checking (odor, color of discharge), gram staining, pH determination and cultivation for bacterial isolations. While the second swab was used for trichomonas cultivation using modified trichomonas media (purchased from high media company-India) and brain heart broth supplemented with L-cysteine and arginine, antimicrobial such as Penicillin G (million unit/ml), Streptomycin (500 000 units/ml) and Nystatin (10 mg/100 ml) were added for each screw sterile vial before specimen soaking. Culture vials were incubated anaerobically using anaerobic candle jar at 35°C, each vial was examined daily for one week before being considered negative. Statistical analysis using Chi-square and t-student test were applied for determination of variances among variable parameters within the study, P-value 0.05 is considered to be significant.

Results:

The rate of Trichomoniasis was (20.49%). According to women age's higher rate (28.57 %) was found among women aging from 51 to 60 years, while low rate (15 %) was recorded among women aging from 41 to 50 years, as shown in table 1.

Table two clarifies the role of laboratory stains, solutions and cultivation on special protozoa media. High rate was found by using wet preparation of (0.85 %) NaCl followed by (14.9%) and (11.18 %) by using vital stain and Giemsa stain respectively. While culture method reveal low rates of parasite growth (3.72%) and (1.24%) using modified trichomonas media and enriched brain heart broth. Wet preparation of normal saline exert high rate of accuracy, specificity and sensitivity compared to other preparations and culturing methods.

Table three shows that high rates of trichomonal infection were recorded with greenish discharge, pH ranged from 4 to 6 and patients suffering from burning sensation, itching and back pains the rates were (40 %), (77.78 %), (62.79%), (62.26%) and (57.17%) respectively.

With regard to body design (body height and weight) in relation to trichomonal infection table four shows that high rates (22.22%), (31.03%) of trichomonas were recorded in samples of short stature below 155 cm and thin (below 55 kg) women. (*P* value <0.05).

Table five shows the rate of trichomonal infection in relation to residency of women enrolled in the study, high rate (25 %) of trichomonas trophozoites were recorded in vaginal discharges from the center of Kirkuk province(urban area) compare to 12.28 % in samples of women from rural area.

According to women occupations, the following rates were obtained (66.66 %), (24.32%) and (18.18%) in adolescent, officers and housewives respectively. (*P* value<0.001), *P* value<0.05).

The causative agents for vaginal discharge other than trichomonas was *Gardnerella vaginalis* that recorded in high rate (34%) among women aging from 21 to 30 years while it was not recorded in specimens of women aging from 51 to 60 years and those aging 61 years and above , (P<0.05).

Fungal thrushes caused by the yeast *Candida albicans* also seen in some vaginal discharges as pure or inform of co-exist with trichomonas in the present study, table 7 reveal high rate of candidiasis among women aging from 21 to 30 years, while low rate (7.69 %) was recorded in samples of patients aging from 11 to 20 years, *P* <0.05.

Table (1): The distribution of *Trichomonas vaginalis* among women is according to age.

Age groups/years	Total No. Examined	%	No. of Positive	% of positive	Chi^2 values
15---20	33	20.49	6	18.18	0.043
21--- 30	50	31.05	12	24.00	0.51
31--- 40	47	29.19	9	19.14	0.088
41 --- 50	20	12.42	3	15.00	10.04
51--- 60	7	4.34	2	28.57	32.64 *
≥ 61 y	4	2.28	1	25	20.34
Total	161	100	33	20.49	*P<0.05

Table (2): Frequency of *Trichomonas vaginalis* trophozoites according to lab.stains and cultures, and efficacy of lab.tests for detecting trichomoniasis.

	Parasitological stains and solutions			Culture media	
	0.85 % NaCl	Giemsa stain	Vital stain	Modified T.V	Enriched brain heart
No. of Positive cases	33	18	24	6	2
% of positive cases	20.49	11.18	14.90	3.72	1.24
Efficacy of tests					
Sensitivity %	79.51	54.54	37.5	10.34	3.12
Specificity %	100.00	86.33	76.30	20.76	20.12
Accuracy	98.17	82.29	89.94	74.88	71.55

Total number examined=161 Number positive =33 samples.

Table (3): Distribution of *Trichomonas vaginalis* according to colour, *pH* of vaginal discharge and the dominant signs and symptoms.

Colour of disgharge	Total No. exam	No. (%) of infected cases.	pH of vaginal discharge in infected cases			Chi^2 Values
			3-4	4-6	6-8	
Colorless	24	0	0	0 0	0 0	0.0
White	46	6 (13.04%)	1(16.66%)	4(66.68%)	1(16.16%)	2.42
Green	40	16 (40%)*	2(12.5%)	12(75%)	2(12.5%)	1.0
Yellow	31	9 (29.03%)	1(11.11%)	7(77.78%)	1(11.11%)	4.07
Blood	20	2 (9.52%)	0 0	2(100%)	0 0	5.49
Total	161	33 (20.49%)	4(12.12%)	26(78.78%)	3(9.09%)	P<0.05

Signs and symptoms						
	Back pain	Itching	Dysuria	Burning sensation	Sweating	Fever
Frequent No.	28	53	15	43	16	12
Tricomonas +ve	16	33	3	27	5	5
(%) of positive	57.17	62.26	20	62.79	31.25	41.66

Table (4): *Trichomonas vaginals* distribution according to body length and mass.

Body parameters	Body height (Cm)				Body weight (Kg)			
Results	Tall	Moderate	Short	Total	Thin	Moderate	Obese	Total
No.Exam.	22	94	45	161	58	73	30	161
Percentage	13.66	58.38	27.96	100	36.02	45.34	18.64	100
No.+ve	4	19	10	33	18	9	6	33
Percentage +ve.	18.18	20.21	22.22*	20.49	31.03**	12.32	20	20.49

Tall is above than 165 cm. Moderate=155-165 cm short= below 155cm
Thin= below 55kg Moderate= 56 to70 Kg Obese= above than 70 kg.
*, ** P<0.05 Chi 2 *=1.27 **=0.909

Table (5): Frequency of trichomoniasis in relation to women residency.

Residency	Total No. Examined	(%)	No. of infected women	%	t-values P<0.01
Urban	104	64.59	26	25.00	2.36 *
Rural	57	35.41	7	12.28	1.9
Total	161	100.0	33	20.49	2.29
Occupations					
	No. Exam	%	No. of infected women	%	
House wives	121	75.15 **	22	18.18	
Officer	37	22.98	9	24.32	
Adolescent	3	1.87	2	66.66	
Total	161	100	33	100	

*P<0.01 and **P<0.05.

Table (6): Frequency of *Gardnerella vaginalis* among women according to age.

Age group/years	Total number exam	Percentages %	No. Positive +ve	Percentages +ve	Chi^2 values
15---- 20	33	20.49	6	18.18	0.26
21---- 30	50	31.05	17	34.00	8.90 *
31---- 40	47	29.19	6	12.76	2.91 * *
41----50	20	12.42	4	20.00	0.17
51----60	7	4.34	0	0.00	0.00
≥ 61	4	2.28	0	0.00	0.00
	161	100	33	20.49	*, **P<0.05

Table (7): Frequency of *Candida albicans* among women according to age.

Age groups/years	Total No. Exam.	percentages	No. Positive +ve	Percentages +ve	Chi^2 values
15 --- 20	26	16.14	2	7.69	1.077
21 --- 30	57	35.40	15	26.31	1.157 *
31--- 40	46	28.57	10	21.73	0.606
41 --- 50	21	13.04	4	14.28	0.28
51--- 60	7	4.34	1	14.29	0.27
≥ 61	4	2.28	1	25.00	1.002
Total	161	100	33	20.49	*P<0.05

Discussion:

The best way for assessing the efficacy of any laboratory method should be characterized by high rate of sensitivity, specificity and accuracy[10], so obtaining high rate of trichomonaisis in the present study by using wet preparation and Giemsa staining procedures are good guidelines and provident to determination of trichomonaisis among women in this study. In contrast to low rates recorded by using parasite cultivation in artificial media especially brain heart infusion media. From the results of current study it is obvious that the all rate of trichomoniasis is high when it was compared to (8.5%), (10.2%), (16.5%) recorded in Kirkuk city by Ali B M, Sulayiman M E, and Kadir M A[5,13]. Controversy to (61.25%) recorded by Karyaghdi T K in

the same province [14] .The rate of trichmoniasis in our study (20.49%) was higher than (1.33%), (7.5%), (13.0 %) and (19.6 %) recorded in Tikrit, Mosul, Basra and Baghdad respectively by Al-Somaeyday E G, Kharofa W A, Mahdi N K, and Alkaysi A A respectively [15,16,17].

Trichomonaisis rate in Turkey and USA were (40.3 %), (46.9 %) are not in agreement with the rate of present study, Suay A, Mete O, Yayla M *et al* and Shuter J, Bell D and Graham D.Variances in trichomonasis rates can be explained by different causes such as differences in size of the study, age groups, type of the laboratory methods and hygienic condition and sanitation in the study community [19,20]. High rate of trichomoniais among old age women may be explained by high rate of atrophic vaginitis in this age group, while high incidence among women aging from 21 to 30 years may be attributed to an excess sexual activity or to hormonal changes due to usage of contraception[9]. To evaluate role of body indices such as body height and weight, the high rate of trichomonas frequency among short stature and thin weight compare to other design of women can be explained by heavy infection of trichomoniasis and other microorganisms such as *Gardnerella vaginalis* and *Candida albicans* which increases the period of illness definitely that influence immune system that becomes weak. Statistical significant relationships between trichomonas frequency among women from urban area and among house wives in the present study against with that recorded in Kirkuk by Ali B M and that recorded in Al-Najaf by Al-Zubaidy K GH[5,21]. High occurrence of trichomoniasis among officer women may be attributed to use of contraception by them as a step for reducing the conception rate, also some females enrolled the study were adolescent (18.88%) might be due to hormonal changes because they were not adult. *Trichomonas vaginalis* co-infection with *Gardnerella vaginalis* and *Candida albicans* among women aging from 21 to 30 years mostly related to factorial agents such as vaginal douching that reduce normal flora and enhance growth of *Gardnerella vaginalis*, none programmed checking of the intra-uterine device, abuse of antibiotics which increases the propagation of *Candida albicans* in addition to an frequent sexual activity by woman or their husband. The result was agreed with that recorded in Kirkuk and in India by Griebeler M L and Silvina L, Thulker J, Kriplani A and Agrawstudyal N respectively[7,22]. High rate of trichomoniasis finding in greenish vaginal discharges and pH range from 4 to 6 can be related to scientific fact that this parasite prefer and survive in pH ranging from 4 to 6 [7,23]. Regarding more dominancy signs and symptoms burning sensation and itching in the present study can be interpreted by the parasite colonization in the vagina, surface trichomonad protein and movement by the aid of anterior four flagella, in addition to axostyle that extend outside of the parasite causing irritation of the lining tissue of the vagina and evoking mast cells for secretion the chemo-tactic substances which had role in increasing the IgE level mostly ended by pruritus, erosions and sever genital itches[5].

Conclusions:

Under clinical examination women with abnormal vaginal discharges, burning sensation and genital itches should be checked for trichomoniasis using high

sensitive and specific tests: direct wet amount preparations and fixed slide stained with Giemsa stain an addition to adjusting the pH of the vagina and performing germ tube and whiff test for detecting vaginal thrush and bacterial vaginosis caused by *Candida albicans* and *Gardnerella vaginal* respectively.

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