------Raf. Jour. Sci., Vol. 18, No.2, pp.19-26, 2007------

Serological detection of *Trichomonas vaginalis* infection

Ramzia H.A. Rahman
Department of Anatomy
College of Medicine
Mosul University

Wahda Ab.Al-Kharofa
Department of Anatomy
College of Medicine
Mosul University

Saba A.S.H.Al-Sultan
Department of Anatomy
College of Medicine
Mosul University

(Received 25/1/2003, Accepted 1/11/2003)

ABSTRACT

A study on presence of antibodies to ***T. vaginalis*** in serum was done on a group of (67) infected women with ***T. vaginalis***, and (75) pregnant asymptomatic women, (89) non-pregnant asymptomatic women.

A serologic test, done by Ouchterlony revealed that (22.6%) sera of pregnant asymptomatic women and (16.8%) sera of non- pregnant asymptomatic women were positive for somatic killed ***T. vaginalis*** antigens while (59.7%) sera of infected women were positive for somatic killed ***T. vaginalis*** antigens, but (7.4%) of these sera were positive for the soluble antigen of ***T. vaginalis*** antigens.

It was concluded that soluble trichomonal antigens present in association with living flagellates reacting with some sera, while those present in association with dead parasites are common antigens reacting with all the sera, also the study proved the infection with ***T. vaginalis*** occurs in subclinical or asymptomatic cases. The findings presented in this work offer a new tool for epidemiologic studies and open new perspectives for vaccinations.

الكشف السيرولوجي عن الإصابة بطفيلي *المشّعرات المهبلية*

الملخص

تم في هذه الدراسة جمع نماذج الدم (لغرض الحصول على المصول) من (67) إمـرأة مـصابة بطفيلي المشعرات المهبلية، (75) إمرأة حامل و عديمة الأعراض، (89) إمـرأة غيـر حامـل عديمـة الأعراض لغرض الكشف عن وجود الأضداد ضد المستضد المحضر من قتل طفيلي المشعرات المهلبيـة بالحرارة وضد المستصد الذائب المتجمع على سطح الوسط الذي ينمو فيه الطفيلـي بإسـتخدام طريقـة (التلازن المباشر) على السلايد و طريقة (الإنتشار المناعي الثنائي في الأكاروز)، وقد بينَتْ نتائج الدراسة بأن (22.6%) من مصول النساء الحوامل و عديمات الأعراض و (16.8%) من مصول النـساء غيـر

الحوامل و عديمات الأعراض كانت موجبة للمستضد المحضر من طفيلي المشعرات المهبليـة المقتولـة حرارياً بينما (59.7%) من مصول النساء المصابات بالطفيلي كانت موجبة لهذا المستـضد و أن نـسبة (7.4%) من مصول النساء المصابات بالطفيلي كانت موجبة للمستضد الذائب.

بينت هذه الدراسة بأن المستضد الذائب يكون مرتبطاً بالطفيليات الحية و يتفاعل مع بعض أنـواع المصول ، بينما المستضد المحضر من قتل الطفيلي حرارياً يكون عاماً و شائعاً و يتفاعل مع الكل ، كمـا بينت هذه الدراسة أن وجود الأضداد في مصول النساء عديمات الأعراض دلالة على أن الإصـابة بـداء المشعرات المهبلية يحدث بصورة تحت سريرية أو إصابة بدون أعراض.

إن هذا النوع من الدراسة قد يكون وسيلةً جديدةً تستخدم في الكشف عن حدوث الوباء بالطفيلي و ممكن إستخدامها مستقبلاً في صناعة اللّقاح ضد الطفيلي.

INTRODUCTION

Trichomonas vaginalis is a protozoan which parasitized urogenital tissues, and in recent years trichomnasis has emerged as the most common sexually transmitted disease of parasitic origin (Aalvarenga, Cicarelli, 1997 and Bozner, 1997). A variety of immune responses of infection with the uro-genital flagellate ***Trichomonas vaginalis*** have been described, including specific secretory antibody in vaginal secretion IgA and IgM antibody in serum. Polymorphonurclear cell chemotaxis and phagocytosis. Despite these responses chronic infection with the parasite is common and immunity to re-infection is poor (Mason and patterson, 1985).

The variation in this clinical presentation of Trichomonas infection in women, in addition to reports documenting the presence of metronidazole resistant strains of ***Trichomonas vaginalis*** initiated many researchers to undergo avariety of immunological, serological, biochemical and pathological (Quinn and Krieger, 1990) test in trial to distinguish between different strains of the parasite (Azab and Salem, 1992). The number of serotypes reported by such methods, ranged from two to eight in selected areas in Europe, monoclonal antisera were also useful in detecting different antigenic composition of isolates- (Krieger and et al, 1985).

The numbers of serotypes of ***T. vaginalis*** reported by various workers varied from two among seven strains examined to eight among nineteen strains the numbers differed according to the tests used and the district examined (Azab and Salem, 1992).

In recent years, the analysis of isoenzyme dectrophorefic patterns has been used to differentiate between species, subspecies or strains of several parasitic protozoa such ***Plasmodium***, ***Leishmania***, ***Trypanosoma*** and ***Entamoeba histolytica***. Similar studies on ***Trichomonas vaginalis*** started in 1953, by Kupferberg-
(Sargeaunt and Williams, 1978).

MATERIAL AND METHODS

Trichomonas isolates and cultivation:

Trichomonas vaginalis was isolated from vaginal discharges of female patients attending the outpatients Gynaecology Clinics of Al- Batol – Teaching Hospital. Isolates were maintained in vitro at 37 ºC on Oxide Trichomonas media (CM 161) supplemented

with 10% inactivated horse serum, subcultures were done every 48-72 hours (Kharofa, 1999). Preparation of somatic killed ***T. vaginalis*** antigen was prepared by taken 5-10 tubes containing at least 2×10^6 living parasites count by haemosytometer, suspended in 2 ml. of physiological saline and mixed by vortexgenie mixer then centrifuged at 3000 r.p.m. for 30 minutes, this run process was repeated 3 times and in the last run the supernatant was discarded and the sediment was resuspended in 2 ml. of physiological again, the suspension was heated at 60 ºC for 30 minutes and centrifuged the sediment (contain somatic killed ***T. vaginalis***) was resuspended in 2 ml. of physiological saline (Azab and Salem, 1992) the final suspension was used to produce the immune sera. Soluble antigens was prepared by collected culture fluid of ***T. vaginalis*** supernatants from tubes containing 2×10^6 / ml. living parasites.

Preparation of anti sera:

Anti sera against both somatic killed ***T. vaginalis*** and soluble fluid antigens of ***T. vaginalis*** were prepared according to the method of protocol of cooney and kenny (1970), using 4 mice each 2 mice received 5 injections of one type of the antigens above (Salem and Azab, 1992).

Collection of human sera:

Blood sample (2-5) ml. was drawn through a venpuncture from each of 67 infected women, 75 pregnant asymptomatic women and 89 non-pregnant asymptomatic women. Blood samples were left at room temperature for 3 hours then over night at 4 ºC. The serum was separated from the clotted blood by centrifugation and the recovered serum was stored at - 20 ºC until use.

RESULTS

As shown in Table (1) by using SAT somatic killed ***T. vaginalis*** antigens reacted with the following sera, mice anti somatic killed ***T. vaginalis*** antigens serum until the dilution $1:10^7$ ($x^2 = 0.4$, $p > 0.01$), also with serum of infected mice until the dilution $1:10^4$ ($x^2 = 0.4$, $P > 0.01$), also in Table (1) soluble antigens of ***T. vaginalis*** reacted with mice anti soluble antigens of ***T. vaginalis*** serum until the dilution $1:10^5$ ($x^2 = 0$).

As shown in Table (2) by using Ouchterlony gel double immunodiffusion technique. Somatic killed ***T. vaginalis*** Antigens reacted with mice anti somatic killed ***T. vaginalis*** antigens serum until the dilution $1:10^8$ ($x^2 = 3.6$, $p < 0.01$), and with serum of infected mice by ***T. vaginalis*** serum until the dilution $1:10^3$ ($x^2 = 1.6$, $p > 0.01$), also this table shows the soluble antigens reacted with mice anti soluble antigens of ***T. vaginalis*** sera until the dilution $1:10^5$ ($x^2 = 0$).

As shown in Table (3) from the 67 sera samples of infected women there were 31 (46.2%, $x^2 = 0.2$, $p > 0.01$), were positive for somatic killed ***T. vaginalis*** antigens, 13 (4.47%, $x^2 = 16.2$, $p < 0.01$), were positive for soluble antigens of ***T. vaginalis***.

As shown in Table (4) from the 75 sera samples of pregnant asymptomatic women, 17 (22.6%, $x^2 = 13.5$, $p < 0.01$), were positive for somatic killed ***T. vaginalis***

As shown in Table (4) from the 75 sera samples of pregnant asymptomatic were positive for the somatic killed ***T. vaginalis*** antigens and 5 (7.4%, $x^2 = 13.5$, $p < 0.01$), were positive for the soluble antigens of ***T. vaginalis***.

antigens from the 67 sera samples of infected women 40 (59.7%, $x^2 = 0.16$, $p > 0.01$), were positive for the somatic killed ***T. vaginalis*** antigens and 5 (7.4%, $x^2 = 13.5$, $p < 0.01$), were positive for the soluble antigens of ***T. vaginalis***.

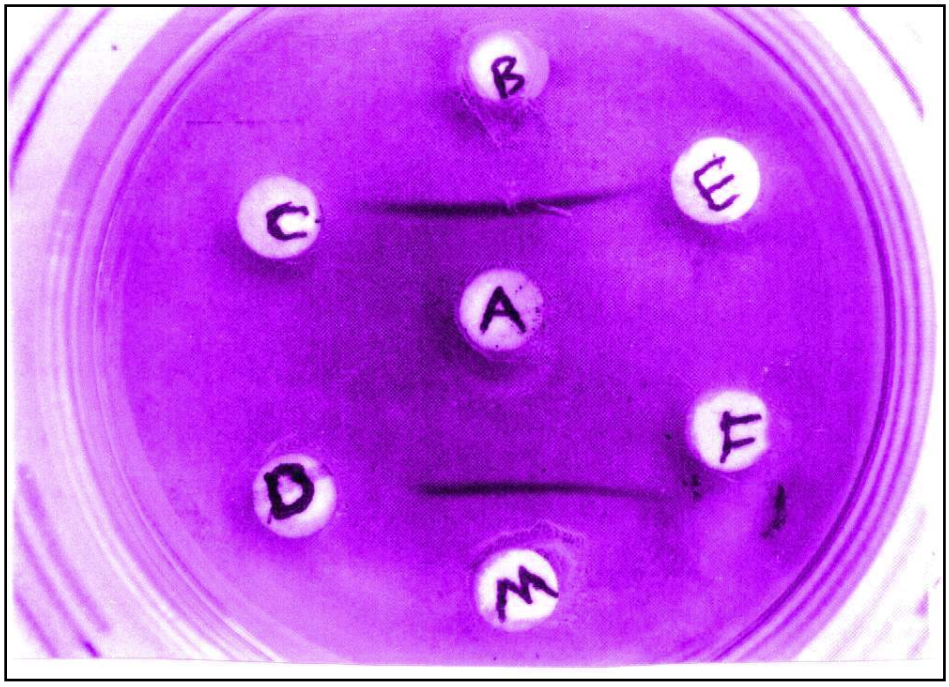

Fig. 1: Ouchterlony double immunodiffusion technique showing a positive result up to dilution $1:10^2$ using serum of infected women with somatic killed ***T. vaginalis*** antigens.
A – somatic killed ***T. vaginalis*** antigens M,B serum of infected women. C, F, E, D negative sera as (negative control).

Table 1: SAT – Slide Agglutination Technique - of mice sera anti somatic killed and soluble antigens of *T. vaginalis*

SAT	Types of antigens					
	Somatic killed ***T. vaginalis*** antigens			Soluble antigens of ***T. vaginalis***		
	1	2	3	1	2	3
1:10	+	-	+	-	+	+
$1:10^2$	+	-	+	-	+	-
$1:10^3$	+	-	+	-	+	-
$1:10^4$	+	-	+	-	+	-
$1:10^5$	+	-	-	-	-	-
$1:10^6$	+	-	-	-	-	-
$1:10^7$	+	-	-	-	-	-
$1:10^8$	-	-	-	-	-	-
$1:10^9$	-	-	-	-	-	-
$1:10^{10}$	-	-	-	-	-	-
x^2	0.4	0	0.4	0	0.4	6.4
p	> 0.01		> 0.01		> 0.01	< 0.01

1. Mice anti somatic killed ***T. vaginalis*** antigens sera.
2. Mice anti soluble antigens of ***T. vaginalis*** sera.
3. Sera of infected mice with ***T. vaginalis*** sera.

Table 2: Ouchterlony method of mice sera anti somatic killed and soluble antigens of *T. vaginalis*

Ouchterlony	Types of antigens					
	Somatic killed ***T. vaginalis*** antigens			Soluble antigens of ***T. vaginalis***		
	1	2	3	1	2	3
1:10	+	+	+	-	+	+
$1:10^2$	+	-	+	-	+	+
$1:10^3$	+	-	+	-	+	-
$1:10^4$	+	-	-	-	+	-
$1:10^5$	+	-	-	-	+	-
$1:10^6$	+	-	-	-	-	-
$1:10^7$	+	-	-	-	-	-
$1:10^8$	+	-	-	-	-	-
$1:10^9$	-	-	-	-	-	-
$1:10^{10}$	-	-	-	-	-	-
x^2	3.6	6.9	1.6	0	0	3.6
p	< 0.01	< 0.01	> 0.01			< 0.01

1. Mice anti somatic killed ***T. vaginalis*** antigens sera.
2. Mice anti soluble antigens of ***T. vaginalis*** sera.
3. Sera of infected mice with ***T. vaginalis*** sera.
4.

Table 3: SAT of women sera anti somatic killed and soluble antigens of *T. vaginalis*

SAT	Types of antigens					
	Somatic killed ***T. vaginalis*** and No. positive			Soluble antigens of ***T. vaginalis*** and No. positive		
	1	2	3	1	2	3
1:10	+(9)	+(8)	+(20)	-	-	+(4)
$1:10^2$	+(5)	+(7)	+(8)	-	-	+(1)
$1:10^3$	+(3)	+(3)	+(4)	-	-	-
$1:10^4$	+(1)	+(2)	+(2)	-	-	-
$1:10^5$	-	-	+(1)	-	-	-
$1:10^6$	-	-	-	-	-	-
$1:10^7$	-	-	-	-	-	-
$1:10^8$	-	-	-	-	-	-
$1:10^9$	-	-	-	-	-	-
$1:10^{10}$	-	-	-	-	-	-
Total positive	13	11	31	0	0	3
Total negative	62	78	36	75	89	64
Total	75	89	67	75	89	67
x^2	7.2	9.8	0.2	0	0	16.2
p	< 0.01	< 0.01	> 0.01			< 0.01

1. Sera of pregnant asymptomatic women.
2. Sera of non-pregnant asymptomatic women.
3. Sera of infected women.

Table 4: Ouchterlony double delusion test of women sera anti somatic killed and soluble antigens of *T. Vaginalis*

Ouchterlony method	Types of antigens					
	Somatic killed ***T. vaginalis*** antigens and No. positive			Soluble antigens of ***T. vaginalis*** and No. positive		
	1	2	3	1	2	3
1:10	+(10)	+(9)	+(33)	-	-	+(1)
$1:10^2$	+(6)	+(3)	+(12)	-	-	+(1)
$1:10^3$	+(2)	+(2)	+(5)	-	-	-
$1:10^4$	+(2)	+(2)	+(1)	-	-	-
$1:10^5$	+(1)	+(1)	-	-	-	-
$1:10^6$	+(1)	-	-	-	-	-
$1:10^7$	+(1)	-	-	-	-	-
$1:10^8$	-	-	-	-	-	-
$1:10^9$	-	-	-	-	-	-
$1:10^{10}$	-	-	-	-	-	-
Total positive	17	15	40	0	0	5
Total negative	58	74	27	75	89	62
Total	75	89	67	75	89	67
x^2	13.5	4.1	0.16	0	0	13.5
p	< 0.01	< 0.01	> 0.01			< 0.01

1. Sera of pregnant asymptomatic women.
2. Sera of non-pregnant asymptomatic women.
3. Sera of infected women.

DISCUSSION

Immunological characters are among those listed for identification and classification of the members of aprotozoan genus, and specifically those serological methods depending on extracts or homonogenates or metabolic products of living parasites (show, 1982).

In the present study, the serological differentiation of locally isolated ***T. vaginalis*** stocks was attempted using whole killed parasites and their culture fluid supernatants as possible sources of trichomonal antigens excreted or secreted by the parasites. The gel double diffusion technique was used by (Goldman and Honigberg, 1968) and by (Stepk Auski and Honigberg, 1972) for somatic antigenic analysis of ***T.gailinae*** stocks using polyvalent rabbit immune sera. As shown in tables (1,2) different serologicaltests were used (SAT and Ouchterlony method), it was found that upto the dilution $1:10^7$(SAT) of mice anti somatic killed ***T.vaginalis*** antigens sera was positive for somatic killed ***T.vaginalis*** Antigens also in table (2) the results obtained indicated that upto dilution $1:10^4$mice anti soluble antigen of ***T.vaginalis*** serum was positive for soluble antigen of ***T.vaginalis*** regarding the others sera the differences were not statistically significant, this could be explained as when immunized mice with somatic killed ***T.vaginalis*** antigens or soluble antigen of ***T.vaginalis*** ,gave higher response for the specific antigen ,since there was cross reaction and similarity between the two antigen . Also the

differences in positively rate might be due to the degree of sensitivity and specificity the serological method . ln table (3,4) by using (SAT) and Ouchterlony method ,the data shows that 59.7% of sera of infected women were positive for the somatic killed ***T.vaginalis*** antigens while 22.6% of sera of pregnant asymptomatic women and 16.8% of sera of non – pregnant asymptomatic women ,were positive for this antigens .

This results agrees with that reported by (Azab and Salem, 1992)who indicated that soluble trichomonal antigens present in association with living flagellates are stock – specific reaction with some sera ,but not all the antitrchomonal hyperimmune sera, while those present in association with dead parasites are common antigens reacting with all the sera, also the reaction between sera of pregnant asymptomatic and nonpregnant asymptomatic women with killed somatic ***T.vaginalis*** antigens, due to presence the antibodies in the sera of these women indicated the infected with ***T.vaginalis*** occurs in subclinical case or due to previous infection with this parasites (petrin and delgaty ,1998)

In tables (3,4) by using SAT and Ouchterlony method the obtained indicated that 4.47% by (using SAT) sera of infected women were positive for the soluble antigens of T.vaginalis this might be due to the reaction occurs between sera of infected women and culture fluid supernatants of ***T.vaginalis*** antigens only when collected from tubes containing either high numbers of living parasites (2×10^6/ml) irrespective of the age of the culture. No, soluble antigens were precipitated from culture fluid containing smaller number of living flagellates (Azab and Salem ,1992).

The presence of free antigens in the culture supernatants of ***T.vaginalis*** was also reported by Mason and Forman (1980), Alderete and Garza (1984) and Mason and Patterson (1985), Mason and Forman (1980) reported that these were large protein molecules released continuously by the live parasites at a rate of about 0.016 mg protein per 1000,000 organisms/ per hour, while the authors suggested that these might be secretary factors released by life trichomonads.

Alderete and Garza (1984), demonstrated that these were surface antigens shed from the live parasites.

In the present study, the differential reactions occurring when the culture fluid supernatants were collected from tubes containing only living parasites, suggest that these were the specific antigens shed from the surface of the living parasite with the appearance of dead flagellates in the culture tubes.

REFERENCES

Alvarenga ,V.L.D.S. and Cicarelli ,R.M.B., 1997 .Interaction on ***Trichomonas vaginalis*** (Brazilian isolates) with Mccoy Monolayers on.J.Protozool .Res .,7: pp.102-106.

Azab, M.E. and Salem, S.A. 1992. “Characterization of Egyptian isolates of ***Trichomonas vaginalis*** I isoenzyme”. J. of the Egypt. Soc. of parasite; 22(3): pp.775-782.

Bozner , P., 1997. Immunological detection and HSP60 Homologs in ***Trichomonas vaginalis*** .J.Parasitol .,83(2): pp.224-229.

Cooney, M.K. and Kenny, G.E. 1970. “Immunoenicity of Rhinoviruses”. Pro. Soc. Exp. Biol. Med., 133: pp.645-650

Kharofa, W.A. 1999. “An epidemiological study and cultivation of ***Trichomonas vaginalis*** in Mosul city”. M.Sc. thesis, college of science, university of Mosul, Iraq. pp.1-75.

Krieger, J.N., Ravdin, j.I. and Rein, M.F. 1985. “Contact-dependent cytopathologenic mechanisms of ***Trichomonas vaginalis***”. Infect. Immun. 50: 778-786 pp.

Mason , P.R., and Patterson ,B.A., 1985 .Proliferalive Response of human Lymphocytes to secretary and cellular antigens of ***Trichomonas vaginalis*** .J.ParasitS., 71(3): pp.265-268.

Quinn, T.C. and Kiger, J.N. 1990. “Trichomoniasis in tropical and geographical medicine”. By warren, K.S. and Mahmoud A.A., second ed., McGrew Hill information Serrice Company.

Salem, S.A. and Azab, M.E. 1992. Characterization of Egyptian isolates of ***Trichomonas vaginalis*** II isoenzyme”. Patterns stereotyping. J. of the Egypt. Soc. of parasite; 22(3): pp. 675-682.

Sargeaunt, P.G. and Willium, J.E. 1987. “Entamoeba coli Trans, Trop, patterns of entamoeba histolytica and Entamoeba coli, Trans, Roy”. Soc. Hyg. 72: pp.164-166.

Shaw, J.J. 1982. “Taxonomy of the genus ***Leshmania*** Traditional view and modern concepts in chemical characterization of ***Leshmania***”. By chance M.L. and Walton, B.C. UNDP, world Bank / WHO.