IMMUNOGLOBULIN LEVELS IN SERUM AND CERVICOVAGINAL SECRETIONS OF PATIENTS INFECTED WITH *TRICHOMONAS VAGINALIS*

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Abstract

Background: Trichomoniasis caused by *Trichomonas vaginalis*, is one of the most common sexually transmitted parasites in the world, accounting for approximately 170 million infections annually. Although the factor behinds such incidence is still not clear, local and systemic host immune response is involved.

Objectives: Accordingly, the present research was planned to determine the level of immunoglobulins (IgA, IgG and IgM) in sera and vaginal washes of women infected with *T. vaginalis*.

Materials and Methods: Fifteen women infected with *T. vaginalis* (culture confirmed) and 15 healthy females were inspected for total level of IgA, IgG and IgM in their sera and vaginal washes by means of single radial immunodiffusion.

Results: The total level of IgA, IgG and IgM in serum and vaginal wash of patients and controls showed no significant difference, with the exception of IgG, which showed a significant increased mean in the sera of patients.

Conclusion: Humoral immune response is important in controlling *T. vaginalis*, with a special reference to IgG.

Keywords: Immunoglobulins, *Trichomonas vaginalis*, serum and vaginal wash.

Introduction

Trichomonas vaginalis is the causative agent of trichomoniasis. It is one of the most common sexually transmitted parasites in the world, accounting for approximately 170 million infections annually [1]. Males harboring *T. vaginalis* may have acute or chronic urethritis or prostitis, but ordinarily have no symptoms and may be unaware of the infection [2]. Females may also harbor the parasite without any symptoms, but they usually have an increased vaginal discharge [3], and the infection may lead to vaginitis, urethritis, cervitis [4] and other complications such as premature labour, low-weight offspring and post-abortion or post-hysterectomy infections [5]. Although the exact factor behinds wide variation in symptomatology in different individuals is still not clear, local and systemic host immune response is involved [6]. Circulating antibodies to *T. vaginalis* have been demonstrated in chronically infected patients [7]. Sera from experimental animals [8] and infected subjects showed high levels of IgA, IgG and IgM anti-*T. vaginalis* antibodies, moreover, specific IgA and IgG antibodies have also been demonstrated in vaginal washes from women with acute trichomoniasis [9]. Therefore, the present research was planned to determine the total levels of IgA, IgG and IgM in sera and vaginal secretions of women infected with *T. vaginalis* for a further understanding of the humoral immune response in such patients.

Materials and Methods

i. ***Vaginal swabs*****:** Three swab specimens were taken from each of 250 adult females (age range: 18 - 52 years) attending the Department of Gynecology at Al-Yarmouk Teaching Hospital with symptoms of heavy vaginal discharges, during the period December 2005 - June 2006. The first swab was subjected to a microscopical wet smear examination [10], while the second was cultured in CPLM medium (Himedia) for the detection of *T. vaginalis* [11]. Then, the cultures were daily

microscopically examined for the presence of parasite, and cultures that were still negative after 7 days of incubation were considered negative and discarded. The third swab was transferred into a test tube containing 0.5 ml phosphate-buffered saline (pH 7.2), and then they were incubated at 37° C for 1 hour before being centrifuged at 3000 rpm for 20 minutes at 4ºC. The supernatant was transferred into 2 ml vial and stored at -20° C until the detection of immunoglobulin levels [10]. Similar samples were taken from 15 healthy females, who had no symptoms of heavy vaginal discharges, and age-matched with the patients.

ii. ***Blood samples:*** Two milliliters of venous blood were collected from each subject in a plain tube. The blood was left at room temperature for 15 minutes to clot, and then it was centrifuged (2000 rpm for 10 minutes) and the serum was collected and frozen at -20ºC until the assessment of immunoglobulin levels.

iii. ***Assessment of Immunoglobulin (IgA, IgG and IgM) Levels:*** The radial immunodiffusion assay was employed to assess the total level of IgA, IgG and IgM in sera and vaginal washes using commercially available kits (Biomaghreb, Tunisia), and the instructions of the manufacturer were followed.

iv. ***Statistical Analysis*****:** The data was given in terms of means ± standard deviations (S.D.), and significant differences between means were assessed be the Least Significant Difference. Additionally, the patients and controls were divided into two groups; the first included subjects with normal immunoglobulin level (NOR), while the second group consisted of subjects who had values above the normal level (ABV). Significant differences between the two groups were assessed by Pearson's Chi-square test.

Results

Based on the finding of vaginal swab culturing, there were 15 positive cases (6%) that showed *T. vaginalis* growth, and the samples (sera and vaginal washes) of these cases together with samples of healthy females (negative culture) were further examined for the assessment of immunoglobulin levels.

The total level of IgA and IgM in sera and vaginal washes showed no significant difference between patients and controls, and the IgG in the vaginal washes showed similar results. However, the total serum level of IgG showed a significant increase in patients as compared to controls (1465.45 ± 588.94 *vs.* 1057.69 ± 384.73; P = 0.03) (Table (1)). Dividing the patients and controls into NOR and ABV groups also revealed no significant differences in the immunoglobulin levels between patients and controls (Table (2)).

Table (1)
Immunoglobulin levels (IgA, IgG and IgM) in sera and vaginal washes in women infected with Trichomonas vaginalis and healthy females.

Immunoglobulins		Immunoglobulin Level (mean ± S.D.; mg/dL)		P Value
		Patients (No. = 15)	Controls (No. = 15)	
Serum	IgA	193.48±98.81	177.63±77.75	N.S.
	IgG	1465.45±588.94	1057.69±384.73	0.03
	IgM	126.97±80.58	146.91±83.15	N.S.
Vaginal Wash	IgA	201.11±132.48	158.65±120.53	N.S.
	IgG	1111.07±399.45	1050.21±316.77	N.S.
	IgM	168.73±244.40	95.95±32.91	N.S.

N.S. Not significant

Table (2)
Observed numbers and percentage frequencies of NOR and ABV groups in women infected with Trichomonas vaginalis and healthy females.

Immunoglobulins			Patients (No. = 15)		Controls (No. = 15)		P value
			No	%	No	%	
Serum	IgA	NOR	12	80.0	14	93.3	N.S.
		ABV	3	20.0	1	6.7	
	IgG	NOR	9	60.0	13	86.7	N.S.
		ABV	6	40.0	2	13.3	
	IgM	NOR	12	80.0	14	93.3	N.S.
		ABV	3	20.0	1	6.7	
Vaginal Wash	IgA	NOR	10	66.7	12	80.0	N.S.
		ABV	5	33.3	3	20.0	
	IgG	NOR	13	86.7	14	93.3	N.S.
		ABV	2	13.3	1	6.7	
	IgM	NOR	14	93.3	15	100.0	N.S.
		ABV	1	6.7	0	0.0	

NOR and ABV: Subjects with normal immunoglobulin level and above normal, respectively.

Discussion

Infection with *T. vaginalis* in humans results in parasite-specific antibodies in the reproductive tract and in most instances, circulating antibodies in the serum [12]; there is also evidence of lymphocyte priming as detected in peripheral blood mononuclear cells [13]. Thus, natural infection with *T. vaginalis* results in priming of acquired immune response. It is generally accepted that IgA is the prominent immunoglobulin in many external secretions in humans and other animals and that secretary IgA plays an important role in immunity to certain viral and bacterial infections of the respiratory and gastrointestinal tracts [14]. Relatively few studies have investigated the immunoglobulins in the cervicovaginal secretions during local infections [15]. The present results approximated the findings of Su [11], who demonstrated that IgG antibody against *T. vaginalis* was detected in 17 (70.8%) secretion samples from *T. vaginalis* infected women and among these, 11.8% showed a high level of IgA, while, 5.9% showed a high level of IgM. In other study, anti-trichomonal IgA antibodies were estimated in serum and vaginal secretions of 25 symptomatic and 25 asymptomatic *T. vaginalis* positive patients before and after treatment and in 25 age-matched controls. Significantly higher levels of antit-richomonad IgA antibodies were found in *T. vaginalis* positive patients when compared to control subjects, especially in vaginal secretions. In addition, a significant decrease in these antibodies was observed after treatment, which

was more pronounced in vaginal secretions. It seems that anti-trichomonal IgA antibodies in serum and more so in vaginal secretions are directly related to and specific to the presence of *T. vaginalis* in the urogenital tract [16]. In front of the present results, there was no significant difference related to IgA and IgG levels in vaginal washes, but patients with immunoglobulines levels above the normal levels in both specimens were observed. These results correlated well with reports conducted in clinical patients [16] and in experimental animals [8] whereby response in infected patients and animals was higher than uninfected ones.

As a conclusion, humoral immune response is important in controlling *T. vaginalis*, with a special reference to IgG.

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الخلاصة

خلفية: داء المشعرات المهبلية الذي يسببه طفيلي المشعرات المهبلية Trichomonas *vaginalis* وهو من أحد الطفيليات التي تنتقل جنسيا في العالم والذي يتسبب في اصابة ما يقارب 170 مليون شخص سنوياً وعلى الرغم من أن العوامل التي تقف خلف حدوث هذا المرض ما زالت غير واضحة الا ان الاستجابة المناعية الموضعية و العامة تدخل ضمن هذه العوامل .

الهدف: وفقا لذلك فان هذا البحث خطط لمعرفة مستوى الكلوبيولينات المناعية IgA و IgG وIgM في مصول و الغسول المهبلية لنساء مصابات بالمشعرات المهبلية.

طرائق العمل: قيس مستوى الكلوبيولينات المناعية IgA و IgG وIgM في مصول و الغسول المهبلية لخمسة عشر امرأة مصابة بالمشعرات المهبلية (مؤكدة بتنمية الطفيلي) و خمسة عشر امرأة سليمة بواسطة اختبار الانتشار المناعي المنفرد.

النتائج: اظهر مستوى الكلوبيولينات المناعية IgA و IgG و IgM في مصول و الغسول المهبلية للمصابات دلالة غير نوعية ما عدا مستوى IgG في المصل و الذي اظهر دلالة نوعية .

الأستنتاج: أن الاستجابة المناعية الخلطية لها اهمية في السيطرة على المشعرات المهبلية خصوصاً IgG .