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#### **ORIGINAL ARTICLE**

# Immunological evaluation of virulence factor Alphα-actinin among women with *Trichomonas Vaginalis*

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#### **Abstract**

**Background:** *Trichomonas Vaginalis* infection is one of the most common sexually transmitted infections in women, and  $\alpha$ -actinin is one of the most important virulence factors for this parasite.

Objective: To evaluate the concentration of alphα-actinin protein in women infected with *Trichomonas Vaginalis* infection and control groups using ELISA technology. **Methods:** Two hundred and sixty four of the participating women were included in our current study, They were going to Al-Manathra Hospital, Al-Najaf Hospital, Al-Furat Hospital, and a few outpatient clinics between January and June of 2023. One hundred and thirty two were included as a control group and one hundred and thirty two were included as patients (they were suffering from vaginal infection *Trichomonas Vaginalis*). All participants in the study underwent direct culture in diamond culture media by taking a swab from the vagina to confirm the presence of the parasite. And also a blood was taken for conducting immunological evaluation by ELISA technique.

**Results:** The results shown that the level of  $\alpha$ -actinin concentration is higher in the serum of patients infected with the parasite T.vaginalis (509.113  $\pm$  26.401 pg/ml) and this increase is statistically significant (P-value <0.0001) compared to its concentration in the control serum (67.939  $\pm$  8.179 pg/ml). The highest mean rank for  $\alpha$ -actinin (78.94 pg/ml) appeared in the (35-44 year) age group, which numbered 35. While the results showed that the lowest mean rank for  $\alpha$ -actinin (55.77 pg/ml) appeared in the (25-34 year) age group, which numbered 30. The level of α-actinin concentration, based on the diagnosis of the clinical condition of patients infected with the parasite, had reached its highest level in the group of patients infected and diagnosed suffering from endometriosis, where was mean rank (77.68Pg/ml). While the lowest increase in the level of  $\alpha$ -actinin was in the group of patients diagnosed with polycystic ovary syndrome (PCOS), where it was the mean rank (55.67 pg/ml). Based on the clinical symptoms, the results showed that the highest mean rank for the level of the virulence factor α-actinin appeared in the group of women suffering from foul smelling, where it reached 82.53 pg/ml. While the lowest mean rank for αactinin (67.31pg/ml) appeared in the group of women who suffer from irregular bleeding.

Conclusions: The virulence factor  $\alpha$ -actinin is significantly and highly secreted in patients infected with the parasite *Trichomonas Vaginalis*,  $\alpha$ -actinin secretion appears to be affected by the woman's age, as the secretion of the virulence factor increases during the period when hormonal balance becomes unattainable in women aged 35-40 years.  $\alpha$ -actinin secretion appears to increase in women diagnosed with endometriosis, this means that vaginal bleeding is an appropriate environment for increased secretion of the virulence factor.

**Keywords:** α-actinin, *Trichomonas Vaginalis* 

#### INTRODUCTION

Trichomonas Vaginalis is a type of microscopic parasite that is responsible for causing trichomoniasis, a sexually transmitted disease [1]. Transmission of T.vaginalis occurs by sexual contact between males and females [2].

It is uncertain how long the incubation period is between becoming infected and being exposed to the parasite. However, estimates vary from four to twenty-eight days. The illness can be transmitted even in the absence of symptoms [3]. The pathogen T.vaginalis causes significant immunological reactions in its hosts [4]. Actinin-F and actinin-T fragments that are derived from T.vaginalis α-actinin have the capacity to induce a mixed (Th1/Th2) humoral and cellular immune response. Because of their high immunogenicity, high antigen specificity, and well-conserved immunogenic epitopes, α-actinins are strong potential vaccine candidates against T.vaginalis infection [5]. Tvα-actinin attaches itself to one or more surface components of the host epithelial cells when T.vaginalis is in its amoeboid phase. and assumes importance in specific T.vaginalis circumstances (that is, following direct actin contact exposure to the host cells) [6].

Tvα-actinin binds to the iron-responsive site of Tvcp RNA, which codes for *T.vaginalis* cysteine proteinase and aids in post-translational iron regulation. Additionally, *T.vaginalis* adheres to the host epithelium by a particular interaction with the glycoproteins in the extracellular matrix basement membrane [7]. Serum from women infected with *T.vaginalis* often contains one of the most prevalent immunogens, α-actinin. Moreover, it was noted that many *T.vaginalis* strains shared

conservations in  $\alpha$ -actinin. Hence, this immunogen may cause matching antibodies to distinct epitopes in various strains of Trichomonas when isolated from a single strain [8,9].

## MATERIALS AND METHODS Participants

Two hundred and sixty-four of the participating women were included in our current study. One hundred and thirty two were included as a control group and one hundred and thirty two were included as patients (they were suffering from vaginal infection Trichomonas Vaginalis). participants in the study underwent direct culture in diamond culture media by vaginal swab to confirm the presence of the parasite. Blood samples were taken immunological evaluation by the ELISA technique.

Written consent was obtained from all patients and controls in this study, and the current study received ethical approval from the Najaf Health Department / Training and Development Center (No.71) and the Department of Medical Laboratories in the College of Health and Medical Technologies/ Kufa (No.172).

#### **Excluded Criteria**

- 1. Patients with a history of immunodeficiency disorders.
- 2. Patients with a history of autoimmune diseases.
- 3. Patients are currently undergoing immunosuppressive therapy.
- 4. Pregnant patients.
- 5. Treated patients with Metronidazole in the past 14 days.
- 6. Age < 15 years and > Age 55 and asexual.

#### **Study Design**

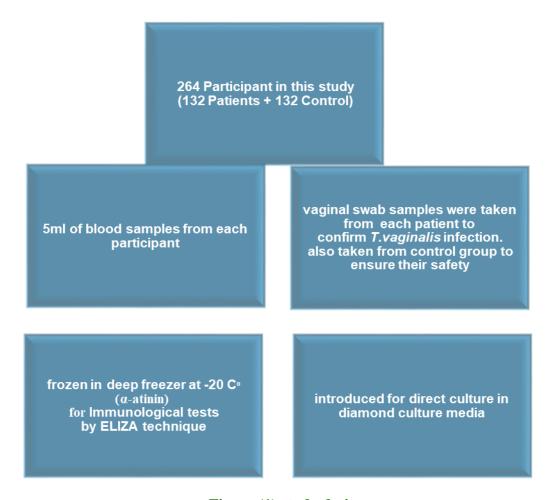


Figure (1) study design

#### **Samples Collection**

Samples were taken from Al-Manathra Hospital, Al-Najaf Hospital, Al-Furat Hospital, and a few outpatient clinics between January and June of 2023. A case-control research design was employed for this investigation. In this study, 264 participants,132 trichomoniasis patients and 132 controls, were given vaginal swaps and had 5 milliliters of blood taken. After inserting a sterile vaginal speculum, swab sample was taken from the posterior fornix or vaginal wall using sterile cotton swap. The swabs were inoculated in liquid

diamond medium for culture.

## Culturing method in Diamond culture media

- 1-After receiving the swab, implanted directly, by rotating the swab in the center, then cut off the protruding part of the swab, and replace the cap loosely.
- 2- The antenna was incubated at 33-37°C for up to 5 days.
- 3. The medium was examined microscopically for the presence of moving *Trichomonas Vaginalis*.

**Enzyme-linked Immunosorbent Assay** 

#### (ELISA)

The concentration of virulence factor  $\alpha$ -actinin (Mybiosource/USA) was measured using ELISA technology based on the manufacturer's instructions. This assay has high sensitivity (9.375pg/ml) and excellent specificity for detection of Alph $\alpha$ -actinin .

# RESULTS AND DISCUSSION Basic Demographics of Cases and Control Subjects

This study was conducted on a total of 264 females aged (15-54 years) enrolled in this study. The first group (case) included female patients *suffering from vaginal trichomoniasis* (132); the second group (control) included females without *vaginal inflammation* (132). Subjects were selected from Al-Manathra Hospital and Al-Najaf Hospital and Al-Furat Hospital, and some outpatient clinics in the period from January 2023 to June 2023.

In our *T.vaginalis* study, we chose to include women aged 15 to over 55 years for the following reasons:

High prevalence during in age range: *Trichomonas Vaginalis* is a common sexually transmitted infection (STI) that primarily affects sexually active people. The prevalence of infection in women between 15 and 55 years is at its highest because women tend to be more sexually active in this age range. This would allow evaluation of infection rates and to determine the associated factors within this population.

Sexual activity patterns: Women between 15 and 55 years are probably more sexually active. This exposes them to the risk of becoming infected with *T.vaginalis* through unprotected sex. The study data will allow evaluation of transmission, risk factors, and the effect of this infection in sexually active females by studying this

particular population.

Vulnerable populations: There are some vulnerable groups who are more prone to infection with STIs, including *T.vaginalis*. These groups include adolescents and women in their reproductive age. Studying these cohorts can help identify risk factors, barriers to care, and potential interventions to prevent and control *T.vaginalis* infection in these populations.

Reproductive effects: *T.vaginalis* infection can have adverse effects on reproductive health, like an increased risk of getting pelvic inflammatory disease (PID), infertility, preterm birth, and other complications. Studying the mentioned group provides a valid evaluation of *T.vaginalis* influence on reproductive health, fertility, and pregnancy outcomes.

Clinical presentation and symptoms: Itching, vaginal discharge, and discomfort are some of the symptoms that could follow a *T.vaginalis* infection. These symptoms might drive women from this particular age group to seek medical attention. Therefore, improve insight can be gained about the clinical presentation along with the diagnostic and treatment strategies of this infection by studying this group.

Overall, selecting females aged between 15 to 55 years for a *T.vaginalis* study allowed a focus on a population at high risk of infection, with reproductive implications, and in need of targeted interventions for prevention, diagnosis, and management of *T.vaginalis*.

Table (1) shows demographic characteristics according to age groups. Most cases appeared in the age group 25-34 (45.46%), while fewer cases appeared in the age 45-54 (12.88%). The other categories (15-24 years and 35-44 years) formed the following number s and percentage: 30

(22.72%) and 25 (18.94%) respectively.

There were five clinical diagnosis groups (PCOS, BOH, Secondary infertility, primary infertility, and endometriosis). The results are shown in Table 1. Most cases were diagnosed with PCOS 33 (25%); 15 cases (11.36%) were diagnosed with secondary infertility. 22 patients (16.67%) were diagnosed with BOH; 31 patients (23.49%) were diagnosed with endometriosis, and 31 patients (23.49%) were diagnosed with primary infertility.

Polycystic ovary syndrome (PCOS) is a hormonal disorder that affects women of reproductive age. PCOS is characterized by hormonal imbalances, irregular menstrual cycles, and enlarged ovaries with small cysts. Women with PCOS often have elevated levels of androgens (male hormones) and insulin resistance, which can contribute to inflammation in the body, including in the vaginal area.

Many factors are associated with vaginal inflammation in PCOS patients, including:

Hormonal disturbance: Women with PCOS often have elevated levels of androgens, such as testosterone. hyperandogenism can lead to increased inflammation in the body, including the vaginal area. PCOS patients experience a hormonal imbalance that might affect the response and inflammatory immune processes. For this reason, women with **PCOS** are vaginal more prone to inflammation.

Insulin resistance: PCOS patients usually have a condition of insulin resistance. This condition raises the insulin levels in the blood. High insulin levels could be a factor connected to chronic inflammation in the body including the vaginal area. High insulin levels can also trigger inflammation by disrupting the hormonal balance in the body.

From another perspective, secondary infertility is a term that refers to the inability to conceive or carry a pregnancy to term after having previously given birth. This condition might have other underlying causes that are not necessarily related to hormonal imbalances and inflammation. Known causes include age-related factors, reproductive health changes, infertility due to a male factor, morphological issues, or other medical conditions that are not associated with PCOS.

Moreover, vaginal inflammation could happen for many reasons other than PCOS and secondary infertility. It can be triggered by factors like hygiene practices, infections, sexual activity, use of certain medications, and underlying health issues.

Overall, the hormonal imbalances and insulin resistance associated with PCOS can contribute to an increased risk of vaginal inflammation in women with this condition, while vaginal inflammation may be less common in women with secondary infertility due to the different underlying causes of infertility in this group.

Table (4-1) clarifies that there may be individual differences in the frequency and severity of symptoms such as irregular bleeding and discomfort during sexual activity, which may lead to similarities between the two groups, where 64 patients (48.48 %) suffered from irregular bleeding, 59 patients (44.69 %) suffered from pain during intercourse.

Also, 34 patients (25.75%) suffered from foul odour; 31 patients (23.48%) suffered from vaginitis; 28 patients (21.21%) suffered from cervicitis; 28 patients (21.21%) suffered from itching; 27 patients (20.45 %) suffered from lower abdominal pain; 20 patients (15.15%) suffered from burning pain on micturition and 19 patients

(14.39%) suffered from dysuria.

The above symptoms may be due to the inflammation and discomfort these diseases generate. A major infection or strong inflammatory response that needs medical treatment may be indicated by inflammation that spreads to the lower abdomen or causes pain during urination, thus, these symptoms may indicate a vaginal infection or a more serious condition.

These symptoms may also be uncomfortable, have an impact on physical and mental health, and reduce quality of life. In order to feel better, patients who experience these symptoms will likely seek medical help.

Untreated vaginal inflammation or infection puts the patient at a risk of developing several complications. Pregnancy problems, infertility, recurring infections, and pelvic inflammatory diseases are some of these consequences. When there are signs such as vaginitis or cervicitis, there is an increased chance of acquiring these issues. This usually occurs when the underlying problem is misdiagnosed or improperly treated.

Although some women experience vaginal inflammation, they may not exhibit the condition's characteristic symptoms. This makes the diagnosis of the underlying cause quite challenging. Women with subclinical or asymptomatic vaginitis may not be diagnosed without special tests if they do not have symptoms such as lower abdominal discomfort, itching, or dysuria.

Females suffering from symptoms including searing pain when urinating, itching, lower abdomen discomfort, cervicitis, and vaginitis may have underlying infections, more severe inflammation, worse quality of life, and a higher chance of multiple and diagnostic difficulties.

Recent global data shows that the prevalence of *T.vaginalis* infection in women between 15-49 years old is 5.3%, while it's 0.6% in men, with a total of 156 million cases per year. This infection could be asymptomatic in women or cause vaginal discharge, vaginitis, pruritus, cervicitis, and dysuria [10].

The *T.vaginalis* infection may cause other conditions like vaginitis, cervicitis, and urethritis, and this may result serious complications such as infertility and a higher susceptibility of infection from human papillomavirus, herpes simplex virus, and cervical cancer. Along with dysuria, itching, burning sensations in the vaginal region, pelvic discomfort, and dyspareunia, women infected with T.vaginalis may also have green or yellow vaginal discharge and an unpleasant stench. The "strawberry cervix," which is regarded as a pathognomonic symptom of trichomoniasis, is a tiny hemorrhagic area that develops on the vaginal membrane in severe instances due to inflammatory responses [11].

A study in Dohuk city examined 425 vaginal swabs collected from women who had vaginal discharge accompanied by pelvic inflammatory disease, vaginitis, and cervicitis. According to this study, young women in the age range between 20 and 25 years had an infection rate of 7.6% which was higher than the rate in other age [12].

In Erbil, trichomoniasis screening was conducted in epidemiological research. The research examined 440 women within the age range of 16-60 years, and showed that the highest infection rate was 4% in the age group of 16-26, whilst the group of 16-26 years had an infection rate of 3.3% and the group of 27-37 years showed a rate of 3.1% [13].

Mahmoud *et al.* did not find any significant symptomatic differences among

females with trichomoniasis (P=0.129) [14].

A study in Egypt by Hussein *et al.* found that the most common symptoms among infected women were dysuria and dyspareunia, while itching and vaginal discharge came after that. No significant differences were observed, except in those with dysuria and dyspareunia [15].

In Babylon, a study aimed to detect the infection in symptomatic women using polymerase chain reaction and wet mount. The results showed that women between 30-

40 years had the highest infection rates [16]. In a Hamdy study, there was a significant correlation between trichomoniasis and dysuria (P=0.003) and dyspareunia (P<0.001). But, there was no correlation found with discharge and itching [17]. Conversely, IBRAHIM *et al.* reported that the presence of vaginal dysuria and discharge had a significant association with trichomoniasis infection, unlike dyspareunia and itching [11].

**Table 1: Demographic Data of Study Groups** 

	Crowns	Patients	Controls
Demographic data	Groups	No. (%)	No. (%)
	15-24 year	30 (22.72)	27 (20.45)
	25-34 year	60 (45.46)	30 (22.73)
Age groups	35-44 year	25 (18.94)	35 (26.52)
	45-54 year	17 (12.88)	40 (30.30)
	Total	132 (100.00)	132 (100.00)
	PCOS	33 (25.00)	-
	ВОН	22 (16.67)	-
Clinical diagnosis	Secondary infertility	15 (11.36)	-
Cillical diagnosis	Primary infertility	31 (23.49)	-
	Endometriosis	31 (23.48)	-
	Total	132 (100.00)	-
	Itching	28 (21.21)	-
	Foul smelling	34 (25.75)	-
	Dysuria	19 (14.39)	-
	Burning pain on micturation	20 (15.15)	-
Symptoms	Cervicitis	28 (21.21)	-
, ,	Irregular bleeding	64 (48.48)	-
	Vaginitis	31 (23.48)	-
	pain during intercourse	59 (44.69)	-
	Lower abdominal pain	27 (20.45)	_

The Level of Alph $\alpha$ -actinin Concentration in The Serum of Patients Infected with the Parasite Trichomonas Vaginals and Controls

According to the results shown in Table 2, it is clear that the level of  $\alpha$ -actinin concentration was higher in the serum of

patients infected with the parasite T.vaginalis (,509.113  $\pm$  26.401 pg/ml) and this increase is statistically significant (P-value <0.0001) compared to its concentration in the control serum (67.939  $\pm$  8.179 pg/ml).

α-actinin is one of the most common

immunomodulators and can be detected in the serum of women suffering from the parasite T.vaginalis. It was also mentioned that  $\alpha$ -actinin is protected across many strains of T.vaginalis. Thus, when separated from a single strain of Trichomonas, this immunogen can stimulate antibodies identical to various epitopes in diverse strains [18, 19].

Many previous studies and research have indicated the importance and key roles played by  $\alpha$ -actinin, as they have proven that this virulence factor actively involves in the process of pathogenicity, plays an essential and important role in the redistribution of cellular actin, and contributes in transforming the parasite into the amoeboid form and form of false extensions. Thus, it plays a wide role in the morphological changes of the parasite [20, 21, 22].

Compared to other studies, our results were consistent with what was reported by [23, 24], where women infected with T.vaginalis were repoted to have a higher level of  $\alpha$ -actinin compared to controls.

Several studies reported that the virulence factor  $\alpha$ -actinin has been noticed on the surface of cells infected with the parasite T.vaginalis. Perhaps the presence of this protein on the surface of cells infected with the parasite and the high immunity to T.vaginalis-alph $\alpha$ -actinin are responsible for the high titers of anti-T.vaginalis-alph $\alpha$ -actinin [25].

In a study by Alderete,  $\alpha$ -actinin is found to be one of the most common detectable immunogens in the serum of female patients with T.vaginalis infection [26]. In addition, it has been noted that having different

T.vaginalis strains did not affect the conservation of  $\alpha$ -actinin through these strains. So, it could induce antibodies against different epitopes in various strains.

There might be an idea of a vaccine development regarding the importance of  $\alpha$ -actinin in the survival and immunogenicity of this parasite. Subunit vaccines can avoid multiple antigens causing autoimmunity by mimicking molecular patterns during host defense. That makes them more popular now than ever [27].

Research by Xie *et al.* demonstrated that  $\alpha$ -actinin could provide partial or complete protection in patients exposed to *T.vaginalis* challenges [5].

Also [5] noted that α-actinin identification is possible by sera from women who are exposed to T.vaginalis, because it is the most common immunogen with high immunogenicity. These are crucial characteristics for a vaccine candidate. An immune response of Th1/Th2 can be bv α-actinin provides induced This significant protection against infection to those who are affected by the parasite.

According to particular studies, Tvaginalis- $\alpha$ -actinin might act as an immune-modulator by increasing the production of IL-10, IL-12, and IL-6 [6], [28].

In addition, the presence of the protein on the cell surface and its strong immunogenic characteristics are the reasons behind the high levels of anti-T.vaginalis alphaactinin antibodies in women with trichomoniasis or in regions with a high prevalence rates [25].

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α-actinin	Mean ± SEM	T	Df	P-value
Trichomoniasis Patients	509.113 ± 26.401	15.96	262	<0.0001 ****
Controls	$67.939 \pm 8.179$			

Table 2: The level of Alphα-actinin Concentration in The Serum of Study Groups

The level of Alph $\alpha$ -actinin concentration in the serum of Trichomoniasis patients according to age groups

The results shown in Table 3 show that the highest mean rank for  $\alpha$ -actinin (78.94 pg/ml) appeared in the (35-44 years) age group, which numbered 35. While the results showed that the lowest mean rank for  $\alpha$ -actinin (55.77 pg/ml) appeared in the (25-34 years) age group, which numbered 30.

While the mean rank level of  $\alpha$ -actinin concentration in the patients' serum depended on the other age groups (15-24 years and 45-54 years) which was their number (27 and 40) reached (70.04 pg/ml and 61.28 pg/ml) respectively.

 $\alpha$ -actinin secretion appears to be affected by the woman's age, as the secretion of the virulence factor increases during the period when hormonal balance becomes disrupted in women aged 35-40 years.

Research on the specific effects of age on

the concentration of  $\alpha$ -actinin in the serum of trichomoniasis patients is fairly limited. Despite that, we should note the importance of  $\alpha$ -actinin in cytoskeletal organization and controlling cell motility.

The risk of getting infections like trichomoniasis gets higher as age advances because the immune system might not be as functional in older individuals. Regarding that, levels of  $\alpha$ -actinin in the serum of older patients with trichomoniasis might be higher than its levels in younger patients.

Moreover, the presence of  $\alpha$ -actinin in the serum might be affected by the changes of hormones and general health with age.  $\alpha$ -actinin levels regulation could be under the influence of hormonal disturbances in older people.

Thus, there are several plausible reasons for this, such as immune response, hormone variations, chronic inflammatory illnesses, and aging-related changes to the vaginal microbiota.

Table 3: The level of Alphα-actinin Concentration in The Serum of Trichomoniasis Patients according to Age Groups

	age	N	Mean Rank	Chi-Square	Df	Asymp. Sig.	
	15-24	27	70.04				
a actinin	25-34	30	55.77	7.044	3	0.071	
α-actinin	35-44	35	78.94	7.044			
	45-54	40	61.28				
	Total	132					

The level of Alph $\alpha$ -actinin Concentration in The Serum of Trichomoniasis Patients according to Clinical Diagnosis

According to the results shown in Table No. 4, it is clear that the level of  $\alpha$ -actinin

concentration, based on the diagnosis of the clinical condition of patients infected with the parasite, reached its highest level in the group of patients infected and diagnosed suffering from endometriosis, where was mean rank (77.68Pg/ml). The reason for this is that Tv $\alpha$ -

actinin binds to the iron-binding element of Tvcp RNA that encodes the T.vaginalis (Cysteine-Proteinase) and thus participates in the process of post-transcriptional iron regulation. Adhesion to the host epithelium occurs via T.vaginalis also by a specific interaction with glycoproteins located outside the cell in the basement membrane. While the lowest increase in the level of  $\alpha$ -actinin was in the group of patients diagnosed with polycystic ovary syndrome (PCOS), where it was the mean rank (55.67 pg/ml), the explanation for this result is also attributed to the fact that women who suffer from polycystic ovary syndrome have few, irregular, or sometimes non-existent menstrual cycles, and menstrual blood is considered a major source of iron [29], which is an important and essential nutrient for the *T.vaginalis* parasite, as it obtains it from the host through very specific receptor mechanisms [28]. Therefore, the lack of iron availability is considered an unsuitable medium for the growth of the *Trichomonas Vaginalis* parasite [11], and therefore, women who suffer from polycystic ovary syndrome are less susceptible to infection with the parasite.

While the level of  $\alpha$ -actinin concentration in the group of patients diagnosed with bad obstetrics history (BOH), primary infertility and secondary infertility was as follows: it was mean rank (75.00 pg/ml, 61.39 pg/ml and 65.33pg/ml) respectively. This difference in height was statistically nonsignificant where it was P-value=0.139.

Table 4: The level of Alphα-actinin Concentration in The Serum of Patients Infected with Trichomonas Vaginalis according to Clinical Diagnosis

	Clinical diagnosis	N	Mean Rank	Chi-Square	df	Asymp. Sig.			
	PCOS	33	55.67		4	0.139 NS			
	ВОН	22	75.00						
α-actinin	Primary infertility	31	61.39	6.949					
	secondary infertility	15	65.33						
	endometriosis	31	77.68						
	Total	132							
PCOS:Poly	PCOS:Polycystic ovaries syndrome, BOH: Bad obstetric history, N; Number, df: degree freedom.								

The level of Alphα-actinin concentration in the serum of patients infected with *Trichomonas Vaginalis* according to clinical symptoms groups

The level of  $\alpha$ -actinin concentration in the serum of patients infected with the parasite *T.vaginalis*, according to the results shown in Table No. 5, was generally higher in the group of women suffering from symptoms, which we indicated in the table with the word (yes), compared to the women who did not show symptoms, which we

indicated in the table with the word (no).

Based on clinical symptoms, the results showed that the highest mean rank for the level of the virulence factor  $\alpha$ -actinin appeared in the group of women suffering from foul-smelling odor, where it reached 82.53 pg/ml.

The lowest mean rank for  $\alpha$ -actinin (67.31pg/ml) appeared in the group of women who suffered from irregular bleeding.

While the level of virulence factor

reached (72.71 pg/ml, 72.79 pg/ml, 80.90 pg/ml, 70.79 pg/ml, 72.42 pg/ml and74.7 pg/ml) in other clinical groups (itching, dysuria, burning pain on micturition, cervicitis, vaginitis and pain during intercourse) respectively.

Thus, the levels of  $\alpha$ -actinin (pg/ml) do not show a significant difference among most groups. The explanation for this can be due to various factors:

Among them is biological variation: alph $\alpha$ -actinin levels can show significant variation within and between study groups, reducing the power to detect important differences. Biological variability can mask

true differences in biomarker levels.

Overlapping pathophysiology: The conditions studied (BOH, endometriosis, PCOS, primary infertility, secondary infertility) may share common underlying mechanisms or pathways that influence alpha-actinin levels. As a result, the differences in alpha actinin levels between the two groups may not be pronounced enough to reach statistical significance.

These results suggest that women with trichomoniasis and other diseases may have different amounts of this biomarker, and that this  $\alpha$ -actinin may be associated with the corresponding conditions.

Table 5: The level of Alphα-actinin Concentration in The Serum of Patients Infected with

Trichomonas Vaginalis according to Clinical Symptoms Groups

	Iricnom	ionas va	iginaiis ac	coraing to	Clinical Syl	mptoms Gro	ups				
	Symptoms groups	N	Mean Rank	Sum of Ranks	Mann- Whitney U	Wilcoxon W	Z	Asymp. Sig. )(2- tailed			
	Itching										
	Yes	28	72.71	2036.00	1282.000	6742.000	-0.969	0.333			
	No	104	64.83	6742.00							
	Total	132									
		dysuria									
	Yes	19	72.79	1383.00	954.000	7395.000	-0.775	0.439			
	No	113	65.44	7395.00							
	Total	132									
				foul smel	ling discharge						
	Yes	34	82.53	2806.00	1087.000	5840.000	-2.951	0.003			
	No	97	60.21	5840.00							
	Total	131									
		burning pain on micturition									
	Yes	20	80.90	1618.00	832.000	7160.000	-1.828	0.068			
α-actinin	No	112	63.93	7160.00				0.008			
	Total	132									
		Cervivitis									
	Yes	28	70.79	1982.00	1336.000	6796.000	-0.668	0.504			
	No	104	65.35	6796.00				0.304			
	Total	132									
		irregular bleeding									
	Yes	64	67.31	4308.00	2124.000	4470.000					
	No	68	65.74	4470.00	2124.000		-0.237	0.813			
	Total	132									
				V	aginitis						
	Yes	31	72.42	2245.00	1382.000	6533.000					
	No	101	64.68	6533.00	1382.000		-0.985	0.325			
	Total	132									
					ng intercourse						
	Yes	59	74.76	4411.00	1382.000	6533.000	-2.231				
	No	73	59.82	4367.00	1362.000			0.026			
	Total	132									

#### **Correlation** between Alphα-actinin and Study groups

Table 6 shows that there is a significant Pearson correlation at the 0.01 level (2tailed), between α-actinin and the foulsmelling (p-value=0.006, r=-0.238). While there is an non significance Pearson correlation between α-actinin and itching (pvalue=0.719, r=-0.032), dysuria value=0.752, r=-0.028), burning pain during (p-value=0.173, r=-0.119), urination cervicitis (p-value=0.608, r=0.045), irregular bleeding (p-value=0.972, r=0.003), vaginitis (p-value=0.477, r=-0.062), pain during intercourse (p-value=0.101, r=-0.143) and age (p-value= 0.350, r=-0.082).

<b>Table 6: Correlation</b>	between Al	phα-actinin	and Study groups

	Correlations									
	Type of correlation	Itching	Dysuria	Foul smelling	Burning pain during urination	Cervivit i	Irregular bleeding	Vaginitis	Pain during intercourse	Age
α- actinin	Pearson Correlation	-0.032	-0.028	238**	-0.119	-0.045	0.003	-0.062	-0.143	-0.082
	Sig. (2- tailed)	0.719	0.752	0.006	0.173	0.608	0.972	0.477	0.101	0.350
	N	132	132	132	132	132	132	132	132	132
**. Corre	**. Correlation is significant at the 0.01 level (2-tailed.(									

#### CONCLUSIONS

- 1. The virulence factor  $\alpha$ -actinin is significantly and highly secreted in patients infected with the parasite Trichomonas Vaginalis.
- 2. α-actinin secretion appears to be affected by the woman's age, as the secretion of the virulence factor increases during the period when hormonal balance becomes unattainable in women aged 35-40 years.
- 3. α-actinin secretion appears increase women diagnosed in endometriosis, this means that vaginal bleeding is an appropriate environment for increased secretion of the virulence factor.

#### **AUTHORS CONTRIBUTIONS**

SA collected samples and data, diagnosed the submitted samples, conducted tests, analyzed the data, and prepared the draft.

RA supervised the study, developed the study design, assisted in data analysis, and corrected the manuscript.

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#### CONFLICT OF INTEREST

A conflict of interest does not exist.

<sup>\*.</sup> Correlation is significant at the 0.05 level (2-tailed.(

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### التقييم المناعى لعامل الضراوة الفا-اكتينين لدى النساء المصابات بالمشعرة المهبلية

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الخلفية والاهداف: تعد عدوى المشعرة المهبلية من أكثر الأمراض شيوعا المنقولة جنسيا بين النساء، ويعتبر ألفا أكتينين أحد أهم عوامل الضراوة لهذا الطفيلي.و تقييم تركيز بروتين ألفا أكتينين في النساء المصابات بعدوى المشعرة المهبلية والمجموعة الضابطة باستخدام تقنية ELISA.

منهجية الدراسة: تم تضمين مائتين وأربعة وستين من النساء المشاركات في دراستنا الحالية، كان يذهبن إلى مستشفى المناذرة، مستشفى النجف، مستشفى الفرات، وعدد قليل من العيادات الخارجية بين يناير ويونيو من عام 2023. تم تضمين مائة واثنان وثلاثون كمجموعة مراقبة ومائة واثنان وثلاثون مريضا (كانوا يعانون من العدوى المهبلية المشعرة المهبلية). خضع جميع المشاركين في الدراسة للزرع المباشر في وسائط زراعة الدايموند عن طريق أخذ مسحة من المهبل للتأكد من وجود الطفيلي. وأيضا تم أخذ عينة دم لإجراء التقييم المناعي بتقنية ELISA.

النتائج: أظهرت النتائج أن مستوى تركيز ألفا-أكتينين أعلى في مصل المرضى المصابين بالطفيلي المشعرة المهبلية ( 26.401 ± 509.113 بيكوغرام/مل وهذه الزيادة ذات دلالة إحصائية (P-value <0.0001) مقارنة مع تركيزه في مصل المجموعة الضابطة (87.939 ± 8.179 ± 8.179 مقارنة مع تركيزه في مصل المجموعة الضابطة بيكوغرام/مل) ظهر في الفئة العمرية (35-44 سنة) والتي بلغ عددها 35 سنة. بينما أظهرت النتائج ظهور أدنى رتبة له الفا اكتينين (55.77 بيكوغرام/مل) في الفئة العمرية (25-34 النتائج ظهور أدنى رتبة له الفا اكتينين بركيز ألفا-أكتينين بناء على تشخيص الحالة المريدية للمرضى المصابين بالطفيل أعلى مستوياته في مجموعة المرضى المصابين و تم تشخيص إصابتهن ببطانة الرحم المهاجرة، حيث كانت الرتبة المتوسطة (77.68 بيكوغرام/مل). في حين أن أقل زيادة في مستوى ألفا-أكتينين كانت في مجموعة المرضى المتوسطة (PCOS)، حيث كانت الرتبة المتوسطة (55.67 بيكوغرام/مل). وبناء على الأعراض المريرية أظهرت النتائج أن أعلى متوسط لمستوى عامل الضراوة ألفا-أكتينين ظهر في مجموعة النساء اللاتي يعانين من رائحة كريهة حيث بلغ 82.53 بيكوغرام/مل. في حين أن أقل متوسط رتبة له الفا اكتينين من رائحة كريهة حيث بلغ مجموعة النساء اللاتي يعانين من نزيف غير منتظم.

الاستنتاجات: يتم إفراز عامل الضراوة ألفا أكتينين بشكل كبير وعالي عند المرضى المصابين بطفيلي المشعرة المهبلية، ويبدو أن إفراز ألفا أكتينين يتأثر بعمر المرأة، حيث يزداد إفراز عامل الفوعة خلال الفترة التي يصبح فيها التوازن الهرموني. لا يمكن تحقيقه عند النساء الذين تتراوح أعمارهم بين 35-40 سنة. يبدو أن إفراز ألفا-أكتينين يزداد عند النساء المصابات ببطانة الرحم المهاحرة، وهذا بعني أن النزيف المهبلي بشكل بيئة مناسبة لزيادة

الكلمات الدالة: ألفا –أكتينين، المشعرة المهبلية.