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Detection of Pro-Inflammatory IL-8 and IL-12 in Iraqi Women Infected with Trichomoniasis

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Abstract

Trichomonas vaginalis is a unicellular flagellated protozoan that resides in female and male genital tract and considered the most prevalent sexually transmitted infectious parasite. The infection rate is relatively equivalent between male and female but trichomoniasis is usually asymptomatic in men. Primary triggering of host inflammatory response to this parasite is not fully understood and most studies address the local reaction of the parasite in female genital tract. In this study, two pro-inflammatory cytokines, IL-8 and IL-12, were investigated in the serum of infected women with *Trichomonas vaginalis* during acute and chronic stages of the disease. The results demonstrated that the level of IL-8 was significantly higher along the acute and chronic disease stages in female patients. However, the level of IL-12 was significantly increased in the acute group but exceptionally decreased in the chronic group of patients, in comparison with the control group. These results indicate that local infection of the vaginal Trichomoniasis may alter the levels of the studied inflammatory cytokines during infection.

Keywords: *T. vaginalis*, immune response, cytokines, vaginitis

التحري عن الانترلوكينات الالتهابية IL-8 و IL-12 في النساء العراقيات المصابات بداء المشعرات

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الخلاصة

طفيلي المشعرات المهبلي هو طفيلي وحيد الخلية سوطي يتواجد في الجهاز التناسلي للإناث والذكور، ويعتبر أكثر الطفيليات المعدية التي تنتقل عن طريق الاتصال الجنسي. معدل الإصابة بهذا الطفيلي عادة متساوي بين الذكور والإناث ولكن داء المشعرات عادة ما يكون غير مصحوب بأعراض عند الذكور. التحفيز الأولي للاستجابة الالتهابية للمضيف لهذا الطفيلي غير مدروس بصورة فصلية إذ تركز معظم الدراسات على التفاعلات الالتهابية للطفيلي في الجهاز التناسلي للإناث. في هذه الدراسة ، تم التحري عن اثنين من الانترلوكينات الالتهابية المحفزة في مصل النساء المصابات بالمشعرات المهبلي هما IL-8 و IL-12 ، وذلك خلال المرحلة الحادة والمزمنة من الإصابة. أظهرت النتائج أن IL-8 كان أعلى بشكل ملحوظ على مدى الإصابة الحادة و المزمنة للإناث المصابات، بينما ازداد IL-12 بشكل ملحوظ عند الإصابة الحادة فقط و انخفضت مستوياته بشكل معنوي عند المصابات في المرحلة المزمنة من المرض، مقارنة مع مجموعة السيطرة. تشير هذه النتائج إلى أن الإصابة الموضعية لطفيلي المشعرات المهبلي قد أثرت على مستويات السابتوكينات الالتهابية المدروسة خلال العدوى.

Introduction

Human trichomoniasis is an obligate extra-cellular infection caused by a unicellular parasite, *T. vaginalis* a teardrop shape parasite which attacks female and male genital tract and is considered as the most non-viral sexually transmitted agent [1, 2]. Recently, the parasite gained further consideration due to of the increase in the infection rate according to the WHO statistics in 2016; the worldwide prevalence of *T. vaginalis* infection is more than 270 million people annually [3]. *T. vaginalis* is endemic in Iraq; during the last decade, many epidemiological studies around many provinces such as Baghdad, Kufa, Najaf and Mosul, confirmed positive diagnosis of infection in women attending governmental hospitals [4, 5]. The disease is usually asymptomatic in men, while in women, the asymptomatic phase is uncommon. The instigation of symptoms starts when white blood cells accumulate in the lower urogenital area and become in contact with the moving trichomonads [6]. Symptomatic female patients may undergo discomfort, dysuria, cervicitis, and vaginitis, associated with unusual vaginal discharge with foul-smell and possible infertility [7, 8]. In pregnant women, *T. vaginalis* infection may lead to preterm labor, abnormal infant weight and, if left untreated, the baby is most likely contracts live trichomonas form the infected mother [9]. Considerate investigation of the mechanism of immune response of host to *T. vaginalis* infection is essential for better understanding the infection strategy, early diagnosis, and treatment of the disease [10].

Host-parasite interaction is complicated in trichomoniasis; it depends on the host immune response towards trichomonads and the virulence factors of the parasite. This leads to pathological consequences by evading the durable physical barrier of the female genital tract after effective cytoadherence of the parasite to epithelial cells and the successful destruction and phagocytosis of target cells to carry chronic infection [11, 12]. Systemic and local innate immunity of both males and females against infection is sex-dependent due to the special mucosal fluids during menstrual cycle and pregnancy in women [13]. Active or passive T-cell mediated immune response of the infected host play a vital role in controlling or exacerbating trichomoniasis. Previous experimental studies showed that *T. vaginalis* infection inhibits the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and its related Th-1 cytokines, such as TNF- α and IL-12 [11, 14]. Experimental mouse infection with *T. vaginalis* demonstrated low level of IL-2 and IFN- γ , both in serum and vaginal secretion. On the other hand, triggering the residing Natural Killer cells by the trichomonads activates the production of IFN- γ , IL-10 and IL-8 by macrophages and cytotoxic T cells [15, 16]. One of the major *T. vaginalis* antigens, Tv α -actinin2, was found to trigger cytokine production in experimental vaginal epithelial cells infection, in which increasing levels of IL-10, IL-12, IL-6 and TNF- α were observed during the first 16 hours of screening [17]. In this study, two pro-inflammatory cytokines were investigated in the serum of women infected with trichomoniasis. The levels of IL-8 and IL-12 were detected in the chronic and acute phases of the disease to inspect the possible immune effects of the parasite during infection.

Materials and methods

Sample collection Blood samples from 114 adult married women suspected with trichomoniasis were collected from different hospitals in Baghdad. Blood was centrifuged at 3000 rpm and serum was collected and stored at -20.

Diagnosis of trichomoniasis

All samples used in this study were diagnosed previously as acute or chronic infections as described earlier [18]. This confirmation test is not commercially available and was designed and purchased in collaboration with BIO-RAD, UK, especially for this study. BIORAD Company has developed a universal indirect ELISA kit for specific anti-*T. vaginalis* IgM and specific anti-*T. vaginalis* IgG for acute and chronic trichomoniasis detection, particularly for the purposes of this study.

Procedures and results of BIORAD indirect ELISA test for IgM and IgG analyses are not shown because they were performed and published earlier [18]. Number of samples was 18 and 16 for acute (IgM) and chronic (IgG), respectively.

Cytokines detection

The pro-inflammatory cytokines, IL-8 and IL-12, were purchased from Sigma Aldrich[®] (USA); the protocol of detection was sandwich ELISA, according to the manufacturer's protocol. Samples were screened in a 96-well plate for pretested women; 18 samples of IgM and 16 of IgG for trichomoniasis. Plates were read at 450 nm by ELISA reader [19]. The control group included 15 serum samples of

apparently healthy women, with no known illness, and screened as above for IL-8 and IL-12 comparison.

Exclusion criteria

Samples from men, single women, and women under 18 years old were excluded in this study. Only married women with positive anti-*T vaginalis* IgM or IgG, in comparison with control groups, were involved.

Statistical analysis

Statistical Package for Social Sciences SPSS (V.21) software was used for t test analysis to detect the significant differences between test and control groups for each parameter, where p value ≤ 0.05 was considered significant.

Results and discussion

IL-8 level in acute and chronic stages

The results showed that IL-8 level was higher in both acute (IgM) and chronic (IgG) groups of infected women in comparison with the healthy group. The mean concentrations of IL-8 were 30.85 pg/ml and 31.34 pg/ml in the acute and chronic groups, respectively, while a lower value in the control group was detected, which was equal to 22.47 pg/ml (Figure-1).

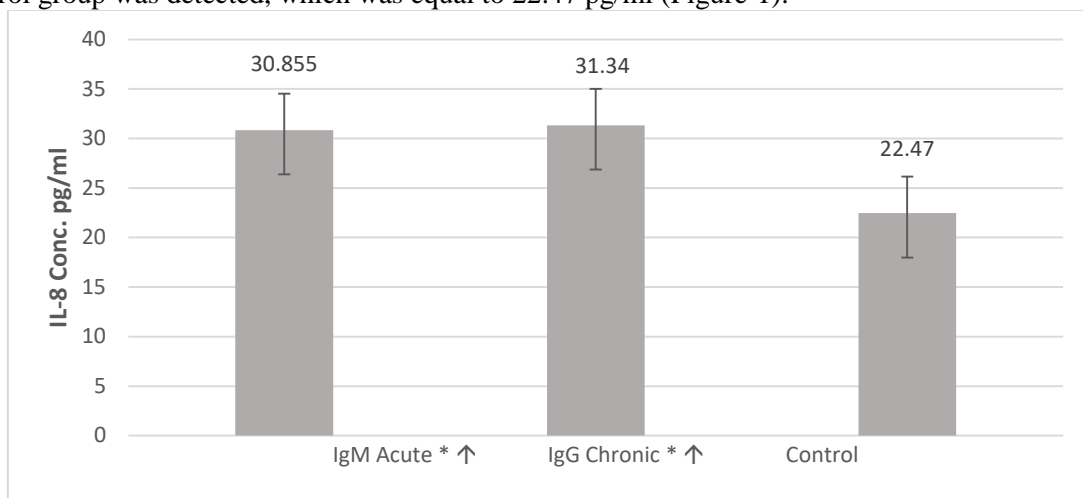


Figure 1-IL-8 level in acute and chronic trichomoniasis in comparison with control women, * = significance ≤ 0.5 .

IL-12 level in acute and chronic stages

The results demonstrated that IL-12 level was higher in patients during the acute stage of the disease (30.055 pg/ml) in comparison with the control group (23.053 pg/ml). Whereas, the results of patients during the chronic stage of the disease exhibited a significance decrease of IL-12 level, with a mean concentration of up to 16.6 pg/ml, as shown in Figure-2.

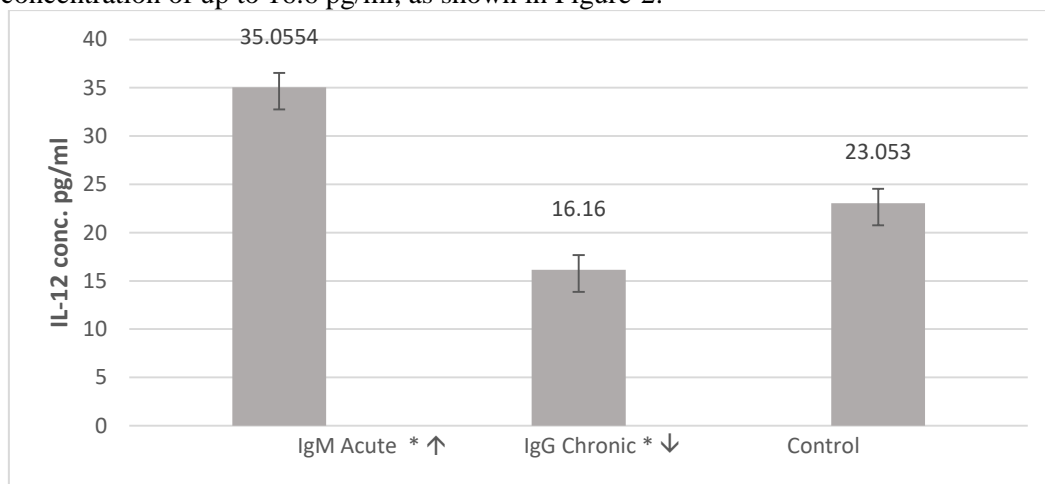


Figure 2-IL-12 level in acute and chronic trichomoniasis in comparison with control women, * = significance p value ≤ 0.5

Furthermore, there was no significant difference between the acute and chronic groups for IL-8 values. On the other hand, the level of IL-12 in the chronic patients was significantly lower than that in the acute group, as shown in Figure-3.

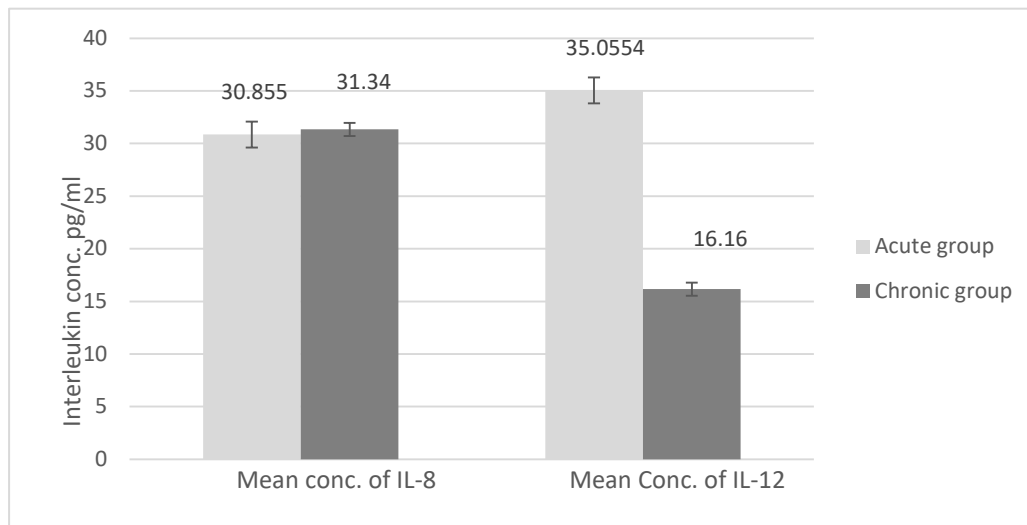


Figure 3-IL-8 and IL-12 mean concentrations in acute and chronic patients.

It was erroneously considered that *T. vaginalis* habitation of the vagina is harmless, until its role in pathogenesis of vaginitis was demonstrated [20]. However, the mechanism of immuno-pathogenesis and how the parasite can evade the immune system, as an extracellular pathogen, is still not well-identified [2]. Several studies evidenced that infection with different sexual pathogens, including sexually transmitted diseases (STD) bacteria and *T. vaginalis*, is associated with increasing risk of human immunodeficiency virus HIV infection [21].

A previous study examined the endo-cervical inflammatory markers of women infected with *T. vaginalis*. They showed that the levels of IL-8, IL-6 and TNFr1 receptor were increased in association with *T. vaginalis* infection, but no correlation was detected for anti-inflammatory IL-10 [22]. The high expression of IL-8 is based on the fact that this local chemoattractant triggers the innate immune neutrophils in vaginal discharges, although it was also linked to high HIV infection risk [21, 23]. Moreover, bacterial lipopolysaccharide (LPS)-activated dendritic cells produce more IL-8 during *T. vaginalis* infection, which generates circulating specific IgG and IgA towards the parasite. In addition, IL-8 was found to develop mucosal irritation [2, 11]. *In vitro* culture of leukocytes proved that *T. vaginalis* stimulated these cells to produce IL-8 and iNO_s. Furthermore, *T. vaginalis* lipophosphoglycan (LPG) was found to induce upregulation of IL-8 mRNA protein [20]. Additional research on IL-8 production in *T. vaginalis* infection confirmed the increased level of IL-8 in symptomatic women with trichomoniasis, after releasing TNF- α and IL-1 β from damaged vaginal epithelial cells, where the concentration was up to 900 pg/ml, suggesting that epithelial cells were provoked by LPG of live trophozoites [1].

Previous studies demonstrated the leukotoxic activity of *T. vaginalis*, in which IL-8 served as the primary inflammatory cytokine in response to infection and it is related to the severity of clinical presentation; in addition, the more damaged target cells occurred by the parasite, the higher release of IL-8 was followed; noting the fact that no phagocytosis is involved in pathogenesis [7, 24]. Moreover, it was verified that *T. vaginalis* promotes human macrophages and neutrophils to produce IL-8 and the suppression of this interleukin and other chemokines facilitated the phagocytosis of host cells by the trichomonads [17, 25].

On the other side, this study demonstrated parallel elevation of IL-12 in the acute group but, surprisingly, it was decreased in the chronic group, in comparison with control. IL-12 is known as a pro-inflammatory cytokine, which prompts the differentiation of Th1. An experimental study found that *T. vaginalis* affects the expression of IL-12 by the inhibition of NF-kB, a mechanism that is elicited by the parasite stimulation of anti-inflammatory macrophage marker IL-10, suggesting a

potent invasion strategy [11, 26]. An experimental research on the macrophage cell line RAW264.7 exposed to *T. vaginalis* showed that mRNAs of IL-12 and TNF- α were over-expressed during the first 2 hours of infection, but down-regulated after 8 hours [25]. rT $\nu\alpha$ -actinin 2, a protein derivative of the parasite, was found to alter the immune response of dendritic cells, as confirmed by suppressing IL-12 but upregulating the anti-inflammatory IL-10 [17]. Nevertheless, innate immunity was found to have a minor effect on the parasite, in which an indistinguishable recurrent infection may occur in sexually active women, also in men suffering from prostate disorder, requiring better understanding of innate immunity towards this extra-cellular parasite [27, 28].

This study concludes a role of the extracellular *T. vaginalis* in modulating the responses of the systemic cytokines IL-8 and IL-12 in response to infection.

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