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TRICHOMONIASIS – AN IMPORTANT COFACTOR OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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ABSTRACT

Trichomonas (*T.*) *vaginalis* is the most common non-viral sexually transmitted infection worldwide. The estimated number of new *T. vaginalis* infections accounts for 276.4 million cases globally. The pathogen induces local inflammation of the lower genitourinary tract, can be involved in premature labour, low birth weight and facilitates HIV-1 transmission via sexual intercourse. In the paper, we present basic epidemiological data on *T. vaginalis* infection, biologic mechanisms by which the pathogen enhances HIV-1 acquisition, principles of modern diagnosis and treatment of the infection.

Key words: *Trichomonas vaginalis, epidemiology, HIV-1, diagnostics, treatment*

EPIDEMIOLOGY OF *TRICHOMONAS VAGINALIS* INFECTION

Trichomonas (T.) vaginalis infection is the most frequent non-viral sexually transmitted infection (STI) worldwide and accounts for one-third of all STIs (1). As it is a non- notifiable infection, only estimates of its incidence and prevalence are available. In 2008, the World Health Organization estimated a global incidence of 276.4 million, and prevalence of approximately 190 millon – an 11% increase in comparison to figures from 2005 (1). Africa (with 384.4 million adults between the ages of 15 and 49) was the region with the highest incidence (59.7 million cases of new infections in 2008) (1). Trichomoniasis affects mainly females in developing countries and in underprivileged societies in developed countries (2). *T. vaginalis* infection is infrequent in Europe, seen mostly among immigrants (3). Scanty publications from Polish Medical Bibliographies indicate that trichomoniasis can be found in 3.8% of asymptomatic pregnant females (4).

Infection rates increase with females' age, which is a striking feature distinguishing trichomoniasis from other curable STIs (2). The average duration of infectiousness lasts 3-5 years in females and only four months in males, due to unspecific immune response and mechanical clearance of male urethra (2). Infections are symptomatic in up to 40% of females and 50% of males. The probability of female-to-male transmission ranges between 4-80% and that of male-to-female – 85-100%, and is similar in symptomatic and asymptomatic infections (2). Women with asymptomatic trichomoniasis serve as a reservoir for continuing disease transmission.

CLINICAL PICTURE OF TRICHOMONIASIS

T. vaginalis infection causes local inflammation of the lower genitourinary tract. The main symptoms reported among females are: vaginitis, sometimes secondary cervicitis. Both males and females can experience urethritis, and balanoposthitis effects males. Untreated trichomoniasis may lead to pelvic inflammatory disease in women and to prostatitis and epididymitis in men. *T. vaginalis* infection in pregnancy can promote premature rupture of membranes, premature labour and lead to low birth weight (5).

T. VAGINALIS INFECTION AND HIV-1 INFECTION

The possible synergy between non-ulcerative lower genitourinary tract infections and human immunodeficiency virus (HIV-1) was discussed since the 1990's (6). Epidemiological studies indicated a two to three-fold increase in HIV-1 infection risk with concomitant *T. vaginalis* infection, even after controlling for high-risk sexual behaviors (6). Up to 20% of new HIV-1 infections could be attributable to the presence of trichomoniasis

(6). Results from meta-analytical studies' revealed that *T. vaginalis* infection was associated with 1.64 – fold higher incidence of HIV-1 infection in women (95% confidence interval – 1.28 – 2.08) (7). Infection with HIV-1 increases the risk of *T. vaginalis* infection and visa versa (9). Other studies have further shown vaginal HIV-1 shedding to be higher in HIV-positive females with concomitant *T. vaginalis* infection, however initiating treatment of *T.vaginalis* prior to antiretroviral therapy reduces viral shedding (8).

Several mechanisms may contribute to the increased risk of HIV-1 infection in trichomoniasis, including: inflammatory response within mucous membranes of the lower genitourinary tract, inhibition of innate immune response, weakening or breach of mucous membranes and the alteration of normal vaginal flora (10).

The excessive immune response can lead to tissue damage, thus increasing the risk of HIV-1 acquisition. A suboptimal immune response may enable survival of HIV-1. *T. vaginalis* infection is usually asymptomatic however can lead to symptoms within the lower genitourinary tract. The immune response associated with vaginal infection draws HIV-1 target cells (CD4+ lymphocytes, monocytes, macrophages) within genitourinary tract, and is likely to be the most important mechanism by which *T. vaginalis* serves as a cofactor of HIV-1 infection (11). The interleukins (IL) concentration, namely that of IL-1ß, IL-8, as well as the amount of neutrophils in the vagina is increased during infection (12-14). IL-8 can activate HIV-1 replication (14). *T. vaginalis* lipophosphoglycans can induce, in a dose-dependent manner, the expression of chemokines, IL-8 and a macrophage inflammatory protein (MIP-3α) or CCL20. MIP-3α can recruit Langerhans-like CD34+ cells to the mucous membrane surface, thus facilitating HIV-1 acquisition (15).

Cationic antimicrobial polypeptides, such as ß-defensins and secretory leukoprotease inhibitor, are the main substances providing the innate immune response in the mucous membrane of genitourinary tract against bacterial, viral (they are also virucidal to HIV-1) or fungal pathogens in the lower genitourinary tract. Their concentration during *T. vaginalis* infection is reduced (16).

The integrity of the epithelium of genitourinary tract serves as the best protection against HIV-1 acquisition via sexual intercourse. The stratified pseudosquamous epithelium of the vagina is the most effective barrier. Junctions between epithelial cells become weaker during *T. vaginalis* infection facilitating migration of Langerhans cells through epithelium (17). Infection causes punctate bleeding of the cervix (known as *colpitis maculosa* or strawberry cervix) that enables the entry of HIV-1. *In vitro* studies showed that *T. vaginalis* infection of polarized cervical epithelial cells induced disruption of epithelial integrity, production of TNF-α and thus initiated the pathway leading to HIV-1 replication (17).

CONTEMPORARY LABORATORY DIAGNOSIS OF *T. VAGINALIS* INFECTION

The choice of laboratory diagnostic method depends on setting and the biological material being tested.

Laboratory methods used to confirm diagnosis of trichomoniasis are aimed at detection of the pathogen, its antigens or genetic material. Various techniques are used including: wet mount preparation, culture, nucleic acid amplification tests (NAATs), enzyme immunoassay or immunofluorescence. In females, self-obtained vaginal or in-office taken swab (sometimes cervical) or first catch urine – FCU are used, whilst in males – the urethral swab or FCU are the preferred techniques. Generally, FCU testing provides the lowest sensitivity.

The wet mount preparation of vaginal swabs or FCU is a method most frequently used in the diagnosis of trichomoniasis as they are easy to perform, provide results rapidly and cost less. Live *Trichomonas* can be identified as flagellated parasitic protozoan, typically pyriform, but occasionally amoeboid in shape, presenting with a quivering (twitching) motility. The above method is not recommended for male patients, as urethral swab contains few live protozoas. Microscopic examination of the wet mount must be performed up to 30 minutes after obtaining the material (18). The sensitivity of the method in cases of vaginitis ranges from 38% to 82% (comparing with the result of culture) but can be as low as 20% after 10 minutes (19). *T. vaginalis* in females can be detected also by Giemza or Gram staining as well as in Papanicolau smear.

Culture, using Diamond's medium or its modifications are considered gold standard diagnostic techniques for *T. vaginalis* infection and have been used for the past 40 years. The sensitivity is approximately 95% (20, 21). The material taken (urethral swab or sediment of FCU) should be incubated for 2 -7 days at 37 ^{o}C, ideally in 5% CO_2, and direct microscopic preparation should be performed every day. The InPouch TV (BioMed Diagnostics, White City, USA) is a commercially available technique that allows both culture and microscopic examination of the specimen (22). Culture is the laboratory diagnostic method of choice in males and in females where *T. vaginalis* was not detected via wet mount and clinical signs or symptoms are suggestive of trichomoniasis, in addition to patients whose symptoms persist after treatment of STIs (18).

Methods involving amplification and detection of genetic material of *T. vaginalis* are the best diagnostic option, useful for both screening (e.g. of women with highest risk groups) and in diagnosis of particular cases.

The sensitivity and specificity of NAATs is better than that of wet mount and culture (21, 23). The TVA5-A6 gene, stable and specific for different *T. vaginalis* strains consisting of 102 base pairs, was amplified and detected with the use of polimerase chain reaction (PCR) in the first NAATs method described (24). Since then several systems using polimerase chain reaction (PCR), for the diagnosis of *T. vaginalis* infection were elaborated, with the use of different primers (21, 23). The average sensitivity of NAATs was 95% (range: 89-100%) and specificity – 98% (range: 95-100%) (21, 23). The only commercially available Food and Drug Administration cleared diagnostic kit for molecular diagnosis of trichomoniasis in females (APTIMA® T. vaginalis assay, GenProbe Inc., San Diego, CA, USA) enables the detection of ribosomal RNA of protozoa and its sensitivity is over 95% and specificity 99% in various biological materials (vaginal or cervical swab, FCU) (25). The small amount of biological material needed for testing, long-term storage and detection of different pathogens at the same time are also advantages of NAATs (25).

In practice, methods that enable the detection of *T. vaginalis* antigens or antibodies produced in the response of the infection (namely: immune enzyme assays, direct or indirect immunofluorescence tests, immunoblotting) are of less importance, as their sensitivity and specificity is not higher than that of culture or PCR (24). The point-of-care tests (e.g. Kalon TV Latex Agglutination Test, Kalon Biological, Guildford, UK), have a sensitivity and specificity similar to that of combined wet mount and culture, results are available within minutes, during consultation, thus enabling prompt treatment (26).

TREATMENT OF *T. VAGINALIS* INFECTIONS

Detection of the pathogen using any of the aforementioned tests, irrespective of presence or absence of symptoms, warrants trichomoniasis treatment (27). Treatment must also be offered to the current sexual partner of infected person (27). The treatment of choice are nitroimidazoles: metronidazole (orally 400 mg or 500 mg BID for 5 – 7 days or a single dose of 2 g) or a single dose of tinidazole 2 g. Topical treatment with metronidazole is not recommended for trichomoniasis. In relation to pregnancy metronidazole is classed as category B (animal studies have revealed no evidence of harm to the fetus, but no adequate, well-controlled studies among pregnant women have been conducted) and tinidazole - category C (animal studies have demonstrated adverse events, and no adequate, well-controlled studies in pregnant women have been conducted). About 2-5% of *T. vaginalis* strains can be resistant to recommended metronidazole doses but are sensitive to tinidazole or higher metronidazole doses (e.g. 400 TID mg for 5 – 7days). The test of cure is unnecessary for men and women who became asymptomatic after treatment or who were initially asymptomatic (27).

In summary, *T. vaginalis* infection, is an important, underestimated and poorly reported cofactor for the acquisition of HIV-1 infection via sexual intercourse. The lack of epidemiological data on trichomoniasis and limited laboratory diagnostic methods of the infection applied in Poland are of importance and require improvement.

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