

TRICHOMONAS VAGINALIS – A RISK FACTOR FOR CERVICAL CANCER

LILIANA PUSTAN^{1*}, OCTĂVIȚA AILIEȘE², SIMONA DUNCA²

Keywords: parasitic protozoa, cellular atypia, genome, trichomoniasis

Abstract: *Trichomonas vaginalis* is the human parasite most widely spread in the world. The national statistics shows that it occurs in 20 to 25% of the women in Romania. The latest research studies (Carlton, J.M. *et al*, 2007) have proved that persistent infections with *Trichomonas vaginalis* increase the risk of HIV infection, cervical cancer, and serious complications in premature births. This study was conducted over a period of five years (2004 - 2008). From the 9117 cases examined, 220 were diagnosed with inflammations caused by *Trichomonas vaginalis*. Approximately 6.4% of the infected cases exhibited cellular atypia. The purpose of this study was to determine the incidence of atypia associated with trichomoniasis in the cervicovaginal smears examined during the Babes-Papanicolaou tests. Another important objective was to correlate different aspects of the pathogenic mechanism of the parasite with the information of the *Trichomonas vaginalis* genome, as well as morphopathological characteristics of the cervicovaginal cells.

INTRODUCTION

In the last years, *Trichomonas vaginalis* has regained the interest of the specialists in microbiology throughout the world. The genetic researches have shown that the genome of the parasite is similar to that of *Drosophila*, with 160 million building blocks of DNA, approximately twice as many as the human genome. In addition, this genome, which dictates the parasitic mode of life, integrates into the genome of the host cell reducing host's immunity to HIV, and prostate, urethral, or cervical cancer (Carlton, J.M. *et al*, 2007). For this reason, the staff of the laboratory run by Dr. Jane Carlton have been working to discover a vaccine against the pathogen. Continuous reinfection with the parasite is the most dangerous as it can cause premalignant initially and then malignant lesions. The only way to prevent the occurrence of such lesions seems to be the detection and removal of this factor with cancer-causing potential from the very early stages of its interaction with the cellular genome (Stark J.R. *et al*, 2009).

Exfoliative cytology is one of the most effective methods to detect and prevent cervical cancer.

Research groups in important laboratories examine the intraepithelial lesions generated by *Trichomonas vaginalis*. Extremely important, for instance, are the research studies of Dr. Jane Carlton from the University of Medicine (New York), Dr. Jennifer Stark from the University of Oxford, and of the research working groups from the microbiology departments of renowned clinics in Germany, Spain, etc.

Fundamental aspects regarding the cellular and genetic characteristics involved in the *Trichomonas vaginalis* - induced metabolism and pathology

The parasite *Trichomonas vaginalis* is a protozoan which ranges in size from 8 to 20 μ , has an irregular shape, sometimes with larger basal part (pyriform), has 1 – 2 flagella (less visible after fixation of slides), is well defined, with a foamy, cyanophilous cytoplasm (greenish grey on a Pap smear) with red granules, and eccentric, possibly red-coloured nucleus.

It is transmitted during sexual intercourse; men also become infected (asymptomatic hosts), but most commonly exhibit urethral infections. In fact, the parasite may reside in the urethra of women, too, from where it reinfects the cervicovaginal segment upon the pH increase. When the lactobacilli have reinstated, the vaginal pH level returns to normal and the parasite disappears from the area since it cannot survive in an acid environment. Hence the conclusion of some specialists according to which some women cure by themselves. As a matter of fact, the parasite present in the urethra reinvas the vagina in the period prior to menses when the pH increases to 7.5 and after the menstrual cycle, when the pH decreases again to 4 – 4.5, *Trichomonas vaginalis* moves back into the urethra. Thus, in the absence of a sustained therapy, continual reinfection occurs.

The parasite adheres to the wall of the cervicovaginal cells and, by a very effective enzyme system, it interferes in the metabolism of the host generating toxins that may lead to cell necrosis. In addition, for survival, the parasite needs very little energy, which it acquires by the glycolysis of cellular glycogen (Arroyo, R. *et al*, 2006). Due not only to the enzymatic action but also to the parasitic genome which cause the host so many damages, some specialists thought even of a method to detect it based on the antigens secreted particularly in young girls (Huppert, J.S. *et al*, 2007). Interpretation for women at menopause is more difficult because their ability to defend is reduced due to the absence of the lactobacilli, which produce an acid pH, not conducive to parasite growth. Moreover, changes occur in terms of vascularisation, similar to the increase in the estrogenic activity: the number of superficial and intermediate cells increases and after treatment,

the atrophic pattern reinstates.

MATERIALS AND METHODS

Biological material. The pathological products required for diagnosis are cervicovaginal exudates collected by authorized personnel under doctor's supervision in gynaecology clinics. The preparation to be examined is obtained by smear spreading on glass slides followed by fixation in absolute ethyl alcohol. The smears are allowed to dry for 10 to 15 minutes, and then sent to the cytology laboratory.

Analytical method. The infections with *Trichomonas vaginalis* are detected by the microscopic examination of cervicovaginal smears. This requires smear staining, which is carried out either by the Papanicolaou test or by the May-Grünwald Giemsa method. Both methods were used in our investigations.

May-Grünwald Giemsa staining. The staining technique used in this study is an in-house method developed by adapting the May-Grünwald-Giemsa method to our laboratory conditions with influences of the Tzanck test (Galatâr, N., 1978). The nucleus of the cells will be coloured in purple-red, while the cytoplasm in shades of blue. The principle is based on the differential staining of the cell nucleus in purple-red and of the cytoplasm in shades of blue, depending on the amount of cytoplasmic RNA. The advantages provided by this method are the rapidity of its performance (it takes less than 10 minutes) and reduced costs (cheaper dyes), our method decreasing even more the price for such staining.

Papanicolaou staining. This staining method has been the most commonly accepted lately since it is considered to show most clearly the aspects of the morphological pathology of the cell and particularly the nucleus (Galatâr, N., 1978).

The Orange G solution is prepared from a dye with the same name, while EA 50 is prepared by mixing the following dyes: light-green, Brun Bismark and eosin yellow. In our study, ready-made kits purchased from authorized companies were used. Upon staining, the nucleus stains in greyish-blue, the cytoplasm of the eosinophilic cells stains in pink to orange, while that of the basophilic cells stains in greenish blue (Galatâr, N., 1978).

Interpretation of smears

The samples thus prepared are screened using an optical microscope with eyepieces of 10 x and objective lens of 20 x. The screening aims at indicating the cellular composition specifying the type of cells on the smear and their state of normality, the inflammatory and microbial components.

RESULTS AND DISCUSSIONS

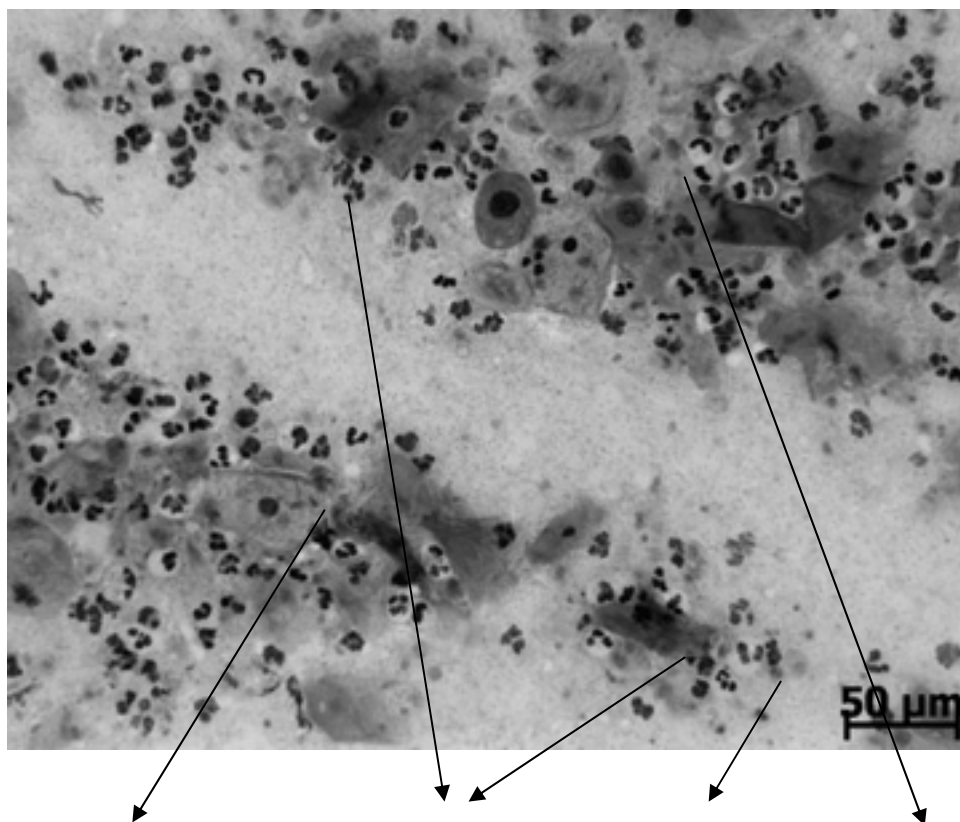
Our study included the screening of 3234 smears in 2004, 1769 in 2005, 922 in 2006, 1281 in 2007 and 1911 in 2008.

The microscopic examination indicates the presence of *Trichomonas vaginalis* infections characterized by a persistent inflammatory process with a rosette-like formation of neutrophil granulocytes surrounding the parasite. The parasite appears well-defined, with irregular shape, and peripheral nucleus. The affected cells exhibit a perinuclear halo quite clear in some cases, while in other cases the smear has a "ground glass" effect (Photo 1).

The following aspects are examined: the inflammatory nature of the smear, proclivity for eosinophilia, presence of reactive parabasal exocervical cells.

In the cervicovaginal mucous, the parasite induces a number of cytological changes (up to cellular atypia) in the cells of the parabasal layer. Moreover, in the superficial layers, it causes: necrosis (as a result of parasite multiplication by simple fission), enlarged nucleus, sometimes increased number of nuclei in a cell (most commonly there are two), hyperchromasia, denuded nuclei, proclivity to eosinophilia or amphophilic behaviour.

The cytodiagnosis is expressed using the Bethesda reporting system. The smears are classified according to the cellular changes found.



eosinophilic cells *Trichomonas vaginalis* neutrophil granulocytes reactive cells

Photo1. Cervicovaginal smear showing inflammation associated with *Trichomonas* infection (x 200)

Most cases (93.6% in our study) show an acute inflammatory process, but with no cellular changes outside the reactive zone; therefore, we classify the smear to the NILM (negative for intraepithelial lesion or malignancy) category, indicating the presence of inflammation.

Cases in which the parasite-induced cellular changes can be classified as mild atypias (6.4% in our study) have also been reported.

The results concerning the incidence of cases of infection with *Trichomonas vaginalis* between 2004 and 2008 are indicated in Figure 1 to Figure 6.

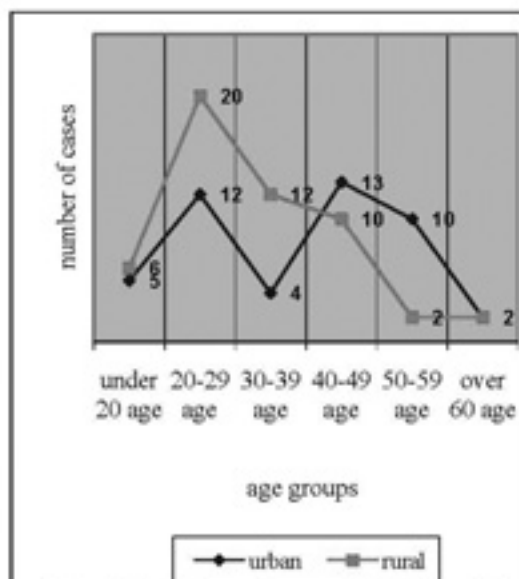


Figure 1. Incidence of cases of infection with *Trichomonas vaginalis* (2004)

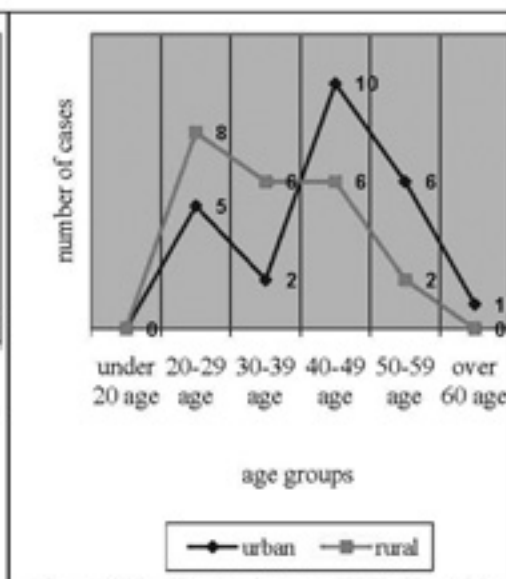


Figure 2. Incidence of cases of infection with *Trichomonas vaginalis* (2005)

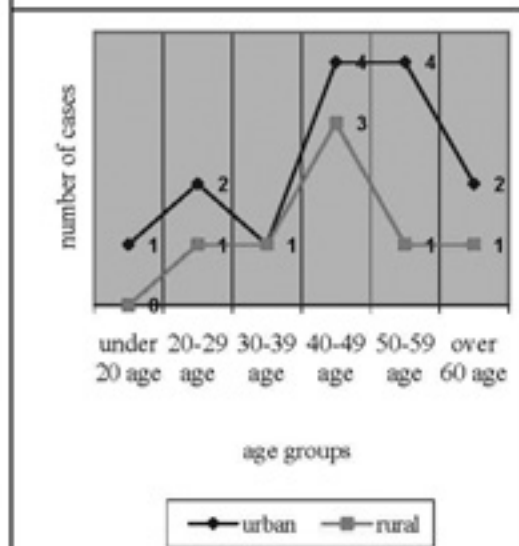


Figure 3. Incidence of cases of infection with *Trichomonas vaginalis* (2006)

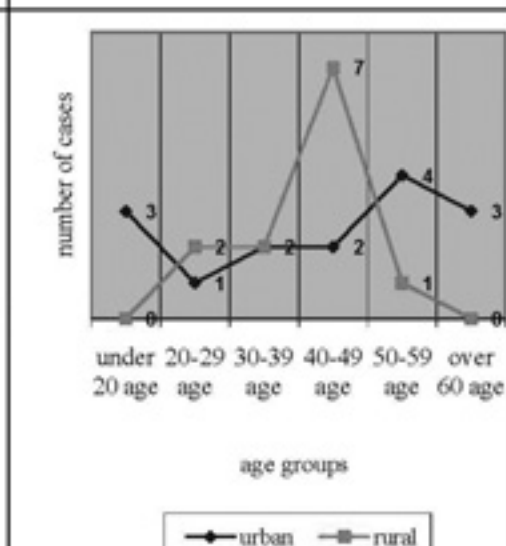


Figure 4. Incidence of cases of infection with *Trichomonas vaginalis* (2007)

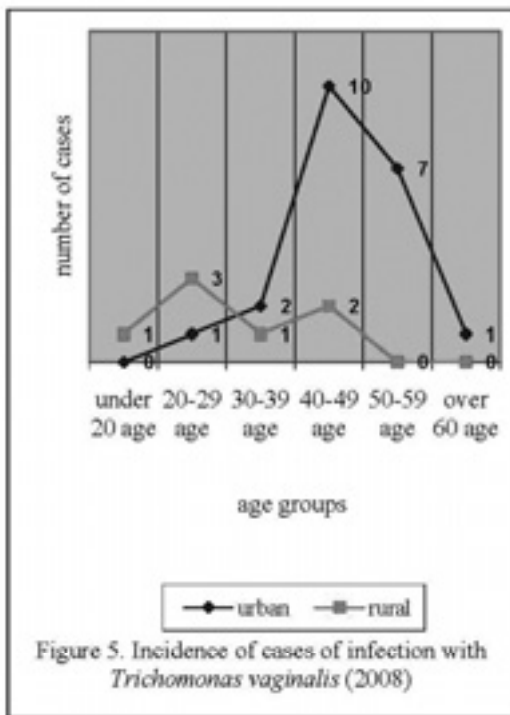


Figure 5. Incidence of cases of infection with *Trichomonas vaginalis* (2008)

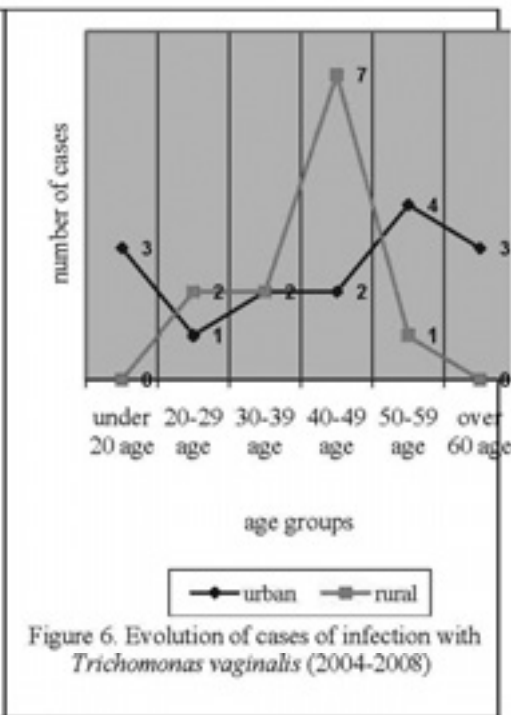


Figure 6. Evolution of cases of infection with *Trichomonas vaginalis* (2004-2008)

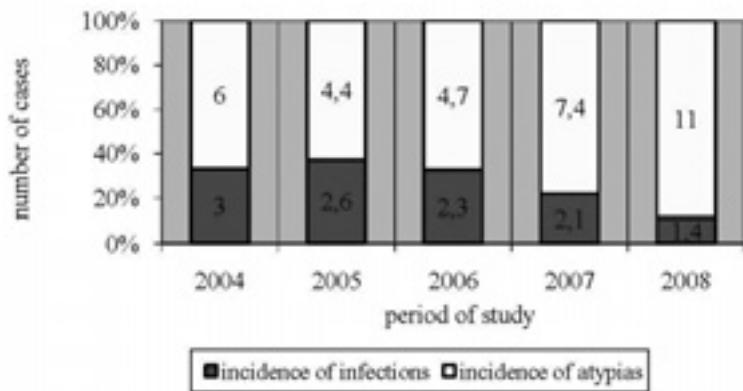


Figure 7. Incidence of infections with *Trichomonas vaginalis* and the incidence of atypias

With little exception, the difference between the incidence of cases infected with *Trichomonas vaginalis* in the rural environment and in the urban one is not significant. We can

With little exception, the difference between the incidence of cases infected with *Trichomonas vaginalis* in the rural environment and in the urban one is not significant. We can

estimate that in the age group comprising subjects under 30, the cases in the rural areas are predominant, while above this age, alternative cases of undetermined significance are found.

The analysis of the evolution of *Trichomonas vaginalis* infection cases shows that the incidence rate has decreased in the young women from all age groups in the last years.

Disturbing is the fact that despite the last years' decrease in the incidence of *Trichomonas* infections, the cases of atypia increased, almost doubling towards the end of the examined period (Fig.7). This is not surprising since the process of becoming malignant is slow, with delayed effects visible only after a number of years. We consider that the result supports our theory according to which reinfection with the parasite may result in premalignant and malignant lesions.

Koss and Woliska concluded in their research studies (1970) that the evolution of cancer does not depend on its association with the presence of the parasite; however, in women with malignancy the incidence of parasitic infection is much higher than in healthy women. After 40 years old, the microbiology research laboratories consider that a vaccine ought to be developed to neutralize this parasite.

Nevertheless, from the cytological point of view, the presence of the *Trichomonas vaginalis* species does not exclude the diagnosis of suspected malignancy when the appearance of the smears indicates such changes.

CONCLUSIONS

Of the number of 9117 cases screened between 2004 and 2008, 220 were found to exhibit inflammations caused by *Trichomonas vaginalis*.

The morpho-pathological examination of the cervicovaginal cells showed that 6.4% of the investigated cases had cellular atypia.

In the last years, a decrease in the incidence of cases of *Trichomonas vaginalis* infection has been noticed, both in young women and in all age groups.

The results of the study showed a twofold increase of the cases of cellular atypia correlated with the slow premalignant and malignant processes.

REFERENCES

- Arroyo, R., Engbring, J., Alderele, J.F. (2006): *Molecular basis of host epithelial cell recognition by Trichomonas vaginalis*. Molecular Microbiology 6(7):853-862.
- Carlton, J.M., Hirt, R.P., Silva, J.C. (2007): *Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis*. Science, New York: 315(5809): 207-12.
- Galatâr, N., Bolba Gh., Chiricuta I., Munteanu, S., Risca, M. (1978): *Îndreptar de citologie pentru diagnosticul precoce al cancerului de col uterin*. Ed. Medicală, București, 48-49.
- Huppert, J.S., Mortensen, J.E. (2007): *Rapid antigen testing compares favorably with transcription – mediated amplification assay for the detection of Trichomonas vaginalis in young women*. Clinical Infection Diseases, 45(2): 194-198.
- Stark R.J., Judson G. (2009): *Prospective Study of Trichomonas vaginalis Infection and Prostate Cancer incidence and Mortality*. JNC of Cancer Institut; 1406-1411, Oxford University Press

- 1) Emergency County Hospital Piatra Neamț - Laboratory of Pathological Anatomy, Traian Avenue, no. 1 Piatra Neamț;
 - 2) "Alexandru Ioan Cuza" University of Iași, Faculty of Biology
- * pustan_liliana@yahoo.com