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Investigation of *In Vivo* Effects of *Trichomonas Vaginalis* on Visceral Organs

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Abstract

Background: Urogenital *trichomoniasis* (UGT) is the most common sexually transmitted disease in Kazakhstan. Association of *T.vaginalis* infection with the development of ascites and multiple visceral abscesses was shown in experiments with intraperitoneal injection of infectious material. The effect of *T.vaginalis* on visceral organs in natural infection is unknown.

Materials and methods: *T.vaginalis* effect on visceral organs was assessed with the model previously developed by us ("The Method of Urogenital *Trichomoniasis* Simulation", patent application No. 06 331 dated April 1, 2016). It was ethically carried out on animals with the approval of the KazNMU Ethics Committee (Registration No. 191). The material for histological and cytological studies was prepared in accordance with the conventional methods.

Result: The changes was found in all visceral organs, most pronounced in hepatic parenchyma: parenchymal degeneration and necrosis of hepatocytes, significant edema with cellular infiltration in the portal tracts and separation of blood plasma from the formed elements in blood vessels representing changes in rheological properties of blood. Expressed changes were also observed in renal tissues. Less pronounced changes were observed in lung tissues.

Conclusions: The results of our study showed high activity of *T. vaginalis* in visceral organs.

Keywords: Urogenital *trichomoniasis*; *Trichomonas vaginalis*; Experimental model; Disturbances in rheological properties of blood

Introduction

Urogenital *trichomoniasis* (UGT) is the most common sexually transmitted infection in Kazakhstan. UGT is characterized by a predominance of mixed infection which is asymptomatic and undiagnosed form [1]. The structural and functional heterogeneity of *Trichomonas vaginalis* (*T. vaginalis*) is well known, which explains the biological properties of the simplest and clinico-pathogenetic features of UGT [2-4]. Study made by Mahdi Nadham and colleagues shows the damaging effect of *T. vaginalis* on the internal organs of mice after intraperitoneal injection of the infection with the development of ascites, multiple abscesses in internal organs, mainly in the liver and spleen [5]. In other mouse models, intraperitoneal injection of *T. vaginalis* infection resulted in visceral necrosis (especially pancreatic and liver cancer), the extent of which is proportional to the level of virulence of the inoculated strain, which can lead to death. Subcutaneous administration of *Trichomonas* leads to the development of a localized abscess at the injection site [6-8]. In the available literature, we have not found any research on the effect on internal organs in the natural pathway of infection.

In this regard, the aim of the study is to study the effect of *T. vaginalis* on internal organs in the urogenital infection pathway on a model of chronic infection.

Materials and Methods

Material

T.vaginalis No8 strain, identified by morphological, cultural, biological characteristics, was used to infect guinea pigs.

Animals

Animals used in this study were 6 adult male guinea pigs weighing 450-500 g. All animals were on a standard dietary/drinking diet prior to the experiment. 2 weeks before the experiment the animals underwent quarantine. To exclude the indigenous *trichomonas* infection, a microscopic and culture study of the urethral microflora was previously carried out. Animals with negative results were included in the study. Animals were divided into 2 groups: 1st (n=4)-experimental and 2nd (n=2)-control.

Model of chronic urogenital trichomonas infection

Modeling of chronic urogenital trichomonas infection was carried out on the developed experimental model "Reproductive system

(guinea pigs)+*T. vaginalis*", simulating the natural course of infection (Method for modeling urogenital *trichomoniasis*, patent application No. 06331 of April 1, 2016). To reproduce the *trichomonas* infection against the background of a decrease in the immune status, guinea pigs were intramuscularly injected with hydrocortisone at a dose of 125 mg/kg of body weight 1 time per day for 2 days. On the following day, the animals of the 1st group received intravenous injection of a suspension containing 1×10^6 trichomonads per 0.5 ml of nutrient medium, animals of the 2nd group received 0.5 ml of nutrient medium.

Methods

Ether overdose was used to euthanize 4 animals of the 1st group on the 30th day (complete spermatogenic cycle). The animals of the 2nd group were euthanized at the same period. The material for histological examination was prepared according to the generally accepted scheme. Smears prints were stained by Romanovsky Giemsa stain. Stained smears were studied using an Axioskop 40 (Carl Zeiss, Germany) microscope with an eyepiece 10x and 4x, 10x objectives.

Ethical consideration

The research was carried out in compliance with the ethical norms for working with animals and with the permission of the Ethics Committee of the Kazakh National Medical University (registration No. 191).

Results

In the study of histological preparations on the 30th day of the experiment, the following materials were obtained:

Lung: Morphology of the lungs from control group was normal. The lung was represented by alveoli with a thin interalveolar septum. Clearance of the alveoli is free, the walls of the bronchial tubes and bronchioles have a thin clear clearance (Figure 1).

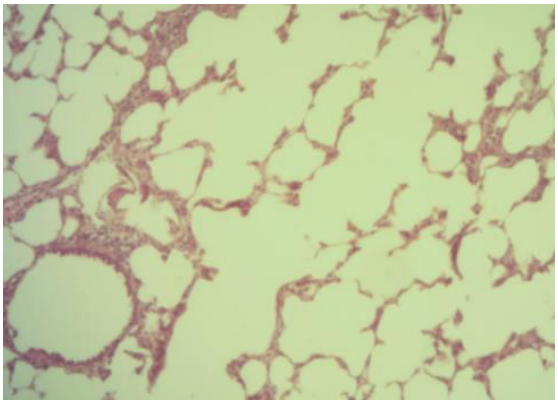

Figure 1: Control group. Lung tissue. Usual histological structure, HE 100x.

In the experiment: a circulatory disturbance was observed in the form of vasoconstriction with perivascular circular cell infiltration (Figure 2). In a cytological study, homogeneous amorphous masses surrounded by round-cell infiltration are detected. There is the cell that does not correspond to the structure of the lung tissue (Figure 3). In the cytoplasm of this cell, elements of consolidation and canning of DNA with cellular reorganization are clearly visible.

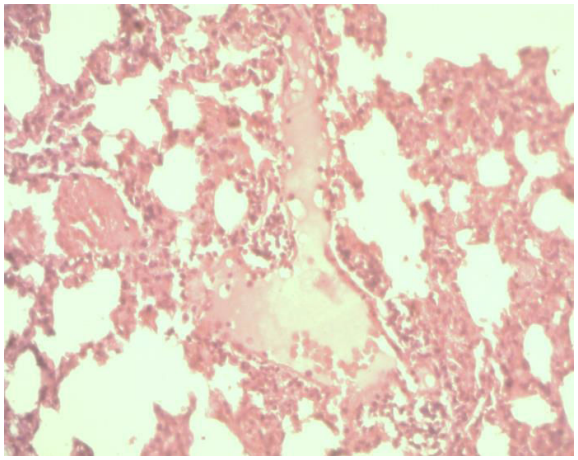

Figure 2: Experimental group. Lung tissue. Plasma proteins in the lumen of the vessel with perivascular circular cell infiltration HE 200x.

Figure 3: Experimental group. Lung tissue. The altered cell in the tissue of the organ. RG stain 1000x.

Liver: The structure of the organ in the control was in accordance with the norm. In the experimental group, parenchymal dystrophy and necrosis of hepatocytes were revealed. Moreover in the portal tracts-pronounced edema with circular cell infiltration, in the vessels-ablation of blood plasma from uniform elements, with aggregation of erythrocytes was detected. These results indicate a violation of the rheological properties of blood (Figure 4).

It is necessary to pay attention to the reaction of Kupffer cells of the liver, which in the form of nodules are grouped in the region of hepatocytes with vesicle-like pale nuclei and diffuse, fuzzy cytoplasm. Visible nodules representing structureless masses are seen around the hepatocytes.

Along with damage to the liver cells proliferative processes were detected with the formation of nodules (Figure 5), grouped in separate areas around the dead hepatocytes.

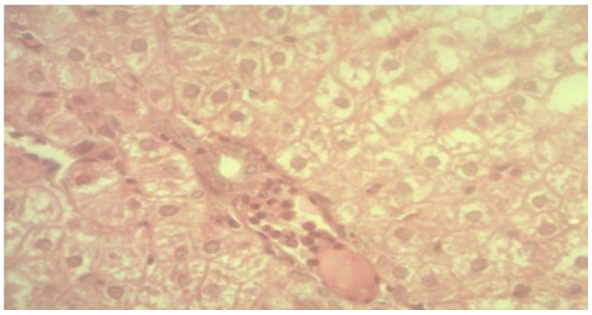

Figure 4: Experimental group. The liver is in the triad region. Blood plasma separated from formed elements, hepatocyte dystrophy. HE 400x.

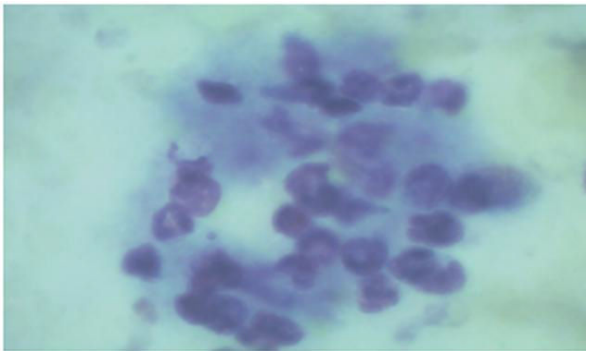

Figure 5: Experimental group. Liver. Productive Nodules. RG staing 100x.

In a cytological study, a cone-shaped cell with a large nucleus and dense chromatin condensation attached to the dead hepatocyte was identified (Figure 6). Morphologically, this cell differs from the structural elements of the liver, which complicates its identification.

Figure 6: Experimental group. Cytology of liver tissue. RG stain 1000x.

Kidney: In control, as well as in other organs, the typical morphology of the organ is preserved. With the preservation of the organ structure, a pronounced edema of the stroma was observed in the experiment; vacuoles filled with cytoplasmic fluid appeared in the cytoplasm of the tubular epithelium. Vasoconstriction with plasma separating from the formed elements and aggregation of erythrocytes was observed, which indicates a violation of the rheological properties of blood (Figure 7).

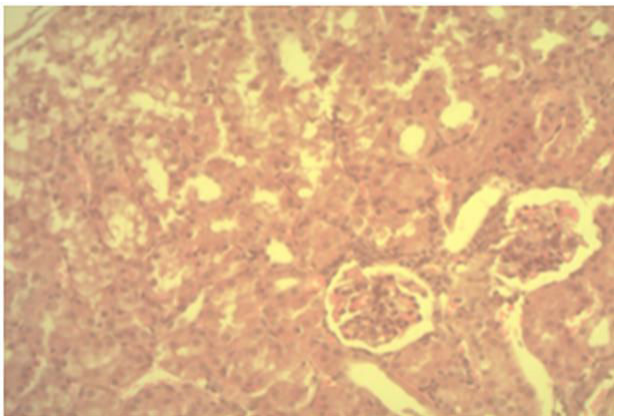

Figure 7: Experimental group. Kidney. Edema, vasoconstriction, dystrophy of tubular epithelium. HE 200x.

Dystrophy of the tubular cells with round-cell infiltration around them, as well as the proliferation of cells with the formation of nodules was observed (Figure 8).

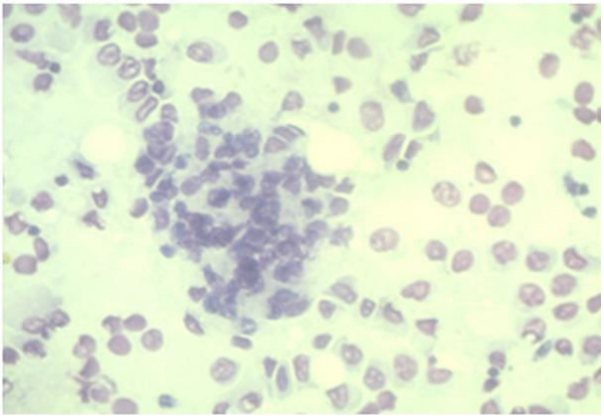

Figure 8: Experimental group. Kidney-nodules from round cells. RG stain 400x.

Discussion

The study of the pathogenesis of *trichomoniasis* has certain complications because of the difficulty of selecting an experimental model on animals [9]. Many types of laboratory animals were tested as a model of *T. vaginalis* infection, but most of them did not meet the requirements for *in vivo* studies due to inability to maintain genital infection, asymptomatic disease, weak immune response, limitations in the content and treatment of certain species [10]. The lack of an adequate model severely limits the ability to conduct standardized, controlled studies on transmission, pathogenesis, immune response,

treatment and the development of a vaccine for *trichomoniasis*. Therefore, in this study, it was decided to investigate the effect of *T. vaginalis* on the internal organs of guinea pigs in the natural pathway of infection.

Summarizing the results of the study, one can ascertain the high activity of *T. vaginalis* in relation to internal organs. The damaging effect of *T. vaginalis* on the tissues and vessels of the parenchymal organs may be due to the high affinity of *T. vaginalis* due to the presence of binding sites with the carbohydrate content (glycoproteins, and mannose or mannose-like residues) on the outer surface of the protozoa [11-13]. The invasive properties of *trichomonas*, their ability to penetrate into tissues [14] are described in the literature, which indicates a systemic defeat of the organism by protozoa. This ability of *trichomonas* with possible spread through blood and lymph was described in the fundamental studies of researchers of the 1960s and 1970s [15-17]. Penetration of *trichomonas* through tissues is possible due to the release of spreading factors by pathogen: hyaluronidase enzymes, neuraminidase, cellular cleavage factor and proteases affecting the cell membrane spectra [18-20]. Violation of the rheological properties of blood, which is observed in the experiment, can be related to the secretion of the hemolytic factor by *T. vaginalis*-a protein responsible for the damage of red blood cells without the preliminary contact of cells; damaging effect is also possible as a result of the obligatory contact of two cells-trichomonad and erythrocyte [21].

Vaginal mucosa is a poor nutrient medium for the parasite. And since *Trichomonas* is not able to synthesize some lipids, it is likely that red blood cells can be the primary source of fatty acids necessary for the parasite. In addition to lipids, iron is the most important product for *T. vaginalis* and can also be consumed through the lysis of red blood cells. *Trichomonas* phagocytizes erythrocytes, in connection with which the hemolytic activity of the parasite correlates with virulence [10].

The invasive properties of pathogens are promoted by the pronounced polymorphism of *trichomonas*, the presence of several adaptive structures for movement, causing translational and rotational movement, as well as movement around the longitudinal axis of the body, which allows the pathogen to move in liquid media such as blood and lymph. The penetration of *T. vaginalis* into the peripheral channel is facilitated by the pronounced immunosuppressive action of the pathogen on the body cells, as well as the decrease in the immunological reactivity of the organism due to background disease. [17].

In the experiment with chronic infection, the development of two alternating processes was revealed: damage and repair (proliferation). Repair is manifested by the proliferation of cells in the form of circular cell infiltration, mainly perivascular.

As a result, the modeling of chronic urogenital *trichomonas* infection in guinea pigs has shown the effectiveness of this method of modeling *trichomoniasis* for experimental investigation of *trichomonas* infection in the natural pathway of infection. Histomorphological examination of tissues in this experiment let us to reveal the destructive effect of *trichomonas* on the internal organs of animals, described earlier in the literature, which makes it possible to use this model for the study of *trichomoniasis*. The study of the problem of UGT is relevant, as there are still many white spots that are unknown in the behavior of the "chameleon" *Trichomonas vaginalis*.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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