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# OPTIMIZATION OF *LAMBLIASIS* MICROSCOPIC DIAGNOSTICS BY THE METHOD OF POLARIZED FLUORESCENCE FOR PATIENTS WITH *ROSACEA* AND *URTICARIAL*

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**Abstract**

**Introduction:** There is little information about diagnosis of concurrent lambliasis in patients with rosacea and urticaria. We used method of polarized fluorescence to diagnose liambliasis, taking into account belonging of macromolecular structures of unicellular parasites Giardia lamblia to the optically active substances with the properties of liquid crystals.

**Material and Methods:** Lambliasis was diagnosed on the basis of feces parasitological research and duodenal contents by methods of light and optic microscopy and polarized fluorescence in 105 patients with rosacea and urticaria. Research results were processed by the method of variation statistics in the Statgraf program by using Student's criterion.

**Results:** Search results of lamblia in patients with rosacea and urticaria depended on the conditions of its holding, patients' preparation and from the previously received basic therapy if it consisted absorbents. Due to the fact that the fluorescence polarization as a physical method does not require the use of any generally toxic, dye- fluorochromes, qualitative cyto fluorescent analysis of lamblia in greeting microdrugs enables to distinguish vegetative forms of cysts.

**Conclussions:** Polarized fluorescence method allows optimize the microscopic diagnosis of lambliasis, increasing its sensitivity. Previous preparation for the laboratory examination of Giardia lamblia is needed for the best exposure of vermin for patients with rosacea and urticaria.

**Key words:** rosacea; urticarial; lamblia; polarized fluorescence

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## Introduction

Studying the pathogenesis of chronic skin diseases, new methods development of diagnostics and treatment of those dermatoses are still one of the priorities in dermatology [1]. The relevance of this problem is caused by increasing proportion of dermatoses severe clinical forms that are resistant to traditional therapies and involving in the pathogenesis of various infectious agents, including some parasites [2-3].

Our previous studies [4] found aggravating effect of some parasites, such as lamblia, on the course of certain skin diseases – rosacea, urticaria. At the same time, the inclusion of complex anti-parasitic drug therapy of „Ornidazole" increases the treatment effectiveness referred to above dermatosis, resulting in reducing the severity of clinical symptoms, rapid disappearance of rash and itching elements. Complete clinical recovery is possible to achieve in 88.6% of patients versus 18.9% in the application of basic therapy [5].

According to our data, the concomitant lambliasis occurs in 67.7% of patients with urticaria and 52.2% of patients with rosacea. The lack of basic therapy efficiency of mentioned above dermatoses, leads to purposeful data examination of patients with presence of concomitant parasitosis.

## Material and Methods

Under the supervision there were 105 patients with rosacea and urticaria on the background of lambliased invasion (lambliasis regarded as concomitant disease), aged from 16 till 69 years, receiving inpatient and outpatient treatment in Ternopil regional Dermatovenerologic Dispensary. The diagnosis of rosacea and urticaria was established clinically.

Lambliasis was diagnosed on the basis of feces parasitological research and duodenal contents by methods of light and optic microscopy and polarized fluorescence with using a fluorescent microscope LYUMAM 8-P 3m with photometric nozzle FMEL-1 for spectral analysis.

Research results were processed by the method of variation statistics in the Statgraf program by using Student's criterion.

**Results**

Search results of lamblia in patients with rosacea and urticaria depended on the conditions of its holding, patients' preparation and from the previously received basic therapy if it consisted absorbents. Preliminary patients preparation during 5-7 days with the use of spasmolitic „No-spa" and bile-expelling drug „Alohol" helped to increase the exposure of cystic forms of lamblias to 87.5% cases compared with 29.8% in patients receiving absorbents.

As you know, macromolecular structures of unicellular parasites Giardia lamblia belong to the optically active substances with the properties of liquid crystals [6]. Membrane lipids, nucleic acids of nuclei of living cells are inherent the ability to induce elliptic light polarization, which shows up the dependency upon a wave-length phenomenon of circular dichroism [7-9]. Due to the fact that the fluorescence polarization as a physical method does not require the use of any generally toxic, dye- fluorochromes, qualitative cyto fluorescent analysis of lamblia in greeting microdrugs enables to distinguish vegetative forms of cysts (Fig. 1, 2).

The most typical distinction of lamblia fluorescence in polarized light should be considered extremely high level of luminescence intensity of cell nuclei parasite. For example, if the glow intensity of nuclear structures of leukocytes which corresponds to the level of cellular bioenergy DNA and RNA, conventionally taken as 100%, so for similar lamblia intracellular structures this index is founded out as higher [10].

Exactly this fact gets the sign of specificity: even with full blocking primary of primary light stream by polarization filters (polarizer and analyzer) on a background of faint fluorescence by other micro objects, for example, leukocytes, lamblia cells shown especially brightly. The specified diagnostic phenomenon is peculiar to the individuals of exciter at any stage of its life cycle.

In polarized light lamblia are glowing bright golden-yellow and greenish-red light (Fig. 1, 2).

Contrary to the conventional rule about the reliability of parasites detection only in freshly („warm") material, we were able to detect lamblia (both vegetative forms and cysts) in duodenal contents, which is stored in the refrigerator in the syringe, within 72 hours after taking the bile - using both traditional light and optic microscopy and polarized-fluorescence method (Fig. 3).

Another characteristic distinction cyto luminescent parasitological analysis is the dependence of the intensity of Giardia lamblia luminescence in polarized light from the place of parasite staying at the time of diagnostic testing. Thus, the highest intensity of luminescence was in parasites from portions of bile A, taken by us at 100%, while it was lowest in portions of bile C - (71,6 ± 5,9)%.

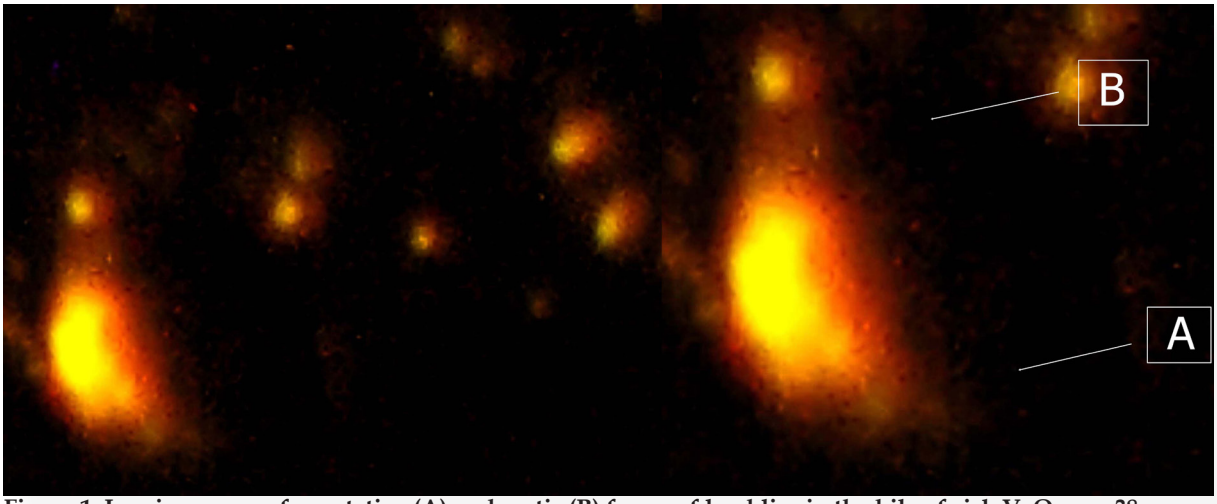


**Figure 1. Luminescence of vegetative (A) and cystic (B) forms of lamblias in the bile of sick V. O., age 38. Diagnosis: rosacea, papulo-pustular form, concomitant lambliasis. Microscope LYUMAM P 8. Ok × 10 lens × 9.**

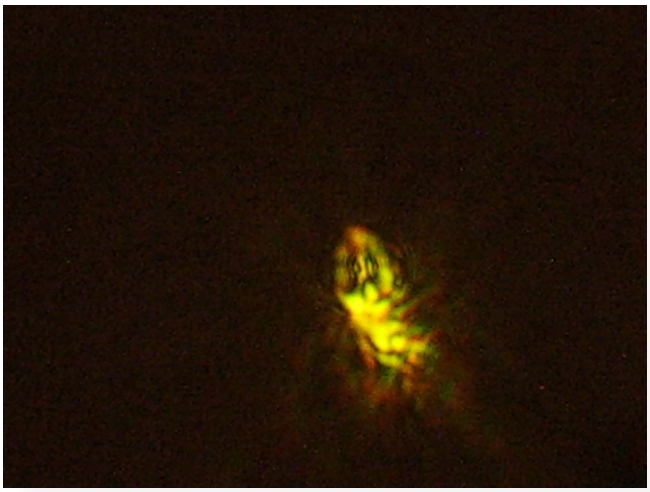

**Figure 2. Polarized fluorescence lamblia in bile (portion A) patient V. O., age 38. (Diagnosis: Chronic urticaria associated lambliasis). Microscope LYUMAM P 8. Ok × 10 lens × 9.**

Thus, the highest intensity of luminescence was in parasites from portions of bile A, taken by us at 100%, while it was lowest in portions of bile C - (71,6 ± 5,9)%. Intermediate level of fluorescence intensity, namely (82,7 ± 5,1)%, took place at research portion of bile B ($p < 0.05$). The resulted distributing of levels of bioenergetics cell parasites from different portions of bile, in our opinion, is a reflection of reactions from the side of parasites on changing of terms in the microenvironment, namely quantitative composition of bile components (Fig. 3).

In bile research by lamblia polarized fluorescence method it was found out (90,3±3,7) % of patients, whereas the traditional method of light-optical microscopy - only (77,1±5,2) % ($p > 0,05$). In addition, lamblia cysts in separate portions of bile in addition to the results obtained using traditional microscopy were found in 10 patients by polarization-fluorescent method.

Informative were the results of spectral analysis of polarized fluorescence lamblia obtained from different portions of bile in diagnostic duodenal intubations. In all samples of bile were observed two characteristic peaks, namely in the area of 600 nm and 750 nm, that corresponds to emission spectra of DNA and RNA (Fig. 4).

Thus, if the spectral peak areas of RNA differ in terms of intensity, so oscillation wavelengths become character for a similar range of DNA sites, that are evidence of landslides fluctuations bioenergy cells associated with processes of adaptation to altered conditions of parasite survival and parasitism.

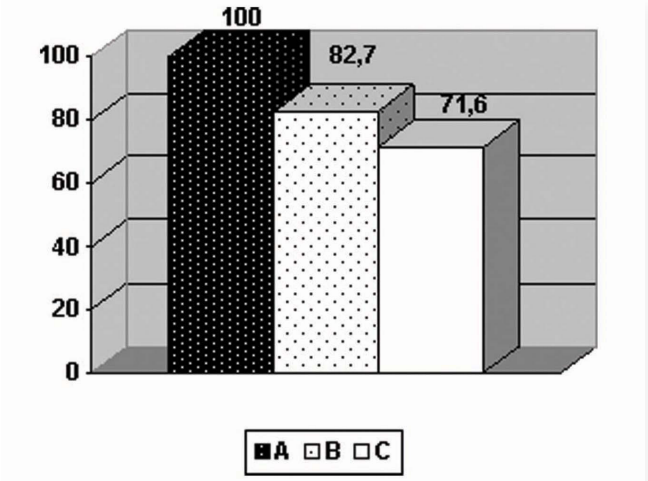


**Figure 3. Dependence of Giardia lamblia fluorescence on their localization in portions of bile.**

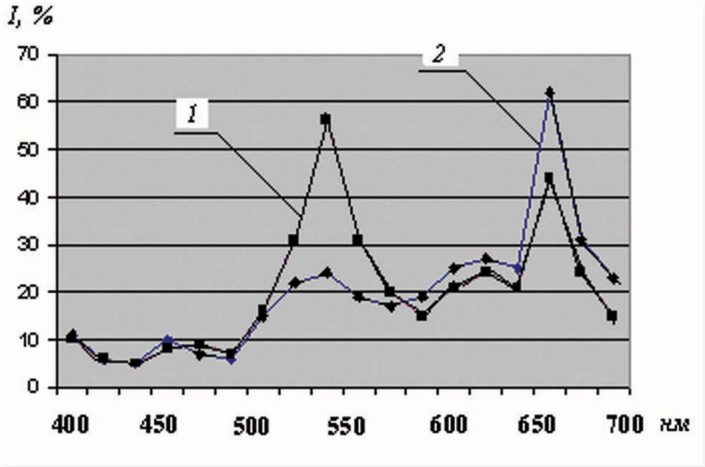


**Figure 4. The spectral composition of the polarized fluorescence vegetative forms (1) and lamblia cysts (2) with the bile of patient with urticaria on the background of lambliais. LUMAM-P 8 3m: lens × 9; FMEL-1, 1.5 mm probe.**

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**Conclusions**

1. The basis of laboratory diagnostics of concomitant lambliais in patients with urticaria and rosacea remains a classic method of faeces scope of Parasites (95.2%).
2. Polarized fluorescence method allows optimize the microscopic diagnosis of lambliasis, increasing its sensitivity.
3. Spectral analysis of native lamblia radiation in polarized light provides methodical possibilities of differentiation vegetative and cystic forms of parasites by registration and evaluating bioenergy cell parasites in accordance with the terms of their experiencing in patients' body with urticaria and rosacea, and therefore contains diagnostic and prognostic information.
4. Previous preparation for the laboratory examination with the use of antispasmodics and bile-expelling drugs and avoidance of taking absorbents is needed for the best exposure of vermin for patients with rosacea and urticaria.

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