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APPLICATION OF THE MICROSCOPIC METHOD IN CUTANEOUS LEISHMANIA DIAGNOSIS

ZASTOSOWANIE METODY MIKROSKOPOWEJ W DIAGNOSTYCE SKÓRNEJ LEISZMANIOZY

Mohammed Wael Daboul

Laboratory Medicine Specialist, Damascus, Syrian Arab Republic
***Corresponding author**:* Dr. Mohammed Wael Daboul *idaboul@scs-net.org*

Our Dermatol Online. 2011; 2(4): 198-202 Date of submission: 10.07.2011 / acceptance: 07.09.2011
Conflicts of interest: None

Abstract
Introduction: Cutaneous leishmania is spreading fast.
This study aims at developing the microscopic method to achieve a full detection of all positive cases of leishmania.
Methods: 50 human cases have been studied by applying microscopic smears stained with Wright stain. Microscopic photos were taken for the presumed unfamiliar figures.
Results: Mononuclear cells with tails are present at a rate of (98%). They are associated with Leishman Donovan (LD) bodies in 50% of the cases. The polygonal figures and the spherical forms are present at the same rate (60%) and are associated with LD bodies in 24% of the cases. The small promastigote like forms are seen at a rate of (76%) and are associated with LD bodies in 26% of the cases. The giant promastigotes like forms are present in (80% of the cases) and are associated with LD bodies in 28% of the cases. Candle flame forms are present in (40% of the cases) and are associated with the LD bodies in 21% of the cases.
Discussion: It is applicable to use those discovered figures in diagnosing cutaneous leishmania.

Streszczenie
Wprowadzenie: Skórne postacie leiszmanii rozprzestrzeniają się szybko.
Badanie to ma na celu opracowanie takiej metody mikroskopowej, aby osiągnąć pełne wykrywanie wszystkich pozytywnych przypadków leiszmanii.
Metody: 50 ludzkich przypadków badano stosując mikroskopijne rozmazy barwione metodą Wrighta. Mikroskopowe zdjęcia zostały wykonane dla przypuszczalnych nowych danych.
Wyniki: Komórki jednojądrzaste z ogonami są obecne w 98%. Są one związane z ciałkami Leishmania Donovani (LD) w 50% przypadków. Wielokątne figury i kuliste formy były obecne w tym samym odsetku (60%) oraz były związane z ciałkami LD w 24% przypadków. Małe, promastigotyczno podobne formy są dostrzeżone w 76% i są związane z ciałkami LD w 26% przypadków. Gigantyczne, promastigotyczno podobne formy są obecne w 80% przypadków i są związane z ciałkami LD w 28% przypadków. Formy typu płomień świecy są obecne w 40% przypadków i są związane z ciałkami LD w 21% przypadków.
Dyskusja: Mikroskopia jest odpowiednia do zastosowania, z wykorzystaniem tych nowych, odkrytych danych w diagnostyce skórnej leiszmanii.

Key words: cutaneous leishmania; cells; flagellates
Słowa klucze: skórna leiszmania; komórki; wiciowce

Introduction

The Mediterranean area is considered one of the most common places that cutaneous leishmania inhabits [1-3]. Cutaneous leishmania was classified according to its spreading area into two classes:
Old world leishmaniasis: which includes Afghanistan, India, Pakistan and the Middle East countries including Syria and south of Turkey.
New world leishmaniasis: They inhabit South American countries [1,3].
More than two million new cases of leishmania are discovered every year [4]. In addition to that, more than 350 million individuals are prone to leishmania infection [5]. It is highly important to find a suitable, manageable with high sensitivity method for leishmania diagnosis in order to identify those new discovered cases and provide the best available treatment.
The direct microscopic method for cutaneous leishmania secretion is considered one of the simplest methods used to detect any possible infection. This method is considered as a reference method for many sources [5-7]. The microscopic method detects the presence of (LD) bodies Leishman Donovan bodies within the macrophages and multi nucleated giant cells or

Langerhan's cells in the skin lesion [8]. (LD) bodies are nothing but the amastigote forms. They appear inside the cytoplasm of those mentioned cells in a spherical or a spindle like shape containing a nucleus and having a dimension between 2-4 microns in diameter. The principle of the microscopic method depends on the appearance of these LD bodies in the intracellular space for those cells. The presence of those LD bodies will confirm the diagnosis. While absence of those bodies may exclude the diagnosis with the infection. This diagnostic method has been discovered by both the scientists (Leishman- Donovan) more than a hundred years ago. Since that discovery, no developmental work or modification has ever been made on the procedure. Though it is a simple one, it is considered less sensitive and can at best, detect about 60-70% of the infected cases. That means that over 30% of cutaneous leishmania infected cases cannot be detected by this microscopic method and are considered as falls negative.

The purpose of this study is to search the possibility of developing and improving this traditional microscopic procedure so that we are able to detect the additional 30% of the undiagnosed cases where the LD bodies do not appear in the microscopic slides.

Procedures and Methods

50 cases have been studied, 43 males and 7 females who attended the laboratory between April 2006 and July 2008. Samples were obtained from the secretions of what was previously diagnosed by consultant dermatologists as cutaneous leishmania infection. Samples were collected as follows:

1.Slit skin smears: The area was cleaned with 0.9% saline or 70% alcohol. We squeezed the edge of the lesion between thumb and forefinger and made shallow 1 mm slits through skin to dermis with a scalpel then scraped the edges to make slide smears, then made smears as thin as possible, air dried, fixed in methanol, and stained with wright stain.

2.Dermal scrapings: We Obtained 2-4 scrapings from different areas of the lesion. We scraped dermis along the necrotic lip with a scalpel, obtaining as much tissue as possible, then making thin smears on slide, air dried, fixed in methanol, and stained with Wright stain.

In the microscopic study, we looked for the unfamiliar cytomorphologies in the smears, when they repeatedly appear in the different infected lesions, that could be of a diagnostic significance. Microscopic photos for confirmation and documentation were produced for those suggested potential figures featured in each related case. A classification was later made for those different figures and cytomorphologies according to their percentage of appearance among the different smears in a related table.

Results

The attached table indicates the count and the appearance of such unfamiliar figures and their percentage of appearance in the smears. The table nr 1 indicates the association of those figures with the LD bodies (the amastigote forms) in the same smear.

From the table above, it is clear that some new, distinct, cytomorphological figures have been discovered. Those new figures were associated with the infected lesions in more than 50% of the cases. The discovered figures are much different in their morphology than the well-known blood components and the skin tissues. That includes cells like fibroblasts, which appear in normal cases. Here is a brief description for those figures while a detailed description is found in the study (The pathological features of cutaneous leishmania) [9].

Mononuclear cells with tail: The percentage of the appearance of those cells in the smears as noticed from the table above in leishmania infection is as high as (98%) figure 1. The cytoplasmic tail is elongated in some cases up to more than 20 microns in diameter (Fig. 2). Both figures 1,2 show the different cytomorphologic manifestations of those cells. It is important to emphasize the amastigote appearance in the same photos as a confirmatory clue. That proves the possibility of using those new discovered figures to elevate the sensitivity and the specificity of the traditional microscopic procedure in detecting cutaneous leishmania.

Polygons multiformies: Those morphologies are 3-8 microns in diameter. They appear with different sides looking in a way like the neutrophile lobe (Fig. 3). The association of such figures with the amastigote form (LD bodies) is in about 24% of the cases.

Spherical forms: They are spherical in shape, very dense, having no surrounding cytoplasm. The dimension of such forms is between 2-8 microns (Fig. 4). They appear in 60% of the studied cases of leishmania. They are associated with the LD bodies in 24% of the cases.

The big flagellates: They look like the small flagellates in shape but their size is huge. Theycan reach 20-30 microns in diameter (Fig. 5). They appeared in 80% of the studied cases and were associated with LD bodies in 28% of the cases studied.

The candle flame forms: They are formed of chromatin mass with a tiny tail protruding out. Their dimension is 2-4 microns (Fig. 6). They show up in the smears in 40% of the studied cases. They are associated with the LD bodies in 22% of the cases.

The small flagellates: Those small flagellates look very much in shape like the promastigote form of the leishmania (figure 7 shows the different morphologies that this flagellate microscopically appears in the smears). Small flagellates appear in 76% of the infected cases and are associated with amastigote forms in 26% of the cases.

Total count of the cases	Mononuclear cells with tail	Multi formed polygons	Spherical forms measure 2-7 microns	Small promastigote like forms <12 microns	Large promastigote like forms>14 microns	Candle flame forms
50	49	30	30	38	40	20
Percentage	98%	60%	60%	76%	80%	40%
Association with LD bodies	25/50	12/50	12/50	13/50	14/50	11/50
Percentage	50%	24%	24%	26%	28%	22%

Table 1. The count and the appearance of unfamiliar figures and their percentage of appearance in the smears. The association of those figures with the LD bodies (the amastigote forms) in the same smear

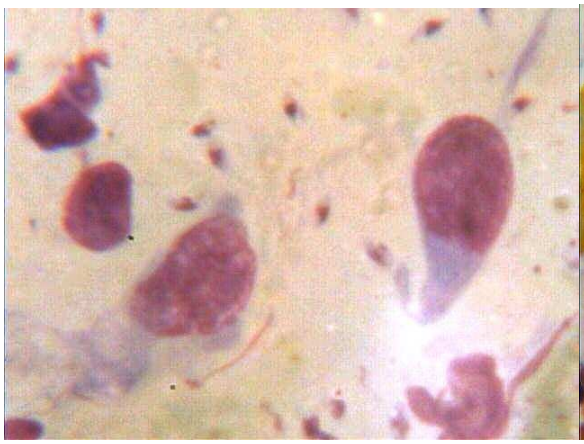

Figure 1. Mononuclear cells with tail

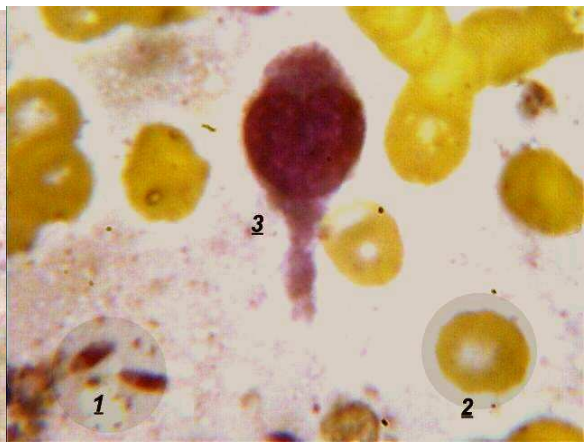

Figure 2. Mononuclear cells with tail

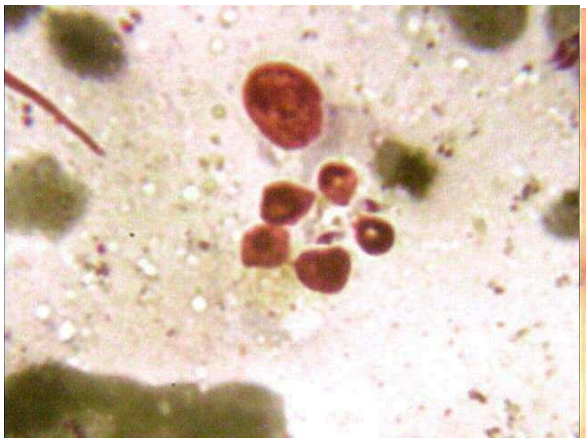

Figure 3. Polygons multiformies

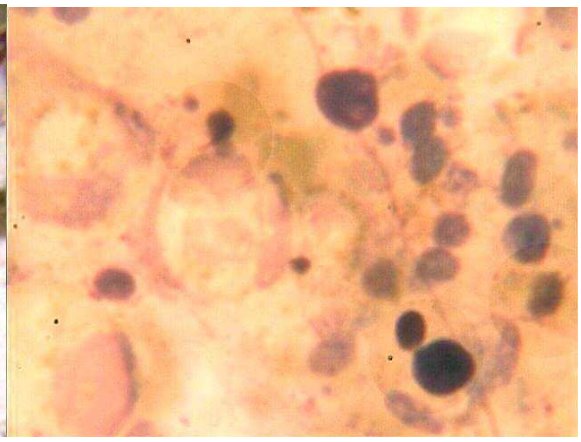

Figure 4. Spherical forms

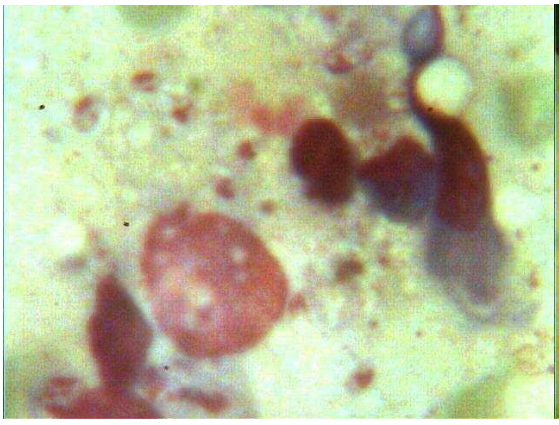

Figure 5. The big flagellates

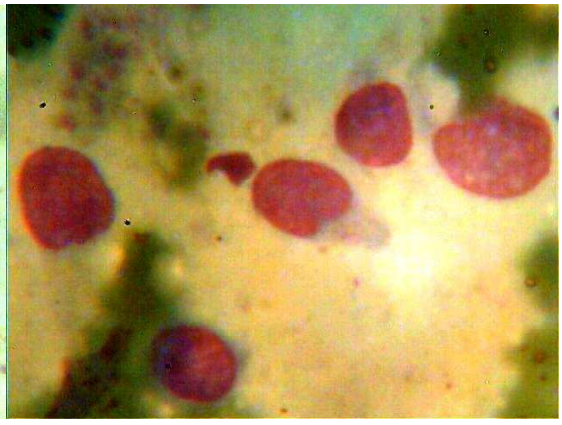

Figure 6. The candle flame forms

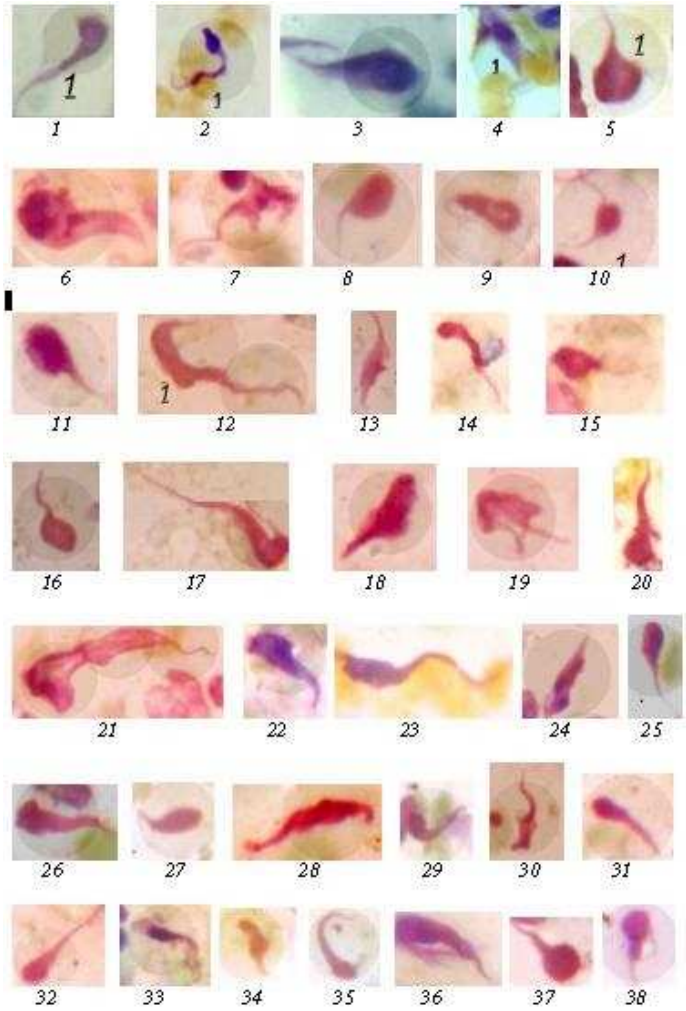

Figure 5. The small flagellates. Different morphologies

Discussion

The organisms with their different cytomorphologies presented above represent a distinguishing mark that could be utilized in diagnosing cutaneous leishmania, especially in cases where the (LD bodies) are totally absent from the microscopic smear. When applying the traditional microscopic reference method that most laboratories rely on, they only detect the LD bodies in the maximum of 70% of the infected cases. It is not yet possible to detect the disease in the other 30% of the positive cases when the LD bodies disappear from the smear. According to many studies [1-6], it seems that at a later stage of the disease process of cutaneous leishmania, the disappearance of such LD bodies together with the macrophages that are infected with, is a fact. The disappearance of such LD bodies then, cannot be explained by the decrease in the sensitivity of the microscopic procedure. It is more or less, due to a real disappearance of such amastigote form during the disease progress. Our study found that in many disease cases of leishmania, even at that stage of the disease process with the (LD bodies) disappearance, the clinical signs continue to show a sharp inflammatory reaction with a deep ulceration, and for a long period still to go [9].

The appearance of such discovered forms of organisms (the mentioned above) in the smear, and their association at different rate with LD bodies in the same smear, is a proof that those cytomorphologic figures are evidence for cutaneous leishmania. And thus, they could be utilized as a diagnostic material for detecting cutaneous leishmania especially in cases when (LD bodies) are not found in the smear . Regardless of those organisms origin, it is obvious that such organisms do not match in their morphology any of the well known cellular components or organelles that are present in the blood or the skin tissues. And because it might still be possible to find these organisms associated with other types of disease, and until we are sure that they are specific for cutaneous leishmania, it is suggested for cutaneous leishmania diagnosis, to consider, not to depend on the appearance of only one type of those organisms in the smear. When LD bodies are absent, it is more applicable to confirm the presence of at least three different types of those organisms in the same smear. In case (LD bodies) are found in the smear, no need then for looking for those organisms.

Conclusion

This study has added a good bonus for the traditional microscopic method in diagnosing cutaneous leishmania. The old method that was used to detect only the presence of LD bodies in the smear is a hundred years old method. It very much lacks sensitivity. And

with the fast spread of the disease in third world countries and with limited resources, it is hoped for an easy, non-costy, fast and reliable method for detecting cutaneous leishmania. The microscopic method when adding those discovered figures and modifications to it, is capable enough to do the job with a 100% sensitivity achievement and almost equal specificity.

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