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Research Article

The Histopathological Criteria of Cutaneous *Leishmania*

Mohammed Wael Daboul*

Laboratory Medicine Department, Daboul Medical Laboratory, Syrian Arab Republic

***Corresponding Author:** Mohammed Wael Daboul, Laboratory Medicine Department, Daboul Medical Laboratory, Syrian Arab Republic.

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Abstract

Background: With the new discovery of the promastigote form and the fiber producing promastigote form in the infected lesion in human with cutaneous *Leishmania* [1], it became important to reconstruct the pathological features of the disease process considering the new findings.

Methods: 42 cases of cutaneous *Leishmania* referred to the laboratory in Damascus between January and October 2022 for microscopic diagnosis and simultaneously rearranged in series according to the approximate time of the lesion appearance in the patient skin. A reconstruction model of the pathology of the disease sequence was established.

Results: Lymphocytes (with tails) are microscopically present in 41 out of the 42 (98%) of the cases referred. Amastigote form microscopically is present in 20 cases out of 42 (48%). Promastigote and fiber forming promastigote microscopically are present in 32 out of the 42 cases (76%). Candle flame form appears in 21 out of 42 in (50%) of the cases and the spherical and polygon forms appear in 25 out of the 42 cases referred at 59% rate.

Conclusion: Neutrophils have a role in eliminating the amastigotes released to the outer cellular fluid. Some subgroups of lymphocytes by converting into giant promastigote like forms have a role to play in the disease process by directing the process into fiber formation and hence controlling the causing factor (the different parasite forms present) and through that controlling or eliminating the disease.

Keywords: *Cutaneous Leishmania; Promastigote; Leishmaniasis; Neutrophils; Lymphocytes*

Introduction

Studies of leishmaniasis that were done before lack many of the pathological features of the disease process. Many pathological events are either unclearly elucidated or uncertain or overlooked without being fully explained. That made the whole pathology of the disease process somewhat ambiguous and unclear.

Cutaneous leishmaniasis presents as a skin ulcer at the site of the sand fly bite and generally heals spontaneously with a scar within three to six months. Histological examination of the cutaneous lesion sometimes reveals extensive subcutaneous lymphohistiocytic infiltrate with clusters of amastigote within histiocytes [2]. Other times it shows granulomatous inflammation with histiocytic infiltrate [3]. Other

histopathological studies indicate lymphocytes and plasma cells abundance in the wet ulcerative lesions. The overlying epidermis is hyperkeratotic and subsequently break down to form an ulcer covered with dried exudate, dead cells and a mixture of live and dead organisms [4]. Through the disease process, over the following months, there is a gradual decrease in the number of amastigotes and macrophages [4], leaving a sporotrichoid granuloma with less lymphocytes, epithelioid cells, multinucleated giant cells and scanty plasma cells in dry nodular lesions [5-7].

From the immunopathologic point of view: The extent of the disease manifestation is a combination of the parasite pathogenesis and the immune host response. The interactions between the parasite virulence factors and the cell-mediated immunity is not fully understood [8]. A study of post-kala-azar dermal Leishmaniasis declared that there is an abundance of CD4, which closely interacts with *Leishmania* antigen present [9]. Another study proposes the presence of three groups of antigenic determinants in the parasite: First group is the invasive/evasive determinants. They help the parasite to establish infection in the host. Second group is the parasite pathoantigenic determinants. The immune response against such determinants results in immunopathology causing the disease symptoms to appear. Third group is the vaccine determinants: When the immune system interacts with those determinants they lead to parasites elimination. A hypothetical model was constructed assuming that the disease virulence is due to interaction between the host's immune system and the *Leishmania* parasite determinants. Recent works revealed the existence of T-cell epitopes in *Leishmania* cytoplasmic molecules elucidating protective immunity [10].

Such studies mentioned above, assume the amastigote as the only form present in vertebrates and hence, it summarizes the whole process of the disease of cutaneous *Leishmania* accordingly. Our findings [1] reveal that beside the amastigote, other forms of the parasite are present and missed by those studies. These findings will add more light to the pathology of the disease process.

Additionally, the pathological features under the microscope have to be a reflection to the immunopathologic interaction between the parasite and the host.

Unfortunately going through the literature presented, we do not see such a reflection. In fact, all what is seen is a mix up of deficient pathological elements timely misarranged describing the whole set of the immunopathologic interaction during the disease course.

Purpose of the Study

The purpose of our study was to reconstruct the pathologic features with the aid of the images in the right sequence by adding the new pathological figures discovered and the new data presented [1] and locating them into their right position of the sequence on step by step basis. This will contribute to better understanding of the pathology of the disease.

Materials and Methods

42 cases referred to the laboratory in Damascus since January 2022 from consultant dermatologists as being clinically diagnosed as cutaneous leishmaniasis.

From each lesion couples of slides were obtained and stained with Wright stain.

A study was done to identify the presence of the lymphocytes with tails among the different samples and cytomorphologically to compare them with each others in the cases referred, and with typical forms of lymphocytes as controls.

Another study was done to identify and compare the different cytomorphologies of the parasite forms (amastigotes, promastigotes, candle flame shapes, polygon forms, spherical forms and fiber forming forms) found and to define their appearance percentage among the different samples.

The approximate time of the lesion appearance in patient skin at the time of the sample collection was investigated through patient inquiry.

With the time aid of the lesion appearance connected with the pathological features for all the 42 samples and simultaneously rearranged in series according to that approximate time and studied, a reconstruction model of the pathology of the disease sequence was established.

Confirmatory microscopic photos were taken for documentation.

Results

From table 1 we found that the lymphocytes (with tails) are present in 98% of the cases. 41 out of the 42 samples referred are having this type of lymphocytes microscopically showing up. The appearance of such lymphocytes among the different samples was not in the same rate. That was related to the disease stage when the sample was collected in the lab.

Total number of samples investigated	42
Total appearance of the lymphocytes (with tails)	41
Percentage of total appearance of the lymphocytes (with tails)	98%

Table 1: *Appearance of the lymphocytes (with tails).*

Table 2 reveals that in 20 cases out of 42 (48%) the amastigote microscopically exists while the existence of the promastigote and the fiber forming promastigote was equal. They are present in 32 out of the 42 cases (76%). The candle flame form appears in 21 out of 42, (50%) of the cases and the spherical and polygon forms appear in 25 out of the 42 cases referred at 59% rate.

Appearance of				
Amastigote	**Promastigote**	**Fiber forming Promastigote**	**Candle flame**	**Spherical and Polygons**
20/42	32/42	32/42	21/42	25/42
Percent 48%	76%	76%	50%	59%

Table 2: *Appearance of amastigotes, promastigotes, fiber forming promastigotes, (Candle flame) and (Polygons and spherical forms).*

The reconstruction model of the disease pathology

Phase one (The lag phase): The disease pathology starts when the infected sand fly during its meal transfers the parasite to the cutaneous area of the human skin. A (lag) incubation period extends from one day up to several months [11]. During which, the promastigote type of the parasite enters the subcutaneous area where it loses its flagella and is engulfed by the macrophage or the phagocyte which has an important role to play. The parasite assumes its multiplication within that cell while the macrophage starts the recognition process.

Phase two: The second phase after the lag phase is (the intracellular amastigote appearance phase) where inside the macrophage; the amastigote begins to show up multiplying and filling the cytoplasm of the phagocytes (Figure 1). Synergistically, the macrophage plays a role as an antigen presenting cell. It introduces the amastigote antigen in its surface to other cells of the immune system meaning the

B, and T lymphocytes and natural killer cells. Microscopically, the lymphocytes are noticed in large numbers surrounding the infected macrophages at this point (Figure 1). Once the parasite is recognized by such cells, the lymphocytes morphologically and microscopically seem to behave somewhat different than the usual lymphocytic immune reaction. The usual immune reaction in cell-mediated immune response is characterized by T cell activation in to helper, suppressor or cytotoxic T lymphocytes, which morphologically show if at all, mild to moderate enlargement changes in those T lymphocytes. The B-lymphocytes usually are increased in size converting last to plasma cells with the nucleus positioned at one pole of the cell. In both cases, morphologically, there are no cytoplasmic protrusion or extension changes seen erupting out of those lymphocytes. Here, may be due to its unique distinction as being the only parasite that infects the macrophage and utilizes it for its own multiplication, major morphologic changes take place on a time basis within the lymphocytes. A polarization of the nucleus and an elongation of the cytoplasm within the lymphocytes are recognized. Later, the nucleus becomes more condensed and the cytoplasm looks like a pale blue tail protruding out from the cell. Afterwards, the nucleus becomes even more condensed like a plain dark piece and it is impossible at that stage to microscopically differentiate any of its nucleus parts. Here the cytoplasmic tail becomes even thinner and more elongating while the whole size of what is believed to be a lymphocyte becomes overall smaller. Then, the cytoplasmic elements inside the lymphocyte tail disappear leaving it like a flagellum looking sheath while the condensed nucleus appears in the other pole. The new-formed structure looks in shape very much like a giant type of a promastigote parasite. From the photos taken for those changes, a schematic diagram of the lymphocytes conversion in a sequence was built up (Figure 1A). In this stage also, macrophages with the amastigotes multiplying inside are markedly noticed. Many of the macrophages become giant in size and some multinucleated cells appear with the amastigotes showing inside their cytoplasm. These phagocytes together with the different lymphocytes constitute the core microscopic features of the second stage (Figure 1). Interestingly enough, many of the macrophages seen also, tend to form elongating cytoplasmic tails protruding out. This phenomenon may be called (The tail phenomena) (Figure 2 and 10). In this stage neutrophils are occasionally seen.

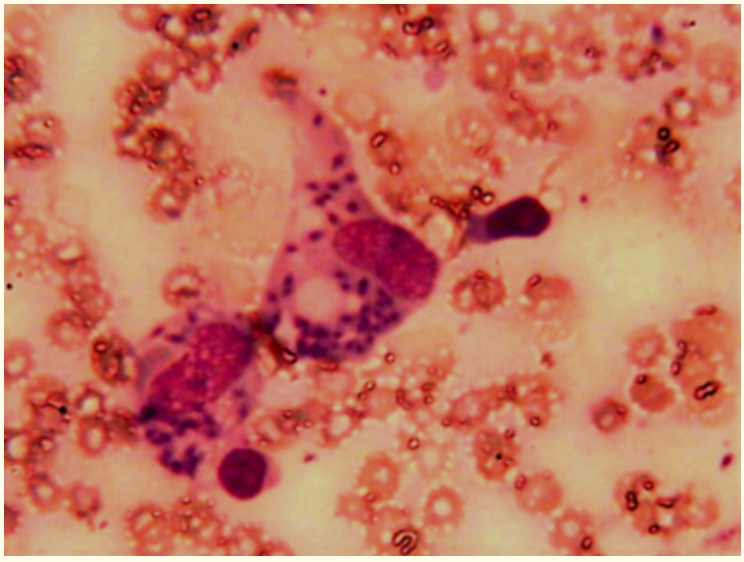

Figure 1: *X400.*

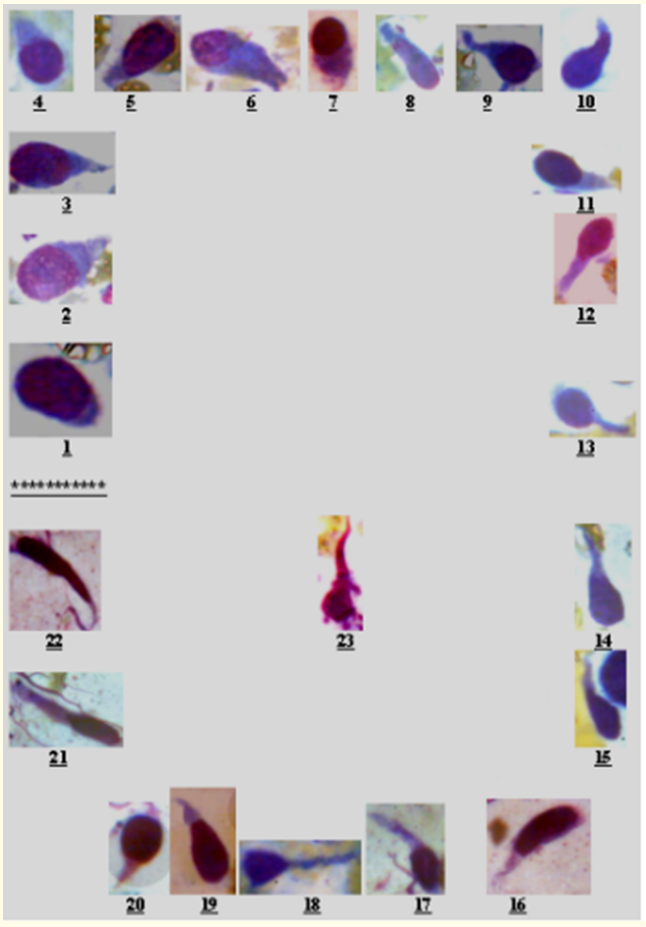

Figure 1A

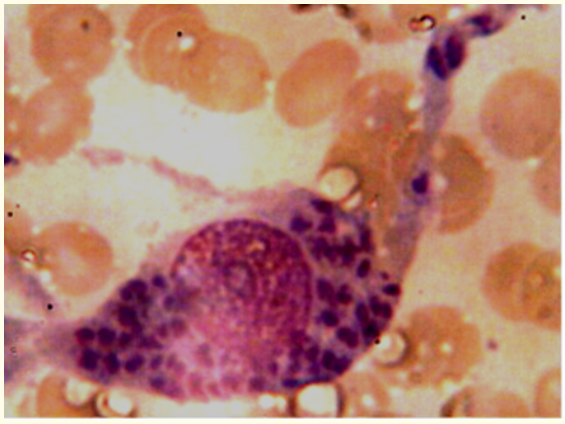

Figure 2: *X1000.*

Reaching the end of this stage, the macrophages membrane tears out and the amastigotes are released in a large number into the extra cellular fluid leaving other microscopic features as they are (Figure 3).

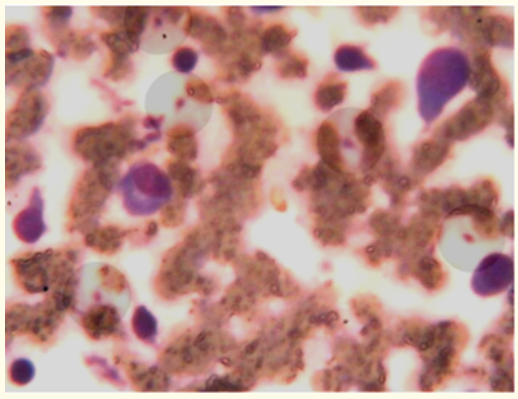

Figure 3: *X400.*

Phase three: (The promastigote appearance phase): In this phase, the already released amastigotes are present in the extra cellular fluid, which stimulate an acute phase inflammatory reaction characterized by neutrophils accumulation for phagocytosing and amastigote killing. Other figures present are decrease numbers of the lymphocytes, plasma cells, and what are known as the lymphocytes with tail, and the existence of what looks like giant promastigotes. We may also see a decrease number of macrophages with the amastigotes inside. At this stage, in the extra cellular fluid, the amastigotes appear at different shapes from round to oval and to spindle while the chromatin inside is either spreading or condensed and taking a polar position. Interestingly, candle flame figures develop out of those amastigotes, taking a spindle shape with a tail protruding out. At a later time on their development, some of those candle flame figures become polygons in shape reaching in maximum the RBCs size. They become difficult to differentiate from regular lymphocytes. Other growing parasites from the candle flame appear round in shape reaching also the RBC size. Some of those candle flame figures and the amastigotes too are phagocytosed by neutrophils. We may later see many destroyed neutrophils as a result of such parasitic neutrophilic interaction (Figure 4). From that time on, some of those candle flame figures that have the spindle shape and the tail become more enlarged taking the morphology of small promastigotes. Later on, the developing promastigotes are manifested at many different cytomorphologies (See figure 2A indicating the amastigote transformation into promastigote forms) making this phase by large, the promastigote appearance phase.

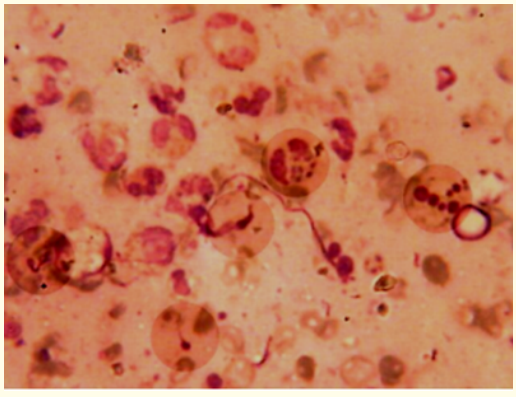

Figure 4: *X400*

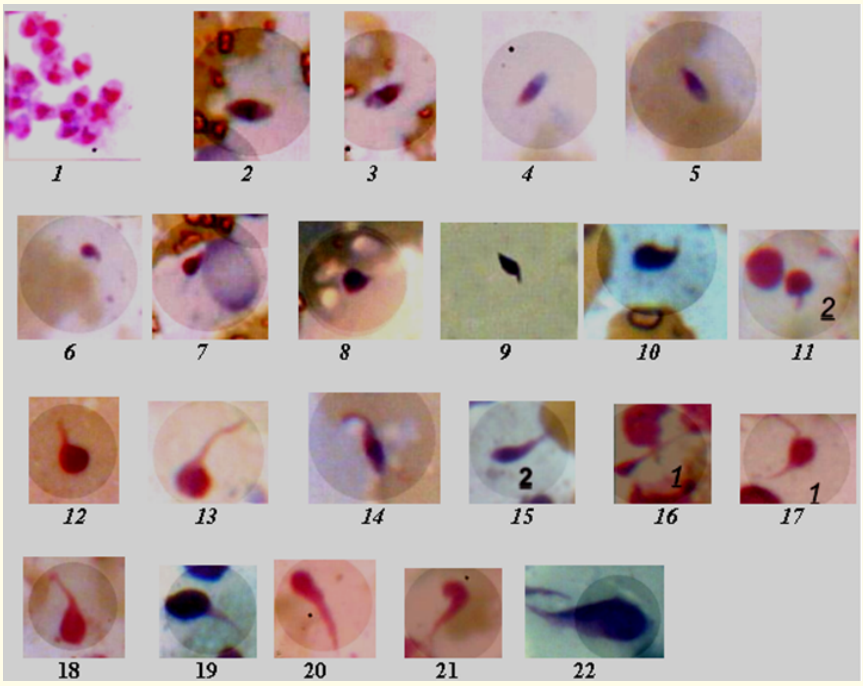

Figure 2A

Phase four: (The amastigote disappearance phase): At this phase the neutrophils have already consumed the extra cellular amastigotes, so the amastigotes are rarely seen in the extra cellular fluid while the macrophages and the multinucleated giant cells with the intracellular amastigotes tend to disappear, leaving the screen at the end of this phase with few neutrophils and plasma cells and less number of the different lymphocytes including the mononucleated cells with tail and the lymphocytes originated giant promastigotes and an increase number of the parasitic promastigotes and polygon figures (Figure 5).

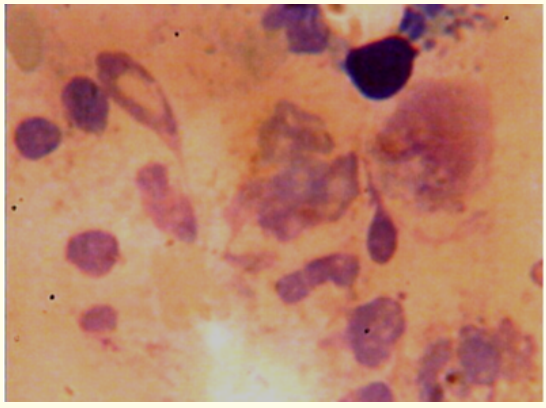

Figure 5: *X1000.*

Phase five (The fiber formation phase): In this phase the parasitic polygons together with the lymphocytic giant promastigotes and the parasitic promastigotes start gathering and establishing the core elements for fiber matrix (Figure 6). Those parasitic promastigotes begin producing thick fiber structure from their flagella area terminated with a thinning nucleus in a middle of a whole fiber (Figure 7). By this way, those formed parasitic promastigotes are involved in fiber formation and got embedded in a center of hairy like fiber elements, causing the disease to be controlled by controlling the causing factor which is the parasite and hence terminating the illness (Figure 3A illustrates the fiber formation on a step bases).

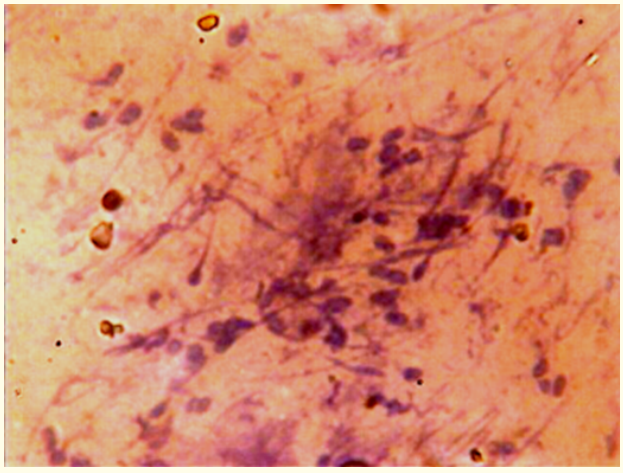

Figure 6: *X200.*

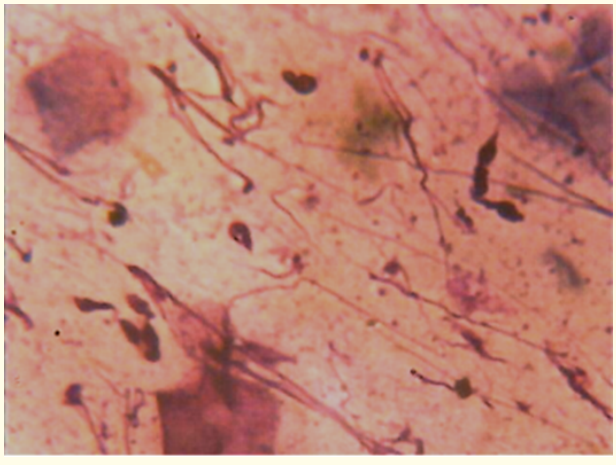

Figure 7: *X400.*

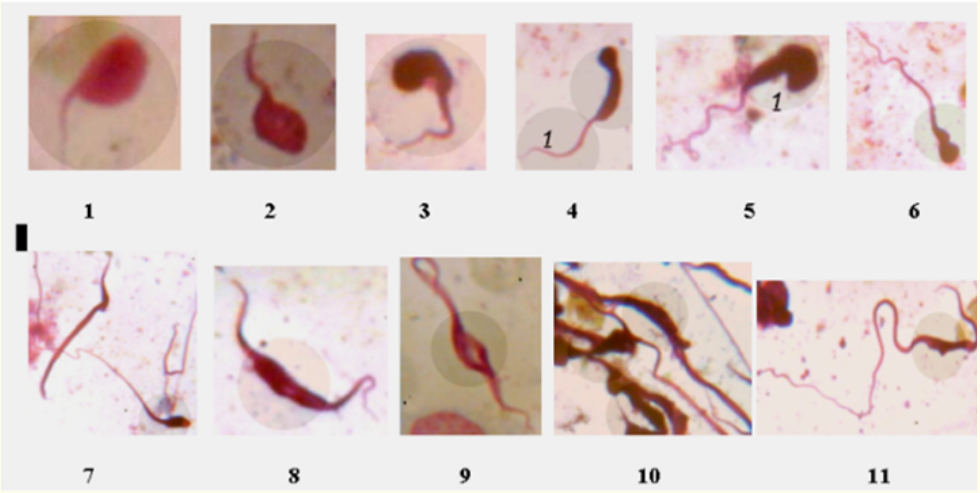

Figure 3A

Discussion

One may question the credibility of such lymphocytes (with tails) whether being originated from lymphocytes or from the parasite during its development sequence. We had this dilemma in mind. Going back through the disease process, the lymphocytes (with tails) appeared early on phase two and that is much earlier before the amastigote had transformed in to any promastigote form. The appearance of the polygon forms was declared in the middle of phase three. Those polygonal forms are the only forms, which may look like and may be morphologically confused with lymphocytes but they appeared at a later time within the disease process. Additionally, the only figures that are seen in the disease initiation process are those macrophages and the different lymphocytes. So, one can assume that these mononucleated cells (with tail) were developed from the lymphocytes.

Second, the question of the origin of the giant promastigotes from those lymphocytes (with tails): From the diagram attached (Figure 1A) and hundreds of photos taken (Figure 8 and 9), one can be sure that such conversion from lymphocytes (with tails) to giant promastigotes took place.

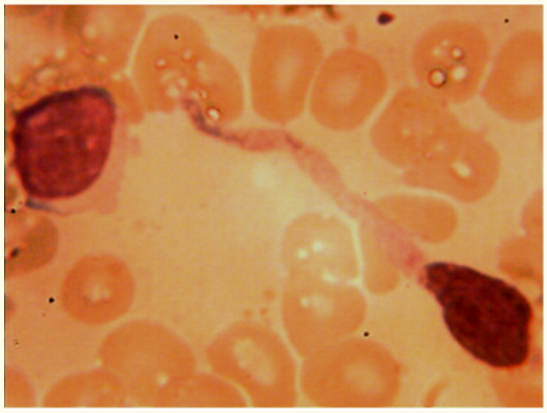

Figure 8: *X1000.*

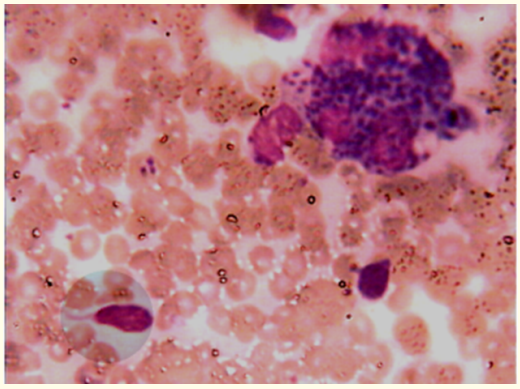

Figure 9: *X400.*

But the question is what role does this conversion play in the disease sequence?

Our hypothesis suggests that from the immunologic point of view, first, the morphology of those mononucleated cell and macrophages (with tails) is unique, that we are uncertain of any such transformation in morphology of either lymphocytes or macrophages like this occurred in any other condition. Second, the enlarged parasitic promastigote developed by the amastigote seems to be so large for the neutrophil to phagocytose. In such case, the alternative process for the disease control goes in the direction of the parasite surrounding and embalming by hairy fiber formation. Here, an immunologic interaction takes place among the three figures, the parasite in one hand and the macrophages in the second, and some subgroup of lymphocytes. This subgroup of lymphocytes becomes activated and is converted to lymphocytes (with tails). In their conversion process to a huge size promastigote, lymphocytes (with tails) had an obvious role to play. That role could be an educational one for the parasitic promastigote towards producing fiber like materials so that the disease tends to control itself.

Other studies totally overlooked the neutrophil roles in the disease process. In fact, neutrophils, are best specialized in phagocytosing process, while macrophages are considered less efficient than PMNs (polymorphonuclear neutrophils) at killing bacteria, and the mechanisms of killing are not as well understood [12]. Accordingly, one can assume that those PMNs must have a strong role in phagocytosing and destroying the amastigotes released after membrane rapture of the infected macrophages (Figure 10). Otherwise, it might not be possible to explain the amastigotes disappearance from the infected tissues after their release in that huge number into the extra cellular fluid.

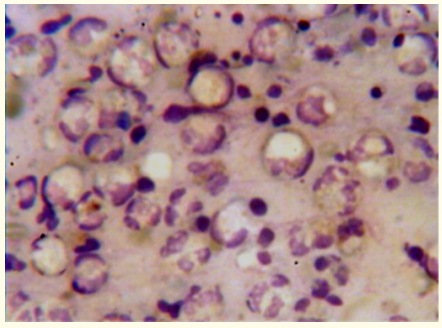

Figure 10: *X400.*

Conclusion

Researchers always relied on one fact that the only type of parasites present in the vertebrate including human being infected with *Leishmania* is the amastigote form and they formulated all their pathological and immunologic understanding of the disease process on such an incomplete fact [2-5].

The existence of such amastigote and promastigote forms together in the vertebrate may modify our understanding to the pathology, life cycle and hence our approach for controlling and treating the disease. Talking about the disease, not only the cutaneous type of leishmaniasis but the visceral leishmaniasis (VL) as well as the mucocutaneous leishmaniasis. All the three parasite disease types share similar parasitic morphology. And though, this study did not check the existence of the promastigotes in the other two types, the mucocutaneous and the visceral leishmaniasis due to the limitation of resources, the similarities in morphology among those different parasites makes it not even possible to differentiate their types by mean of microscopic exam [4]. That permits us to predict similarities to our findings in cutaneous leishmaniasis in both types of parasites infection. Those similar findings are the appearance of such promastigotes at their different stages of development starting from their amastigote transformation into promastigote form and ending with fully mature promastigote appearance inside the infected location of the human host.

Our new discovery with respect to *Leishmania* parasites in general through the cutaneous *Leishmania* species in particular allowed us the following advantages:

1. A better understanding for the disease process in its different stages.
2. A better understanding to the pathology of the disease and the macrophages, neutrophils and lymphocytes role in it.
3. A 100% sensitivity and high specificity for *Leishmania* detection by the microscopic method.
4. A better understanding of the parasite life cycle and the role of both the victor and the host.

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