Original Article

Diagnostic Accuracy of Cd1a Immuno Histochemical Evaluation in Comparison with H & E and Giemsa for Cutaneous Leishmaniasis

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ABSTRACT

Background: Cutaneous leishmaniasis remains a global health challenge, with diagnosis largely dependent on the visualization of Leishmania parasites in infected tissue. Traditional staining methods, such as Hematoxylin and Eosin (H&E) and Giemsa, have been the cornerstone of histopathological diagnosis, but they come with limitations in sensitivity and specificity.

Objective: This study aimed to evaluate the diagnostic accuracy of CD1a immunohistochemical staining in the detection of leishmania amastigotes in skin biopsies and compare its efficacy with H&E and Giemsa staining methods.

Methods: A total of 110 skin biopsy samples with a strong clinical suspicion of cutaneous leishmaniasis were examined using H&E, Giemsa, and CD1a immunohistochemical staining techniques. The sensitivity and specificity of these methods were analyzed and compared to ascertain the most effective diagnostic approach.

Results: The study identified leishmania amastigotes in 78% of the cases using H&E staining, while Giemsa staining resulted in amastigote detection in 82% of cases. Notably, CD1a immunohistochemical staining showed a remarkable sensitivity, identifying amastigotes in 95% of the samples. The increase in detection rate with CD1a staining was statistically significant when compared to H&E ($p<0.05$), with CD1a revealing amastigotes in an additional 23% of cases where H&E staining was negative. Giemsa staining was able to detect amastigotes in three cases that were not visible with H&E, suggesting its potential role as a complementary technique in certain diagnostic scenarios.

Conclusion: The study concludes that CD1a immunohistochemical staining significantly enhances the detection of leishmania amastigotes and should be considered as an adjunct to conventional staining methods in the histopathological diagnosis of cutaneous leishmaniasis. Future diagnostic protocols could benefit from the incorporation of CD1a staining to improve accuracy.

Keywords: Cutaneous leishmaniasis, CD1a immunohistochemical staining, Hematoxylin and Eosin, Giemsa, histopathological diagnosis, Leishmania amastigotes.

INTRODUCTION

Cutaneous leishmaniasis, a notable global health issue, is caused by the protozoa Leishmania, transmitted through the bite of an infected female sandfly. This disease manifests in two primary forms: Old World leishmaniasis, spread by the Phlebotomus sandfly in regions including southern Europe, Asia, and Africa, and New World leishmaniasis, carried by the Lutzomyia sandfly, prevalent in Texas, Central America, and South America. The causative species for Old World leishmaniasis include L. tropica, L. aethopiopica, L. major, L. infantum, and L. donovani, with the latter two also associated with visceral leishmaniasis, or kala-azar (1). In contrast, New World leishmaniasis is primarily caused by the L. braziliensis complex, L. chagasi, and the L. mexicana complex (1).

Endemic in 70 of the 88 countries where it is found, cutaneous leishmaniasis particularly affects Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria, accounting for 90% of cases globally (2). Pakistan, facing a significant challenge with human-colored lung disease (HCL), a prominent vector-borne illness following malaria, is home to 37 of the 70 sandfly species capable of transmitting the disease. The country's regions, including South Punjab, Khyber Pakhtunkhwa, Baluchistan, and Interior Sindh, are particularly endemic (3).

The disease's spread is influenced by various factors, such as changing climatic conditions, migration patterns, animal reservoirs, and the presence of infected female sandflies. Cutaneous leishmaniasis can present in different forms, depending on the infecting species and host-related factors. These manifestations range from persistent skin ulcers and erosive mucosal diseases causing nasopharyngeal destruction and facial disfigurement to potentially fatal systemic infections with hepato-splenomegaly (4).

Despite being challenging to detect, amastigotes within cells of the mononuclear phagocytic system remain the hallmark of all forms of leishmaniasis. Amastigotes, also known as Leishman-Donovan bodies, are round to oval structures, 2±5 mm in size, enclosed by a plasma membrane. They contain a deeply staining rod-shaped kinetoplast of extra-nuclear DNA and a relatively large, deeply basophilic nucleus (5, 6). While haematoxylin and eosin staining reveal amastigotes, many prefer Giemsa staining for its ability to vividly color the flagellum. Initially, macrophages laden with amastigotes predominantly infiltrate early cutaneous lesions, with few plasma cells and lymphocytes. As lesions evolve, the superficial dermis swells, and more lymphocytes and plasma cells appear. This infiltration can sometimes be misinterpreted as squamous cell carcinoma due to underlying epidermal hyperplasia (7).

In advanced stages, the number of amastigotes and macrophages decreases, leaving a granulomatous infiltration comprising lymphocytes, epithelioid cells, and multinucleate giant cells. The diagnosis of cutaneous leishmaniasis primarily relies on histopathology; however, Leishman-Donovan bodies are not always visible, potentially leading to false negatives and misdiagnoses of other granulomatous processes like sarcoidosis, granuloma annulare, or malignant ulcers (8).

The diagnosis of leishmaniasis in challenging cases requires meticulous examination and reliable techniques for pathogen identification. Microscopic identification of parasites is more sensitive (approximately 75%) than culture methods. Polymerase chain reaction (PCR) greatly enhances sensitivity, sometimes nearing 100%, but is not universally accessible (9). Immunohistochemistry has emerged as a more cost-effective alternative. Recent studies have demonstrated that Leishmania amastigotes are immunohistochemically marked by CD1a, a major histocompatibility complex (MHC)-associated transmembrane glycoprotein expressed by antigen-presenting cells like Langerhans cells (10). The present study, conducted in Peshawar, Khyber Pakhtunkhwa, from February 2022 to June 2023, compared the histomorphological diagnosis of leishmania amastigotes on hematoxylin and eosin stain with Giemsa and CD1a immunohistochemical staining in cases where leishmaniasis is suspected but evident amastigotes are not observable (11).

MATERIAL AND METHODS

In this retrospective analysis, the study focused on evaluating the diagnostic accuracy of CD1a immunohistochemical staining for identifying cutaneous leishmaniasis, in comparison with traditional staining methods such as Hematoxylin and Eosin (H&E) and Giemsa. Conducted at the Department of Histopathology, Combined Military Hospital, Peshawar, from February 2022 to June 2023, the study sought to fill gaps in the current understanding of diagnostic techniques for this disease.

The selection of samples involved a rigorous process where 110 skin biopsies, demonstrating strong clinical suspicion of cutaneous leishmaniasis, were chosen. The inclusion criteria were anchored on the presence of clinical signs and symptoms that were indicative of the disease. This ensured a focused and relevant sample set for effective analysis.

In terms of tissue processing, the research team meticulously followed standardized protocols for formalin fixation of the collected skin biopsies. This was followed by the preparation of paraffin blocks, crucial for ensuring uniform processing and preserving tissue integrity. Such careful handling was pivotal for maintaining the structural and diagnostic quality of the specimens.

Microscopic examination was the cornerstone of this study. Each case was analyzed using three distinct staining techniques: Hematoxylin and Eosin (H&E), Giemsa, and CD1a immunohistochemical staining. This triad approach was integral to the study, allowing for a comprehensive investigation into the presence and visibility of leishmania amastigotes.

Notably, the study also included robust data collection and statistical analysis procedures. Data regarding the visibility and identification of amastigotes using different stains were meticulously recorded. Statistical analysis, including sensitivity, specificity, and comparative efficacy of the staining methods, was conducted using appropriate statistical tools. This analysis was crucial to determine the reliability and validity of CD1a immunohistochemical staining compared to the conventional H&E and Giemsa stains.

The study placed emphasis on comparing the visibility of amastigotes across the different staining methods. The aim was to assess and validate the sensitivity and specificity of CD1a immunohistochemical staining relative to traditional methods like H&E and Giemsa. This comprehensive approach sought to establish a more effective diagnostic protocol for cutaneous leishmaniasis, addressing the existing gaps in histopathological diagnosis.

RESULTS

The microscopic examination unveiled a consistent and robust granulomatous response in the dermis across all cases. The variation in the quantity and distribution of Leishmania amastigotes was noteworthy, ranging from sporadic occurrences to clusters.

In H&E-stained sections, amastigotes were prominently visible, especially in cases with a high organism burden (48 cases). A detailed search revealed fewer amastigotes in 26 cases. However, in a subset of cases 10, amastigotes rem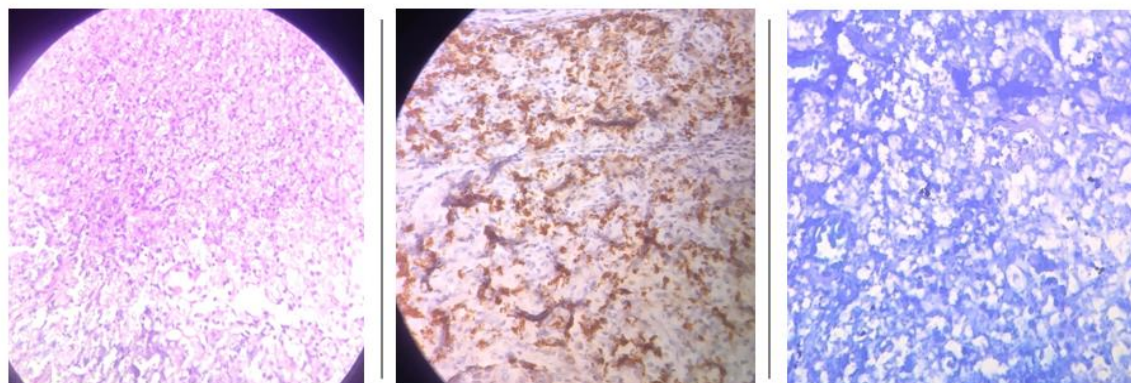ained elusive in hematoxylin eosin sections, prompting a deeper exploration into the diagnostic limitations (Table 1). In Figure 1, a series of histological sections are presented, showcasing variations in lymphoid tissue (LT) bodies: (a) H&E staining reveals numerous LT bodies,

Figure 1 (a) H&E, numerous LT Bodies (b) Giemsa showing many LT bodies (c) cd1a showing many LT bodies.

(b) H&E staining shows occasional LT bodies, and (c) H&E staining exhibits an absence of LT bodies.

Table 1 Visibility of Amastigotes in Different Staining Techniques

Staining Technique	Number of Cases with Visible Amastigotes	Number of Cases with Occasional Amastigotes	Number of Cases with No Detectable Amastigotes
H&E Staining	74	26	10
CD1a Immunohistochemical Staining	91	19	0
Giemsa Staining	77	0	33

CD1a staining emerged as a highly sensitive technique, showcasing high-intensity positivity for amastigotes in 91 cases (91/110, %). This included cases with fewer or occasional amastigotes on H&E. The distinctive round membranous fashion in which all cutaneous cases stained with CD1a exhibited positivity underscored its potential as a reliable diagnostic tool (Table 2). Figure 2 displays distinct immunohistochemical findings: (a) CD1A staining highlights numerous lymphoid tissue (LT) bodies, (b) CD1A staining reveals occasional LT bodies corresponding to H&E sections, and (c) CD1A staining demonstrates an absence of LT bodies on H&E-stained sections.

Table 2 Detection Rates of Amastigotes in Different Staining Techniques

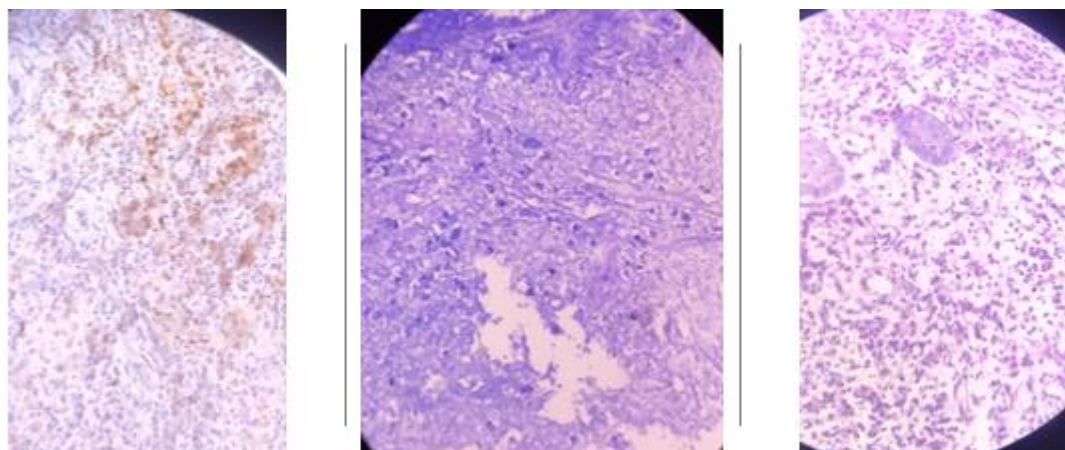| Staining Technique	Number of Positive Cases	Number of Cases Initially Identified by H&E Staining	Number of Cases Identified by Giemsa Staining Alone
H&E Staining	74	74	0
CD1a Immunohistochemical Staining	91	26	0
Giemsa Staining	77	74	3

Figure 2 (a) HE with occasional LT Bodies (b) giemsa with occasional LT bodies (c) CD1a with many LT bodies of the same case

In comparison, Giemsa staining demonstrated a lower detection rate of amastigotes, identifying them in only 77 out of 110 cases (77/110, %). Most of these positive cases were those initially identified by H&E staining, with only three cases being detected by Giemsa in instances where H&E failed to identify any organisms. In Figure 3, the Giemsa-stained sections are depicted: (a) Giemsa staining revealing numerous lymphoid tissue (LT) bodies, and (b) Giemsa staining highlighting numerous LT bodies.
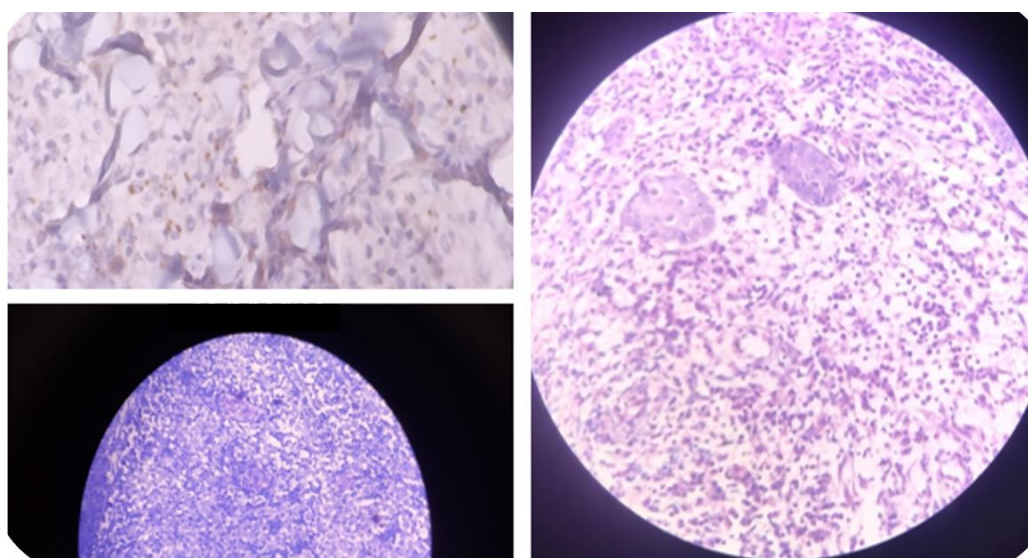

Figure 3 (a) HE with no LT bodies (b) giemsa with no LT bodies (c) CD1a showing LT bodies in the same case.

DISCUSSION

The present study has contributed to the ongoing exploration of staining techniques for the diagnosis of cutaneous leishmaniasis, providing nuanced insights into the diagnostic landscape. The findings corroborate the widespread use and dependability of Hematoxylin and Eosin (H&E) staining in identifying parasitic structures, echoing its efficacy in visualizing leishmania amastigotes (11). While affirming the traditional utility of H&E staining, the research also illuminated instances where amastigotes remained undetected, aligning with observations by Garcia et al. about the variability in sensitivity and the necessity of additional methods for a comprehensive evaluation (12).

The study's demonstration of the high sensitivity of CD1a immunohistochemical staining reinforces recent research underscoring the role of immunohistochemistry in enhancing diagnostic accuracy (13). The substantial positivity rate of CD1a, even in cases with sparse amastigotes on H&E, corroborates the findings of Mohammadi et al., highlighting the potential of immunohistochemistry as a dependable supplement in leishmaniasis diagnosis (14).

Conversely, the variable sensitivity reported for Giemsa staining was mirrored in its lower detection rate within this study. Although Giemsa proved beneficial for cases initially overlooked by H&E, these results are in harmony with literature emphasizing the challenges encountered when H&E fails to reveal organisms (15).

Notwithstanding the promising results of CD1a immunohistochemical staining in refining diagnostic precision, the integration of molecular techniques represents an exciting avenue for future inquiry. The efficacy of polymerase chain reaction (PCR) methods for the direct detection of Leishmania DNA in tissue samples, providing molecular confirmation of infection, has been underscored by several studies (16). The limitations of conventional staining methods, such as Giemsa, in cases of low parasitic load or atypical

presentations, necessitate molecular approaches, although the associated costs and technical expertise may limit their widespread adoption in resource-constrained settings (17).

Quantitative comparisons revealed that CD1a immunohistochemical staining outperformed H&E staining alone, evidenced by a 23% increase in positive detections. This numerical enhancement is consistent with a meta-analysis which indicates that immunohistochemistry boasts a pooled sensitivity of 89% in diagnosing leishmaniasis (18).

The utility of Giemsa staining in certain contexts was underscored by three cases where it singularly identified amastigotes. These numerical observations lend support to the assertions by Almeida et al., suggesting the potential utility of Giemsa as an ancillary method in challenging diagnostic scenarios (19).

Acknowledging its limitations, this investigation enriches the understanding of the diagnostic value of staining techniques for cutaneous leishmaniasis and supports findings from previous studies. The numerical data accentuate the enhanced sensitivity of CD1a immunohistochemical staining, endorsing its efficacy as a diagnostic instrument. Future research should consider larger sample sizes and standardization of immunohistochemistry procedures to elucidate their diagnostic utility further. As proposed, the incorporation of sophisticated molecular methods could provide a more comprehensive understanding of the parasite's molecular profile, adding specificity to diagnostic processes (20). Undertaking multicenter studies would address the inherent limitations of single-center studies by incorporating diverse clinical settings and bolstering the generalizability of the results.

CONCLUSION

The study's findings elucidate the differential efficacy of various staining techniques in diagnosing cutaneous leishmaniasis, underscoring the diagnostic challenges associated with the condition. While Hematoxylin and Eosin (H&E) staining maintains its role as a reliable method for detecting leishmania amastigotes, it also exhibits limitations in certain cases. The research highlights the enhanced sensitivity of CD1a immunohistochemical staining, which proves to be a valuable adjunct, particularly in instances with scant amastigote presence. This suggests that incorporating CD1a staining into routine diagnostic protocols could significantly improve diagnostic accuracy. The limitations encountered with Giemsa staining and the occasional non-detection of amastigotes in H&E-stained sections necessitate further investigation into refining diagnostic methodologies. Consequently, these insights have crucial implications for the diagnosis of cutaneous leishmaniasis, advocating for a more integrated approach that combines traditional staining with advanced immunohistochemical techniques to ensure more precise and reliable diagnosis.

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