



Original Article

Correlation between Histopathological Findings, CD4 Counts, and Treponeme Quantity in Microscopic Sections and Secondary Syphilis in HIV Positive Individuals

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ARTICLE INFO

Key Words:

Infectious agents, Warthin-Starry stain, HIV, immune-histochemistry, Syphilis

How to Cite:

Manzoor, M., Amin, A., Hussain, S., Zia, R., Sarwar, A., Shabir, M. M., Ilah, I.- u, Mir, H., Bangash, S. A., & Muthanna, F. (2022). Correlation Between Histopathological Findings, CD4 Counts, and Treponeme Quantity in Microscopic Sections and Secondary Syphilis in HIV Positive Individuals. *Pakistan BioMedical Journal*, 5(1), 253-256. <https://doi.org/10.54393/pbmj.v5i1.320>

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ABSTRACT

Syphilis is not common, although HIV-infected people are far more likely to be infected than the general population. This may be caused by weakened cellular immunity due to HIV infection. This study aimed to find out the correlation between CD4 concentration and T. Pallidum occurrence.

Methods: A assessment of a single institution identified ten patients with secondary syphilis diagnosed through a skin biopsy, positive syphilis serology, and high CD4 levels. There were 14 samples obtained from 13 individuals. Immunohistochemistry (IHC) was used to detect the presence of treponemes and CD4 levels in skin samples. These findings have also been compared with results obtained from detecting T. Pallidum by IHC in silver stain sections (Warthin-Starry). A comparison of the histological characteristics of each sample has also been made. IHC was used to determine the number of treponemes on the samples. **Results:** Silver stain had a sensitivity of 9 percent whereas IHC detected the treponemes at 64 percent. (p-value of 0.04). Spirochetes high incidence found only in people with less than 250 cells/ml of CD4 (>100 per 10 HPF). **Conclusions:** The utmost persistent histological outcome was low to highly lymphoplasmocytic infiltration. However, this research did show that a high spirochete count is associated with a low CD4 count (less than 250 cells/ml). In comparison to Warthin-Starry staining results, IHC staining for T. pallidum has shown superior results.

INTRODUCTION

In the period from 2005 to 2013, syphilis infections among Americans more than doubled from 2.9 to 5.3 per million. Approximately all syphilis cases in the U.S. in 2013 were reported to have been among males. The most adversely affected group (MSM) is the one that has interacted with

other men. In 2012, data from 34 states showed that 84 percent of men with syphilis had sex with a partner, and 70 percent of them had sexual contact with a partner prior to getting the disease. There has been a long-established connection between HIV and syphilis. During a study

conducted in the cities of United States of America, 45 percent of 357 male syphilis patients were found to have HIV. Due to the fact that the human immunodeficiency virus and syphilis were classified as STD, that should be treated as such. According to a study of STD and HIV surveillance data, adolescents who had a history of gonococcal infections had a significantly higher risk of contracting HIV than adolescents who did not have a history of STDs. Those with genital tract infection like syphilis have a lower likelihood of preventing HIV transmission due to a weaker skin barrier. Some evidence has been found from previous literature that the syphilis is suppressed by cellular immunity. CD4+T-cells, also known as helper T-cells, are found in a variety of cells, including lymphocytes and macrophages, and they play a role in their function. A function for CD8+ lar immunity in humoral immunity may be shown in the activation of B cells to release cytokines that attract either T cells, culminating in an antibody-mediated response. study believe that helper T cells are important in eliminating syphilis in its early stages, and that those with decreased cellular immunity, such as HIV patients, are more prone to develop secondary and tertiary stages. It is supported by the results of Arroll et al., who observed elevated Inter leukin-2 and IFN gamma in rabbits infected by *T. Pallidum*, which is congruent with a helper T cell response, when compared to controls. The researchers hypothesised that HIV patients with low CD4 counts would have a harder difficulty limiting *T. pallidum* development, which would result in an increase in the amount of spirochetes in the bloodstream. With this study, the researchers wanted to look at the histopathological results in skin-biopsies taken from HIV and syphilis infected individuals. It was also our goal to get insight into the pathophysiology of the comorbidities by comparing the number of treponemes present on the *T. pallidum* immunohistochemistry slides with T-cell counts of the participants. Finally, the sensitivity of *T. pallidum* immunohistochemistry was evaluated in comparison to silver-staining's.

METHODS

From June, 2022 to November, 2022, we analysed the files of patients with final diagnoses of "syphilis" and "skin" at a single tertiary care centre and identified 14 biopsy specimens (two of which were from one patient). The presence of syphilis was confirmed by skin biopsies in each of the patients. Twelve males and two women, ranging in age from 25 to 51, were in the group. HIV was found in 10 of the 14 patients who had skin biopsies a month after they were taken, indicating that they were infected. 17 days after the skin biopsies, the CD4 counts were obtained. Two of the 10 patients were not on HAART when they were first

diagnosed with syphilis and had no idea they had HIV until the syphilis was discovered. Five years apart, one of the HIV-positive persons developed secondary syphilis in the same area of the body. We analysed 13 HIV and syphilis biopsies for this inquiry. Biopsies were necessary in this case. peri-anal region, thigh, arm, abdomen, and chest; peri-anal area; thigh and arm; and sacrum. There is a way to collect CD4 T-cell counts electronically. Additionally, endothelial cell hyperplasia and edoema of the dermis were examined histologically, as were band-like, superficial, or deep perivascular inflammation. Using an immunohistochemistry stain specific to *T. pallidum*, the tissues were all examined under the microscope. Pre-diluted rabbit polyclonal antibody from Biocare Medical, Concord, CA was used to label *T. pallidum*. An incubation time of 16 minutes at 37°C was achieved by Medical Systems, Tucson, Arizona, USA, using the Ultra. The Ventana Optiview DAP polymer kit is required for both benchmarking and detection. At 500x magnification, treponemes were counted in ten sections of dyed *T. pallidum* sections. Spirochete numbers seem to naturally divide between specimens with high and low levels of CD4, according to the findings of this study. For this reason, the cut-off value of 250 cells/ml was established as the threshold for opportunistic infections. Nine Warthin-Starry and two Steiner silver stains were performed during the initial diagnostic test, and all were negative. Silver-staining sections were compared to immunohistochemical stained sections for every sample using McNemar's test. In order to perform the statistical analyses, SAS software was employed.

RESULTS

Every case had a moderate to severe response to inflammation, which was the most often seen histopathologic result. The majority of a 400-field is made up of cells. In the lymphoplasmacytic infiltration, inflammatory cites and plasma cells predominated over other types of cells. On the other hand, icepick epithelial hyperplasia (discovered in six out of eleven patients) and endothelial hyperplasia (found in nine out of eleven patients) were investigated in a less systematic manner. There were no ulcers seen in any of the samples. Table. 01 shows the summarized histological results. However, spirochetes were found in just one of 11 silver-stained *T. pallidum* biopsies in this study. Immuno-Histo-Technique has a treponeme detection sensitivity of 64 percent, while silver stain has a treponeme detection sensitivity of just 9 percent. *T. pallidum* treponemes were found in three to over 200 cases, according to immunohistochemistry. Treponemes were graded based on the patient's CD4 levels (>100 treponemes in 10 hpfs cells/ml and 250 cells/ml) and

the patient's CD4 counts. All of the samples had less than 250 cells per millilitre. Patients with CD4 levels of 250 or above saw an increase in the mean number of spirochetes ($p = 0.03$, t-test). Individuals with low CD4 levels have lower CD4 counts than patients with high CD4 counts findings are summarized in Table 1.

Histological Characteristics	Number of Occurrence (n=13)	Occurrence in Percentages
Spongiosis	(Moderate to severe) 2/11	18%
Ulceration	0/13	0%
Acute, inflammation in epidermis		
Serum/crust	(4/13)	36%
Interface change	(3/13)	27%
Dyskeratosis	(7/13)	64%
Ice-picked epithelial hyperplasia	(5/13)	45%
Band-like inflammation in dermis		
Superficial perivascular	(6/13)	54%
Inflammation		
Moderate to Severe degree of	(0/13)	0%
Inflammation (most of $\times 400$ field)	(11/13)	100%
With inflammatory cells	(9/13)	82%
Endothelial hyperplasia	(2/13)	18%
Papillary dermal edema		
Deep perivascular inflammation	(7/13)	64%
From zero to mild degree of		
Inflammation		
(fewer inflammatory	(10/13)	90%
cells, filling $\times 400$ field)	(8/13)	73%

Table 1: Thirteen Syphilis and Human Immuno-Deficiency Virus Histopathological Outcomes

Patients	Count of CD4 T-Cells (cells/ μ l)	Count of spirochetes
A	18	200
B	62	200
C	76	0
D	127	122
E	152	0
F	246	200
G	258	3
H	377	0
I	397	8
J	426	0
K	484	12
L	485	12
M	487	12

Table 2: Details of Immunohistochemistry of Pallidum

DISCUSSION

This research had employed immunohistochemistry to measure *T. pallidum* density in skin samples, regardless of CD4 levels. *T. pallidum* immunohistochemistry outperformed silver-based immunohistochemistry in terms of sensitivity. We looked at the biopsies for signs of secondary syphilis such as icepick hyperplasia of the epithelium, edoema of the endothelial cells, and inflammation of lymphoplasmacytic lymph nodes. Moderate to significant lymphoplasmacytic infiltration was the utmost prevalent histopathological outcomes in these Human Immunodeficiency Virus and syphilis-infected individuals. In immunosuppressed people with severely

reduced level of CD4 T-cell numbers, skin biopsies showed mild to high response of inflammation. As a result, samples with negative syphilis immunohistochemistry were shown to have extensive inflammatory infiltrates in contrast to those with positive results. According to the findings, sample aspiration from immunocompromised only showed high levels of treponemes (100 treponemes/10 hours post-infection) which aids to the notion that CD4+ T-cells are required for *Treponema* spirochete restriction. The fact that sample extraction from persons with low CD4 counts below only contained significant amounts of treponemes (100 treponemes/10 hpfs) lends support to the notion that CD4+ T-cells are essential for *Treponema* spirochete restriction, according to the findings. The results of a 22-year examination of the records of a big tertiary care hospital revealed that just 14 people had syphilis confirmed by skin biopsy, with 10 of them also having HIV. The low number of reported incidences are most fairly owing to the low level of STDs in USA, as in 2014 it was only 6 incidences per million. 1 Furthermore, skin biopsy is not necessary from all STD infected individuals; the only way to determine whether or not they have the disease is via serology. Despite this, we maintain our optimism. The research conducted on this small sample of patients provides insight into the role of cellular immunity in the fight against Syphilis and other infections. Beyond the expensive immune-histo-techniques, cost effective histological stained are used. This study, on the other hand, reveals that *T. pallidum*-IHC is a far more accurate method of detecting this spirochete than standard silver stains in this situation. The comparison of *T. pallidum* immunohistochemistry to silver-staining in *T. pallidum*, are in agreement with our results. In stomach biopsies, it has been shown that the *Helicobacter pylori* immunohistochemistry stain is much more effective than silver-staining. Most patients with CD4 counts more than 250 cells/ml had a low quantity of spirochetes in their biopsies, which was consistent with previous findings. The most sensitive staining process should be used as a consequence. Our findings suggest that classic bacterial silver stains should be utilised to identify organisms for which IHC is not yet accessible i.e., *Bartonella* species, until immunohistochemistry becomes available.

CONCLUSION

An infiltration of moderate to severe lymphoplasmacytic cells was the most common observation on histological examination (LPI). Despite the fact that the research sample size was limited, CD4 cell counts less than 250 cells/ml were related with increased quantities of spirochetes in the blood. The IHC stain for *T. pallidum* was shown to be superior to the Warthin–Starry stain.

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