Concordance of Acid-Fast Stain Result and Histopathologic vs Clinical Diagnosis of Leprosy: A Three-year Retrospective Study in a Tertiary Government Hospital and Sanitarium in the Philippines

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ABSTRACT

Objectives. In the Philippines, there has been a lack of information on the concordance between classifications of Hansen's disease or leprosy clinically, histopathologically, and with AFS results. The study ultimately aimed to determine the concordance between the clinical diagnosis, histopathological results, and AFS results of patients with leprosy seen at the Dr. Jose N. Rodriguez Memorial Hospital and Sanitarium (DJNRMHS).

Methods. This is a descriptive, retrospective, single-center study conducted at the DJNRMHS, a tertiary government hospital and one of the last remaining sanitaria in the country located in northern Metro Manila in the Philippines. The study reviewed and included all the patient records from the years 2017-2019 which included skin biopsy results and slit-skin smear with AFS. Leprosy patients were then classified based on the following classifications: World Health Organization (WHO) and Ridley-Jopling classifications; and the concordance of clinical diagnosis vs the histopathologic findings and clinical diagnosis vs AFS results were determined using kappa testing.

Results. A total of 48 patients from 2017-2019 were included in the study analysis. Based on the WHO classification, 3 (6.3%) presented clinically with paucibacillary (PB) leprosy and 45 (93.7%) with multibacillary (MB) leprosy. The slit-skin smear with AFS results of these patients ranged from 0 to 4 with the majority being 0. PB results are composed of 28 (58.3%), while MB is at 20 (41.7%). The paucibacillary forms had the highest agreement percentage at 66.7% (2/3) and multibacillary had the lowest percentage of agreement at 19/45 (42.2%). The overall data analysis showed an agreement of 21/48 (43.8%), considered no agreement (kappa = 0.0195, p = 0.05). Using the Ridley-Jopling classification, patients can be clinically stratified with most comprising lepromatous leprosy (LL) at 19 (39.6%) and indeterminate spectrum having the least with only 2 (4.2%). The histopathologic result of these patients reported a majority of LL comprising 24 (50%) and the indeterminate spectrum comprising the least with 2 (4.2%) reported. The inde-terminate and tuberculoid spectrum were those with the highest percentage of agreement: 2/2 (100%) and 5/5 (100%), respectively. The borderline lepromatous spectrum presented an agreement of only 4/10 (40%), and thus

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the lowest agreement. The overall data analysis showed an agreement of 36/48 (75%), considered moderate agreement (kappa = 0.661, p = 0.05).

Conclusion. In the findings of this study, AFS can suffice only for the detection but not for the accurate classification of the different leprosy spectra of patients based on its low overall agreement. On the other hand, histopathology yielded moderate agreement with clinical classification. It is therefore highlighted that AFS, histopathology, and clinical findings are needed to properly detect and classify leprosy patients, leading to appropriate management and treatment.

Keywords: Leprosy, Hansen's Disease, concordance, slit-skin smear, skin biopsy, histopathology, clinical diagnosis, descriptive study

INTRODUCTION

Leprosy, or Hansen's disease, has been present since the start of civilization. Although curable, it continues to be a major health problem in countries. Leprosy results from infection with the *Mycobacterium leprae* bacillus, which produces a chronic granulomatous infection in humans.[1]

Leprosy has a variety of clinical, microbiological, and pathological findings, and it is diagnosed based mainly on the presence of skin lesions, loss of sensitivity, and neural thickening. The various clinical presentations are determined by the different levels of cellular immune response to *M. leprae*, which are expressed through different pathophysiological mechanisms, with particular signs, symptoms, progression, prognosis, and contagion that have allowed numerous classifications.[2] The classification scheme described by Ridley & Jopling (1966) has remained the most popular. This approach classifies leprosy patients into lepromatous leprosy (LL), borderline lepromatous leprosy (BL), borderline (BB), borderline tuberculoid (BT), tuberculoid leprosy (TT), and indeterminate (I) based on immunological, histological, and microbiological characteristics – of which the lepromatous pole has an increased humoral immunity and the tuberculoid pole has heightened cell-mediated immunity.[3]

The World Health Organization (WHO) promoted a primarily clinical method of diagnosing and categorizing patients (WHO, 1994) in response to the issue of poor implementation of leprosy control programs caused by variable quality of skin smears in many regions. This method, which was primarily intended for practical usage, categorizes individuals as multibacillary(MB) if they have more than five lesions, whereas paucibacillary(PB) if there are fewer than five lesions.[1] However, there are a number of drawbacks to counting the skin lesions or positivity in AFS, it was found by Croft et al. that using the WHO classification alone was found to be only 89% sensitive and 88% specific at detecting smear-positive.[4] To prevent treatment failure and take into account clinical and operational considerations, all smear-positive patients at any site were categorized as MB, whereas all smear-negative cases were categorized as PB.[5] There is an agreement between the WHO classification and the Ridley and Jopling classification of only about 77.6% since the WHO classification tends to overestimate the number of MB cases.[6]

However, these classifications present important differences regarding sensitivity and specificity, and thus require critical analysis for their application, especially in regions that are considered endemic. The standard procedure to support leprosy diagnosis includes the identification of the causative organism by slit-skin smears with acid-fast stain (AFS), histopathology, or polymerase chain reaction.[7,8] Many endemic nations have only limited access to laboratory facilities for slit-skin smears with AFS and histopathology.[9]

It was shown in different international studies that there are difficulties in establishing the correct classification, and have also demonstrated a lack of concordance between the clinical and histopathological classifications.[10,11] Furthermore, the simplified classification adopted by the WHO is not predictive of the correct histopathological classification, which raises the need for a combination of clinical diagnosis accompanied by direct smear microscopy and histopathological examination of the lesion, especially in endemic regions.[12,13]

Dr. Jose N. Rodriguez Memorial Hospital and Sanitarium (DJNRMHS) was once home to a Leper colony but is now in a strategic position that continually aids people afflicted with leprosy not only in its locality but from people all over the Philippines with a prevalence rate of 0.31 (<1/10000).[14] Being designated as one of the specialty centers and an end-referral hospital, the DJNRMHS is now equipped with facilities for the proper diagnosis of leprosy.

MATERIALS AND METHODS

Objectives

The study furthermore aims to determine if there is concordance between the clinical diagnosis, histopathological results, and AFS results of leprosy patients versus the Ridley-Jopling classification and the WHO classification.

Study Design

This is a descriptive, retrospective, single-center study conducted at DJNRMHS, a tertiary government hospital and one of the last remaining sanitaria in the country located in northern Metro Manila in the Philippines. The study reviewed and included all the patient records from 2017-2019 which included skin biopsy results and slit-skin smear with acid-fast stain. Patients with leprosy were then classified based on two classifications: WHO (PB and MB) and Ridley-Jopling classifications (I, TT, BT, BB, BL, LL).

Participant Selection

The study only included all the patient records and not actual patients seen in the DJNRMHS Department

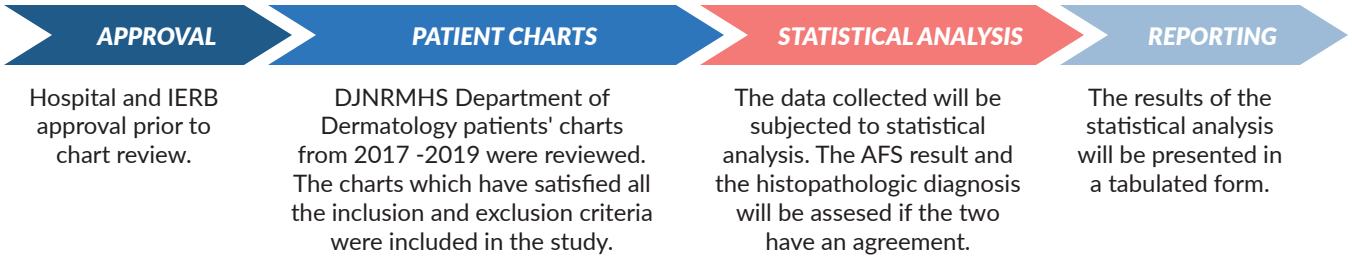

Figure 1. Flow chart of protocol.

of Dermatology from 2017-2019. A patient record was included in the study if the patient's record included both a slit-skin smear with AFS result done by a board-certified medical technologist and a skin punch biopsy interpreted by a board-certified dermatopathologist/pathologist. Other patient charts that have fulfilled the criteria above but have incomplete or missing charts, patients who have completed prescribed therapy and labeled "release from treatment" who underwent repeat biopsy for any reason, and patients who underwent either AFS or skin punch biopsy but have no recorded results were not included in the study.

Data Collection and Ethical Considerations

The methodology for this study is seen in Figure 1. Only patient charts were used, no actual patient interaction nor interventions were done. Prior to looking into the patient's charts, proper Institutional Ethics Review Board (IERB) and hospital's record section permission were secured. Since the study involved physical patient records, privacy and confidentiality were of utmost importance, these were kept under proper storage and security in the Dermatology Department of DJNRMHS. Patient's charts for review were seen by the investigators and were checked for completeness of the criteria set out, namely: inclusion of slit-skin smear with AFS result and histopathologic report. The names of participants were not included in the Microsoft Excel to ensure their privacy. At the end of the data collection period, the primary investigator placed the collected data in a password-protected folder. This encoded data was sent to the statistician of this study for data analysis. Charts were returned to their original folders and stored in the proper storage.

Sampling Method

Complete enumeration sampling was used for leprosy patients from 2017 to 2019.

Study Procedure

A total of 148 Hansen's disease patient charts were produced in the Department of Dermatology from 2017-2019, of which only 48 patient charts fulfilled the criteria set and were included in the study and data analysis (Figure 2). Data of interest such as the patient's clinical data and demographics, slit-skin smear with AFS results, and

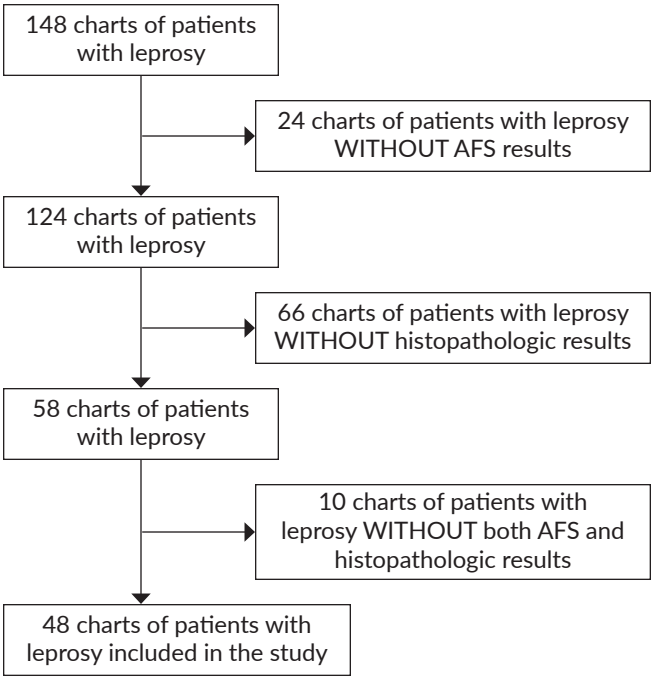

Figure 2. Flow chart of selection process.

histopathologic report were noted and categorized based on the WHO and Ridley-Jopling classifications; these were encoded in a Microsoft Excel spreadsheet.

Data Analysis

The collected data was subjected to statistical analysis by STATA® v17 statistical software. Demographic data were interpreted in absolute percentages as to their means, standard deviations, and their absolute percentage. Data in terms of the WHO and Ridley-Jopling classifications were then tested to the level of concordance between the clinical diagnosis and histopathological results using the agreement and kappa test which was applied to evaluate the concordance of the overall results.

All data gathered were composed of calculating absolute percentage frequencies for the categorical variables and organizing the results into tabular form through descriptive analysis and agreement between variables. The diagnostic agreement between the clinical classification and the AFS result as well as the histopathological type was calculated

by dividing the number of concordant cases (cases agreed by both AFS and clinical classification) by the total number of patients.

The agreement between the AFS result, histopathologic result, and the clinical diagnosis of the patients was determined by attaining their kappa values and interpretations as follows: <0-0.20, no agreement; 0.21–0.39 minimal agreement; 0.40–0.59, weak agreement; 0.60–0.79, moderate agreement; 0.80–0.90, strong agreement; and >0.90, almost perfect agreement.[15] The significance level used for the analyses was 95% (p = 0.05).

Table 1. Demographic and Clinical Characteristics

Characteristics	Results* (n = 48)
Age, years	38 (5 to 71)
10 and below	5 (10.4)
11-20	5 (10.4)
21-30	16 (33.3)
31-40	8 (16.7)
41-50	5 (10.4)
51-60	7 (14.6)
61-70	1 (2.1)
71-80	1 (2.1)
Sex	
Male	34 (70.8)
Female	14 (29.2)
Place of origin	
Caloocan	19 (39.6)
Other cities	12 (25)
Outside Metro Manila	17 (35.4)
WHO Classification Clinically	
Paucibacillary	3 (6.3)
Multibacillary	45 (93.7)
AFS Classification	
Paucibacillary	28 (58.3)
Multibacillary	20 (41.7)
Ridley-Jopling Classification Clinically	
Indeterminate Type	2 (4.2)
Tuberculoid Type	5 (10.4)
Borderline Tuberculoid	7 (14.6)
Borderline	5 (10.4)
Borderline Lepromatous	10 (20.8)
Lepromatous Leprosy	19 (39.6)
Ridley-Jopling Classification Histopathologically	
Indeterminate Type	2 (4.2)
Tuberculoid Type	7 (14.6)
Borderline Tuberculoid	6 (12.5)
Borderline	5 (10.42)
Borderline Lepromatous	4 (8.3)
Lepromatous Leprosy	24 (50)

**Mean ± standard deviation, (minimum to maximum) or frequency (%)*

RESULTS

The age of the patients included ranged from 5 to 71 with a mean age of 38. The majority of the subjects included in the study are male making up 34 (70.8%) of the sample population. Most patients are residents of Caloocan City, which makes up 39.6%. Out of the total 48 subjects included in the study, 3 (6.3%) presented clinically with paucibacillary leprosy, and 45 (93.7%) with multibacillary leprosy and lepromatous leprosy made up the majority 19 (39.6%) when classified under the Ridley-Jopling classification clinically. The AFS results of the patients seen in the study ranged from 0 to 4, with 0 as the most frequently occurring, when classified under the WHO classification, paucibacillary is composed of 28 (58.3%), while multibacillary is 20 (41.7%). The Ridley-Jopling classification clinically stratified the patients with most comprising the lepromatous leprosy 19 (39.6%) and indeterminate having the least with only 2(4.2%). The biopsy result reported a majority of lepromatous leprosy comprising 24(50%) and the indeterminate type comprising the least 2(4.2%) reported in the Ridley-Jopling spectrum as seen in Table 1.

Table 2 shows the evaluation of the concordance between the clinical classification and AFS classification of the 48 patients. The paucibacillary forms were those with the higher percentage of agreements at 2/3 (66.7%) and multibacillary had the lower percentage of agreements at 19/45 (42.2%). The data analysis showed an overall agreement of 21/48 (43.8%), which was considered no agreement (kappa = 0.01818, p = 0.05).

Table 3 shows the evaluation of the concordance between the clinical classification and histopathological classification of the 48 patients. The indeterminate and tuberculoid types were those with the highest percentage of agreement: 2/2 (100%) and 5/5 (100%), respectively. The borderline lepromatous form presented an agreement of 4/10 (40%) and thus the lowest agreement. The data analysis showed an overall agreement of 36/48 (75%), which was considered moderate agreement (kappa = 0.661, p = 0.05).

Table 2. Concordance between Clinical Diagnosis and Acid-Fast Smear (AFS) based on the World Health Organization Classification

Clinical classification	Classification based on acid-fast smear		Total	Agreement, n (%)
	Paucibacillary	Multibacillary		
Paucibacillary	2	1	3	2/3 (66.7%)
Multibacillary	26	19	45	19/45 (42.2%)
Total	28	20	48	21/48 (43.8%)

Kappa=0.01818, p=0.05

Table 3. Concordance between Clinical Diagnosis and Histopathologic Result based on the Ridley-Jopling Classification

Clinical classification[a]	Histopathologic classification[a]						Total	Agreement
	I	TT	BT	BB	BL	LL		
I	2	0	0	0	0	0	2	2/2 (100%)
TT	0	5	0	0	0	0	5	5/5 (100%)
BT	0	1	5	1	0	0	7	5/7 (71.4%)
BB	0	0	0	3	0	2	5	3/5 (60%)
BL	0	0	0	1	4	5	10	4/10 (40%)
LL	0	1	1	0	0	17	19	17/19 (89.5%)
Total	2	7	6	5	4	24	48	36/48 (75%)

Kappa=0.661, p=0.05

[a] *I, indeterminate; TT, tuberculoid type; BT, borderline tuberculoid; BB, borderline; BL, borderline lepromatous; LL, lepromatous leprosy*

DISCUSSION

Ages 21 to 30 are thought to be the peak age group for the economically active population, and it is during this time period that leprosy-related impairments and disabilities have the greatest impact on work and social environments, leading to not only financial losses for the affected person and his or her community but also psychological losses.[16]

The prevalence of males vs females is reflective of other studies where males had better treatment-seeking behavior and fewer social problems faced by the patients thus leading to better disease detection. Likewise, men were more likely to be detected through passive modes than women. It was hypothesized that it might be because men mostly worked outside the home and gained more information compared to women.[17-19]

In terms of place of origin, the majority of the patients were from Caloocan City (39.6%) which is explained by the proximity to the hospital and the history of the place as a previous leper colony.[20]

These findings may reflect the neglect and stigma of the disease. The high percentage of patients with WHO clinical classification of MB leprosy (93.7%) is a manifestation of which patients are afraid to get early consultation and treatment. It is hypothesized that patients in the study did not seek help when the first symptoms appeared, causing delays in diagnosis, progression of the disease, and development of disabilities. This is echoed by a recent study which states that newly diagnosed leprosy patients have obvious physical defects, suggesting a significant delay in case detection. The same study also mentioned that up to 70.2% of those who experience early signs of leprosy did not seek any medical actions.[21] With these numbers, it is still true that leprosy continues to be a significant public health concern up to this day in the country. The National Leprosy Control Program (NLCP) by the Department of Health has already been in place since 1986 with the goal of achieving a leprosy-free Philippines by the year 2030 by ensuring the provision of comprehensive, integrated quality leprosy services at all levels of healthcare in the country, but sadly is still a vision in our country.[22]

Agreement and concordance can be seen as negligible between AFS and the clinical diagnosis. Although the AFS was done by board-certified medical technologists, a lot of factors are to be considered such as but not limited to: the experience of the medical technologist in performing the procedure, assessing AFS results, and the selection of the correct site where smears were to be taken. As such there could be discrepancies since this technique is highly subjective and operator-dependent.[23] It is imperative, therefore, to learn and get acquainted with the procedure for the collection of AFS since every step in the collection is key to proper diagnosis. This finding can also be explained by another study which states: skin smears taken to detect intradermal AFB have high specificity but low sensitivity because about 70% of leprosy patients are smear negative[24] and confirms the study of Mac-Fiberesima et al. which states that slit skin smears are a rapid and inexpensive method of diagnosis but their diagnostic accuracy is low[25]. Nevertheless, skin smears are important because they identify the most infectious patients and those at higher risk of relapse.

Clinically, patients classified as borderline tuberculoid (BT) leprosy were different from tuberculoid type (TT) in that BT leprosy lesions were more raised lesions with altered sensation, numerous nerve involvement, and uneven distribution. Poorly defined hypopigmented plaques were used to diagnose borderline leprosy (BB) cases. Lepromatous leprosy (LL) and BL were the classifications given to cases with numerous asymmetrical nodular lesions and symmetrical shining nodular lesions, respectively. Histopathological analysis revealed granulomas with varying numbers of Langhans giant cells along the superficial vascular plexus for BT and TT. The absence of Langhans large cells, few lymphocytes, active macrophages, and noticeable cutaneous edema were the histopathological hallmarks of BB. BL cases displayed foamy macrophages and extensive lymphocytic infiltrates, while LL cases revealed the presence of the Grenz zone and Virchow cells.[26] In the study, the histopathologic report was based on the Ridley-Jopling classification, BL and LL spectra have the highest total agreement of 75%, and the I and TT spectra have a perfect agreement with regards the clinical diagnosis. This fits in the range published

by the study of Chen et al. which states that the specificity of skin biopsy specimens and histopathological examination ranges from 70% to 72%, but the sensitivity ranges from 49% to 70%.[27] It yielded excellent agreement in BL and LL types of leprosy and good agreement with LL type which limits slit-skin smear study when these types are considered clinically.[28]

The clinical assessment finding of lesions with decreased sensation, thickened or enlarged peripheral nerve with loss of sensation, and histological findings together with the AFS is considered as the "gold standard" in detecting and diagnosing leprosy but thought of as very time-consuming.[29] This is consistent with the study done by Premalatha et al. which described a true correlation between AFS and histopathology.[30]

Limitations

In the study, the authors acknowledge the possible selection bias due to the strict inclusion and exclusion criteria. The study however is still representative of the total population since it took into account all patients with leprosy seen in DJNRMHS. The authors were also limited to three years of available patient records.

CONCLUSION

It was seen that there is no concordance between AFS and clinical diagnosis; this shows that AFS can suffice only for the detection but not for the accurate classification into the different leprosy spectrum of patients. On the other hand, histological diagnosis showed moderate agreement, and therefore this study highlights histopathological diagnosis can be used as the basis of detection and classification. The authors still recommend that AFS together with skin biopsies are needed to increase accuracy in the detection and proper classification of leprosy types, which will in turn lead to appropriate management and treatment in future patients suspected of leprosy in the Philippine setting.

Recommendations

A further study allowing more samples can be done by covering more years and including other sanitaria which have the capacity to do both slit-skin smears and skin biopsies of leprosy patients.

Statement of Authorship

Both authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

Both authors declared no conflicts of interest.

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