Diagnosing Leprosy in Infants:  
A Histopathological Challenge using Several Staining Technique  

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Abstract: Leprosy in infants is a rare case compared to adults. There is an excellent variety of the clinical cutaneous finding in leprosy. Leprosy diagnostic is based on the classical cardinal signs, the presence of acid-fast bacilli which is obtained by slit skin smear and skin biopsy. The gold standard to diagnose leprosy is the identification of *Mycobacterium leprae* (*M. leprae*) bacilli using Hematoxylin and Eosin (H&E) and Fite Faraco (FF) stains. Periodic acid-Schiff (PAS) and Gomori (Grocott) methenamine-silver (GMS), known to give the positive result for fungal staining, revealed the other function is becoming the alternative staining for *M. leprae*. Hereby, we report a case of a six-month-old male patient who presented unusual clinical findings that were never considered as leprosy initially. Histopathological examination with several stains was performed, and a diagnosis of lepromatous leprosy was obtained.  

1 INTRODUCTION  
The occurrence of leprosy during infancy is uncommon. Infants are believed to be the most vulnerable group to infection with *M. leprae* due to their immature immunity and exposure to the member of the family who has leprosy. (Narang T et al., 2018) WHO data showed 210,671 newly diagnosed leprosy cases during 2017, 16,979 were children, represented almost 7,5% of all new cases (5-14 years old). (Narang T et al., 2018) During 2002, based on the data, Indonesia has 21,2% (95% CI: 12,5–29,9%) newly detected leprosy cases in children 6-16 years old. (Bakker et al., 2002) Our data showed 25 new cases leprosy in children (5-14 years old) from 150 patient’s visit in the Dermatology Department of Dr. Cipto Mangunkusumo Central National General Hospital from 2017 to 2018, but none of them were infants.  
The spectrum of leprosy in children or infants has reported mostly to be tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), and indeterminate (I) forms. Lepromatous leprosy (LL) type is rare in children or infants. The great variety of presentations made the diagnosis of leprosy a problematic challenge. The most likely portal of entry is considered to be the upper respiratory tract, but other possibilities through direct contact. (Girdhar et al., 1989) Hematogenous dissemination occurs in LL. (Sabin et al., 2005) Leprosy diagnostic is based on the clinical characteristics, finding acid-fast bacilli (AFB) in slit skin smear, and histopathology. (Narang T et al., 2018) Hematoxylin and Eosin (H&E) and FF stains are the chosen method for AFB staining. The other study reported PAS and GMS stains could stain *Mycobacteria* besides the previously known main staining for fungal. Nevertheless, there is still limited literature concerning the results of these stains when applied to other *Mycobacteria* species (Csillag, 1960; Wright et al., 2017; Schinietz et al., 2018). We have recently highlighted the AFB positive result by using PAS and GMS stains in the case of an infant's LL. Hence, the use of these stains may be considered to diagnose leprosy.  

2 CASE  
A six-month-old baby boy was consulted from the Pediatric Department, on October 2018, with
complaints of erythematous collarette fine-scale papules, plaque, and slightly nodules on the face, chest, abdomen, arms, legs, buttocks and soles since three months ago. The lesion was not accompanied by pain. He was not seen scratching the lesion. Alloamnesis with the patient’s mother, he had a recurrent fever before the lesion appeared on the skin. Pityriasis lichenoides chronica, atypical pityriasis rosea, and psoriasis Vulgaris were initially chosen to become the differential diagnosis based on alloamnesis and physical examination.

Skin biopsy was performed on November 2018 with a histopathological picture of granulomas with numerous foamy cells in the lower dermis. There were many vacuoles containing slightly grey-basophilic colored material, including polymorphonuclear (PMN), extravasation of red blood cells, thickened vessel walls, and visible granulomas surrounded nerves structures, suggesting the diagnosis of lepromatous leprosy. Additional staining using the FF, showed numerous AFB, mostly in the solid forms. Periodic acid-Schiff and GMS were also performed, gave a positive result for bacilli inside the cytoplasm of foamy cells. Acid-fast bacilli finding in histopathology was followed by slit skin smears examination. This was obtained positive on both ears, with 4/6 bacterial index and 0% morphological index.

The patient was diagnosed with Severe Combined Immune Deficiency (SCID) and malnourished by the Pediatric Department since he was five months old. He was the second child of two siblings, with full-term delivery. He obtained incomplete immunization. He lives in one house together with his father, mother, mother’s sibling, and grandmother.

Figure 1. Erythematous to hyperpigmented papule-plaque-nodule with collarette scale in November 2018

Figure 2. Positive AFB on both ear lobes was revealed from slit skin smear

Figure 3. Histopathology, H&E staining, 100x, granuloma in the lower dermis
3 DISCUSSION

Children or infants have a four-fold risk of developing leprosy in the presence of leprosy close contact in the neighborhood, and this risk increases to nine-fold if there is a contact with leprosy patient within the household. (Jain et al., 2014) The occurrence of leprosy and its complication are related to changes in the host’s immune response. Individuals who are immunosuppressed could be more at risk of developing leprosy. (Massone, 2012) Individuals with sufficient exposure to *M. leprae* may develop a broad range of clinical manifestations. The bacillary proliferation and hematogenous dissemination occur in LL. (Sabin et al., 2005).

Initially, the cutaneous lesion was confusing with the other dermatological ailments. Several differential diagnoses were chosen, but a primary diagnosis was not established yet. The clinical findings were never considered as leprosy, hence the skin biopsy was done to obtain a definite diagnosis. Papules, plaque, nodules skin lesions did not appear as a typical lesion of leprosy, therefore slit skin smear examination was done considering this infant was not able having the nerve examination. The result did not show globi as in the histopathology. The number of AFB should be in the same quantity as in the smear according to the bacterial index of granuloma, such as +6 shows many clumps globi (>1000 bacilli). (Massone, 2012). Possibly because of the incision was not deep enough, since the granuloma is in the deep dermis.

Leprosy diagnostic has generally been performed based on a cardinal sign of clinical criteria (anesthetic skin lesions, enlarged or thickened peripheral nerves) and the presence of AFB from tissue smears stained by modified Ziehl-Neelsen staining. These great varieties of presentations in infants made the diagnosis of leprosy as a challenge. Histopathology is the gold standard to diagnose leprosy, particularly in an early stage of leprosy. The infant’s histopathology determined LL, in accordance with nodular granuloma in the dermis, macrophage cytoplasm were loaded with bacilli or globi with large quantities of lipids, which on staining give the appearance of foamy cells (Virchow cells), and the infiltrate was separated from the epidermis by collagenous fiber bands (Unna band) giving the appearance of subepidermal clear zone (Narang T et al., 2018; Weedon, 2012). In a systematic review examined 24 children histopathologically, the majority showed characteristic features of BT leprosy (19/24), three were BL leprosy, and two were indeterminate leprosy. (Jain et al., 2014). Histological confirmation is mandatory in all cases of leprosy because the gold standard to diagnose leprosy is the identification of *M. leprae* bacilli using H&E and FF stains. The gold standard to diagnose leprosy is strictly related to three significant points such as well done and deep, 6 mm punch biopsy performed at the right site of the lesion, well-cut, stained H&E and FF slides, also detailed clinical information. (Massone, 2012; Xavier Jnior JCC et al., 2016)

Job-Chacko modified FF staining for *M. leprae* enables the better chance of detecting the bacteria in
the early leprosy granuloma. Histopathology, carried out by FF staining, displays the sensitivity of 74.6% and positive predictive value of 85.9% and negative predictive value of 56.7%. This study shows that diagnosis was revealed with FF staining, better than other stain. (Reja AHH et al., 2013) Based on authors experience and other literature, FF staining is often negative even in multibacillary leprosy. (Adiga et al., 2013; Joshi, 2014)

*Mycobacteria* is challenging to demonstrate by the Gram technique as they possess a capsule containing a long-chain fatty acid (mycolic acid) which makes them hydrophobic. *Mycobacteria* are PAS-positive due to the carbohydrate content of their cell walls. However, this positivity is evident only when the large concentration of the microorganisms are present. When these organisms die, they lose their fatty capsule and consequently their carbol fuchsin positivity. The carbohydrate can still be stained by GMS reaction, which may prove useful when acid-fast procedures fail, particularly if the patient is already receiving therapy. These organisms are acid- and alcohol-fast but are usually easily identified as contaminants by their appearance as clumps, or floaters. (Morris GB et al., 2015)

Although GMS and PAS often used to identify fungal infection, it is important to recognize their other function of non-fungal staining in order to obtain the correct diagnosis and guide appropriate clinical management. Identified non-fungal organisms, which are stained with GMS, include parasitic worms, virally infected cells, acid-fast bacilli, partially acid-fast bacilli, and non-acid-fast bacteria. Gomori methenamine-silver and PAS stains have also been reported to stain *M. leprae*. (Xavier Junior JCC et al., 2013) In this case report, GMS and PAS were useful to stain the bacilli inside the foamy cells. Wright reported three of the nine cases, which also revealed as non-fungal using GMS and PAS stains. Organisms were not clinically suspected or the number of organisms was sparse and having any difficulty to visualize with routine staining methods. (Csillag, 1960). The other study also reported the prominent PAS and GMS positivity in a case of cutaneous *Mycobacterium avium* complex (MAC) infection, and organisms show the higher intensity of staining with GMS compared to routine AFB staining. Strong staining of *M. tuberculosis* and *M. leprae* has been reported with GMS with a modified silver stain. (Periodic acid-Schiff method selectively stains some carbohydrates and carbohydrate compounds such as glycogen, starch, mucin, and chitin. Csillag also reported PAS-positive material, in all strain of *Mycobacteria*. PAS-positive smears often contain clearly distinguishable vacuolated cells, though these might only be present in small numbers. The reaction was strong and the material colored intensely red, although the study did not include *M. leprae*. In the challenging cases, as in this case, clinical manifestation showed some variety of unusual cutaneous lesion in an infant with immunocompromised. Leprosy was initially unexpected as the differential diagnosis. (Csillag, 1960).

4 CONCLUSION

In children, leprosy is dominated by borderline type to tuberculoid type leprosy. Unusual case leprosy in infant showed atypical cutaneous lesion. Hence leprosy was not an unexpected differential diagnosis. As seen in this case, the diagnosis of lepromatous leprosy was established by histopathological examination. Leprosy could be diagnosed by slit skin smear and histopathology due to the difficulty in conducting the sensitivity test, primarily in children under ten years of age, and the need for knowing other dermatoses commonly found in childhood as the differential diagnosis. This case was solved through the histological analyses using leprosy routine stains and also alternative stains. This case report showed that GMS and PAS were also considered choices to stain AFB in leprosy besides H&E and FF. Although GMS and PAS are not specific for AFB, it can stain bacteria inside the cytoplasm of foamy cells in leprosy.

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