

## A Retrospective Study of the Significance of Ziehl-Neelsen Staining Technique for the Diagnosis of Hansen's Disease in a Low Cost Setting

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### Abstract

Hansen's disease otherwise known as Leprosy is an ancient disease that is tropically neglected. It's manifestation is often tied to the anger of the god's especially in the underdeveloped and developing countries such as Nigeria. The disease is caused by the aerosolized bacilli of *Mycobacterium leprae* when breathed. It can easily be contacted by people of all ages with weakened immune system. Two hundred and two patients accessing care in University of Port Harcourt Teaching Hospital (UPTH) Choba in Obio-Akpor Local Government Area of Rivers State were included in the study. Slit Skin samples were collected from noodles on the left and right ear, right and left legs, left and right hands, thighs and back and stained with Ziehl-Neelsen stain. Age group between 31 - 45 years was statistically significant  $X^2 = 18.28$ ,  $p = 0.001$  but there was no statistically significant association observed between respondents sex and the prevalence of Hansen's disease ( $X^2 = 0.00$ ;  $p = 0.991$ ). Degree of presence of *M. leprae* was statistically significantly higher for Back in Exact No, 37.21% ( $X^2 = 17.60$ ;  $p = 0.001$ ), Toes in 2+, 30.23% ( $X^2 = 17.91$ ;  $p = 0.005$ ), and Ear Lobes in 3+, 23.26% ( $X^2 = 14.80$ ;  $p = 0.002$ ). No statistically significant association was observed between respondents site of Hansen's disease and 1+ degree of the presence of *M. leprae* ( $X^2 = 7.17$ ;  $p = 0.07$ ). Hansen's disease is a threat and disability to human life. Creation of awareness, community education, recruiting and training of volunteer community workers, prompt diagnosis and treatment will reduce the prevalence and consequently eradicate the disease. This can be achieved by the provision of a strong political will.

**Keywords:** Hansen Disease; Ziehl-Neelsen Stain; Diagnosis; Skin Slit

### Introduction

Hansen's disease otherwise known as Leprosy is an ancient disease that is tropically neglected. It's manifestation is often tied to the anger of the god's especially in the underdeveloped and developing countries such as Nigeria. Most unfortunate conditions had befallen several innocent people who are deformed especially in their lower and upper limbs. Others are affected with black spots, noodles and inflammatory lesions on their faces, ear, back, thighs, and other parts of the body. Hansen's disease is caused by the aerosolized bacilli of *Mycobacterium leprae*. The disease can easily be contacted by people of all ages with weakened immune system. A special staining technique known as the Ziehl-Neelsen stain is used to identify the acid fast bacilli under the microscope. It is often misdiagnosed as a result of ignorance and lack of awareness. According to [1] 200,000 to 300,000 cases have been reported globally and in Nigeria [2] 2576 cases were reported. It is worrisome to discover that Nigeria is currently faced with this devastating health condition irrespective of the funding support given by several donors to combat HIV and Malaria.

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As concerned citizens continue to bother on how to control and totally eradicate the disease, there is lack of political will. In addition, stigmatization and superstition has rendered so many abandoned and homeless making the situation worse. Nevertheless, leprosy has gained attention like never before. The German Leprosy and Tuberculosis Relief Association in Nigeria that is committed to the disease has gained tremendous results in diagnosis and treatment. Notwithstanding, leprosy remains a disease of public health concern in Nigeria as those affected are constantly deprived of their rights in the society [3]. The broad aim of this current study is to show the significance of Ziehl-Neelsen staining technique for the diagnosis of Hansen's disease.

### Materials and Methods

#### Study Area

The study area was University of Port Harcourt Teaching Hospital (UPTH) Choba in Obio-Akpor Local Government Area of Rivers State. UPTH is a tertiary Institution that promotes routine Medical investigation, Training and Research.

#### Method of collection

Slit Skin samples were collected from noodles on the left and right ear, right and left legs, left and right hands, thighs and back. The affected areas were firstly swabbed with cotton wool soaked in 70% alcohol. Thereafter, a lancet was used to pinch the skin severally. The initial fluid emanating from the area was wiped off with cotton wool. The area was pressurized to allow fluids which were smeared on a grease free slide labeled with the patient's identification number. This same process was repeated on all affected sites previously mentioned. The slides were left to air dry before staining with Ziehl-Neelsen's staining technique.

#### Staining Process

The smears were covered with Carbol Fuchsin stain and heat fixed using a Bunsen burner under the slide. This was done until vapour began to rise. The stain was allowed to remain on the slide for five minutes and later washed off with water. Acid alcohol (1%) was used to decolorize the smear until no red colour was seen. Clean water was used to flood the slides which will wash off any remaining dye. Methylene blue was used as counter stain for 2 minutes which was thereafter washed off with water. The back side of the slides were wiped clean with a paper towel, placed in a draining rack for the slides to air dry.

#### Microscopic examination

The 100x oil immersion objective was used to examine the slides microscopically. The Acid fast bacilli (AFB) appeared red (hot pink) while Non-acid Fast bacilli (NAF) appeared colorless with a blue background.

#### Microscopic Grading

Grading was done according to the quantity of bacilli seen per 100 fields. When no AFB were seen after examining 100 fields, it was considered as No AFB Seen. AFB ranging from 1 - 9 was reported exactly as the actual number seen. Every 10- 99 AFB seen in 100 fields was reported as 1+. Every 1-10 AFB seen per field in at least 50 fields was reported as 2+. AFB seen above 10 per field in at least 20 fields was reported as 3+.

**Sample Size:** A total of 202 patients were screened for Hansen's disease over a period of two years who accessed health in the tertiary facility mentioned previously.

#### Research Methods

A retrospective study design was adopted to conduct this research. Data was collected from the Laboratory register of University of Port Harcourt Teaching Hospital from June 2016 to June 2018.

**Inclusion Criteria**

All patients sent to the Laboratory for diagnosis of Hansen’s disease were included in the study.

**Exclusion Criteria**

All patients without complete demographic history were not included in the study.

**Ethical Approval**

Ethical approval was obtained from the Ethical Committee of University of Port Harcourt Teaching Hospital.

**Results**

Age	AFB Microscopy } Freq (%) n = 202		Total	X <sup>2</sup> (p-value)
	Positive	Negative		
0 - 15	13 (10.07)	17 (23.28)	30 (14.85)	18.26 (0.001)*
16 - 30	36 (27.90)	9 (12.32)	45 (22.28)	
31 - 45	45 (34.88)	25 (34.24)	70 (34.65)	
46 - 60	24 (18.60)	7 (9.58)	31 (15.35)	
61 - 75	11 (8.52)	15 (20.54)	26 (12.87)	
Total	129	73	202	

**Table 1:** Prevalence of Hansen’s disease among respondent’s by age groups.

\*Statistically significant (p < 0.05).

Age	AFB Microscopy } Freq (%) n = 202		Total	X <sup>2</sup> (p-value)
	Positive	Negative		
Male	48 (37.21)	28 (38.36)	76 (37.62)	0.00 (0.991)
Female	81 (62.79)	45 (61.64)	126 (62.38)	
Total	129	73	202	

**Table 2:** Prevalence of Hansen’s disease among respondent’s by Sex.

No statistical significance.

Sites of Hansen’s Disease	Degree of presence of M. leprae				Total
	Exact No	1+	2+	3+	
Ear Lobes	23 (17.83)	40 (31.01)	36 (27.91)	30 (23.26)	129 (100.0)
Fingers	31 (24.03)	46 (35.66)	33 (25.58)	19 (14.73)	129 (100.0)
Toes	23 (17.83)	46 (35.66)	39 (30.23)	21 (16.28)	129 (100.0)
Back	48 (37.21)	60 (46.51)	13 (10.08)	8 (6.20)	129 (100.0)
X <sup>2</sup> (p-value)	17.60 (0.001)*	7.17 (0.07)	17.91 (0.005)*	14.80 (0.002)*	

**Table 3:** What is the distribution of the disease in the various sites?.

\*Statistically significant (p < 0.05).

## Discussion

The use of Ziehl-Neelsen staining technique for diagnosing Hansen's disease in our setting was very significant. The prevalence of the disease amongst respondents by age was studied. Age group between 31 - 45 years was statistically significant  $X^2 = 18.28$ ,  $p = 0.001$  (Table 1). This is somewhat different from the report given by Jennifer AT, *et al* [4]. In their report, across all age groups, the prevalence rate of leprosy was found to be higher in females than in males 0.82 versus 0.53 per 10,000. However, there was no statistically significant association observed between respondents sex and the prevalence of Hansen's disease ( $X^2 = 0.00$ ;  $p = 0.991$ ) in our study table 2. In Nigeria, the proximity in accessing health is mostly a challenge. People rather depend on self and traditional medication. It could be because of a default in knowledge and practice bearing in mind the perception of people about the disease. Ability to identify signs and symptoms of the disease by care givers is another outstanding challenge. Another reason is location of health facilities [5]. There is hardly a community based leprosy program to sensitize the public about the disease, mode of contact and possible treatment. Education is the best that can be offered to people especially concerning a prevailing health challenge that threatens their comfort. Health facilities are sited far away from the communities basically in the rural areas. Transportation to access health ultimately becomes a challenge no wonder the disease is linked to poverty. Today Polio is eradicated because of the war that was raged against it at the community level. We suppose that if a similar force is pulled against leprosy, it will suddenly become a thing of the past. Overcrowding could be another possible source for this disease. We consider it so because the mode of transmission is synonymous to tuberculosis where secretions from infected people that have been aerosolized are inhaled [6,7]. Maintaining personal hygiene is another promoter to healthy life. This notwithstanding, those affected by this disease are likely to be among them that are presented with this health challenge. In any case, cleanliness and decency in keeping a healthy lifestyle can be a barrier to disability and deformity [10].

Asymptomatic presentation of the signs and symptoms of the disease is another challenge for early diagnosis [8,9]. Clinicians mostly find it difficult to rule out Hansen's disease when the clinical signs are absent. It becomes imperative for a strong political will to train and retrain health workers to enable them identify the disease. We also considered the distribution of the bacilli at various sites. Degree of presence of *M. leprae* was statistically significantly higher for Back in Exact No, 37.21% ( $X^2 = 17.60$ ;  $p = 0.001$ ), Toes in 2+, 30.23% ( $X^2 = 17.91$ ;  $p = 0.005$ ), and Ear Lobes in 3+, 23.26% ( $X^2 = 14.80$ ;  $p = 0.002$ ). No statistically significant association was observed between respondents site of Hansen's disease and 1+ degree of the presence of *M. leprae* ( $X^2 = 7.17$ ;  $p = 0.07$ ). This is a direction to laboratory personnel to bear these sites in mind during skin slit collection. They should consider it a point of necessity to learn and get acquainted with the procedure for collection of skin slits because every step in the collection is key to proper diagnosis.

## Conclusion

Hansen's disease is a threat and disability to human life. Creation of awareness, community education, recruiting and training of volunteer community workers, prompt diagnosis and treatment will reduce the prevalence and consequently eradicate the disease. This can be achieved by the provision of a strong political will to enhance implementation.

## Conflict of Interest

There was no conflict of interest indicated by any of the contributing authors.

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