Fluorescence Microscopy for Detection of *M. leprae* in Tissue Sections [1]

Harshadrai J. Jariwala and Subhash S. Kelkar [2]

Fluorescence microscopy of material stained by Auramine-Rhodamine is a favored method for detecting *M. tuberculosis* in clinical specimens ([7]). Its superiority with Ziehl-Neelsen stained material is due to the contrast of fluorescent microorganisms shining brightly against a dark background. This makes it possible to screen for microorganisms even with a 20x objective, reduces observer fatigue and increases speed and accuracy. Moreover, observation at these low magnifications makes it possible to correlate the distribution of organisms *vis a vis* the inflammatory granuloma. Perhaps the only disadvantages are the cost of the equipment and the sophistication required in its correct use. Faulty optical alignment and improper use of the Koehler principle can lead to very poor illumination.

Leprosy is an important disease in socioeconomically backward countries. A reliable diagnosis hinges around demonstration of the microorganisms in clinical material. In 1952, Gohar ([3]) described the advantages of fluorescence microscopy for detecting *M. leprae* in smears. Kuper and May ([4]) introduced the combination of Auramine and Rhodamine. We were, however, surprised to find that there were hardly any reports on the use of fluorescence microscopy in the diagnosis of leprosy. Nerurkar and Khanolkar ([6]) also used it for smears. The last report was that of Locordaire ([5]) who found the fluorescence method to be very much inferior when compared with the Fite-Faraco method in detecting *M. leprae* in tissue sections.

We describe here our experiences with fluorescence microscopy for detecting *M. leprae* in tissue sections. Material was simultaneously studied by the Fite-Faraco method. Also the feasibility of fluorescence microscopy in determining the Bacterial Index and the Morphologic Index was assessed.

MATERIALS AND METHODS

Subjects. Fifty leprosy cases attending either the J. J. Hospitals or the Pushpa Vihar leprosy colony were studied. They were classified on the basis of clinical features, a biopsy, and the presence of acid-fast organisms according to the five-group system of Ridley and Jopling ([9]). Lepromin testing with armadillo lepromin was done in 22 cases. The cases covered as wide a range in variety and in duration of treatment as possible. A biopsy of an active lesion was obtained from each case and used in the study.

Fluorescence microscopy for M. leprae. Paraffin sections 5μm thick were cut from each tissue. Ribbons containing five serial sections were taken on clean glass slides. Neither egg albumin nor any other adhesive was used to fix the ribbon on the slide. Staining with Auramine and Rhodamine was as described by Kuper and May ([4]) with the following modifications: the staining period was extended to 20 minutes; the decolorizer was 0.5% HCl in 70% alcohol; dehydration after staining was with absolute alcohol, no other concentration being used; clearing in xylol was omitted and the sections were mounted in glycerol. Sections were examined immediately with a fluorescence microscope (PZO, Warzsawa) which had an HBO 50 high pressure mercury arc discharge. Excitation was with blue-violet rays obtained with two BG 12 primary filters and an Abbe condensor was used. An OG.1 barrier filter was placed in the eye piece. All five sections on the slide were carefully screened with a 20x objective. The color and morphology of fluorescing material was confirmed with a 40x objective. *M. leprae* appeared as rod-shaped, golden-yellow structures whose length was equal to or smaller than the diameter of a lymphocyte. All fluorescing objects not having this typical morphology were considered to be nonspecific. Sections showing acid-fast organisms were further examined with a 2 mm oil-im-

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[2] H. J. Jariwala, M.B., B.S., D.P.B., M.D., Lecturer in Microbiology; and S. S. Kelkar, M.B., B.S., M.D., Professor and Head, Department of Microbiology, The Grant Medical College, Byculla, Bombay 400 008, India. Correspondence should be addressed to Dr. Kelkar.

mersion lens and the Bacterial Index of the leproma determined ([8]). Further, the number of solidly fluorescing organisms was determined and the Morphologic Index calculated ([1]). At least 200 bacilli were scanned.

Conventional staining of tissue sections. A duplicate slide with five serial sections was stained by the modified Fite-Faraco method ([2]) and studied with a light microscope using a 2 mm oil-immersion lens. Acid-fast organisms were looked for and, if present, the Bacterial Index and the Morphologic Index determined.

RESULTS

Table 1 summarizes the results. The fluorescent method was more sensitive and detected acid-fast organisms in 22 cases while the Fite-Faraco method detected them in only 20 cases. These figures, however, do not indicate any statistically significant advantage of the former method. Table 2 compares the findings for the Bacterial Index and the Morphologic Index in the 20 cases in which microorganisms were detected by both methods. There was no difference in the Bacterial Index. The Morphologic Index, however, was much higher by the fluorescence method than by the Fite-Faraco method. Quantitatively the average of the Morphologic Index as determined by fluorescence microscopy was one and a half times higher than that determined by the Fite-Faraco method. Figure 1

TABLE 1. *Acid-fast bacilli in paraffin sections of biopsies by both fluorescence and the Fite-Faraco methods.*

Type of leprosy	No. of cases	Fluorescence positive	Acid-fast positive
Indeterminate	1	0	0
TT	10	0	0
BT	11	4	3
BL	4	4	4
LL	24	14	13
Total	50	22	20

TABLE 2. *Bacterial and Morphologic Indices as determined by fluorescence microscopy and the Fite-Faraco method. All 20 cases positive by both methods are listed.*

No.	Type of leprosy	Bacterial Index		Morphologic Index	
		Fluorescence	Fite-Faraco	Fluorescence	Fite-Faraco
1	BT	1+	1+	19	26
2	BT	1+	1+	8	14
3	BT	1+	1+	42	51
4	BL	4+	4+	0	0
5	BL	5+	5+	8	30
6	BL	2+	2+	0	5
7	BL	6+	6+	10	10
8	LL	2+	2+	0	0
9	LL	2+	2+	0	0
10	LL	3+	3+	0	0
11	LL	6+	6+	?[a]	?[a]
12	LL	3+	3+	4	9
13	LL	5+	5+	39	48
14	LL	5+	5+	0	5
15	LL	5+	5+	18	28
16	LL	5+	5+	0	0
17	LL	3+	3+	0	2
18	LL	6+	6+	5	10
19	LL	5+	5+	28	32
20	LL	6+	6+	?[a]	?[a]
			Mean	10	15

[a]Morphologic Index could not be determined because the clumping and density of the organisms was so great that individual density of staining could not be made out.

illustrates the appearance of acid-fast microorganisms under the oil-immersion lens by the fluorescence method.

DISCUSSION

The fluorescence method was superior to the Fite-Faraco method in detecting acid-fast bacilli in paraffin sections of biopsies of patients with leprosy. Both the cases in which organisms were detected only by fluorescence microscopy had very few organisms. Of the five serial sections taken on one slide, only three showed organisms and even these numbered only two or three in the whole section. It is possible, therefore, that the sections stained by the Fite-Faraco method did not contain any acid-fast organisms. The other possibility is that the fluorescence method is more sensitive. It appeared to be useful in those sections where the number of microorganisms was very few. This is a situation which is very important from the clinical point of view. Some differences in sensitivity of the two methods is apparent from the Morphologic Index of the organisms as obtained by the two staining methods. Fluorescence microscopy revealed more organisms with uniform staining and the Morphologic Index, on an average, was one and a half times higher than that obtained by the conventional method.

FIG. 1. Skin biopsy from lepromatous leprosy having a Bacterial Index of 5+ stained by Auramine-Rhodamine. The majority of organisms are stumpy rods and show irregular staining. Even with this method the Morphologic Index could be assessed (fluorescence, oil-immersion, × 1,000).

The ease and speed of observation with the fluorescence method should be emphasized. The organisms stand out and because of the 20x objective, the field covered is 16 times larger than that seen by the oil-immersion lens. An average section can, therefore, be scanned in two or three minutes. This is an important practical advantage. In all laboratories, tedious microscopic work devolves on the lowest cadre of technician who often fails to do justice to the task. Fluorescence microscopy reduces the tedium and makes it more likely that the task will be faithfully carried out. Moreover, because of the low magnification it is possible to orient the location of organisms with respect to the lesion and the faintly outlined adenexal and vascular structures in the tissues. Lastly, both the Bacterial Index and the Morphologic Index can be determined without much difficulty. It takes a little time to form impressions of organisms with uniform or irregular fluorescence.

Some disadvantages of the fluorescence method may be mentioned. There was a lot of fading and the sections had to be examined on the same day. Permanent preparations were not possible. A dark room is essential. Proper application of the Koehler system of illumination and optical alignment is essential. Locordaire ([5]) found the fluorescence method to be very inferior to the Fite-Faraco method in the detection of *M. leprae* in tissue sections. In 30 biopsies as many as 26 were positive by the Fite-Faraco method and only 10 by the fluorescence method. This author found artifacts due to phenol to be a serious problem. We did not have this problem; artifacts were few and could be easily differentiated by their buffy color as contrasted with the golden-yellow glow of the bacteria.

The staining time used in the present study was more than that described by Kuper and May ([4]). This was determined by trial and error. Albumin was not used to fix the section because this did induce artifacts ([5]). Mounting in glycerol was much better than mount-

ing in "Deepex" which reduced fluorescence considerably.

SUMMARY

The fluorescence method was compared with the Fite-Faraco method for detecting acid-fast microorganisms in paraffin sections of cases of leprosy. Biopsies were obtained from 50 cases of leprosy covering all varieties and at varying stages of treatment. The fluorescence method was better than the Fite-Faraco method; 22 biopsies showing acid-fast organisms in fluorescence microscopy and 20 in the Fite-Faraco method. Its superiority was evidenced in two cases in which the organisms were very scanty. Fluorescence microscopy can also be used to determine the Bacterial Index and the Morphologic Index of organisms. The Morphologic Index, however, was one and a half times higher than that obtained by the Fite-Faraco technic. The ease and speed of fluorescence microscopy appear to be a great advantage.

RESUMEN

Se comparó un método de fluorescencia con el método de Fite-Faraco para detectar microorganismos ácido-resistentes en cortes de biopsias incluidas en parafina obtenidas de pacientes con lepra. Las biopsias se obtuvieron de 50 casos de lepra que incluían todas las variedades y diferentes estados del tratamiento. El método fluorescente fue mejor que el de Fite-Faraco; 22 biopsias mostraron organismos ácido-resistentes por microscopía de fluorescencia y 20 por el método de Fite-Faraco. Su superioridad fue evidente en 2 casos en los cuales los organismos fueron muy escasos. La microscopía de fluorescencia puede usarse también para determinar los Índices Bacteriológico y Morfológico de los microorganismos. El I.M., sin embargo, fue 1.5 veces mayor que el obtenido por la técnica de Fite-Faraco. La facilidad y rapidez de la microscopía de fluorescencia, parecen ser grandes ventajas.

RÉSUMÉ

On a comparé la méthode à la fluorescence avec la méthode de Fite-Faraco pour la détection de micro-organismes acido-résistants dans des coupes parafinées provenant de malades de la lèpre. On a récolté 50 biopsies provenant de cas de lèpre, couvrant toutes les variétés de la maladie et les différentes étapes du traitement. La méthode à la fluorescence est meilleure que la méthode de Fite-Faraco; 22 biopsies montraient des organismes acido-résistant à la microscopie fluorescente, et 20 seulement par la méthode de Fite-Faraco. Cette supériorité était particulièrement évidente dans 2 cas où les organismes étaient très rares. La microscopie à fluorescence peut également être utilisée pour déterminer l'Index Bactérien et l'Index Morphologique des organismes. L'Index Morphologique, néanmoins, était une fois et demie plus élevé par la méthode à la fluorescence que par la technique de Fite-Faraco. La facilité et la rapidité de la microscopie à fluorescence se révèle très avantageuse.

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